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INGRID CONCEIÇÃO DANTAS GUERRA

EFICÁCIA DE REVESTIMENTOS A BASE DE QUITOSANA E ÓLEOS ESSENCIAIS DE *MENTHA* SPP. NO CONTROLE DE FUNGOS PATÓGENOS PÓS-COLHEITA EM FRUTOS

RECIFE

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Tese apresentada ao Programa de Pós-Graduação em Nutrição do Centro de Ciências da Saúde, da Universidade Federal de Pernambuco para obtenção ao grau de Doutor em Nutrição.
Área de Concentração: Ciência de Alimentos.

Orientador: Prof. Dr. Evandro Leite de Souza

Co-Orientadora: Profª Dra. Marta Suely Madruga

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RECIFE

2015

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Louis Pasteur

RESUMO

Frutas são ricas em vitaminas, minerais, fibras e outros compostos que trazem benefícios à saúde. O conhecimento desses benefícios tem proporcionado aumento no consumo nos últimos anos, pois, além do interesse em fitoconstituintes os consumidores têm buscado produtos de elevada qualidade e seguros para o consumo. Devido à alta perecibilidade da matéria-prima e às falhas ocorridas nas diferentes fases da cadeia pós-colheita, grande parte dos vegetais produzidos no Brasil são desperdiçados. Dentre os diversos fatores envolvidos as doenças ocasionadas por fungos fitopatógenos se destacam. O controle de doenças fúngicas pós-colheita é comumente realizado através da aplicação de fungicidas, de elevado custo de produção e apresentados como ameaça à saúde pública e ao meio ambiente. Tendo em vista a problemática apresentada, no presente estudo foi avaliada a eficácia de revestimentos elaborados com quitosana (QUI) e óleos essenciais de *Mentha piperita* L. (MPOE) ou *Mentha x villosa* Huds (MVOE) como alternativa para o controle de infecções causadas pelos fungos *Aspergillus niger, Botrytis cinerea, Penicillium expansum* e *Rhizopus stolonifer* em tomates cereja e uvas de mesa durante o armazenamento em temperatura ambiente e em baixas temperaturas. A quitosana foi obtida da carapaça do camarão *Litopenaus vannamei,* em meio alcalino. Os óleos essenciais foram obtidos por arraste de vapor. Para o preparo do revestimento, o polímero de quitosana foi diluído em ácido acético sob agitação por 6 horas, seguido da adição do óleo e agitação por mais 18 horas em presença de glicerol como agente dispersante. As Concentrações Inibitórias Mínima da quitosana e de cada óleo foram determinadas por meio da macrodiluição em caldo. Os efeitos dos revestimentos sobre as características fúngicas (crescimento micelial radial e germinação espórica), físico-químicas (perda de peso, firmeza, cor, acidez e sólidos solúveis) e sensoriais (aceitação e intenção de compra) dos frutos durante a armazenagem também foram avaliados. A concentração inibitória mínima (CIM) de QUI contra todos os fungos-teste foi de 8 mg / mL, ao passo que a CIM para ambos MPOE e MVOE foi de 5 µL / mL. Combinações de QUI (8 e 4 mg/mL) e MPOE (QUI-MPOE) ou (QUI-MVOE) (5, 2,5 e 1,25 µL/mL) inibiu fortemente a germinação de esporos e o crescimento micelial dos fungos estudados. Os revestimentos compostos de QUI-MPOE ou QUI-MVOE retardaram o crescimento dos fungos causadores de mofos ensaiados em frutos artificialmente infectados durante a armazenagem em temperatura ambiente (12 dias) ou em baixa temperatura (24 dias). Os revestimentos ensaiados também preservaram a qualidade de frutos de tomate cereja e uvas de mesa durante a armazenagem, em termos de características físico-químicas e sensoriais. Nas uvas de mesa, houve uma melhoria nos valores de firmeza, cor L* e cor h* indicando frutos mais brilhosos e um possível retardo no desenvolvimento do "*browning*" das uvas revestidas com QUI-MPOE ou QUI-MVOE em relação aos frutos controle. Estes resultados indicam que os revestimentos que compreendem CHI-MPOE ou CHI-MVOE, apresentam-se como alternativa promissora para inibir a infecção de fungos pós-colheita em tomate cereja e uvas de mesa durante o armazenamento sem afetar a qualidade desses frutos.

Palavras chave: *Mentha*. Revestimento comestível. Tomate cereja. Uvas de mesa. Fungos fitopatogênicos. Tratamento pós-colheita.

ABSTRACT

Fruits are rich in vitamins, minerals, fiber and other compounds that provide health benefits. Knowledge of these benefits has provided an increase in vegetable consumption in recent years because in addition to the interest in phytochemicals that benefit the health and nutritional value, consumers have sought high quality products and safe for consumption. Due to the high perishability of the raw material and the failures occurred in different stages of post-harvest chain, most of the vegetables produced in Brazil are wasted. Among the many factors involved diseases caused by fungi pathogens in fruits stand out, they result in significant economic losses. Control of post-harvest fungal diseases commonly is achieved through the application of fungicides, increase the cost of production and presented to public health and the environment. In view of the problems presented, in the present study we evaluated the efficacy comprising shrimp chitosan (CHI) and *Mentha piperita* L. (MPEO) or *Mentha x villosa* Huds (MVEO) essential oils as an alternative for the control mold infections caused by *Aspergillus niger*, *Botrytis cinerea*, *Penicillium expansum* and *Rhizopus stolonifer* of cherry tomatoes and table grapes during storage at room temperature and low temperatures. Chitosan was obtained from the shell of the shrimp *Litopenaeus vannamei* in alkaline medium. The essential oils were obtained by vapor dragging. To prepare the coating, the polymer chitosan was diluted acetic acid with stirring for 6 hours, followed by addition of the oil and shaken for 18 more hours in the presence of glycerol as a dispersing agent. The Minimum Inhibitory Concentrations in chitosan and each oil were determined by the broth macrodilution. The effects of the coatings on the fungal characteristics (radial mycelial growth and esporic germination), physico-chemical (weight loss, firmness, color, acidity and soluble solids) and sensory (acceptance and purchase intention) of the fruit during storage were also evaluated. The minimum inhibitory concentration (MIC) of CHI against all the test fungi were 8 mg / mL, whereas the MIC for both MPOE and MVOE was 5 uL / mL. Combinations CHI (8 and 4 mg / mL) and MPOE (CHI-MPOE) or (CHI-MVOE) (5, 2.5 and 1.25 uL / mL) strongly inhibited spore germination and mycelial growth of of target fungi. The coatings comprising CHI-MPEO or CHI-MVEO delayed the growth of mold-causing fungi in artificially infected fruits during storage at either room (12 days) or low temperatures (24 days). The assayed coatings preserved the quality of cherry tomato fruits and table grapes during storage, in terms of physical, physicochemical and sensory attributes. In table grapes, there was an improvement in the firmness values, color L * and h * color indicating more glossy fruit and a possible delay in the development of "browning" of grapes coated with CHI-CHI-MPOE or MVOE compared to control fruits. These results indicate that coatings comprising CHI-MPEO or CHI-MVEO represent promising post-harvest treatments to inhibit common postharvest mold infections in cherry tomato fruits and in table grapes during storage without affecting the quality of these fruits.

Key words: *Mentha*. Edible coating. Cherry tomato. Table grapes. Phytopathogenic fungi. Post-harvest treatment.

LISTA DE ILUSTRAÇÕES

LISTA DE TABELAS

ARTIGO 1

ARTIGO 2

SUMÁRIO

1 APRESENTAÇÃO

Com uma área plantada de aproximadamente 1,9 milhões de hectares e produção de 40 milhões de toneladas ao ano, o Brasil ocupa a terceira posição em produção de frutos no mundo (FAO, 2014). Apesar da produção elevada, um dos aspectos que ainda precisam ser enfrentados na cadeia de produção e comercialização de frutos no país, consiste na diminuição das perdas nas fases de produção, colheita, embalagem, transporte e pós-colheita, as quais podem chegar à ordem de 20 a 50% em algumas variedades como o tomate cereja (*Solanum lycopersicum* L.) e a uva de mesa (*Vitis labrusca* L.). (RODRIGUES, 2009).

Tanto o tomate cereja quanto a uva de mesa são frutos bem aceitos por serem excelentes fontes de fitoquímicos e por sua destacada capacidade antioxidante resultando em potenciais benefícios a saúde (PINHEIRO et al., 2013; KRIKORIAN et al., 2012; CADEZ, ZUPAN, RASPOR, 2010; MERÍN et al, 2014). Nestes frutos, consideráveis perdas pós-colheita com consequente diminuição da qualidade de mercado são causadas em decorrência da ação de fungos fitopatógenos. O ataque destes microrganismos aos frutos resulta na ocorrência de doenças superficiais, destruição dos tecidos, resultando em redução da sua qualidade e da vida de prateleira, tornando-os menos atrativos ou não comercializáveis (MOSS, 2002).

Entre os fungos fitopatógenos mais comuns responsáveis pela deterioração pós-colheita em tomate e uva em todo o mundo estão o *Botrytis cinerea, Penicillium expansum, Aspergillus niger* e *Rhizopus stolonifer* (FAGUNDES et al, 2014;. LIU et al, 2007; WANG et al, 2010; ABDOLAHI et al, 2010;. SANTOS et al, 2012;. SOUSA et al, 2013;. DO et al., 2015). A principal forma de controle da ação destes fungos no meio agrícola é a utilização de fungicidas sintéticos. Com efeito, fungicidas são aplicados em frutas durante o período pós-colheita, e estes produtos não afetam adversamente a aparência ou a qualidade dos frutos tratados (AMIRI et al., 2008). No entanto, o uso indiscriminado e excessivo de fungicidas sintéticos em culturas tem sido uma das principais causas para o desenvolvimento de populações resistentes a agentes patogênicos fúngicos, fazendo com que seja necessária a utilização de maiores quantidades de antifúngicos na agricultura e, por conseguinte, a detecção de quantidades aumentadas de resíduos tóxicos nos produtos alimentares (CABRAL, PINTO, PATRIARCA, 2013).

Nos últimos anos, as preferências dos consumidores têm se direcionado para os alimentos que contêm níveis mais baixos de conservantes químicos e exibam características mais frescas e naturais. Assim, os pesquisadores têm buscado alternativas mais seguras para reduzir o uso de

fungicidas sintéticos em frutas e vegetais (LIU et al., 2007; OLIVEIRA et al., 2014; SANTOS et al., 2012).

Revestimentos comestíveis são considerados uma tecnologia ambientalmente correta para estender a vida de prateleira de frutas e legumes (FAGUNDES et al., 2014). Em tomates e uvas, a aplicação de revestimentos comestíveis antifúngicos tem-se centrado principalmente na utilização de formulações à base de quitosana. Os revestimentos baseados em quitosana foram eficazes para controlar doença fúngica causada por *Alternaria alternata*, *Colletotrichum* spp.; *B. cinérea* e *P. expansum* (REDDY et al., 2000; MUÑOZ e GARCÉS, 2009; BADAWY e RABEA, 2009; LIU et al, 2007). Também têm sido realizados testes com revestimentos onde se utiliza a quitosana em combinação com outras substâncias anti-microbianas não-químicas, tais como extratos de plantas, óleos essenciais e compostos fenólicos (ELSABEE e ABDOU, 2013). Dentre essas substâncias os óleos essenciais têm recebido muita atenção como inibidores de uma vasta variedade de fungos fitopatogênicos e são geralmente reconhecidos como seguros para as doses normalmente usadas em alimentos (BURT et al., 2004).

Óleos essenciais de diversas espécies têm sido utilizados na indústria de alimentos devido ao seu potencial antimicrobiano e antioxidante, com destaque para os advindos das espécies do gênero *Mentha* (TYAGI e MALIK, 2011; RIAHI et al, 2013). Dentre suas diversas espécies o óleo da *Mentha piperita* e da *Mentha x villosa* Hudson tem apresentado destacado efeito na inibição de fungos pós-colheita isoladamente (LIMA et al, 2014; RIAHI et al, 2013).

A aplicação de quitosana e alguns óleos essenciais provaram ser eficazes no controle de infecções de fungos em uvas de mesa (SANTOS et al, 2012;. SOUSA et al, 2013; OLIVEIRA et al, 2014). No entanto, os estudos de verificação da eficiência da incorporação de óleo essencial de *Mentha piperita* e de da *Mentha x villosa* Hudson em revestimentos de quitosana para controlar infecções causadas por fungos patogênicos pós-colheita são escassos. Assim, o objetivo do presente estudo foi avaliar a eficácia da aplicação combinada de quitosana e do óleo essencial de *Mentha piperita* e da *Mentha x villosa* Hudson como tratamento pós-colheita para evitar infecções fúngicas causadas por *A. niger, B. cinerea, P. expansum* e *R. stolonifer* em frutos de tomate cereja e uvas de mesa e verificar os efeitos dos revestimentos testados sobre as características físicas, físico-químicas e sensoriais dos frutos-teste.

2 PERGUNTAS CONDUTORAS E HIPÓTESE

2.1 PERGUNTAS CONDUTORAS

O revestimento composto de quitosana extraída de camarão *Litopenaeus vannamei* e óleo essencial de *Mentha piperita* L. ou de *Mentha x villosa* Huds em concentrações inibitórias e subinibitórias apresenta ação antifúngica frente a *A. niger*, *B. cinerea*, *P. expansum* e *R. stolonifer* em meio sintético (ensaios *in vitro*) e em tomates cereja (*Solanum lycopersicum* L.) e uvas de mesa (*Vitis labrusca* L.) contaminadas *in vitro*?

A aplicação do revestimento composto de quitosana e óleo essencial de *Mentha piperita* L. ou de *Mentha x villosa* Huds em concentrações inibitórias e subinibitórias mantém ou melhoram aspectos de qualidade física, físico-química e sensorial de tomates cereja (*Solanum lycopersicum* L.) e uvas de mesa (*Vitis labrusca* L.) durante o armazenamento em temperatura ambiente e de resfriamento?

2.2 HIPÓTESE

O revestimento de quitosana adicionado de óleo essencial de *Mentha piperita* L. ou de *Mentha x villosa* Huds em concentrações inibitórias e subinibitórias apresenta efetividade no controle do crescimento dos fungos patógenos pós-colheita como *A. niger*, *B. cinerea*, *P. expansum* e *R. stolonifer* em meio sintético e quando aplicados em frutos de tomates cereja (*Solanum lycopersicum* L.) e uvas de mesa (*Vitis labrusca* L.), assegurando a manutenção ou melhora dos seus aspectos de qualidade física, físico-química e sensorial quando armazenados em temperatura ambiente e de resfriamento.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Avaliar a eficácia da aplicação dos revestimentos compostos por quitosana e do óleo essencial de *Mentha piperita* L. ou de *Mentha x villosa* Huds no controle de infecções causadas por fungos patógenos pós-colheita em tomates (*Solanum lycopersicum* L.) e uvas de mesa (*Vitis labrusca* L.), bem como, os efeitos desta aplicação sobre a qualidade destes frutos.

3.2 OBJETIVOS ESPECÍFICOS

- Produzir e caracterizar a quitosana obtida do resíduo industrial (carapaça) do camarão *Litopenaus vannamei*;
- Determinar a Concentração Inibitória Mínima - CIM da quitosana e de óleos essenciais de *Mentha piperita* L. e de *Mentha x villosa* Huds sobre cepas de *A. niger, B. cinerea, P. expansum* e *R. stolonifer.*
- Avaliar a eficácia da aplicação combinada da quitosana e dos óleos essenciais de *Mentha piperita* L. ou de *Mentha x villosa* Huds sobre o crescimento micelial e germinação de esporos fúngicos;
- Verificar o efeito da aplicação do revestimento no controle de infecções causadas pelos fungos-teste em tomates cereja (*Solanum lycopersicum* L.) e uvas de mesa (*Vitis labrusca* L.);
- Analisar o efeito da aplicação dos revestimentos sobre os parâmetros físicos, físico-químicos e sensoriais dos frutos durante o armazenamento em temperatura ambiente e de resfriamento.

4 REFERENCIAL TEÓRICO

4.1 QUALIDADE PÓS-COLHEITA DE TOMATE CEREJA

O tomateiro é uma dicotiledônea, pertencente à ordem Tubiflorae, família *Solanaceae*, gênero Solanum e subgênero Eulycopersicon, (ALVARENGA, 2004). A grande variabilidade existente no gênero *Lycopersicon* tem possibilitado o desenvolvimento de cultivares para atender as mais diversas demandas do mercado de tomate, seja para o processamento ou para o consumo *in natura* (GIORDANO et al., 2000). Os frutos de tomate tipo "cereja"(*Lycopersicon esculentum* var. *Cerasiforme* ou *Solanum lycopersicum L.*) está entre a gama de espécies de tomates cultivados e consumidos no Brasil, tendo crescido em importância nos mercados das grandes cidades. Tem forma redonda, periforme ou ovalada, com uma coloração que varia entre amarelo e vermelho, com peso variando de 5 a 30g (FILGUEIRA, 2000). Este tipo de tomate é utilizado *in natura* como adorno, aperitivo e na confecção de pratos diversos, por serem pequenos e delicados, no qual trazem novos sabores e decoração dos pratos (MACHADO et al., 2003).

A cultura do tomateiro para mesa ou para consumo *in natura* tem sido uma importante fonte de emprego ao longo de toda sua cadeia produtiva, representando uma oferta de serviços para cerca de 300 mil pessoas (ABCSEM, 2009). Com um crescimento acima da média mundial, o Brasil é atualmente o oitavo maior produtor de tomate do mundo (FAO, 2014). Dados recentes sobre a produtividade brasileira apontam valores de produção de cerca de cinquenta e nove toneladas por hectare, embora alguns produtores mais tecnicamente qualificados tenham alcançado rendimento superior a 100 toneladas por hectare. O custo de produção do tomate é um dos maiores em toda atividade agrícola e apresenta grande variabilidade, ficando entre R$ 30 mil e R$ 55 mil por cada hectare plantado, dependendo do pacote tecnológico utilizado (ABCSEM, 2009).

O tomate cereja é caracterizado, principalmente, por suas propriedades sensoriais, pelo excelente sabor e pela atrativa coloração vermelha e uniforme (ROCHA et al., 2009). Apresenta em sua composição baixo teor de massa seca, baixo valor calórico, alto conteúdo de vitamina C e cálcio, sendo boa fonte de ácido fólico. Além destes, o tomate contém outros componentes como vitamina E, vitamina K e flavonóides (BALDWIN et al., 1998). A composição de cada componente pode se alterar devido a alguns fatores como, origem genética, grau de amadurecimento, data de colheita, condições do solo e comdições climáticas (ALVARENGA, 2004).

Após a colheita, o tomate apresenta-se como um fruto altamente perecível, pois apresenta elevado conteúdo de água, estando sujeito às variações de temperatura e umidade relativa do ambiente onde se encontra. A perda de água ocasiona perda de massa e danifica a aparência do fruto (CHIUMARELLI e FERREIRA, 2006). Por sua elevada perecibilidade as perdas pós-colheitas chegam a 21%.

Em tomates cereja as principais doenças pós-colheita são ocasionadas por diferentes fungos patogênicos, resultando em perdas econômicas importantes. *Botrytis cinerea, Penicillium expansum, Aspergillus niger* e *Rhizopus stolonifer* causam infecções, denominadas cinza, azul, preto e podridão-mole, respectivamente. Estes fungos estão entre os patógenos mais comuns responsáveis pela deterioração pós-colheita em frutos de tomate em todo o mundo (FAGUNDES et al, 2014;. LIU et al, 2007; WANG et al, 2010; ABDOLAHI et al, 2010).

No intuito de proteger o fruto da ação dos microorganismos, medidas de controle são tomadas visando controlar a ocorrência destes patógenos que causam perdas e oneram a produção. Tais medidas são adotadas e realizadas de forma não planejada por meio de métodos químicos (PICANÇO et al., 2004; ALVES et al., 2008), por meio do uso de fungicidas sintéticos o que explica o fato de o tomate estar no grupo dos cultivares onde mais se utilizam agrotóxicos. Estima-se que são feitas entre 30 e 35 pulverizações de pesticidas na cultura, utilizando-se aí herbicidas, inseticidas, fungicidas, acaricidas e bactericidas (BETTIOL et al., 2004).

O controle químico, pela aplicação de fungicidas sintéticos apresenta desvantagens como aumentado custo de produção, perigos para os manipuladores, preocupação acerca dos resíduos em alimentos e ameaça a saúde pública (ISMAIL; ZHANG, 2004). A preocupação pública destes riscos tem despertado o interesse na descoberta de protetores de culturas mais seguros com vista à substituição de pesticidas químicos sintéticos. Uma alternativa emergente tem sido o uso de protetores naturais com potencialidade fungitóxica, os quais devem ter baixa toxicidade em mamíferos, menos efeitos deletérios sobre o ambiente, e ampla aceitação pública (HAMILTON-KEMP et al., 2000).

4.2 QUALIDADE PÓS-COLHEITA DE UVA

O sistema agroindustrial da uva apresenta significativa importância econômica atualmente em decorrência dos inúmeros empregos nos setores de insumos para atividade, produção, processamento, distribuição e serviços de apoio (SOUSA, 2013). Em 2010 a área

mundial destinada ao plantio de uvas representou 7.20 milhões de hectares, havendo um total produzido de 58,21 milhões de toneladas de frutos. De acordo com o relatório da FAO em 2012, no ano de 2010 o Brasil ocupava o 20º lugar no ranking mundial da área cultivada com videiras e o 14º lugar na produção de uvas tendo aumentado em 27,45% sua produção em relação ao ano 2000. Atualmente a China é o maior produtor de uvas no mundo seguida de Itália, Estados Unidos, Espanha e França (MELLO, 2012).

A produção de uvas de mesa no Brasil pode ser dividida em dois grupos: um formado pelas uvas finas (*Vitis vinifera* L.), representado principalmente por cultivares como a Itália e suas mutações (Rubi, Benitaka e Brasil), Red Globe, Red Meire, Patrícia e as sem sementes (Centennial Seedless, Superior Seedless ou Festival, Thompson Seedless, Perlette, Catalunha e Crimson Seedless); e outro pelas uvas comuns ou rústicas (*Vitis labrusca* L.), cuja representante principal é a cultivar Niágara Rosada (NACHTIGAL, 2003).

Outra representante das uvas rústicas de mesa (*Vitis labrusca* L.) é a cultivar americana ´Isabel´, uva tinta altamente fértil, de colheita abundante e com poucas intervenções de manejo, trazida ao Brasil pelos imigrantes italianos (CAMARGO et al., 2010). A uva ´Isabel´ tem o sabor característico da espécie, adaptando-se a todos o seus usos, sendo consumida como uva de mesa e utilizada para a destilação ou para a elaboração do vinagre e como matéria prima para a fabricação de doces e geléias (CAMARGO, 2005). Apresenta cachos com bagas bastante aglomeradas, rápido amadurecimento, fácil degrana, rápida desidratação e baixa resistência pós-colheita sendo susceptível às infecções fúngicas (ARTÉS-HERNÀNDEZ e TOMÁS-BARBERÁN, 2006).

A deterioração de uvas pode ser desencadeada por fatores fisiológicos, físicos ou patológicos que podem ocorrer na vinha (pré-colheita) ou após a colheita (ZOFFOLI; LATORRE; NARANJO, 2009), sendo a podridão o principal fator limitante do prolongamento da sua vida útil (DEL NOBILE et al., 2008). Consideráveis perdas pós-colheita ou diminuição da qualidade de mercado de uvas são causadas em decorrência da ação de fungos fitopatógenos, sendo a deterioração ocasionanda por *Botrytis cinerea* a mais comum (GONZÁLES-RODRIGUEZ et al., 2011). Outros agentes patogênicos tornam-se importantes em temperaturas mais quentes, e geralmente aparecem durante o transporte ou comercialização logo após os frutos serem retiradas do armazenamento a baixas temperaturas ocasionando podridão mole (*Rhizopus stolonifer*), podridão negra (*Aspergillus niger)* e podridão azul *(Penicillium sp),* principalmente (FAGUNDES et al., 2014; SANTOS et al., 2012).

A ocorrência de doenças fúngicas em cultivares de uvas de mesa pode provocar grandes perdas e tornar-se fator limitante à viticultura, quando medidas adequadas de controle não são adotadas. A suscetibilidade das principais cultivares plantadas, as condições ambientais favoráveis ao desenvolvimento de patógenos, além do manejo inadequado da cultura, fazem com que o cultivo da videira só se viabilize com a aplicação maciça de fungicidas, aumentando os custos de produção, os riscos de intoxicação dos trabalhadores e de contaminação do ambiente (NAVES; GARRIDO; SÔNEGO, 2006). Diante disso, novas alternativas tecnológicas de produção e proteção destes frutos devem ser avaliadas e implementadas, caso sejam viáveis de uso, com o objetivo de melhorar a produtividade e qualidade do fruto, minimizando as agressões ao meio ambiente e evitando riscos à saúde humana, além das reduções de perdas pós-colheita (SOUSA et al., 2013)

4.3 CONTROLE DA DETERIORAÇÃO DE FRUTOS POR FUNGOS FITOPATÓGENOS

Sabe-se que os índices de perdas pós-colheita no Brasil são elevados, em função de uma série de fatores, como as distâncias existentes entre as regiões produtoras e os mercados distribuidores, e os custos adicionais da implantação de uma infraestrutura de pós-colheita adequada. Conhecer e controlar essas alterações passa a ser condição essencial para a conservação e o aumento da vida útil desses produtos. Na cadeia de frutos para exportação, a qualidade alcançada para o consumidor final é resultado da qualidade do gerenciamento de cada elo da cadeia: produtor, exportadores, importadores, atacadistas e varejistas (CARVALHO, 2003; NEVES, 2009).

Durante o período denominado pós-colheita, os produtos que não são manipulados adequadamente e/ou tratados com inibidores microbianos eficientes, podem perder a qualidade para o consumo. O declínio da resistência natural das frutas pode ativar infecções quiescentes e aumentar a incidência de doenças (TERRY; JOYCE, 2004). Dessa forma, o controle das doenças em pós-colheita é uma atividade imprescindível para manter a qualidade e aumentar a vida de prateleira dessa fruta, especialmente quando o período entre a colheita e o consumo for amplo (LINS et al, 2011). Diante disso, observa-se que o potencial de conservação de um fruto está diretamente relacionado não só ao manejo adequado, mas também ao seu ponto de colheita e aos tratamentos fitossanitários e de campo, que podem interferir na deterioração desses frutos (CHITARRA; CHITARRA, 2005).

A colonização por fungos em frutas pode determinar perdas quantitativas, principalmente quando ocorre o rápido ataque dos patógenos aos tecidos sadios; ou perdas

qualitativas, que são decorrentes de efeitos deteriorativos como descolorações, manchas e produção de odores desagradáveis (BENATO, 2002). A presença de doenças pós-colheita é de extrema importância na segurança alimentar, em termos da sanidade do consumidor e no tempo de vida útil de produtos colhidos (CHITARRA; CHITARRA, 2005).

Segundo Gutierrez; Barry-Ryan; Bourke (2009), as doenças dos frutos e hortaliças são os principais danos pós-colheita, uma vez que impedem o consumo e a comercialização do fruto. Além disso, um fruto deteriorado serve de inóculo para outros frutos, ou seja, eles provocam danos indiretos nas plantações pela introdução rápida em novas áreas onde anteriormente não existia a doença, comprometendo a qualidade dos frutos, vegetais e grãos colhidos e armazenados (NÓBREGA, 2004). Isto ocorre devido aos fungos que produzem esporos ou fragmentos de hifas, que são espalhados pelo vento ou por respingos de água de chuva e de irrigação. Outras estruturas dos fungos permitem a disseminação e sobrevivência de diversas espécies na ausência da planta hospedeira, desta forma, esses patógenos podem sobreviver de uma estação para outra, associados a plantas (vivas ou mortas), ao solo (em restos de lavoura em decomposição), a sementes ou a insetos (LOPES; ÁVILA, 2005).

Os fungos apresentam uma grande versatilidade para crescerem em substratos e em condições que outros micro-organismos não são capazes de se reproduzir, como: crescimento em condições de atividade de água (Aw) reduzida (dentro do limite de 0,65 até 0,99); crescimento em condições de pH reduzido; crescimento em uma ampla faixa de temperatura (< 0 °C a 40 °C); utilização de uma grande versatilidade de substrato, como fontes de carbono, nitrogênio e energia; capacidade de esporulação e disseminação em diferentes condições (TANIWAKI; SILVA, 2001). Um fator agravante que contribui para o desenvolvimento desses seres vivos é a característica de, na ausência de um ambiente favorável, oxidar glicose formando água para criar o ambiente propício ao seu desenvolvimento. Isso significa que, mesmo com relação a pequenas quantidades de umidade, havendo o crescimento de fungos, esses propiciarão umidade para o crescimento de mais micélios (JONES, 2005).

Mesmo na ausência de sinais claros de infestação, como a visualização de esporos, pode haver uma infestação imperceptível, e estes esporos causam algumas doenças respiratórias, o que se torna um grande risco para as pessoas que se expõem a eles. E, mesmo sob processos onde esses esporos são destruídos, as toxinas permanecem, uma vez que são bastante resistentes aos tratamentos térmicos e químicos. Tais toxinas são denominadas micotoxinas, as quais em frutas colonizadas por fungos têm despertado preocupação (DRUSCH; AGAB, 2003), pois constituem ameaça potencial à saúde, sendo tóxicas para

animais e causadoras de mutagênese e carcinogênese em células humanas (YIANNIKOURIS; JOUANY, 2002). Outros fatores ambientais, tais como estresse oxidativo, injúrias causadas por insetos e as condições do plantio também podem influenciar na formação de micotoxinas nos produtos agrícolas (BAYMAN; BAKER; MAHONEY, 2002; JAYASHREE; SUBRASMANYAM, 2000).

Quatro fungos em particular estão relacionados com grandes perdas e com grande poder de resistência a diversas condições ambientais: *Penicillium expansum, Botrytis cinerea, Aspergillus niger* e *Rhizopus stolonifer*. O *P. expansum* é o principal agente causal do mofo azul (BAERT et al., 2008), esse fungo encontra-se difundido em todas as regiões, e seus esporos podem permanecer viáveis de uma safra para outra, em sacolas de colheita, caixas de madeira e em câmaras de armazenamento (SANHUEZA, 2004). Além da infecção de frutas, esse fungo é também responsável pela produção de patulina (COELHO et al, 2011;. LIMA et al, 2011), uma micotoxina que provoca genotoxicidade em células de mamíferos (MOSS; LONG, 2002). *Botrytis cinerea* é o agente causal do mofo cinzento, que causa perdas de importância econômica, não apenas na pré-colheita, mas também durante o transporte e armazenamento quando o agente patogênico aproveita a maior umidade relativa e as temperaturas menores para afetar as defesas do hospedeiro (fruto). No início da infecção uma área circular é visível no fruto seguido de formação de micélio e esporificação abundante com cor variando de branco a cinza dependendo da quantidade de luz disponível (BAUTISTA-BAÑOS, MOLINA e BARRERA NECHA, 2014). *A. niger* é o agente causador da podridão negra. A infecção deste agente patogênico em frutos caracteriza-se pelo amolecimento do local infectado seguido do desenvolvimento do mofo escuro que corresponde as estruturas de frutificação dos fungos ao longo do tempo causando grandes perdas para o produtor (LICHER et al., 2002)

A podridão-mole, causada pelo fungo *Rhizopus stolonifer*, causa sérias perdas nas fases pós-colheita de transporte e comercialização. O fungo é altamente destruidor, com grande capacidade saprofítica, exigindo ferimentos para sua penetração, após a qual rapidamente coloniza o fruto, causando uma podridão-mole e aquosa, geralmente não incidindo em frutos imaturos, sendo raramente vistos no campo (CARVALHO et al., 2009). As infecções em frutos caracterizam-se por zonas alagadas rapidamente cobertas por um micélio grosso formando uma massa de esporângios pretos em suas pontas (BAUTISTA-BAÑOS, MOLINA e BARRERA NECHA, 2014).

Para reduzir estes prejuízos, métodos físicos, químicos e biológicos vêm sendo empregados, visando o controle deste grupo de doenças. O controle químico, pela aplicação

de fungicidas sintéticos, permanece sendo a principal medida para reduzir a incidência de doenças pós-colheita em frutos. Estes compostos podem ser utilizados de forma isolada, combinados em misturas, ou aplicados separadamente em seqüencia (ISMAIL; ZHANG, 2004).

O controle químico de fungos patógenos em vegetais através do amplo uso de fungicidas sintéticos apresenta significantes desvantagens, incluindo aumentado custo de produção, perigos para os manipuladores, preocupação acerca de resíduos em alimentos, ameaça à saúde pública e ao meio ambiente (FERNANDEZ et al., 2001; SOROUR; LARINK, 2001). Ainda, devido ao desenvolvimento de resistência por parte dos fungos fitopatógenos, muitos dos compostos fungitóxicos sintéticos amplamente empregados estão tornando-se gradualmente menos efetivos (PARANAGAMA et al., 2003).

O amplo uso de fungicidas no mundo é variável, embora seja estimado que anualmente cerca de 23 milhões de quilos destes compostos sejam aplicados em vegetais, sendo geralmente aceito e difundido pelos produtores, que a produção e mercado destes produtos perecíveis não seriam possíveis sem o seu uso. Carcinogenicidade, teratogenicidade, alta e aguda toxicidade residual, longo período de degradação, poluição ambiental, influência sobre os caracteres organolépticos dos alimentos, e efeitos colaterais em humanos são os principais fatores que têm restringido o uso de fungicidas químicos no controle da deterioração pós-colheita (CABRAL, PINTO e PATRIARCA, 2013).

A preocupação pública destes riscos tem despertado o interesse na descoberta de protetores de culturas mais seguros com vista à substituição de pesticidas químicos sintéticos. Os métodos biológicos se constituem em alternativas viáveis em relação ao método químico tradicional, principalmente em função de não deixarem resíduos tóxicos nos produtos tratados. O biocontrole, caracterizado como o uso de organismos e/ou seus produtos derivados ou metabólitos para prevenção de doenças em vegetais, é ecologicamente viável, normalmente seguro, e pode prover proteção por um longo prazo para a cultura (SAN-LANG et al., 2002).

Alguns agentes empregados no processo de biocontrole têm se mostrado eficientes como alternativa a fungicidas sintéticos na prevenção da deterioração pós-colheita de vegetais, a citar compostos de aroma (UTAMA et al., 2002), ácido acético (SHOLBERG et al., 2000), glucosinolatos (MARI et al., 2002), própolis (LIMA et al., 1998), óleos essenciais (SANTOS et al., 2012; SOUZA et al., 2013), extratos vegetais (MAGRO et al., 2006; LATHA et al., 2009) e quitosana (CAPDEVILLE et al., 2002; SANTOS et al., 2012; OLIVEIRA et al., 2014). Dentre as substâncias supracitadas, a utilização da quitosana e de

óleos essenciais, como alternativa para o controle de patógenos pós-colheita tem atraído a atenção de pesquisadores (SANTOS et al., 2012; SUN et al., 2014) A atividade antimicrobiana é uma das mais importantes bioatividades da quitosana e dos óleos essenciais, e estudos relataram que estes compostos podem reduzir o crescimento de fungos fitopatógenos, que são prejudiciais para o campo, na produção de frutas e vegetais (HERNANDEZ-MUÑOZ et al., 2006; BADAWY; RABEA, 2009).

4.4 PROPRIEDADES DA QUITOSANA E USO COMO REVESTIMENTO

O polissacarídeo quitosana é obtido a partir da desacetilação da quitina que é o maior constituinte de exoesqueletos de crustáceos, como o camarão. Este heteropolímero natural é composto por unidades β-1,4 D-glucosamina ligadas a resíduos de N-acetilglucosamina, sendo encontrado na parede celular de fungos e exoesqueletos de crustáceos (SYNOWIECKI; AL-KHATTEB, 2003; STAMFORD et al., 2007). Trata-se de um produto de baixa toxidez, capaz de formar géis com atividade antimicrobiana. Esses são atributos que fazem com que a quitosana seja estudada para aplicação em pós-colheita de frutos, tanto in natura, quanto minimamente processados (ASSIS e SILVA, 2003).

Por se tratar de um polímero natural, biodegradável, extremamente abundante e atóxico, a quitosana tem sido proposta como um material potencialmente atraente para usos diversos, principalmente em engenharia, biotecnologia e medicina. As indicações mais comuns são seu emprego como alimento funcional (DAMIAN et al., 2005), analgésico (OKAMOTO et al.; 2002), redutor de lipídeos plasmáticos (MUZZARELLI et al., 2006; ZHANG et al., 2008; LIU; ZHANG e XIA, 2008), substância hipoglicêmica (YAO; HUANG; CHIANG, 2008), removedor de sujidades no tratamento de água (ZENG; WU; KENNEDY, 2008), carreador de fármacos de liberação controlada (BOONSONGRIT; MUELLER; MITREVEJ, 2007), regeneração de tecidos epiteliais (KIM et al., 2008). Este polímero também pode ser utilizado como filme plástico antimicrobiano para cobrir frutas e legumes frescos (JIANG; LI, 2001, SANTOS, 2012).

Apesar da vasta gama de possíveis aplicações da quitosana em produtos alimentícios, destaca-se aqui seu uso como agente conservante no sentido de impossibilitar ou atrasar a deterioração microbiana ou enzimática dos alimentos, considerando-se suas propriedades físico-químicas e seu reconhecido potencial antimicrobiano.

Pesquisas têm demonstrado que esses polímeros são capazes de inibir o crescimento de bactérias como *Staphylococcus aureus, Escherichia coli, Salmonella typhimurium,*

Streptococcus faecalis, Pseudomonas aeruginosa, Listeria monocytogenes (ZHENG; ZHU, 2003; CHUNG et al., 2004; YADAV; BHISE, 2004) dentre outras e de fungos como *Rhizopus* sp e *Cladosporium* sp, *Alternaria alternata* (CHEN et al., 2014); *Colletotrichum gloeosporioides* (MUÑOZ e GARCÉS, 2009), *Penicillium expansum* e *Botrytis cinerea* (LIU et al., 2007); *Aspergillus niger* (SEBTI et al., 2005).

O mecanismo de ação da quitosana sobre os micro-organismos não está completamente elucidado, porém diversas hipóteses têm emergido. Alguns pesquisadores correlacionam a atividade antimicrobiana da quitosana à formação de complexos polieletrolíticos, uma vez que seus grupos amínicos protonados, provavelmente, se ligam à superfície celular, carregada negativamente, interferindo na atividade celular e na permeabilidade da membrana causando perda de componentes intracelulares, sem a formação de poros (AVADI et al., 2004; TSAI; HWANG, 2004; YADAV; BHISE, 2004; KONG et al., 2010; SANTOS, 2012, ELSABEE E ABDOU, 2013). Além disso, pode haver o comprometimento da geração de energia pela desestabilização celular da cadeia transportadora de elétrons, interferindo no aporte adequado de oxigênio, forçando as células a produzirem energia anaeróbica, desencadeando a disfunção do aparelho celular (KONG et al., 2010). Outra ação seria a penetração da quitosana em células vivas, levando à inibição de várias enzimas, interferindo na síntese de mRNA e de proteínas (RABEA et al., 2003; ZHENG; ZHU, 2003). Ou ainda, formação de camada impermeável ao redor da célula, bloqueando o transporte intracelular (EATON et al., 2008).

Como revestimento de vegetais a quitosana pode apresentar dupla função na interação patógeno-hospedeiro, podendo apresentar atividade antifúngica como retardar o crescimento micelial, diminuir a germinação de conídios e acarretar em alterações morfológicas no tubo germinativo de algumas espécies de fitopatógenos (LIU et al., 2007) bem como induzir a ativação de mecanismos bioquímicos de defesa do vegetal como o aumento na produção de compostos fenólicos e a diminuição da ação de enzimas como a polifenoloxidase (BADAWI e RABEA, 2009).

Estudos realizados utilizando-se a quitosana como revestimento em frutos, em diferentes concentrações, mostram resultados satisfatórios tanto em relação ao potencial antifúngico, como também como agente de manutenção ou mesmo de promoção de melhorias na qualidade dos vegetais revestidos. Em seus estudos com tomate e tomate-cereja, Muñoz e Garcés (2009); Badawi e Rabea (2009) e Chen et al. (2014) avaliaram o potencial antifúngico da quitosana como protetor de tomate frente a *Colletotrichum gloesporioides*, *Botritys cinere*a e *Alternaria alternata*, respectivamente, e concluíram que o revestimento de quitosana inibiu

o crescimento micelial, a germinação de esporos e a elongação do tubo germinativo dos espécimes fúngicos estudados. Também observaram o efeito protetor dos frutos revestidos contra as infecções causadas pelas estirpes fúngicas nos tomates artificialmente contaminados independente da condição de armazenamento, associando esse efeito protetor também a indução de marcadores bioquímicos de defesa nos frutos induzidos pela quitosana. Este mesmo efeito foi observado por Meng et al. (2008) em seu estudo com uvas contaminadas por *Botrytis cinerea.*

Apesar do efeito antimicrobiano das soluções de revestimento estarem relacionados à concentração, os estudos com frutos diversos demonstram o mesmo comportamento antifúngico e de manutenção ou melhora das características físico-químicas e sensoriais dos frutos revestidos durante a vida-de-prateleira mesmo em concentrações inferiores (CHIEN; SHEU; YANG, 2007; SANGSUWAN; RATTANAPANONE; RACHTANAPUN, 2008).

4.5 POTENCIAL ANTIMICROBIANO DOS ÓLEOS ESSENCIAIS DE *MENTHA* SPP

Dentre muitos compostos estudados com vistas a um potencial uso no controle do crescimento de fungos fitopatogênicos em frutos, os óleos essenciais têm apresentado destacáveis resultados (TZORTZAKKIS; ECONOMAKIS, 2007). Óleos essenciais são compostos voláteis complexos produzidos em diferentes partes (folhas, caule, frutos, folhas e flores) de plantas aromáticas, sendo estocados em células secretórias, cavidades, canais, células epidérmicas ou tricomas glandulares (BURT, 2004). Estes compostos têm grande importância para os vegetais, provendo resistência a doenças e ação de micro-organismos fitopatógenos, além de atuarem como inibidores de germinação, na atração de polinizadores, além de inibirem a perda de água e aumento da temperatura (FENG; ZHENG, 2007).

Atualmente, várias espécies de *Mentha* e OE são exploradas em setores diversificados, incluindo alimentos, agronomia, cosmético, medicina e farmácia. Na indústria de alimentos, os OES de espécies de *Mentha*, principalmente o de *Mentha piperita* L., são utilizados como agentes aromatizantes em alimentos e bebidas (BURT, 2004) e são comumente exploradas devido ao potencial antioxidante e propriedades antimicrobianas destas substâncias (TYAGI e MALIK, 2011; RIAHI et al, 2013). A *Mentha piperita* é um híbrido de *Mentha aquatica* e *Mentha spicata.* Comercialmente, é a mais importante espécie de Mentha. O óleo essencial de *Mentha piperita* é um dos mais populares e amplamente utilizados por causa de seus componentes majoritários o Mentol (30-50% do óleo) e a Mentona (14-32%). O mentol é uma substância cerosa, cristalina utilizada para diversos fins médicos, tais como, para aliviar a

irritação da pele, queimaduras solares e aliviar o congestionamento nasal enquanto a mentona é utilizada em perfumaria, como agente saborizante ou aromatizante (KUMAR et al., 2012).

A atividade antimicrobiana dos óleos essenciais depende da sua composição química e suas atividades biológicas são frequentemente atribuídas aos seus componentes principais (TEIXEIRA et al., 2012; AIT-OUAZZOU et al., 2012). Alguns autores, no entanto, referem que a atividade antimicrobiana se deve ao equilíbrio entre os componentes majoritários e os demais que aparecem em menor proporção (AIT-OUAZZOU et al, 2012; RIAHI et al, 2013).

Vários autores investigaram o potencial inibitório de mentol contra fungos. O mentol foi testado contra *Fusarium verticillioides* utilizando a técnica de susceptibilidade no agar semi-sólido e o crescimento foi reduzido em 75% quando utilizado 200 ppm de mentol (DAMBOLENA et al., 2008). Em uma outra experiência, o vapor de óleo de hortelã-pimenta e dois dos seus principais componentes (mentol e mentona) foram avaliadas em relação a dois fungos (*Sclerotinia sclerotiorum* e *Mucor* spp.) em um sistema fechado. Os resultados indicaram que o mentol só foi encontrado como sendo o composto responsável pelas propriedades antifúngicas de óleo de hortelã-pimenta, ao passo que mentona isoladamente não apresentou qualquer efeito em *Botrytis cinerea* e *Monilinia fructicola* (TSAO e ZHOU, 2000).

A *Mentha x villosa* Hudson é um híbrido das plantas aromáticas de *M. spicata* L. e *M. suaveolens* Ehrh. comumente usada na medicina popular brasileira (LAHLOU et al., 2002), e é conhecida popularmente como Hortelã de- Tempero, Hortelã-de-Folha-miúda, Hortelã-do-Brasil, Hortelã-Pimenta-Rasteira, sendo também designada por *Mentha crispa* L (ARRUDA et al., 2006). O constituinte químico majoritário presente de 30 a 75% no óleo essencial dessa espécie (*Mentha x villosa*) é o 1,2-epoxi-pulegona, também denominado óxido de piperitenona ou rotundifolona (RADÜNZ, 2004; ARRUDA *et al*., 2006; MARTINS *et al*., 2007). Segundo MARTINS et al., (2007), outros constituintes como: mentol, mentofurona, pineno, limoneno e cânfora, tanino, ácidos orgânicos, flavonóides e heterosídios também foram encontrados no óleo essencial de *Mentha x villosa*.

As informações etnobotânicas de diversos estudos da medicina popular atestam a utilização da *Mentha villosa* nas afecções bucais (SOUZA; FELFILI, 2006); diarréias, cólicas intestinais, cólicas menstruais (ALMEIDA et al., 2003; RADÜNZ, 2004) e nos tratamentos das amebíases, giardíases e tricomoníases (MONTE; OLIVEIRA; BRAZFILHO, 2001; RADÜNZ, 2004).

Os óleos essenciais têm atraído interesse científico pelo fato de caracterizarem-se como produtos naturais reconhecidos como seguros - GRAS, possuir amplo espectro de atividade antimicrobiana, e apresentar eficácia no controle de micro-organismos patogênicos e

deteriorantes de importância em vegetais (GUTIERREZ et al., 2009; OMIDEYGI et al., 2007). Além disso, esses materiais naturais de plantas apresentam efeitos baixos de indução de resistência em microorganismos (MARINELLI et al., 2012). São constituídos, principalmente, por fenilpropanóides e terpenóides, sendo que os últimos se sobressaem. Essas classes de substâncias são frequentemente alvos de interesse de pesquisadores que veem neles uma fonte promissora de princípios ativos diretos ou precursores na síntese de outros compostos de maior importância e valor agregado, como, o eugenol, citral, citronelal, mentol entre outros (DE LA ROSA; ALVAREZ-PARRILLA; GONZALEZ-AGUILAR, 2010).

Esses compostos são hidrofóbicos e o seu sítio de ação é a membrana celular da célula microbiana. Eles se acumulam na bicamada lipídica causando desarranjo na função e na estrutura da membrana penetrando na célula, onde exercem atividade inibitória no citoplasma celular e provocam lise e liberação do ATP intracelular, aumentando sua permeabilidade, com posterior liberação de constituintes intracelulares vitais, e danos em seus sistemas enzimáticos (TURINA et al., 2006). De modo geral, a ação dos compostos fenólicos sobre os fungos compreende coagulação citoplasmática, desorganização dos conteúdos celulares, ruptura da membrana plasmática, inibição das enzimas fúngicas, distúrbio da funcionalidade do material genético, culminando na inibição da germinação espórica e do crescimento micelial (BURT, 2004; LIU et al.; 2007; HERNÁNDEZ-LAUZARD et al.; 2008; RABEA et al.; 2009; MENG et al.; 2010).

Os compostos lipofílicos são eficientemente absorvidos pelos fungos, pois o micélio fúngico apresenta larga natureza lipofílica, associado à grande área relativa superficial em comparação ao volume do fungo (REGNIER et al.; 2008; SANTOS, 2012). O mecanismo subjacente à ação antifúngica de óleos essenciais serve como interruptor entre a fase vegetativa e reprodutiva do desenvolvimento fúngico. Os compostos presentes nos óleos essenciais impactam a esporulação por impedir o desenvolvimento micelial (reconhecida como ‘plataforma’ que suporta a produção espórica) e/ou a percepção/transdução de diferentes sinais fisiológicos envolvidos na síntese de moléculas do funcionamento da forma vegetativa com vistas ao desenvolvimento da forma reprodutiva (TZORTZAKIS; ECONOMAKIS, 2007). Como conseqüência da supressão da produção espórica, resultante do tratamento com o óleo essencial, ocorre limitação da propagação do patógeno, diminuindo a liberação de esporos pela atmosfera do ambiente e superfícies.

A quiralidade da biomembrana dos componentes do óleo desempenha papel essencial na organização e funções biológicas da membrana celular (BOMBELLI et al.,2008). Diferenças estruturais leves em alguns compostos são suficientes para alterar as características

físicas e/ou químicas e, portanto, modificar a atividade antifúngica. Os compostos lipofílicos também podem afetar a capacidade de certos compostos se acumularem no interior da membrana provocando alterações nas características físico-químicas de certas moléculas. Quando monoterpenos tornam-se menos solúveis em água, eles podem facilmente interagir com a membrana de raiz e perturbar a integridade, e, portanto, causar uma despolarização rápida (MAFFEI et al., 2001).

Ressalta-se que, ao se decidir pela aplicação de OE em alimentos, é fundamental se considerar o impacto sobre as características sensoriais dos produtos durante o armazenamento, uma vez que a concentração do OE necessária para o estabelecimento da eficácia antimicrobiana pode resultar em características organolépticas desagradáveis ao consumo (GUTIERREZ; BARRY-RYAN; BOURKE, 2009). Considerando estes aspectos, o uso dos OES associados a outros métodos na inibição do crescimento microbiano em alimentos tem sido objeto de investigação e aperfeiçoamento, visto que se mostram como alternativa para reduzir as perdas pós-colheita e proporcionar ao mercado consumidor alternativas mais saudáveis sem implicações sensoriais dado a possibilidade de uso de menores concentrações de óleo essencial (SOUZA et al., 2007).

5 MATERIAL E MÉTODOS

Os experimentos foram conduzidos no Laboratório de Microbiologia e Bioquímica de Alimentos (análises microbiológicas) e Laboratório de Técnica Dietética (Análise Sensorial) pertencentes ao Departamento de Nutrição, Centro de Ciências da Saúde, Universidade Federal da Paraíba, Laboratório de Análises de *Flavour* (análise da composição dos óleos essenciais) e Laboratório de Análises de Alimentos (análises físico-químicas dos frutos), pertencentes ao Departamento de Engenharia de Alimentos, Centro de Tecnologia da Universidade Federal da Paraíba e no Laboratório de Combustíveis e Materiais - LaCom (análises de caracterização da quitosana) pertencente ao Departamento de Química, Centro de Ciências Exatas e da Natureza da Universidade Federal da Paraíba.

5.1. MATERIAL

Os frutos de tomate-cereja (*Solanum lycopersicum* L.) e as uvas (*Vitis labrusca* L.), foram obtidos comercialmente maduros (com cor vermelha e roxa, respectivamente) na EMPASA (Empresa Paraibana de Abastecimentos e Serviços, João Pessoa, Brasil). Os frutos foram adquiridos sem nenhum sinal visível de dano mecânico ou infecção fúngica, sendo selecionados e padronizados de acordo com o tamanho, cor, aparência e forma. Antes dos ensaios, todos os frutos foram lavados e higienizados por meio da imersão em uma solução de hipoclorito de sódio (1 mL / 100 mL, pH 7,2 ajustado com NaOH a 1 M), durante 15 minutos. Em seguida foram enxaguados em água destilada esterilizada e secos em uma cabine de fluxo laminar previamente esterilizada.

Aspergillus niger URM 5162 *(A. niger*), *Botrytis cinerea* URM 2802 (*B. cinerea*), *Penicillium expansum* URM 3396 (*P. expansum*) e *Rhizopus stolonifer* URM 3482 (*R. stolonifer*) foram obtidos a partir da coleção de culturas da Micoteca URM (Centro de Ciências Biológicas, Universidade Federal de Pernambuco, Recife, Brasil). Para os ensaios de atividade antifúngica foram utilizados repiques das culturas estoque subcultivadas em tubos de ensaio contendo ágar Sabouraud (Himedia, India) incubados a 25°C durante sete dias para suficiente esporulação. Os esporos fúngicos foram colhidos através da adição de soro fisiológico estéril no meio de crescimento fúngico, e a suspensão obtida foi filtrada em tripla camada de gaze estéril para retenção dos fragmentos de hifas. Em seguida o número de esporos presentes na suspensão foi determinado através de contagem em hemocitômetro. A concentração obtida de esporos foi ajustada com soro fisiológico estéril para prover um

inóculo fúngico de aproximadamente 10^6 esporos/mL (RASOOLI e ABYANETH, 2004; RASOOLI e OWLIA, 2005).

As carapaças de camarão utilizadas para a extração da quitosana foram obtidas a partir de uma única espécie [*Litopenaeus vannamei* (Boone 1931) - *L. vannamei*] e foram gentilmente cedidas pela empresa Aquamaris Aquicultura S / A (João Pessoa, Brasil).

As folhas de *Mentha piperita* L. (*M. piperita*) e *Mentha x villosa* Huds (*M. villosa*) foram coletadas sempre pela manhã (antes das 8:00 horas), no Horto de Plantas Medicinais do Laboratório de Tecnologia Farmacêutica, da Universidade Federal da Paraíba (João Pessoa, Brasil), em julho de 2011 (7º08'29 ' 'S, 34º50'48"W). As espécies de *M. piperita* e *M. villosa* foram identificadas e registradas no Herbário Prisco Bezerra da Universidade Federal do Ceará (Fortaleza, Brasil) sob os números 14.423 e 14.996, respectivamente.

Para a extração de cada óleo essencial, as folhas frescas da *M. piperita* e de *M.villosa*, foram lavadas para a retirada de resíduos e partículas indesejáveis, numa média de 10 kg por coleta, trituradas em processador elétrico, à temperatura ambiente, e em seguida transferidas para um balão volumétrico de seis litros com água destilada suficiente para cobri-las. O processo extrativo se deu através de arraste por vapor de água em aparelho de Clevenger adaptado. O período de extração durou em média 8 horas e foram obtidos óleos de coloração amarelada e odor característico. Os óleos essenciais obtidos foram dessecados com sulfato de sódio anidro e armazenados em recipientes âmbar, bem vedados e conservados a uma temperatura constante de 4°C (CRAVEIRO et al., 1981; MATOS, 1996ba).

5.2 MÉTODOS

5.2.1 Extração de quitosana das carapaças do camarão *L.vannamei*

Para a obtenção da quitosana, as carapaças de camarão foram lavadas em água corrente para remover resíduos orgânicos solúveis, proteínas aderentes e outras impurezas. Em seguida as carapaças foram recolhidas, fervidas em água destilada durante 1 h para remover os tecidos restantes e secas em estufa (Fanem, modelo 320, Brasil) a 160 ° C durante 2 horas. Após a secagem as carapaças foram moídas para formar um pó fino usando um moinho padrão (Modelo TE-330, Tecnal, São Paulo, Brasil) (KUCUKGULMEZ et al., 2011).

Para remover o carbonato de cálcio (principal componente inorgânico em carapaças de camarão), as carapaças foram imersas em 1,7 N de HCl (1:30 w / v) durante 6 h. A extração foi realizada a temperatura ambiente (25 ° C) com agitação (150 rpm). O resíduo foi lavado

diversas vezes de modo a remover os resíduos minerais. Após a desmineralização prosseguiu-se com a desproteinização que foi realizada por meio da imersão do resíduo desmineralizado em NaOH 2,5 N a 65 ° C durante 6 h. Subsequentemente, o material obtido foi filtrado, lavado em água destilada até atingir pH neutro e seco (160 ° C, 2 h). A conversão de quitina para quitosana foi realizada através de desacetilação. Para este fim, a quitina foi misturada com NaOH 2,5 N (1:50 w / v, 120 ° C, *overnight*), filtrou-se, lavou-se com água deionizada até o filtrado atingir pH neutro e secou-se (90 ° C, 2 h) (KUCUKGULMEZ et al., 2011). A quitosana obtida na forma de pó foi embalada a vácuo e armazenada sob refrigeração (7 ° C) até à sua utilização nos ensaios.

5.2.2 Identificação dos constituintes dos óleos essenciais

Os componentes individuais de cada óleo essencial foram identificados através de cromatografia em fase gasosa e espectrometria de massa (GC/MS) usando um Varian Saturn Cromatógrafo de Gás 2000R Modelo 3800 equipado com espectrômetro de massa de modelo 2000R-2000 e uma coluna capilar DB-5 (60 mx 0,25 mm x 0,25 um) sob as seguintes condições analíticas: impacto de elétrons, 70 eV; gás de transporte, Hélio; taxa, 1,0 mL/min de fluxo; temperatura do forno programada de 60 a 240°C a 3°C / min; temperatura do injetor, 240°C; temperatura do detector, 230°C; razão de separação 20/1. O volume injetado foi de 1,0 mL de uma solução contendo aproximadamente 0,1 mL de cada óleo essencial em 1,0 mL de acetato de etilo. Cada componente foi identificado após a comparação dos espectros de massa de cada composto com a base de dados de GC/MS (NIST / EPA / NIH de Massa Espectral Database Versão 1.7). Os componentes foram quantificados, depois normalizadas as áreas e expressos em percentagem total de área (%) (ADAMS, 2001).

5.2.3 Caracterização físico-química da quitosana

Para determinar o grau de desacetilação (DD), a amostra de QUI foi submetida a espectroscopia na região do infravermelho usando um espectrofotômetro (Bomem Michelson FT-IR, modelo MB-102). Os espectros foram obtidos na região de 400 - 4.000 / cm^{-1} utilizando 2 mg de QUI previamente seca durante a noite a 60 ° C sob pressão reduzida e cuidadosamente misturados com 100 mg de KBr para produzir discos de 0,5 mm de espessura. Com base no espectro de infravermelho, o DD foi determinado utilizando a relação de absorvância A1655 / A3450 e calculado de acordo com a seguinte equação:

DD (%) = 97,67 - [26,486 (A1655 / A3400)] (ROBERT, 1992).

Para determinar o perfil térmico, 5 mg de QUI foi preparada em um cadinho de platina. As análises termogravimétricas (TGA) foram realizadas em uma termobalança (Shimadzu TGA-50) a temperaturas na gama 28-900 ° C sob uma atmosfera de ar dinâmica (velocidade de fluxo de 30 mL / min) e uma taxa de aquecimento de 10 ° C / min. Calorimetria exploratória diferencial (DSC) foi realizada utilizando um calorímetro (Shimadzu, modelo DSC-50WS) após aquecimento das amostras a partir de 25 ° C a 500 ° C sob um fluxo contínuo de N2 (50 ml / min) a uma taxa de aquecimento de 10 ° C / min (ROBERT, 1992).

Os padrões de raios X foram determinados utilizando um difratômetro da Shimadzu modelo XD3A. O índice de cristalinidade do CHI foi calculada usando a seguinte equação

Cri = (1θ H - 1θ A) / A x 100 1θ (Equação 1),

1θ onde M = pico (correspondente à intensidade máxima), e A = 1θ pico menor (segundo a difracção amorfo).

5.2.4 Produção da solução de quitosana e óleos essenciais

As soluções de quitosana foram obtidas por dissolução do polímero (16 mg/mL) em ácido acético (1 mL/100 mL) por 24 horas em temperatura ambiente sob agitação (120 rpm) (SANTOS et al., 2012). Em seguida foram feitas sucessivas diluições seriadas (1:1) em caldo Sabouraud (Himedia, India), obtendo-se soluções com diferentes concentrações (8,0; 4,0; 2,0 mg/mL). Para assegurar que a atividade anti-fúngica foi devido a quitosana e não o ácido acético, o pH de todas as soluções utilizadas nos ensaios antifúngicos foi ajustado para 5,0 por meio da adição de NaOH 1N. As soluções de cada óleo essencial foram obtidas após a dissolução de cada óleo (80 uL / mL) em caldo de Sabouraud contendo Tween 80 [1%, v / v (Himedia, Índia)] como um agente de estabilização (SANTOS et al., 2012), com a sucessiva diluição (1: 1) no mesmo caldo para se obter soluções de diferentes concentrações (20, 10, 5, 2,5, 1,25, 0,06 e 0,03 mL / mL). Tween 80, na concentração mais elevada, obtida após a diluição em caldo Sabouraud (0,5%, v / v), não inibiu o crescimento das estirpes de ensaio.

Quando da aplicação combinada de quitosana e cada óleo essencial, inicialmente foi realizada a dissolução da quitosana (4,0 mg/mL) em ácido acético (1 mL/100 mL) sob constante agitação (120 rpm) por seis horas a temperatura ambiente. Em seguida, foram adicionadas as diferentes concentrações de óleo essencial (5; 2,5; 1,25 µL/mL), seguindo-se o processo de agitação por 18 horas adicionais a temperatura ambiente. Nos ensaios de

aplicação da solução formada pela combinação de quitosana e óleos essenciais como filmes em frutos, foi adicionado glicerol (2 mL/100 mL) como agente plastificante no mesmo momento da incorporação de cada óleo essencial a solução filme (OJAGH et al., 2010).

5.2.5 Determinação da Concentração Inibitória Mínima (CIM) de QUI e OES

Os valores da CIM foram determinados utilizando macrodiluição em caldo. Inicialmente, 1 ml de uma suspensão da estirpe fúngica teste foi inoculada em 4 mL de caldo Sabouraud, em seguida foi adicionada 4 ml de soluções/emulsões contendo diferentes concentrações de CHI, MPOE ou MVOE (com uma concentração ajustada para 10 ml). O sistema foi incubado a 25 ° C durante 7 dias, e no final do período de incubação, a menor concentração de CHI, MPOE ou MVOE (maior diluição) que não exibiu crescimento do fungo visível foi considerado a CIM (SANTOS et al., 2012; SHARMA e TRIPATHI, 2008).

5.2.6 Efeitos da QUI e OES no crescimento micelial radial dos fungos

Para a avaliação da ação da QUI em combinação com o MPOE ou MVOE frente ao crescimento micelial radial em fungos, utilizou-se a técnica do envenenamento do substrato de crescimento (diluição em meio sólido). Para este fim, foi tomada uma alíquota de 2 mm, de cada estirpe fúngica com crescimento de 7 dias cultivadas em agar Sabouraud a 28 ° C, e colocada no centro de uma placa de petri de agar Sabouraud (Himedia, Índia) contendo QUI e MPOE ou MVOE nas concentrações inibitórias ou subinibitórias e incubadas a 28 ºC durante 7 dias. Como ensaio controle, as estirpes fúngicas foram cultivadas em meio de crescimento sem a adição de QUI, MPOE ou MVOE foram igualmente testados (OLIVEIRA et al., 2014). Os resultados foram expressos como as taxas de percentagem de inibição de crescimento micelial radial (mm) em diferentes pontos de tempo de exposição (3, 5 e 7 dias) em relação ao ensaio controle.

5.2.7 Efeitos da QUI e OES na germinação de esporos de fungos

Alíquotas de 0,1 ml de cada suspensão de esporos (aproximadamente 10^6 esporos / ml) obtido a partir de uma cultura de 7 dias, cultivadas em agar Sabouraud (28 ° C) foram colocados em tubos Eppendorf contendo 0,1 ml de uma solução contendo diferentes quantidades de QUI e MPOE ou MVOE. Subsequentemente, 0,1 ml do sistema foi colocado

no centro de uma lâmina de vidro esterilizada e incubadas numa câmara úmida a 28 ° C durante 24 h. Cada lâmina foi fixada e tratada com o corante azul lactofenol algodão. A germinação de esporos foi observada em microscopia óptica e cerca de 200 esporos de cada lâmina foram contados. A eficácia da inibição da germinação de esporos foi avaliada depois de comparar o número de esporos germinados em meios contendo QUI e MPOE ou MVOE com os obtidos no ensaio de controle, no qual as soluções de QUI-EOS foram substituídas por caldo Sabouraud (FENG e ZENG, 2007). Um esporo (conídio) foi considerado germinado quando o tubo de germinação desenvolvido era pelo menos duas vezes o seu diâmetro original, e os resultados foram expressos como percentagem de inibição de taxas de germinação de esporos, quando comparada com o ensaio controle.

5.2.8 Efeitos da QUI e OES sobre o crescimento superficial (infecções fúngicas) em tomates-cereja e uvas de mesa

Para a avaliação do potencial de inibição da QUI em combinação com MPOE ou MVOE nos frutos (tomate-cereja ou uva) inicialmente os frutos foram imersos em 500 mL de uma solução de inóculo (aproximadamente 10^6 esporos / mL) de cada estirpe fúngica durante 1 minuto sob agitação suave usando um bastão de vidro estéril. Em seguida, os frutos foram imersos em 500 ml da solução de revestimento contendo diferentes concentrações de QUI MPOE ou MVOE sob agitação suave usando um bastão de vidro estéril durante 1 minuto. Os frutos foram secos sobre um filtro de nylon para drenar o excesso de líquido, dentro de uma câmara de fluxo laminar estéril e quando secos, foram embalados em recipientes de polietileno com tampa. Um grupo de frutos foi armazenado à temperatura ambiente (25 ° C), enquanto que o outro grupo foi armazenado em temperatura de refrigeração (12 ° C). O ensaio controle foi realizado com a imersão dos frutos na solução de inóculo seguida da imersão em água destilada estéril adicionada de glicerol (2 g / 100 mL). Cada tratamento incluiu 40 frutos. Em diferentes intervalos de tempo de armazenamento (temperatura ambiente 1, 4, 8 e 12 dias; baixa temperatura 1, 6, 12, 18 e 24 dias), os frutos foram analisados quanto à presença de sinais de infecção fúngica (FENG e ZENG, 2007; LIU et al, 2007). Os resultados foram expressos como percentual de frutos infectados nos diferentes intervalos de tempo analisados.

Os sintomas característicos definidos para o bolor negro (causado por *A. niger*) foi o escurecimento e amolecimento do local infectado no fruto seguindo-se o desenvolvimento do mofo escuro; os sintomas característicos para o mofo cinzento (causado por *B. cinerea*) foram áreas circulares mais escuras seguida de esporificação abundante que variou do branco ao

cinza; os sinais de infecção característicos para mofo azul (causada por *P. expansum*) foram áreas circulares mais escuras que em outras partes dos frutos com esporificação posterior abundante, cuja cor variou de branco para azul ou cinza; e os sinais de infecção característicos para a podridão mole (causadas por *R. stolonifer*) foram áreas rapidamente cobertas por filamentos grossos com micélio peludo cinza formando uma massa de esporângios preto em suas pontas (BAUTISTA-BAÑOS et al, 2014; LICHTER et al, 2002).

5.2.9 Análises físicas e físico-químicas dos frutos

Nos mesmos intervalos de tempo e temperaturas de armazenamento utilizados nos testes de infecção fúngica, os frutos de tomate-cereja e uva foram avaliados quanto aos parâmetros gerais de qualidade física e físico-química descritos a seguir. Todos os ensaios foram realizados em triplicata.

- ✓ **Perda de Peso:** Os frutos foram separados em grupos de 40 unidades para cada tratamento, pesados em balança analítica e em seguida foram dispostos em bandejas, de modo que permaneceram separados em proporções iguais (controle e tratamento) para posterior acompanhamento da diferença de peso final durante o armazenamento. Os resultados foram expressos em porcentagem, relativo à massa inicial do produto (ALI et al., 2010; OLIVEIRA et al., 2014).
- ✓ **Cor:** A cor da casca foi medida em três diferentes posições equatoriais do fruto, através do Sistema CIE*Lab* (L*a*b*); ângulo Hue (*h*ab*) e croma (*C*ab*) em colorímetro Minolta Modelo CR-300 (Osaka, Japão), de acordo a Comissão Internacional de Iluminação (CIE, 1986). Antes da medição de cor, o colorímetro foi calibrado com uma placa de cerâmica branca padrão (L * = 96; a * = 0,14 *, b * = 1,63). Para a medição foi usado com o iluminante D65 (luz do dia normal) a um ângulo de 10 ° (ALI et al., 2010; OLIVEIRA et al., 2014).
- ✓ **Acidez titulável:** De cada tratamento foram tomadas amostras de 10 gramas que foram misturadas a 25 mL de água destilada previamente fervida e em seguida trituradas em triturador elétrico (Arno, São Paulo, Brasil). O sobrenadante da mistura foi levado a titulação com NaOH a 0,1N utilizando-se a fenolftaleína como indicador de modificação de pH. Os resultados foram expressos como mmol H + / 100 g de fruta (ALI et al., 2010; OLIVEIRA et al., 2014).
- ✓ **Sólidos Solúveis**: De cada fruto e de cada tratamento foram tomadas cinco gramas de amostra e trituradas em triturador elétrico (Arno, São Paulo, Brasil). Com auxílio de

uma pipeta graduada coletou-se o suco da amostra triturada e levou-se ao leitor de um refratômetro digital (Modelo HI 96801, Hanna Instruments, São Paulo, Brasil). Os resultados foram expressos como ºBrix (ALI et al., 2010; OLIVEIRA et al., 2014).

- ✓ **Firmeza:** A firmeza foi determinada por meio da medição da força necessária para perfurar um orifício no fruto em cada dia de armazenamento utilizando-se um texturômetro universal TA.XT plus *Texture Analyser* (STABLE MICRO SYSTEMS®, 1997), equipado com probe 1/8 de 3mm de diâmetro operando a uma velocidade de 20 mm/minuto e distância de 30mm. As forças foram registradas em *software* (STABLE MICRO SYSTEMS®, TE32L, versão 4.0, Surrey, Inglaterra), e os resultados foram expressos em Newton/mm (ALI et al., 2010; OLIVEIRA et al., 2014).

5.2.10 Análise sensorial dos frutos

Os frutos de tomate cereja ou uva tratados com QUI, MPOE ou MVOE foram submetidos a testes de aceitação e de intenção de compra em diferentes intervalos de tempo (1, 4, 8 e 12 dias) com frutos armazenados a baixa temperatura (12ºC) (para garantir a segurança microbiológica das amostras oferecidas aos painelistas). Sessenta provadores não treinados participaram dos testes de aceitação e intenção de compra. As análises sensoriais foram realizadas após aprovação no Comitê de Ética em Pesquisa do Hospital Universitário Lauro Wanderley (protocolo 712.884/2014). As análises foram realizadas sob condições de temperatura e iluminação controladas em cabines individuais. Cada um dos participantes recebeu uma unidade de tomate ou uva tratadas com os diferentes revestimentos CHI-EOs servido em pratos descartáveis brancos, codificadas com um número de três dígitos aleatórios servidos monadicamente, acompanhados de biscoito água e sal e água para serem utilizados entre as amostras de modo a limpar o paladar, da ficha de avaliação e do termo de consentimento livre e esclarecido. Para avaliação da aceitabilidade de aparência, cor, sabor, textura, sabor residual e avaliação global foi utilizada numa escala hedônica estruturada de nove pontos ancorados em 1 (desgostei muitíssimo), 5 (nem gostei nem desgostei) e 9 (gostei muitíssimo). A intenção de compra foi avaliada em uma escala hedônica estruturada de cinco pontos ancorados em 1 (certamente não compraria), 3 (talvez comprasse/talvez não comprasse) e cinco (certamente compraria). (SANTOS et al., 2012) .

5.2.11 Análise estatística

Todas as análises foram realizadas em triplicata, em três repetições e os resultados expressos como a média dos dados obtidos em cada repetição. As análises estatísticas foram realizadas utilizando estatística descritiva (média e desvio padrão) e inferencial (testes ANOVA, seguido pelo teste de Tukey ou Kruskal-Wallis) para determinar diferenças estatisticamente significantes ($p \leq 0,05$) entre os tratamentos. Para as análises estatísticas, o software Sigma Stat computacional 2,03 foi utilizado.

6 RESULTADOS E DISCUSSÃO

Como resultado desse estudo foram elaborados dois artigos científicos:

Artigo 1: Coatings comprising chitosan and *Mentha piperita* L. or *Mentha x villosa* Huds essential oils to prevent common postharvest mold infections and maintain the quality of cherry tomato fruits, publicado no periódico **International Journal of Food Microbiology,** v. 214, p.168-178, 2015. Este periódico tem fator de impacto de 3.155, sendo classificado como Qualis A1 na área de Nutrição pela CAPES 2012.

Artigo 2: Impact of composite coatings containing chitosan and *Mentha* essential oils on mold occurrence and quality of table grapes foi enviado para publicação no periódico **Postharvest Biology and Technology**. Esta revista tem fator de impacto de 2.628, sendo classificada como Qualis B1 na área de Nutrição pela CAPES 2012.

Os dois artigos estão apresentados no Apêndice A e no Apêndice B.

7 CONCLUSÃO

Os resultados obtidos demonstraram que a aplicação combinada de QUI obtida de camarão (*L. vannamei*) e MPOE ou MVOE em concentrações subinibitórias exibiram fortes efeitos antifúngicos contra *A. niger*, *B. cinerea*, *P. expansum* e *R. stolonifer*, com base na inibição do crescimento micelial radial e inibição da germinação de esporos fúngicos. A aplicação de QUI e MPOE ou MVOE compreendendo diferentes revestimentos em frutos de tomate cereja e uva de mesa infectados artificialmente atrasou o aparecimento de sinais de infecções durante o armazenamento em temperatura ambiente e em baixas temperaturas. Ainda, os revestimentos testados não afetaram negativamente os aspectos físicos, físico-químicos e sensoriais de tomates cereja e uvas durante o armazenamento em temperatura ambiente e em baixas temperaturas. Nas uvas, atributos específicos de qualidade de cor e firmeza foram melhorados quando revestidas com QUI-MPOE ou QUI-MVOE. Estes achados são suficientes para considerar o potencial de aplicação de revestimentos compostos de QUI de camarão e MPOE ou MVOE em concentrações reduzidas como potenciais tratamentos pós-colheita para controlar as infecções fúngicas, reduzir as perdas pós-colheita e prolongar a vida de prateleira de tomates cereja e uvas de mesa.

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APÊNDICE

APÊNDICE A – Artigo 1: Coatings comprising chitosan and *Mentha piperita* L. or *Mentha x villosa* Huds essential oils to prevent common postharvest mold infections and maintain the quality of cherry tomato fruits, publicado no periódico **International Journal of Food Microbiology,** v. 214, p.168-178, 2015.

Coatings comprising chitosan and *Mentha piperita* L. or *Mentha x villosa* Huds essential oils to prevent common postharvest mold infections and maintain the quality of cherry tomato fruits

Running-title: Anti-mold effects of chitosan and *Mentha* essential oils

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Abstract

In the present study, we evaluated the efficacy of coatings comprising shrimp chitosan (CHI) and *Mentha piperita* L. (MPEO) or *Mentha x villosa* Huds (MVEO) essential oils to control mold infections in cherry tomato fruits (*Solanum lycopersicum* L.) caused by *Aspergillus niger*, *Botrytis cinerea*, *Penicillium expansum* and *Rhizopus stolonifer*, during storage at room (25 ºC for 12 days) and low temperatures (12 ºC for 24 days). The effects of the coatings on the physicochemical and sensory characteristics of cherry tomato fruits during storage were also assessed. The minimum inhibitory concentration (MIC) of CHI against all test fungi was 8 mg/mL, whereas the MIC for both MPEO and MVEO was 5 µL/mL. Combinations of CHI at 4 mg/mL and MPEO or MVEO at 2.5 or 1.25 µL/mL strongly inhibited the mycelial growth and spore germination of target fungi. The coatings comprising CHI and MPEO or CHI and MVEO at the different tested concentrations delayed the growth of mold-causing fungi in artificially contaminated tomato fruits during storage at either room or low temperatures. The assayed coatings preserved the quality of cherry tomato fruits during storage, in terms of physicochemical and sensory attributes. These results indicate that coatings comprising CHI and MPEO or CHI and MVEO represent promising post-harvest treatments to prevent common postharvest mold infections in cherry tomato fruits during storage without affecting the quality of these fruits.

Keywords: *Mentha* spp., chitosan, edible coating, tomato, phytopathogenic fungi, post-harvest treatment

1. Introduction

In the last decade, consumers have shown increasing interest in natural healthy fresh fruits and vegetables. Cherry tomatoes (*Solanum lycopersicum* L.) are popular and well-accepted fruits and excellent sources of phytochemicals (e.g., lycopene, beta-carotene, polyphenols and quercetin), with highlighted antioxidant capacities and potential health benefits (Pinheiro et al., 2013). As a climacteric fruit, the cherry tomato has a relatively short postharvest life, generally limited through transpiration, postharvest diseases, increased ripening and senescence (Fagundes et al., 2014).

These fruits are susceptible to postharvest diseases caused by different pathogenic fungi, resulting in important economic losses. *Botrytis cinerea* Pers. (1794),

Penicillium expansum Link (1809), *Aspergillus niger* Tiegh. and *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill. cause diseases commonly termed gray, blue, black and soft-rot mold, respectively, in cherry tomato fruits. These fungi are among the most common pathogens responsible for postharvest decay in tomato fruits worldwide (Fagundes et al., 2014; Liu et al., 2007; Wang et al., 2010).

The first step to control fungal contamination in fruits involves the application of fungicides in the field. Indeed, fungicides (e.g., benzimidazoles, aromatic hydrocarbons, and sterol biosynthesis inhibitors) are applied on fruits during the postharvest period, and these products do not adversely affect the appearance or quality of the treated fruits (Amiri et al., 2008). However, the indiscriminate and excessive use of synthetic fungicides on crops has been a major cause for the development of resistant fungal pathogen populations, prompting the use of greater amounts of antifungals in agriculture and consequently the detection of increased amounts of toxic residues in food products (Cabral et al., 2013).

In recent years, the preferences of consumers have tended towards foods that contain lower levels of chemical preservatives (or antimicrobials) and exhibit more fresh-like and natural characteristics. Thus, researchers have been investigating safer alternative substances to reduce the use of synthetic fungicides in fruits and vegetables (Liu et al., 2007; Oliveira et al., 2014; Santos et al., 2012). Edible coatings (primarily comprising proteins, lipids or polysaccharides) are considered an environmentally friendly technology to extend the shelf-life of fruits and vegetables through reductions in moisture loss and respiration rate, thereby preventing physical damage and enhancing product appearance (Fagundes et al., 2014).

For cherry tomato fruits, the post-harvest application of edible antifungal chitosan-based coatings has been considered, in which the coating-formulation comprises chitosan (CHI) alone or in combination with other non-chemical antimicrobial substances, such as plant extracts, essential oils and phenolic compounds (Elsabee and Abdou, 2013). CHI is derived from chitin through deacetylation in alkaline media, and this product is considered a natural, non-toxic copolymer comprising β-(1–4)-2-acetamido-D-glucose and β-(1–4)-2-amino-D-glucose units (Elsabee and Abdou, 2013). Chitin (primarily composed of poly(β-(1–4)-2-acetamido-D-glucose) is an abundant, naturally occurring biopolymer found in the exoskeleton of crustaceans, fungal cell walls and other biological materials (Guerra-Sánchez et al., 2009).

Essential oils (EOs) are naturally synthesized in different plant organs as secondary metabolites and are characterized as oily aromatic liquids extracted from aromatic plant materials (Asbahani et al., 2015). EOs have received much attention as inhibitors of a wide variety of phytopathogenic fungi and are generally recognized as safe (GRAS) at the doses typically used in foods (Burt et al., 2004). In addition, these natural plant materials present low resistance-inducing effects in microorganisms (Marinelli et al., 2012). Currently, several *Mentha* species and associated EOs are exploited in diversified sectors, including food, agronomy, cosmetic, medicine and pharmacy. In the food industry, the EOs of *Mentha* species, primarily the *Mentha piperita* L. essential oil (MPEO), are used as flavoring agents in foods and beverages (Burt, 2004) and are commonly exploited due to the antioxidant and antimicrobial properties of these substances (Tyagi and Malik., 2011; Riahi et al., 2013). *Mentha* × *villosa* Hudson is an aromatic plant hybrid of *M. spicata* L. and *Mentha suaveolens* Ehrh., commonly used in Brazilian folk medicine (Lahlou et al., 2002). MPEO and *Mentha x villosa* Huds essential oil (MVEOs) have shown promising results in the inhibition of food-related fungi (Lima et al., 2014; Riahi et al., 2013).

CHI-based coatings have been effective for controlling black spot disease caused by *Alternaria alternata* (Reddy et al., 2000), anthracnose caused by *Colletotrichum* spp. (Muñoz et al., 2009) and gray and blue mold infections caused by *B. cinerea* and *P. expansum*, respectively, in tomato fruits (Badawy and Rabea, 2009; Liu et al., 2007). However, studies verifying the efficacy of the incorporation of MPEO or MVEO in CHI-based coatings to control a variety of pathogenic fungi that cause different post-harvest mold infections in tomato fruits are scarce or nonexistent. Thus, the aim of the present study was to assess the efficacy of the combined application of CHI and MPEO or MVEO as a postharvest treatment to prevent common mold infections caused by *A. niger*, *B. cinerea*, *P. expansum* and *R. stolonifer* in cherry tomato fruits. Assays were performed to (1) determine the minimum inhibitory concentration of CHI, MPEO and MVEO, (2) evaluate the inhibitory effects of different combinations of these substances at subinhibitory concentrations in laboratory media and different coatings on cherry tomato fruits against fungi and (3) verify the effects of the tested coatings on the physicochemical and sensory characteristics of cherry tomato fruits.

2. Material and methods

2.1 Material

Commercially mature (with red color) cherry tomato fruits (*Solanum lycopersicum* L.) were obtained from EMPASA (Supplies and Services Company of Paraíba, João Pessoa, Brazil). Cherry tomato fruits with no visible sign of mechanical damage or fungal infection were selected and standardized according to homogeneous size, appearance color and shape. Prior to the assays, the fruits were surface-disinfected via immersion in a sodium hypochlorite solution (150 ppm, pH 7.2 adjusted using 1 M NaOH) for 15 min, washed with sterile distilled water and dried for 2 h in a safety cabinet.

A. niger URM 5162, *B. cinerea* URM 2802, *P. expansum* URM 3396 and *R. stolonifer* URM 3482 were obtained from the University Recife Mycology Culture Collection (Center for Biological Sciences, Federal University of Pernambuco, Recife, Brazil). The stock cultures were subcultured in Sabouraud agar (Himedia, India) at 25 ºC for seven days to facilitate sufficient sporification. The fungal spores were collected in a sterile saline solution (0.85 g/100 mL NaCl) in Sabouraud broth (Himedia, India), and the resulting suspension was filtered through a triple layer of sterile gauze to retain the hyphal fragments. The number of spores present in the suspension was quantified using a hemocytometer. The spore concentration was adjusted with sterile saline solution to yield an inoculum of approximately 10^6 spores/mL (Rasooli and Owlia, 2005).

The shrimp shells, from a single species of shrimp [*Litopenaeus vannamei* (Boone 1931) – *L. vannamei*] used for CHI extraction were kindly provided by Aquamaris Aquaculture S/A (João Pessoa, Brazil).

Mentha piperita L. (*M. piperita*) and *Mentha x villosa* Huds (*M. villosa*) leaves were collected at the Medicinal Plants Garden, Institute of Research in Drugs and Medicines, Federal University of Paraíba (João Pessoa, Brazil) in July 2011 (7º08’29’’S,34º50’48’’W). Voucher specimens for *M. piperita* and *M. villosa* have been deposited at the Herbarium Prisco Bezerra, Federal University of Ceará (Fortaleza, Brazil) under numbers 14423 and 14996, respectively.

2.2 Extraction of EOs

A total of 10 Kg of each *M. piperita* or *M. villosa* leaves were subjected to hydrodistillation using a Clevenger apparatus, with a proportion of approximately 200 g of fresh leaves for 800 mL of distilled water (Mkadden et al., 2009). Subsequently, the EOs were dried using anhydrous sodium sulfate, filtered and stored in screw-crapped

amber flasks (4 ºC) until tested and analyzed. The EOs obtained from both plant species presented yellowish coloration and a characteristic odor.

2.3 Extraction of CHI from shrimp shells

The shrimp shells were washed under running warm tap water to remove soluble organics, adherent proteins and other impurities. The shells were collected, boiled in distilled water for 1 h to remove the remaining tissues and dried in an oven (Fanem, model 320, Brazil) at 160 °C for 2 h to make the shells more brittle and cause the breakdown of the crystalline structure of chitin. The dried shells were ground into a fine powder using a standard grinder (Model TE-330, Tecnal, São Paulo, Brazil) (Kucukgulmez et al., 2011).

To remove the calcium carbonate (main inorganic component in shrimp shells), the shells were immersed in 1.7 N HCl (1:30 w/v) for 6 h. The extraction was performed at room temperature (25 ºC) with stirring (150 rpm) (Kucukgulmez et al., 2011). The deproteinization of chitin was performed through immersion in 2.5 N NaOH at 65 °C for 6 h. Subsequently, the obtained material was filtered, washed and dried (160 °C, 2 h). The conversion of chitin to CHI was performed through deacetylation. To this end, the chitin was mixed with 2.5 N NaOH (1:50 w/v, 120 ºC, overnight), filtered, washed with deionized water until the filtrate was neutral and dried (90 °C, 2 h) (Kucukgulmez et al., 2011). The obtained CHI in powder form was vacuum-packaged and stored under refrigeration (7 ºC) until use in subsequent assays.

2.4 Identification of the constituents of EOs

The individual constituents of EOs were identified via gas chromatography/mass spectrometry (GC/MS) using a Varian Saturn Gas Chromatograph Model 2000R 3800 equipped with mass spectrometer model 2000R-2000 and a DB-5 capillary column (60 m x 0.25 mm x 0.25 µm) under the following analytical conditions: electron impact, 70 eV; carrier gas, Helium; flow rate, 1.0 mL/min; oven temperature programmed from 60 to 240 °C at 3 °C/min; injector temperature, 240 °C; detector temperature, 230 °C; split ratio 1/20. The injected volume was 1.0 mL of a solution containing approximately 0.1 mL of EO in 1.0 mL of ethyl acetate. Each component was identified after comparing the mass spectra of each compound with the GC/MS database (NIST/EPA/NIH Mass Spectral Database Version

1.7). The components were quantified after normalizing the areas and expressed as a percentage of the total area (%) (Adams, 2001).

2.5 Characterization of CHI

To determine the deacetylation degree (DD), the CHI sample was subjected to infrared (IR) spectroscopy using a spectrophotometer (Bomem Michelson FT-IR, model MB-102). The spectra were obtained in the region from 400-4,000/cm using 2 mg of CHI previously dried overnight at 60 °C under reduced pressure and thoroughly blended with 100 mg of KBr to produce 0.5 mm-thick disks. Based on the infrared spectra, the DD was determined using the absorbance ratio A1655/A3450 and calculated according to the following equation: DD (%) = 97.67 – [26.486 (A_{1655}/A_{3400})] (Robert, 1992).

To determine the CHI thermal profile, 5 mg of CHI was prepared in a platinum crucible. Thermogravimetric analyses (TGA) were performed in a term balance (Shimadzu TGA-50) at temperatures ranging from 28 - 900 ºC under a dynamic air atmosphere (flow rate 30 mL/min) and a heating rate of 10 °C/min. Differential scanning calorimetry (DSC) was performed using a calorimeter (Shimadzu, model DSC-50WS) after heating the samples from 25 °C to 500 °C under a continuous flow of N_2 (50 mL/min) at a heating rate of 10 °C/min. The X-ray patterns were determined using a Shimadzu model XD3A diffractometer. The crystallinity index of the CHI was calculated using the following equation (Robert, 1992):

$CrI = (1\theta\ M - 1\theta\ A) / 1\theta\ A \times 100$ (Equation 1),

where 1θ M = peak (corresponding to the maximum intensity), and 1θ A = minor peak (second to the amorphous diffraction).

2.6 Production of CHI and EOs solutions/emulsions and coatings

The CHI solutions were prepared after dissolving the polymer (16 mg/mL) in acetic acid (1 mL/100 mL) for 24 h at room temperature with stirring (120 rpm) (Santos et al., 2012). The CHI solutions were serially diluted (1:1) in Sabouraud broth to obtain solutions of different concentrations (8.0, 4.0, 2.0, 1.0, 0.5, 0.25 and 0.125 mg/mL). To ensure that the antifungal activity was due to the CHI and not acetic acid, the pH of all of the CHI solutions used in the antifungal assays was adjusted to 5.0 through the addition of 1 M NaOH. MPEO and MVEO emulsions were obtained after dissolving the

substance (80 µL/mL) in Sabouraud broth containing Tween 80 (1%, v/v) as a stabilizing agent (Santos et al., 2012), with successive dilutions (1:1) in the same broth to obtain solutions of different concentrations (20, 10, 5, 2.5, 1.25, 0.06, 0.03 and 0.015 µL/mL). Tween 80, at the highest final concentration obtained after dilution in Sabouraud broth (0.5%, v/v), did not inhibit the growth of the test strains.

For the combined application of CHI and EOs, CHI (4.0 mg/mL) was initially diluted in acetic acid (1 mL/100 mL) with constant stirring (120 rpm) for 6 h at room temperature. Subsequently, different MPEO or MVEO amounts were added (final concentration of 2.5 or 1.25 µL/mL), followed by stirring for an additional 18 h at room temperature. To assay the application of the solution formed by combining CHI and EOs to coat cherry tomato fruits, glycerol was added (2 mL/100 mL) as a plasticizer as the EO was incorporated into the coating-forming solution (Ojagh et al., 2010).

2.7 Determination of the Minimum Inhibitory Concentration (MIC) of CHI and EOs

The MIC values were determined using broth macrodilution. Initially, 1 mL of a suspension of the test fungal strain (approximately 10^6 spores/mL) was inoculated into 4 mL of Sabouraud broth (with concentration adjusted to 10 mL) and 5 mL of the solutions/emulsions containing different concentrations of CHI (8.0, 4.0, 2.0, 1.0, 0.5, 0.25 and 0.125 mg/mL), MPEO or MVEO (20, 10, 5, 2.5, 1.25, 0.06, 0.03 and 0.015 µL/mL) were added. The system was incubated at 25 °C for 7 days, and at the end of the incubation period, the lowest CHI, MPEO or MVEO concentration (highest dilution) that exhibited no visible fungal growth was considered the MIC (Santos et al., 2012; Sharma and Tripathi, 2008).

Based on these MIC values, subinhibitory concentrations of CHI (1/2 MIC) and each of the tested EOs (1/2 MIC and 1/4 MIC) were applied in different combinations to examine the effects on fungal mycelial growth, spore germination and the application as coatings on cherry tomato fruits.

2.8 Effects of CHI and EOs on fungi mycelial growth

The inhibition of CHI in combination with MPEO or MVEO on radial fungi mycelial growth was determined using the poisoned substrate technique (dilution in solid medium). To this end, a 2-mm plug obtained from a 7-day-old test fungal culture, grown on Sabouraud agar at 28 ºC, was placed on the center of Sabouraud agar in Petri dishes - containing CHI and MPEO or MVEO in different combinations and incubated

at 28 ºC for 7 days. As controls, fungal cultures cultivated in growth media without CHI or EOs were similarly tested (Oliveira et al., 2014). The results are expressed as the percent inhibition rates of mycelial radial growth (mm) when the fungi were treated with CHI and MPEO or MVEOs for different incubation periods (3, 5 and 7 days) in relation to the control treatment.

2.9 Effects of CHI and EOs on fungal spore germination

Aliquots of 0.1 mL of each spore suspension (approximately 10^6 spores/mL) obtained from a 7-day-old culture grown on Sabouraud agar (28 °C) were placed in Eppendorf tubes containing 0.1 mL of a solution containing different amounts of CHI and MPEO or MVEO. Subsequently, 0.1 mL of the system was placed at the center of a sterile glass slide and incubated in a moist chamber at 28 °C for 24 h. Each slide was fixed and treated with lactophenol cotton blue stain, and spore germination was observed through light microscopy. Approximately 200 spores were counted on each slide. As controls, suspensions of fungal spores were cultivated in growth media without CHI or EOs were similarly tested. The results are expressed as the percent inhibition of spore germination after comparing the number of germinated spores in media containing CHI and MPEO or MVEO with those obtained in the control treatment (Feng and Zeng, 2007). A spore (conidium) was considered germinated when the developed germ tube was at least twice its original diameter.

2.10 Effect of CHI and EOs on mold growth in cherry tomato fruits

The cherry tomato fruits were first immersed in 500 mL of an inoculum solution (approximately 10^6 spores/mL) of the test fungal strain for 1 min, with gentle stirring using a sterile glass stem, and left to dry in a biosafety cabinet for 1 h (25 ºC). Subsequently, the fruits were immersed in 500 mL of the coating solution containing different CHI and MPEO or MVEO concentrations with gently shaking using a sterile glass stem for 1 min. The fruits were air-dried on a nylon filter to drain the excess liquid, packed in a polyethylene container and covered with a lid. One group of fruits was stored at room temperature (25 °C), whereas the other group was stored at low temperature (12 °C). In addition, a control experiment was performed in which CHI and EOs solutions were replaced with sterile distilled water containing glycerol (2 g/100 mL). Each treatment included 40 fruits. At different storage time intervals (room temperature 1, 4, 8 and 12 days; low temperature 1, 6, 12, 18 and 24 days), the fruits

were examined for the presence of signs of fungal infection (mold appearance) (Feng and Zeng, 2007; Liu et al., 2007). The results were expressed as the storage time for appearance of visible signs of mold infection and the percent of infected fruit (disease incidence) at the end of the assessed storage time.

The characteristic symptoms for black mold (caused by *A. niger*) was darkening and softening of the fruit infected site, followed by breaking the shell and development of a dark mold; the characteristic infection signs for gray mold (caused by *B. cinerea*) was darker circular areas than were softer than the other fruit parts, with subsequently abundant sporulation, whose color ranged from white to gray; the characteristic infection signs for blue mold (caused by *P. expansum*) was darker circular areas where the fruit tissues were softer than the other fruit parts, with subsequently abundant sporification, whose color ranged from white to blue or gray; and the characteristic infection signs for soft-rot (caused by *R. stolonifer*) was watery areas quickly covered by coarse, with gray hairy mycelia forming a mass of black sporangia at their tips (Romanazzi and Feliziani, 2014; Lichter et al., 2002).

2.11 Physicochemical analyses of cherry tomato fruits

The cherry tomato fruits were evaluated for weight loss and general quality parameters, such as color, firmness, soluble solids (SS) and titratable acidity (TA) at the same storage periods evaluated for fungal infection (five fruits from each fruits group were randomly chosen and submitted for analyses at each pre-established storage period). The SS content (in a drop taken from a 5g-macerated fruit sample) was determined using a digital refractometer (Model HI 96801, Hanna Instruments, São Paulo, Brazil), and the results were expressed as ºBrix (Ali et al., 2010). The TA (in 10 g of a macerate fruit sample that was previously mixed with 15 mL of boiled distilled water) was determined using phenolphthalein as an indicator with 0.1 N NaOH, and the results were expressed as mmol H^+/100 g of fruit (equivalent of citric acid) (Ali et al., 2010). To determine the weight loss during storage, the weight of the fruit was monitored at different storage time intervals, and the fruit weight loss in each assessed period was calculated as a percentage of the initial weight (Ali et al., 2010). The skin color was measured at three different equatorial positions of the fruit using the CIE*Lab* system (L * a * b *). The hue angle (h^*_{ab}) and chroma (C^*_{ab}) were measured in a CR-300 colorimeter (MINOLTA Co., Osaka, Japan) using a 10-mm quartz cuvette, consistent with the International Commission on Illumination (CIE, 1986). The CIE*Lab*

color scale (L*a*b*) was used with a D^{65} illuminant (standard daylight) at a 10° angle. Using reference plates, the apparatus was calibrated in the reflectance mode, and the specular reflection was excluded. The firmness was determined using a 3-mm diameter probe (1/8) coupled to a TA-XT2 Texture Analyzer (Stable Micro Systems, Haslemere, UK), and the results were expressed as N/mm (Ali et al., 2010).

2.12 Sensory analysis of cherry tomatoes

Cherry tomato fruits treated with CHI and MVEO or MPEO were subjected to acceptance and preference tests at different time intervals (1, 4, 8 and 12 days) throughout the low temperature storage (to ensure the microbiological safety of the samples offered to the panelists). Sixty untrained tasters participated in the acceptance and preference tests. The sensory analyses were performed after approval through an Ethics Research Committee (protocol 712.884/2014). The analysis was performed under controlled temperature and lighting conditions in individual booths. Each panelist received one unit of tomatoes treated with the different CHI-EOs coatings served on disposable white plates coded with a random three-digit number. The fruit samples were simultaneously served in a blinded and random sequence immediately after their removal from low temperature storage. The tasters were asked to eat a salty biscuit and drink water between samples to avoid aftertaste effects. For the preference test, the tasters were asked to select the most and least appreciated samples based on overall evaluation. The intent to purchase was assessed on a five-point structured hedonic scale ranging from one (certainly would not buy) to five (certainly would buy). For the acceptability of appearance, color, flavor, texture, aftertaste and overall assessment, a nine-point structured hedonic scale was used, ranging from one (dislike very much) to nine (like very much) (Santos et al., 2012).

2.13 Statistical analysis

All analyses were performed in triplicate in three replicates, and the results were expressed as the mean of the data obtained in each replicate. Statistical analyses were performed using descriptive statistics (mean and standard deviation) and inferential tests (ANOVA followed by Kruskal-Wallis's test for data of mycelial growth, spore germination and mold occurrence in fruits or Tukey's test for data of physicochemical and sensory parameters of fruits) to determine statistically significant differences ($p \leq$

0.05) between treatments. For the statistical analyses, the computational Sigma Stat software 2.03 was used.

3. Results

3.1 Identification of constituents in MPEO and MVEO

GC-MS analysis of MPEO and MVEO resulted in the identification of 27 and 28 compounds, respectively, in amounts greater than 1% (Table 1). L (-)-menthol (30.31%) was the major constituent in MPEO, followed by isomenthone (26.70%), menthol acetate (8.52%), eucalyptol (7.03%), menthofuran (5.47%), limonene (5.17%), menthone (4.67%) and caryophyllene (3.93%). Rotundifolone (70.2%) was the major compound in MVEO, followed by limonene (6.42%), β-pinene (4.30%), eucalyptol (4.29%) and α-pinene (3.53%). The other identified constituents in MVEO and MPEO ranged from 0.06 – 2.25% and 0.09 –1.77%, respectively.

3.2 Characterization of CHI

The IR spectra of CHI from *L. vannamei* was characteristic of shrimp CHI. The most significant regions of the spectrum were those that show OH-stretching bands between 3340 and 3480 cm^{-1}, C=O axial deformation of amide I between 1661 and 1666 cm^{-1}, N-H angular deformation between 1583 and 1594 cm^{-1}, CH3 angular deformation between 1310 and 1313 cm^{-1}, CN axial deformation of amide approximately 1425 cm^{-1}, CN-amino groups axial deformation between 1308 and 1380 cm^{-1} and bands of polysaccharides structure in region of 890 – 1156 cm^{-1} (Fig. 1). The presence of an absorption band at 1159 cm^{-1} confirmed that there was no impairment of the macromolecular structure, indicating no depolymerization during CHI extraction/production. Based on the IR spectra, the CHI obtained from *L. vannamei* possesses a DD of 83%.

The TGA of CHI showed a characteristic pattern of degradation that occurred in three phases, wherein the first stage correlates with dehydration, and the second stage, initiated near 132 °C, with a peak temperature of approximately 335 ºC, correlates with the decomposition of the polymer and formation of charred residue. The third stage, initiated at 260 °C, was correlated with the consumption of carbonized residues. Differential scanning calorimetry analyses to evaluate the physical and chemical decomposition during CHI transitions showed distinct thermal events: the first event was endothermic, associated with the evaporation of water, with a peak temperature at

71.7 ºC; and the second event was exothermic, with a peak temperature at 316.4 ºC (data not shown).

According to the results of X-ray diffraction (Fig. 2), the degree of crystallinity of CHI obtained from *L. vannamei* was 71%. According to the crystallographic letter (ICDD 00-035-1974), the identity of CHI was confirmed based on the diffraction peaks at angles 10º and 20º 2Θ, as observed in the present study. The graphic details of the IR-spectrum and X-ray diffraction analyses of CHI from *L. vannamei* in the present study are provided as supplementary information.

3.3 In vitro antifungal effects of CHI and EOs

The MIC for CHI was 8.0 mg/mL against all tested fungi, whereas the MIC for both MPEO and MVEO was 5.0 µL/mL. During the seven days of incubation, the different combinations of CHI and MPEO (CHI-MPEO) and CHI and MVEO (CHI-MVEO) strongly inhibited (90.2 – 97.5% and 90.1 – 94.2%, respectively) the growth of *A. niger, B. cinerea, P. expansum* and *R. stolonifer* compared with the control treatment (Table 2). The different combinations of CHI-MPEO or CHI-MVEO induced similar inhibition ($p > 0.05$) of fungal mycelial growth.

Consistent with results of mycelial growth inhibition, the application of CHI-MPEO or CHI-MVEO at the different test combinations induced the strong inhibition of spore germination for all tested fungal strains (Table 3). The inhibition of spore germination by exposure to the different combinations of CHI and EOs was greater than 75% compared with the number of spores germinated in the control experiment (control treatment consistently presented spore germination rates higher than 90%; data not shown). No differences ($p > 0.05$) were observed among the percent of spore germination inhibition through CHI-MPEO or CHI-MVEO at all assessed combinations. However, the highest rates ($p \leq 0.05$) of spore germination inhibition were observed when CHI and EOs were combined at concentrations of 4 mg/mL and 2.5 µL/mL, respectively.

3.4 Antifungal effects of CHI and EOs coatings on cherry tomato fruits

When cherry tomato fruits were artificially contaminated with *A. niger, B. cinerea, P. expansum* or *R. stolonifer* and coated with solutions comprising CHI-MPEO or CHI-MVEO, visible signs of mold infection were delayed compared with those of the control fruits throughout the duration of the storage period at both room and low

temperatures (Table 4). The cherry tomato fruits artificially infected with the test postharvest pathogenic fungi separately and treated with the different coatings of CHI-MPEO or CHI-MVEO displayed no visible signs of mold infection throughout the 24 days of storage at low temperature. The cherry tomato fruits not coated with CHI-MPEO or CHI-MVEO, but contaminated with *A. niger* and subsequently stored at low temperature exhibited visible signs of mold infection on the 6th day of storage (55 - 58% of infected fruits), whereas the fruits infected with *B. cinerea, P. expansum* or *R. stolonifer* exhibited visible signs of mold infection only on the 12th day of storage (42 - 46% of infected fruits) when stored at low temperature. The uncoated cherry tomato fruits infected with *A. niger, B. cinerea, P. expansum* or *R. stolonifer* and stored at room temperature exhibited visible signs of mold infection on the 4th day of storage (70 - 75% of infected fruits).

The cherry tomato fruits stored at room temperature and coated with CHI-MVEO or CHI-MPEO displayed visible signs of mold infections caused by *A. niger, B. cinerea* and *R. stolonifer* infections on the 12th day of storage (35 – 40% of infected fruits), whereas signs of mold infections caused by *P. expansum* were visible after the 8th day of storage (30 - 35% of infected fruits).

The infection rates by all test mold-causing fungi in tomato fruits coated with CHI-MPEO or CHI-MVEO and stored at room temperature was smaller than 40 % at the end of the storage period; whereas for the uncoated tomato fruits the infection rates were greater than 70% (Table 4). In general, the prevention of mold infection in the cherry tomato fruits caused by CHI and EOs directly varied with the amount of EO in the test coatings and decreased over time.

3.5 Effects of CHI and EOs on the physicochemical and sensory characteristics of cherry tomato fruits

The physicochemical changes in the cherry tomato fruits uncoated or coated with CHI-MVEO or CHI-MPEO were evaluated during storage at room and low temperatures (Table 5 and Table 6, respectively). The cherry tomato fruits treated with coatings comprising CHI-MPEO or CHI-MVEO at both tested amounts presented no differences ($p > 0.05$) for all assessed parameters compared with those of the uncoated fruits during storage at both room and low temperatures, with exception of weight loss. However, differences ($p \leq 0.05$) among the detected values for the same monitored

quality attribute and fruit group (coating treatment) were observed over the different assessed time points during storage at room or low temperatures.

The cherry tomato fruits coated with CHI-MPEO or CHI-MVEO and stored at low temperatures exhibited weight loss rates similar ($p > 0.05$) to those of the uncoated fruits for up to 12 days of storage. However, thereafter, the weight loss rates were lower ($p \leq 0.05$) in the fruits coated with CHI-MPEO or CHI-MVEO. Lower ($p \leq 0.05$) weigh loss rates for the coated fruits stored at room temperature were observed by the 4th day of storage and persisted during the later assessed storage time points compared with those of the uncoated fruits. Overall, the cherry tomato fruits coated with CHI and EOs and stored at room temperature exhibited greater ($p \leq 0.05$) weight loss during storage than the coated fruits stored at low temperatures. Still, for both the coated and uncoated fruits, the firmness notably decreased ($p \leq 0.05$) with increasing storage time (Table 5 and Table 6).

The cherry tomato fruits uncoated and coated with CHI-MPEO or CHI-MVEO and stored at either room or low temperatures exhibited the highest TA and SS values until the 8th and 12th day of storage, respectively, and these values decreased ($p \leq 0.05$) at the later assessed storage time intervals. The uncoated and coated cherry tomato fruits were predominantly red throughout storage at both room and low temperatures. The a* and b* values (positive values close to zero) increased ($p \leq 0.05$) over time, resulting in decreased lightness as a consequence of the enhancement in fruit opacity. Furthermore, under all treatment conditions, a decrease ($p \leq 0.05$) in the h* value with concomitant increase in the C* value, provoked changes in color from red/orange to red over time. Additionally, an increase in the lightness (L* value) was observed when the fruits were coated with CHI-MPEO or CHI-MVEO at all tested combinations (Table 7 and Table 8).

The cherry tomato fruits, uncoated or coated with CHI-MPEO or CHI-MVEO, were subjected to acceptance, purchase intention and preference tests during 1, 4, 8 and 12 days of storage at low temperature (data not shown). The uncoated and CHI-MPEO- or CHI-MVEO-coated cherry tomato fruits received similar scores ($p > 0.05$) for all assessed sensory attributes (appearance, color, flavor, texture, aftertaste and overall assessment) at different storage time periods. The coated and uncoated fruits groups received scores in the “liked slightly” or “liked moderately” categories for all sensory parameters tested. When the panelists were asked to indicate the intent to purchase the cherry tomato fruits for all groups and assessed storage periods, the responses were

generally “possibly purchase”. Similarly, there was no difference ($p > 0.05$) in preference between the treatment groups for the duration of the storage time.

Moreover, no difference ($p > 0.05$) was observed between the values for all assessed quality parameters in the fruits treated with coatings containing the two different tested amounts of each MPEO or MVEO. Overall, no differences ($p > 0.05$) were observed between the values for all assessed quality parameters in the fruits treated with coatings containing the same amount of MPEO or MVEO.

4. Discussion

In recent years, researchers have examined safe alternative substances to reduce or even replace the use of synthetic fungicides in fruits, and edible coatings comprising CHI and EOs have been considered as a feasible technology to control postharvest decay in fruits, reflecting the antifungal properties, biodegradability and absence of toxic effects on fruits of these compounds (Cabral et al., 2013). Considering the potential bioactive properties of MPEO and MVEO (Asbahani et al., 2015), in the present study, we assessed the efficacy of these EOs when applied in combination with the biopolymer CHI (obtained from *L. vanammei*) to generate different coatings to control the growth of different post-harvest pathogenic fungi that cause mold infections in cherry tomato fruits.

The major constituents identified in MPEO were menthol (30.3%) and the methol precursor isomenthone (26.7%), consistent with the findings of previous studies on the composition of different samples of MPEO (Tyagi and Malik, 2011). The menthol content is paramount to MPEO quality, and characteristic organoleptic properties (pleasure “like-mint flavor”) of MPEO are predominantly derived from menthol (Davis et al., 2005). In MVEO, the major constituent was rotundifolone (70.15%), an oxygenated monoterpene also referred to as piperitenone oxide, which has been reported to characterise the volatile oil of some chemotypes of *Mentha* sp., such as *M. villosa* (Lorenzo et al., 2002). Studies to determine the individual constituents of MVEO also detected roduntifolone as a major constituent (70.96% - 80.80%) (Lima et al., 2014; Lorenzo et al., 2002; Teles et al., 2013). Roduntifolone is an important compound associated with some of the biological properties of MVEO (Lahlou et al., 2002; Lima et al., 2014).

Among many characteristics, the DD is one of the most important chemical characteristic of CHI, and this factor influences the performance of the CHI molecule in

different biological functionalities (Kucukgulmez et al., 2011). Thus, previous studies (Li et al., 1992; Kucukgulmez et al., 2011) have suggested that the term CHI should present better biological functionalities when the DD of the analyzed molecule is above 70%. In the present study, the DD of CHI extracted from *L. vannamei* was determined as 83%, consistent with the recommended range and specifying the extent of the deacetylation process.

CHI, MPEO and MVEO exhibited MIC of 8 mg/mL, 5 µL/mL and 5 µL/mL, respectively, against *A. niger*, *B. cinerea*, *P. expansum* and *R. stolonifer*. The detected MICs of the tested CHI were lower than those previously reported for commercial CHI (10 mg/mL) against *R. stolonifer* (10 mg/mL) (Santos et al., 2012) and *P. expansum* (10 – 20 mg/mL) (Wang et al., 2014). In contrast, the detected MICs of MPEO and MVEO were higher than those previously reported for the same EOs against *Aspergillus* species (2.5 - 4.0 µL/mL) (Sokóvic et al., 2009). This discrepancy suggests that the MIC could most likely be associated with the intrinsic characteristics of the assayed antifungal compounds, such as the physicochemical properties (primarily the DD) of the CHI molecule and the different major compounds forming the tested EO samples.

In light of the obtained MICs, CHI at 4 mg/mL and MPEO and MVEO at 2.5 and 1.25 µL/mL were used in further antifungal assays. The CHI concentration was set at 4 mg/mL (1/2 x MIC) because this value was the lowest tested concentration capable of forming a viscous solution with coating features to permit application as a coating for cherry tomato fruits. The MPEO and MVEO concentrations were set at 2.5 and 1.25 µL/mL (1/2 MIC and 1/4 x MIC, respectively) because researchers have stated that EOs could be incorporated at low concentrations in coating-forming solutions to minimize the impact of these compounds on olfactory perception of consumers (Perdones et al., 2012).

The combined application of CHI-MPEO or CHI-MVEO at different subinhibitory concentrations strongly inhibited the mycelial growth and spore germination of all test fungal strains in synthetic media. Previous studies have reported the efficacy of CHI to inhibit the mycelial growth and spore germination of *B. cinerea, P. expansum, R. stolonifer* (Li et al., 2009; Santos et al., 2012; Wang et al., 2014). Examination of the inhibitory effects of commercial CHI on *B. cinerea* and *P. expansum* observed that the spore germination was completely inhibited when CHI was incorporated into the growth media at 5 and 10 mg/mL, respectively (Li et al., 2009).

The antifungal properties of CHI have been always associated with the effects of this compound on the fungal cell wall and cell membrane. It has been suggested that the

damage to the fungal membrane might reflect interactions between the positive amino groups of CHI with the negatively charged phospholipids of the plasma membrane, resulting in a change in the permeability of plasma membrane (Laflamme et al., 1999). However, researchers have proposed that the uptake of CHI into fungal cells is energy-dependent, rather than through endocytosis or passive diffusion. Thus, once CHI enters the cytoplasm, this compound translocates to the nucleus of fungal cells and interferes with RNA and protein synthesis (Palma-Guerrero et al., 2008; Ali et al., 2013). Specifically, it has been proposed that the efficacy of CHI in inhibiting pathogenic fungi in fruits could be also related to its eliciting property inducing the increased production of defense-related enzymes (e.g. polyphenoloxydase and peroxidase) in CHI-coated fruits (Liu et al., 2007; Edirisinghe et al., 2014).

Researchers have also reported the efficacy of MPEO and MVEO on the inhibition of different phyotpathogenic fungi, including *A. niger, Penicillium digitarum, A. niger, B. cinerea, Rhizopus oryzae* and *Alternaria alternata* (Lima et al., 2014; Riahi et al., 2013; Tyagi and Malik, 2011). The antimicrobial properties of EOs are often attributed to the major components of these compounds (Ait-Ouazzou et al., 2012). The structural functional group plays an important role in the biological activity of the molecules (constituents) forming EOs. Menthol and rotundifolone are cyclic and oxygenated monoterpenes, respectively. Studies have demonstrated that monoterpenes play essential roles in the disorganization of cell membrane structures, causing depolarization and physical or chemical alterations, thereby disrupting fungal metabolic activities (Ait-Ouazzou et al., 2012). However, minor components (such as, limonene, α-pinene, β pinene, caryophyllene) also significantly influence the antimicrobial properties of the EOs from *Mentha* species (Mahboubi and Haghi, 2008), likely through the establishment of synergistic interactions (Ait-Ouazzou et al., 2012; Riahi et al., 2013).

The application of different coatings comprising CHI-MPEO or CHI-MVEO on cherry tomato fruits inhibited the growth of all target mold-causing fungi in artificially contaminated fruits during storage at room or low temperatures. However, in fruits stored at low and room temperatures, the coatings were less effective at controlling *A. niger* and *P. expansum*, respectively, than the other tested fungi. Although CHI, MPEO and MVEO displayed each the same MIC against all the postharvest pathogenic fungi tested in synthetic media, this result might reflect the differences in the sensitivity of various fungi to CHI and EOs when applied as a coating on fruits and the pathogenesis

of different fungi during host-pathogenic interactions (Oliveira et al., 2014). A previous study noted that CHI was more effective in controlling the gray mold caused by *B. cinerea* than the blue mold caused by *P. expansum* in tomato fruits stored at room or low temperature (Liu et al., 2007).

Similar to the findings of the present study, researchers have suggested that the antifungal activity of CHI and some EOs (either alone or in combination) on fruits increases with decreased storage temperature (Santos et al., 2012; Oliveira et al., 2014). Storage at low temperatures slows physiological processes in fruits, and pathogens have weaker pathogenicity at low temperatures, resulting in a decreased incidence of decay and signs of mold infections compared with that of fruit stored at room temperature, which rapidly decays. The suppression of decay through CHI-based coatings could also partially reflect a delay in the senescence of cherry tomato fruits as resistance to fungal infection in fruits might be enhanced through slower senescence (Oliveira et al., 2014).

Considering that the physicochemical and sensory properties of fruits are the essential characteristics of the general qualities that influence the shelf life and acceptance of these foods by consumers, this study assessed the effects of different coatings comprising CHI-MPEO and CHI-MVEO on these attributes in cherry tomato fruits during storage. The fruits coated with CHI-MPEO or CHI-MVEO lost less weight during storage at both room and low temperatures than the uncoated fruits, although the coated fruits stored at room temperature exhibited the greatest weight loss during storage. Weight loss during respiration primarily results from transpiration and the loss of carbon atoms from fruit in each cycle of respiration. However, the cherry tomato fruits coated with CHI-MPEO or CHI-MVEO exhibited comparatively lower weight loss, likely reflecting the enhanced structural continuity of the applied coatings and hydrophobic nature of the incorporated EOs as effective barriers against gases, moisture and solute movement (Das et al., 2013). Studies on the effects of coatings comprising CHI and different concentrations of *Cymbopogon citratus* D.C. Stapf. (lemongrass) EOs on bell pepper demonstrated that the EO concentration directly affected the water vapor permeability, and the incorporation of EOs into edible coatings is desirable as these compounds enhance moisture retention properties (Ali et al., 2015).

Color is one of the most important quality parameters of tomato fruits, affecting the purchase decision of consumers. The main color quality of tomato fruits is redness as this character is affected through lycopene content (Ali et al., 2010). In the present study, the application of the CHI-MVEO and CHI-MPEO coatings presented no

negative effect on lycopene formation in the cherry tomato fruits, even when the fruits were exposed to low temperatures as the color of these fruit changed from orange-red to darker red during storage. Moreover, the tested coatings did not affect ethylene synthesis, which causes the degradation of chlorophyll and production of carotenoids (e.g., lycopene in tomato fruits), promoting changes in fruit color (Ali et al., 2010). In the tomato fruits coated with CHI and EOs, an increase in luminosity was observed compared with that of the uncoated fruits. Higher luminosity has been described for CHI-coated fruits, which presents luminosity and high transparency of CHI films.

Notably, the results of the analysis of the physicochemical parameters of the cherry tomato fruits assessed in the present study are consistent with the data from previous studies on healthy tomato fruits (Tigist at al., 2013). The results for the coated and uncoated tomato fruits showed decreasing firmness during storage and an initial increase in TA and SS, followed by a decrease at later storage time points. The softening of fruit during maturation reflects changes in the structure/composition of the cell wall and intracellular components, involving the enzymatic hydrolysis of pectin and starch (Ali et al., 2010). The detected changes in TA in cherry tomato fruits could reflect the fact that citric acid is the predominant organic acid in tomato fruits, accounting for approximately 90% of the acidity in these fruits. During the maturation period, tomato fruits undergo an initial increase in the amount of citric acid, with an expected decrease over time until full maturity (Ali et al., 2010). For SS, the values commonly detected for different varieties of tomato fruits range from 4 to 8 ºBrix, which similarly present an initial increase during maturation, followed by a decrease over time (Tigist at al., 2013). The application of the tested coatings did not change the sensory characteristics of the cherry tomato fruits during storage. These findings are notable as the different tested coatings comprising CHI-MPEO or CHI-MVEO showed strong inhibitory effects against post-harvest pathogenic fungi, with no noticeable unsatisfactory effects on the sensory properties of the cherry tomato fruits during storage.

5. Conclusions

The results of the present study showed that the combined application of CHI from shrimp (*L. vannamei*) and MPEO or MVEO at subinhibitory concentrations exhibited strong antifungal effects against *A. niger*, *B. cinerea*, *P. expansum* and *R. stolonifer*, based on the radial mycelial growth and inhibition of spore germination. The

application of CHI and MPEO or MVEO comprising different coatings on artificially contaminated fresh cherry tomato fruits delayed the appearance of signs of common mold infections in fruits during storage at both room and low temperatures. Still, the tested coatings did not negatively affect the physicochemical or sensory aspects of cherry tomato fruits during storage at room or low temperatures. These findings reveal the potential of coatings comprising CHI and MPEO or MVEO to prevent mold infections and reduce post-harvest losses in these agricultural commodities. Finally, further studies are needed to evaluate the efficacy of the tested composite coatings in controlling incipient (already established) mold infections in (wounded) cherry tomato fruits.

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Figure captions.

Figure 1. Infrared (IR) spectrum of chitosan from shrimp (*L. vannamei*).

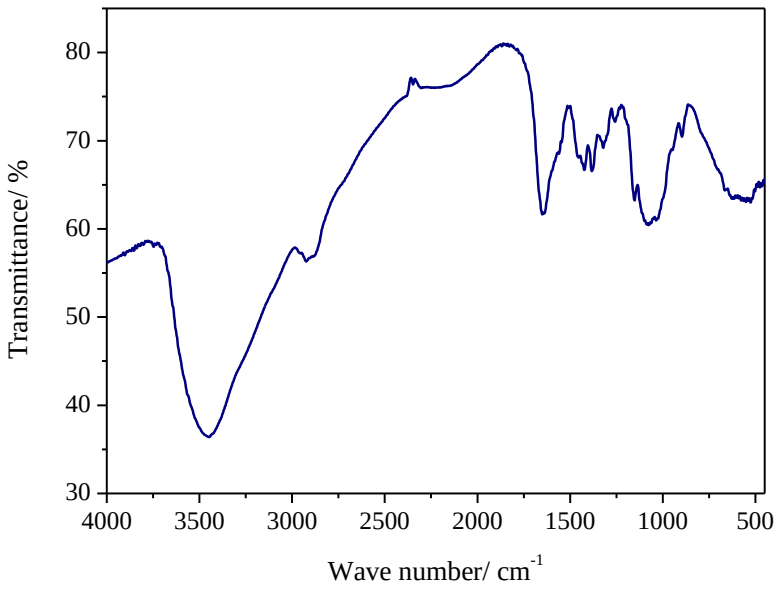

Figure 2. X-ray diffraction of chitosan from shrimp (*L. vannamei*)

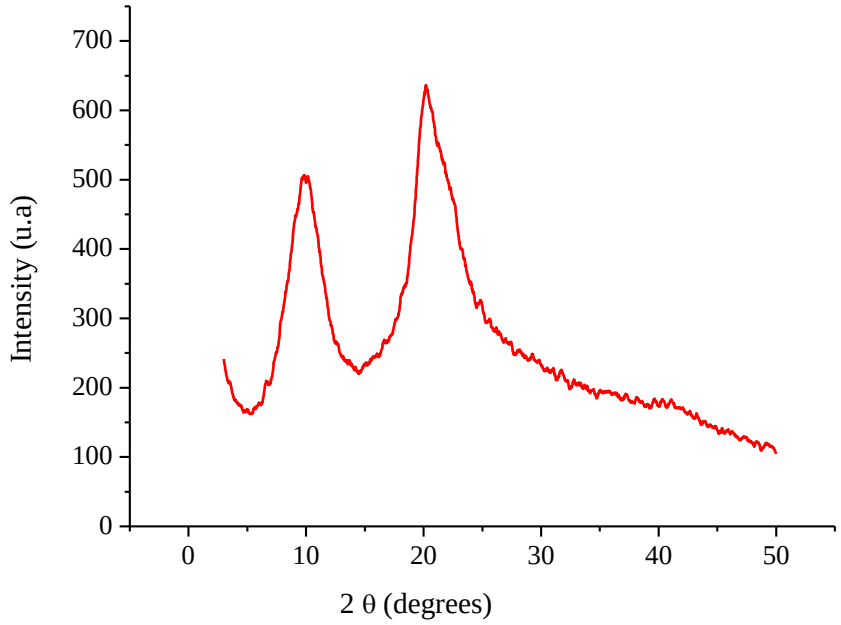

Table 1. GC-MS analysis of the essential oils from *Mentha piperita* L. (MPEO) and *Mentha x villosa* Huds (MVEO).

MPEO				**MVEO**			
Peak	**LRI***	**Constituents**	**%**	**Peak**	**LRI***	**Constituents**	**%**
1	951	1-R-α pinene	1.25	1	886	p-xylene	0.59
2	990	β-phellandrene	0.55	2	936	α-pinene	3.53
3	999	β-pinene	2.25	3	966	camphene	0.73
4	1032	p-mentha-1,4(8)-diene	0.19	4	986	sabinene	1.77
5	1040	o-cymene	0.48	5	995	β-pinene	4.30
6	1044	L-limonene	5.17	6	1005	3-octanol	0.92
7	1051	eucalyptol	7.03	7	1017	(+)-camphene	0.15
8	1072	γ-terpinene	0.32	8	1037	o-cymene	0.13
9	1101	terpinolene	0.13	9	1041	limonene	6.42
10	1176	isomenthone	26.7	10	1045	β-phellandrene	0.09
11	1184	menthofuran	5.47	11	1048	eucalyptol	4.29
12	1186	menthone	4.67	12	1052	β-ocimene	0.57
13	1189	L-(-)-menthol	30.3	13	1099	terpinolene	0.12
14	1209	17-octadecynoic acid	0.06	14	1110	1,6-octadie-3-ol,3,7dim.	0.49
15	1260	pulegone	1.01	15	1122	3-octanol, acetate	0.54
16	1278	piperitone	0.32	16	1196	borneol	0.18
17	1285	menthol, acetate	8.52	17	1264	carvone	0.33
18	1396	cedrene	0.14	18	1388	rotundifolone	70.2
19	1407	β-bourbonene	0.39	19	1395	α-amorphene	0.16
20	1443	β-cubebene	0.20	20	1405	β-bourbonene	0.73
21	1446	caryophyllene	3.93	21	1426	cinerolone	0.62
22	1455	β-copaene	0.15	22	1430	α-gurjunene	0.44
23	1478	α-amorphene	0.17	23	1445	caryophyllene	0.86
24	1483	α-caryophyllene	0.24	24	1460	α-farnesene	0.18
25	1508	germacrene D	0.20	25	1481	humolene	0.30
26	1529	γ-muurolene	0.13	26	1486	β-cubebene	0.49
27	1541	δ-cadinene	0.09	27	1496	germacrene D	0.71
28	-	-		28	1546	calamenene trans	0.21

* Linear Retention Index, calculated relative to the retention time of a mixture of n-alkane (C7 - C25) in a DB-5 column

Table 2. Percent inhibition of the radial mycelial growth of post-harvest pathogenic fungi in solid medium after exposure to chitosan (CHI) and *M. piperita* essential oil (MPEO) or *M. villosa* Huds essential oil (MVEO) at different concentrations.

Treatments	**MPEO**			**MVEO**		
	Days of incubation			**Days of incubation**		
	3	**5**	**7**	**3**	**5**	**7**
	A. niger			***A. niger***		
CHI (4 mg/mL) + EO 2.5 µL/mL	97.5% (± 1.0)	95.3% (± 1.8)	95.1% (± 1.8)	93.8% (± 1.7)	93.6% (± 2.0)	93.2% (± 1.9)
CHI (4 mg/mL) + EO 1.25 µL/mL	94.1% (± 0.9)	92.1% (± 2.0)	92.1% (± 1.7)	93.7% (± 1.1)	92.5% (± 1.9)	92.3% (± 1.9)
	B. cinerea			***B. cinerea***		
CHI (4 mg/mL) + EO 2.5 µL/mL	95.3% (± 1.2)	95.2% (± 1.6)	94.1% (± 2.0)	92.6% (± 1.8)	92.4% (± 1.8)	90.3% (± 2.8)
CHI (4 mg/mL) + EO 1.25 µL/mL	92.4%(± 1.8)	92.3% (± 1.8)	90.2% (± 2.4)	91.5% (± 1.8)	91.3% (± 1.2)	90.4% (± 2.0)
	P. expansum			***P. expansum***		
CHI (4 mg/mL) + EO 2.5 µL/mL	92.5% (± 1.2)	91.4% (± 2.3)	90.3% (± 2.1)	91.4% (± 1.3)	91.2% (± 1.4)	91.5% (± 1.2)
CHI (4 mg/mL) + EO 1.25 µL/mL	92.6% (± 1.3)	91.5% (± 1.7)	90.4% (± 2.2)	91.3% (± 1.2)	90.1% (± 1.9)	90.6% (± 1.4)
	R. stolonifer			***R. stolonifer***		
CHI (4 mg/mL) + EO 2.5 µL/mL	92.2% (± 1.1)	90.2% (± 2.4)	90.5% (± 2.0)	94.2% (± 1.2)	92.2% (± 1.9)	92.7% (± 1.3)
CHI (4 mg/mL) + EO 1.25 µL/mL	91.3% (± 1.9)	91.4% (± 2.0)	91.6% (± 2.0)	92.1% (± 1.2)	92.3% (± 1.4)	90.8% (± 1.9)

CHI: Chitosan, EO: Essential oil

The results are expressed as the percent inhibition rates of mycelial radial growth compared with the control treatment (0 µL/mL of chitosan and essential oil)

The diameter of the radial mycelial fungal growth in control treatments were always greater than 15, 25 and 40 mm after 3, 5 and 7 days of incubation, respectively, for all tested fungi

No superscript letters were presented because no differences ($p > 0.05$) were detected among the mean values (for each fungus submitted to the different treatments and for the same treatment during the assessed incubation period) according to Kruskall-Wallys's test.

Table 3. Percent inhibition of postharvest pathogenic fungal spore germination in liquid medium after exposure (24 h, 28 ºC) to chitosan (CHI) and *M. piperita* essential oil (MPEO) or chitosan and *M. villosa* Huds essential oil (MVEO) at different concentrations.

Treatments	***A. niger***	***B. cinerea***	***P. expansum***	***R. stolonifer***
CHI 4 mg/mL + MPEO 2.5 µL/mL	87.2% (± 1.3) [a]	86.1% (± 1.3) [a]	86.4% (± 1.4) [a]	87.8% (± 1.8) [a]
CHI 4 mg/mL + MPEO 1.25 µL/mL	76.3% (± 1.3) [b]	77.2% (± 1.1) [b]	75.3% (± 1.4) [b]	76.1% (± 1.4) [b]
CHI 4 mg/mL + MVEO 2.5µL/mL	90.4% (± 1.4) [a]	87.3% (± 1.4) [a]	88.2 %(± 1.3) [a]	89.6% (± 1.4) [a]
CHI 4 mg/mL + MVEO 1.25µL/mL	79.5% (± 1.3) [b]	78.4 %(± 1.3) [b]	77.5% (± 1.4) [b]	77.2% (± 1.6) [b]

The results expressed as percent inhibition rates of spore germination compared with the control treatment (0 µL/mL of chitosan and essential oil)

The approximate numbers of germinated spores in control treatments were in a range of 8.2×10^5 to 9.2×10^5 spores/mL for all tested fungi

[a - b] For each trial, different superscript letters in the same column denote differences ($p \leq 0.05$) among the mean values (for the same treatments) according to Kruskall-Wallys's test.

Table 4. Occurrence of mold infection in cherry tomato fruits uncoated and coated with chitosan (CHI) and *M. piperita* essential oil (MPEO) or *M. villosa* Huds essential oil (MVEO), followed by storage at room (25 °C, 12 days) or low temperature (12 °C, 24 days).

Treatments	**Days of storage for detection of first signs of mold infection**		**Percent of infected fruits at the end of the storage time**	
	Room temperature	**Low temperature**	**Room temperature**	**Low temperature**
	MPEO			
	A. niger			
Control	4th	6th	70% (± 3%) [a]	55% (± 4%)
CHI 4 + MPEO 2.5	12th	nd	31% (± 2%) [b]	nd
CHI 4 + MPEO 1.25	12th	nd	38% (± 3%) [b]	nd
	B. cinerea			
Control	4th	12th	71% (± 4%) [a]	42% (± 5%)
CHI 4 + MPEO 2.5	12th	nd	33% (± 4%) [b]	nd
CHI 4 + MPEO 1.25	12th	nd	38% (± 3%) [b]	nd
	P. expansum			
Control	4th	12th	74% (± 4%) [a]	45% (± 4%)
CHI 4 + MPEO 2.5	8th	nd	33% (± 3%) [b]	nd
CHI 4 + MPEO 1.25	8th	nd	35% (± 4%) [b]	nd
	R. stolonifer			
Control	4th	12th	74% (± 5%) [a]	44% (± 3%)
CHI 4 + MPEO 2.5	12th	nd	30% (± 3%) [b]	nd
CHI 4 + MPEO 1.25	12th	nd	35% (± 3%) [b]	nd
	MVEO			
	A. niger			
Control	4th	6th	71% (± 5%) [a]	55% (± 5%)
CHI 4 + MVEO 2.5	12th	nd	36% (± 4%) [b]	nd
CHI 4 + MVEO 1.25	12th	nd	39% (± 4%) [b]	nd
	B. cinerea			
Control	4th	12th	73% (± 5%) [a]	46% (± 5%)
CHI 4 + MVEO 2.5	12th	nd	32% (± 4%) [b]	nd
CHI 4 + MVEO 1.25	12th	nd	36% (± 3%) [b]	nd
	P. expansum			
Control	4th	12th	75% (± 5%) [a]	45% (± 4%)
CHI 4 + MVEO 2.5	8th	nd	33% (± 4%) [b]	nd
CHI 4 + MVEO 1.25	8th	nd	35% (± 3%) [b]	nd
	R. stolonifer			
Control	4th	12th	75% (± 4%) [a]	42% (5%)
CHI 4 + MVEO 2.5	12th	nd	32% (± 4%) [b]	nd
CHI 4 + MVEO 1.25	12th	nd	39% (± 3%) [b]	nd

Control: 0 µL/mL of chitosan and essential oil + 2 g/100 mL of glycerol;
CHI 4 + MPEO 2.5: CHI 4 mg/mL + MPEO 2.5 µL/mL; CHI 4 + MPEO 1.25: CHI 4 mg/mL + MPEO 1.25 µL/mL; CHI 4 + MVEO 2.5: CHI 4 mg/mL + MVEO 2.5 µL/mL; CHI 4 + MVEO 1.25: CHI 4 mg/mL + MVEO 1.25 µL/mL; nd: not detected.
[a - b] For each trial, different superscript letters in the same column denote differences ($p \leq 0.05$) among the mean values (for the same treatments) according to Kruskall-Wallys's test.

Table 5. Mean values of the physicochemical quality parameters in cherry tomato fruits uncoated and coated with chitosan (CHI) and *M. piperita* L. essential oil (MPEO), followed by storage at room (25 °C, 12 days) or low temperature (12 °C, 24 days).

Treatments	**Room temperature (25 °C)**				**Low temperature (12 ºC)**				
	Days of storage				**Days of storage**				
	1	**4**	**8**	**12**	**1**	**6**	**12**	**18**	**24**
	Weight loss (%)								
Control	1.85(±0.26)^{aA}	3.01(±0.64)^{bA}	6.02(±0.63)^{cA}	8.56(±0.25)^{dA}	1.22 (±0.29)^{dA}	2.15(±0.43)^{cA}	3.56(±1.63)^{cA}	5.68(±1.58)^{bA}	6.45(±0.83)^{aA}
CHI 4 + MPEO 2.5	0.98(±0.44)^{aB}	1.45(±0.89)^{bB}	4.12(±0.87)^{cB}	5.42(±0.99)^{dB}	0.75(±0.18)^{dB}	1.65(±0.27)^{cB}	2.76(±0.86)^{bB}	3.76(±1.86)^{bB}	4.93(±0.93)^{aB}
CHI 4 + MPEO 1.25	0.97(±0.51)^{aB}	1.65(±0.44)^{bB}	4.44(±0.21)^{cB}	5.78(±0.53)^{dB}	069(±0.37)^{dB}	1.44(±0.19)^{cB}	2.67(±0.93)^{bB}	3.54(±1.75)^{bB}	4.88(±0.89)^{aB}
	Firmness (N/mm)								
Control	7.67(±0.76)^{c}	6.62(±0.46)^{b}	6.24(±0.36)^{b}	5.18(±0.65)^{a}	8.01(±0.49)^{c}	7.77(±0.34)^{c}	7.87(±1.36)^{c}	6.94(±1.95)^{b}	5.36(±0.28)^{a}
CHI 4 + MPEO 2.5	7.28(±0.24)^{b}	6.33(±0.98)^{b}	6.47(±0.46)^{b}	5.42(±0.45)^{a}	8.40(±0.38)^{c}	7.06(±0.72)^{b}	7.68(±0.58)^{b}	7.74(±1.68)^{b}	6.48(±0.39)^{a}
CHI 4 + MPEO 1.25	7.65(±0.11)^{b}	6.64(±0.44)^{b}	6.50(±0.87)^{b}	5.78(±0.69)^{a}	8.69(±0.47)^{c}	7.94(±0.91)^{b}	7.23(±0.39)^{b}	7.26(±1.42)^{b}	6.51(±0.98)^{a}
	Titrable acidity (mmol H^+/100 g of fruit)								
Control	61.2(±0.47)^{c}	56.2(±1.46)^{b}	55.0(±0.89)^{a}	54.8(±0.78)^{a}	65.7(±0.38)^{c}	63.6(±0.27)^{c}	56.2(±1.04)^{b}	55.1(±1.02)^{b}	52.1(±1.93)^{a}
CHI 4 + MPEO 2.5	61.2(±1.69)^{c}	59.4(±0.56)^{b}	56.6(±0.94)^{a}	55.7(±0.23)^{a}	67.7(±0.47)^{c}	64.4(±0.56)^{c}	59.3(±0.98)^{b}	58.8(±1.34)^{b}	54.1(±1.92)^{a}
CHI 4 + MPEO 1.25	62.8(±0.56)^{c}	57.6(±0.67)^{b}	57.2(±1.56)^{a}	54.2(±1.23)^{a}	63.9(±1.48)^{c}	62.9(±1.74)^{c}	59.9(±1.34)^{b}	58.9(±1.93)^{b}	54.2(±1.25)^{a}
	Soluble solids (ºBrix)								
Control	8.00 (±0.01)^{c}	7.28(±0.01)^{b}	7.11(±0.01)^{b}	6.94(±0.01)^{a}	7.47(±0.01)^{b}	7.29 (±0.01)^{b}	6.93(±0.01)^{a}	6.90(±0.02) ^{a}	6.94(±0.01)^{a}
CHI 4 + MPEO 2.5	7.43 (±0.01)^{c}	7.05(±0.01)^{b}	6.90(±0.01)^{b}	5.81(±0.01)^{a}	7.57(±0.01)^{c}	7.24 (±0.01)^{c}	6.71 (±0.02)^{b}	6.50(±0.02)^{b}	5.81(±0.01)^{a}
CHI 4 + MPEO 1.25	8.07(±0.0.1)^{c}	7.30(±0.01)^{b}	7.11(±0.01)^{b}	6.51(±0.01)^{a}	7.67(±0.02)^{b}	7.58 (±0.01)^{b}	6.63 (±0.02)^{a}	6.50 (±0.01)^{a}	6.51(±0.01)^{a}

Control: 0 µL/mL of chitosan and essential oil + 2 g/100 mL of glycerol

CHI 4 + MPEO 2.5: CHI 4 mg/mL + MPEO 2.5 µL/mL; CHI 4 + MPEO 1.25: CHI 4 mg/mL + MPEO 1.25 µL/mL

$^{a-c}$ For each trial, different superscript letters in the same row denote differences ($p \leq 0.05$) among the mean values (for the same treatments) according to Tukey's test.

$^{A-B}$ For each trial, different superscript letters in the same column denote differences ($p \leq 0.05$) among the mean values (for the same treatments) according to Tukey's test.

Table 6. Mean values of the physicochemical quality parameters in cherry tomato fruits uncoated and coated with chitosan (CHI) and *M.* h*villosa* Huds essential oil (MVEO), at different concentrations, followed by storage at room (25 °C, 12 days) or low temperature (12 °C, 24 days).

Treatments	**Room temperature (25 °C)**				**Low temperature (12 ºC)**				
	Days of storage				**Days of storage**				
	1	**4**	**8**	**12**	**1**	**6**	**12**	**18**	**24**
	Weight loss (%)								
Control	1.76(±0.62)^{cA}	2.22(±0.77)^{cA}	4.54(±0.62)^{bA}	7.02(±0.65)^{aA}	1.02(±0.35)^{dA}	2.22(±0.22)^{cA}	3.56(±0.67)^{bA}	4.87(±0.54)^{bA}	5.78(±0.32)^{aA}
CHI 4 + MVEO 2.5	0.99(±0.44)^{dB}	1.77(±0.44)^{cB}	2.92(±0.42)^{bB}	5.78(±0.88)^{aB}	0.86(±0.48)^{dB}	1.67(±0.45)^{cB}	2.77(±0.84)^{bB}	3.33(±0.23)^{bB}	4.78(±0.44)^{aB}
CHI 4 + MVEO 1.25	0.92(±0.15)^{dB}	1.54(±0.51)^{cB}	3.011(±0.15)^{bB}	5.92(±0.99)^{aB}	0.88(±0.39)^{dB}	1.78(±0.31)^{cB}	2.96(±0.91)^{bB}	3.22(±0.65)^{bB}	4.92(±0.43)^{aB}
	Firmness (N/mm)								
Control	7.11(±0.86)^{b}	6.70(±0.46)^{a}	6.62(±0.36)^{a}	6.20(±0.65)^{a}	8.91(±0.29)^{c}	7.06(±0.34)^{c}	7.55(±1.36)^{c}	6.94(±1.95)^{b}	5.36(±0.28)^{a}
CHI 4 + MVEO 2.5	7.43(±0.24)^{c}	6.39(±0.98)^{b}	6.38(±0.46)^{b}	5.45(±0.45)^{a}	8.50(±0.48)^{c}	7.54(±0.72)^{c}	7.54(±0.58)^{c}	6.74(±1.68)^{b}	5.48(±0.39)^{a}
CHI 4 + MVEO 1.25	7.30(±0.11)^{c}	6.52(±0.44)^{b}	6.05(±0.87)^{b}	5.78(±0.69)^{a}	8.26(±0.57)^{c}	7.56(±0.91)^{c}	7.91(±0.39)^{c}	6.26(±1.42)^{b}	5.51(±0.98)^{a}
	Titrable acidity (mmol H^+/100 g of fruit)								
Control	56.2(±1.46)^{b}	55.0(±0.99)^{b}	52.8(±0.88)^{a}	50.3(±0.57)^{a}	65.7(±0.38)^{c}	63.6(±0.27)^{c}	56.2(±1.04)^{b}	55.1(±1.02)^{b}	52.1(±1.93)^{a}
CHI 4 + MVEO 2.5	55.4(±0.56)^{b}	56.6(±0.84)^{b}	51.5(±0.33)^{a}	50.3(±0.69)^{a}	67.7(±0.47)^{c}	64.4(±0.56)^{c}	59.3(±0.98)^{b}	58.8(±1.34)^{b}	54.1(±1.92)^{a}
CHI 4 + MVEO 1.25	57.0(±0.67)^{b}	57.2(±0.36)^{b}	51.7(±0.23)^{a}	50.8(±0.66)^{a}	63.9(±1.48)^{c}	62.9(±1.74)^{c}	59.9(±1.34)^{b}	58.9(±1.93)^{b}	54.2(±1.25)^{a}
	Soluble solids (ºBrix)								
Control	7.11(±0.01)^{c}	6.40(±0.01)^{b}	6.40(±0.01)^{b}	5.41(±0.01)^{a}	5.80(±0.01)^{a}	5.47 (±0.01)^{b}	5.93(±0.01)^{b}	4.23(±0.2)^{a}	4.01(±0.01)^{a}
CHI 4 + MVEO 2.5	7.43 (±0.01)^{c}	6.86(±0.01)^{b}	5.81(±0.01)^{a}	5.50(±0.01)^{a}	5.70(±0.01)^{b}	5.17 (±0.01)^{b}	5.71(±0.02)^{b}	4.47(±0.2)^{a}	4.81(±0.01)^{a}
CHI 4 + MVEO 1.25	7.30(±0.0.1)^{c}	6.39(±0.01)^{b}	6.11(±0.01)^{b}	5.70(±0.01)^{a}	5.80(±0.01)^{b}	5.33 (±0.01)^{b}	5.63(±0.02)^{b}	4.30(±0.1)^{a}	4.51(±0.01)^{a}

control: 0 µL/mL of chitosan and essential oil + 2 g/100 mL of glycerol

CHI 4 + MVEO 2.5: CHI 4 mg/mL + MVEO 2.5 µL/mL; CHI 4 + MVEO 1.25: CHI 4 mg/mL + MVEO 1.25 µL/mL

$^{a-c}$ For each trial, different superscript letters in the same row denote differences ($P < 0.05$) among the mean values (for the same treatments) according to Tukey's test.

$^{A-B}$ For each trial, different superscript letters in the same column denote differences ($p \leq 0.05$) among the mean values (for the same treatments) according to Tukey's test.

Table 7. Mean values of the color parameters in cherry tomato fruits uncoated and coated with chitosan and *M. piperita* essential oil (CHI-MPEO), at different concentrations, followed by storage at room (25 °C, 12 days) or low temperature (12 °C, 24 days).

Parameters	Days of storage	CHI-MPEO		Control
		CHI 4 + MPEO 2.5	CHI 4 + MPEO 1.25	
	Room temperature			
L	1	36.70(±0.19) [a]	35.78(±0.97) [a]	37.30(±0.51) [a]
	4	36.06[a](±0.54)	36.72(±0.37) [a]	36.11(±0.56) [a]
	8	38.53[b](±1.22)	38.81(±1.12) [b]	37.32(±1.77) [a]
	12	39.58[b](±0.45)	38.64(±1.09) [b]	36.69(±0.12) [a]
a	1	16.24(±0.82) [a]	16.11(±0.37) [a]	16.23(±0.99) [a]
	4	16.40(±0.57) [a]	16.24(±0.31) [a]	16.88(±1.16) [a]
	8	17.08(±0.59) [b]	18.20(±0.32) [b]	18.78(±0.90) [b]
	12	18.32(±0.40) [b]	18.61(±0.30) [b]	18.69(±0.37) [b]
b	1	15.65(±0.17) [a]	15.91(±0.61) [a]	15.99(±0.19) [a]
	4	16.33(±0.73) [a]	16.57(±0.28) [a]	16.16(±0.63) [a]
	8	17.53(±0.17) [b]	17.43(±0.33) [b]	18.94(±0.09) [b]
	12	18.13(±0.03) [b]	17.75(±0.61) [b]	18.09(±0.23) [b]
***h**ab**	1	46.85(±0.68) [a]	46.48(±0.07) [a]	47.40(±2.27) [a]
	4	45.25(±0.91) [a]	44.12(±0.75) [b]	44.44(±0.98) [b]
	8	42.61(±1.27) [b]	42.94(±0.45) [c]	43.57(±1.33) [b]
	12	42.81(±1.03) [b]	41.04(±1.69) [c]	42.90(±0.16) [c]
C*ab	1	23.17(±0.44) [a]	23.47(±0.07) [a]	21.83(±1.06) [a]
	4	23.93(±0.67) [a]	24.30(±0.75) [a]	22.64(±0.20) [a]
	8	24.94(±0.86) [a]	26.40(±0.45) [b]	24.33(±0.78) [b]
	12	25.99(±0.04) [b]	27.39(±1.69) [b]	25.79(±0.61) [b]
	Low temperature			
L	1	35.97(±1.20) [a]	35.88(±0.49) [a]	35.81(±0.25) [a]
	6	36.33(±1.38) [a]	35.72(±0.77) [a]	35.74(±0.94) [a]
	12	37.70(±0.40) [b]	37.43(±1.30) [b]	35.48(±1.12) [a]
	18	38.81(±0.40) [b]	38.64(±0.59) [b]	35.99(±0.66) [a]
	24	38.92(±0.69) [b]	38.98(±1.03) [b]	35.38(±0.20) [a]
a	1	14.99(±1.11) [a]	15.82(±0.18) [a]	17.14(±0.23) [a]
	6	15.45(±0.47) [b]	17.29(±1.74) [b]	17.71(±0.03) [a]
	12	17.46(±0.55) [c]	17.01(±1.78) [b]	18.13(±0.73) [b]
	18	18.35(±1.83) [c]	17.74[b](±1.51)	18.46(±0.69) [b]
	24	18.65(±1.09) [c]	20.26(±0.96) [c]	19.50(±0.97) [c]
b	1	17.47(±0.55) [a]	16.79(±0.48) [a]	15.43(±0.25) [a]
	6	17.79(±0.57) [a]	18.68(±1.30) [b]	17.89(±0.65) [b]
	12	18.31(±0.88) [b]	18.27(±1.12) [b]	17.93(±0.71) [b]
	18	19.94(±1.48) [b]	19,77(±0.61) [b]	18.84(±1.65) [b]
	24	21.24(±0.85) [c]	20.00(±0.03) [c]	22.42(±1.84) [c]
***h**ab**	1	48.12(±1.53) [a]	48.32(±1.68) [c]	45.52(±1.30) [a]
	6	48.16(±0.46) [a]	46.46(±0.44) [a]	45.62(±0.09) [a]
	12	44.97(±0.19) [b]	44.79(±0.60) [a]	44.68(±1.36) [a]
	18	44.35(±0.97) [b]	43.13(±0.13) [b]	42.46(±0.54) [b]
	24	41.72(±0.03) [c]	41.74(±0.43) [b]	41.57(±0.41) [b]
C*ab	1	23.12(±0.78) [a]	22.82(±0.24) [a]	22.02(±0.30) [a]
	6	23.34(±0.34) [a]	24.34(±0.57) [b]	22.88(±0.30) [a]
	12	24.88(±0.04) [a]	24.35(±1.33) [b]	24.92(±0.56) [b]
	18	26.89(±0.48) [b]	27.99(±0.71) [c]	26.41(±1.02) [b]
	24	29.11(±0.22) [c]	29.07(±0.40) [c]	29.57(±0.70) [c]

control: 0 µL/mL of chitosan and essential oil + 2 g/100 mL of glycerol
CHI 4 + MPEO 2.5: CHI 4 mg/mL + MPEO 2.5 µL/mL; CHI 4 + MPEO 1.25: CHI 4 mg/mL + MPEO 1.25 µL/mL

[a - c] For each trial, different superscript letters in the same column denote differences ($p \leq 0.05$) between values obtained for different days of storage according to Tukey's test.

Table 8. Mean values of the color parameters in cherry tomato fruits uncoated and coated with chitosan and *M. villosa* essential oil (CHI-MVEO), at different concentrations, followed by storage at room (25 °C, 12 days) or low temperature (12 °C, 24 days).

Parameters	Days of storage	CHI-MVEO		Control
		CHI 4 + MVEO 2.5	CHI 4 + MVEO 1.25	
	Room temperature			
L	1	37.53(±1.62) [a]	37.84(±1.51) [a]	35.30(±0.51) [a]
	4	37.26(±1.12) [a]	36.81(±0.71) [a]	35.11(±0.56) [a]
	8	37.83(±0.23) [a]	37.10(±0.18) [a]	35.32(±1.77) [a]
	12	38.21(±0.32) [a]	38.89(±0.04) [a]	35.69(±1.07) [a]
a	1	13.27(±0.13) [a]	11.12(±0.32) [a]	12.29(±0.47) [a]
	4	14.50(±0.60) [a]	12.96(±1.10) [a]	13.53[a](±0.35) [a]
	8	15.51(±0.66) [b]	14.05(±1.33) [b]	14.22[a](±0.18) [b]
	12	17.54(±1.56) [c]	17.90(±0.49) [c]	17.83(±0.49) [c]
b	1	14.47(±1.24) [a]	15.64(±0.09) [a]	14.72(±0.80) [a]
	4	15.27(±1.09) [a]	16.67(±0.64) [b]	16.32(±0.42) [b]
	8	16.73(±0.63) [b]	17.88(±0.56) [b]	17.96(±1.33) [b]
	12	19.14(±1.09) [c]	19.36(±0.16) [c]	18.14(±0.22) [c]
***h**ab**	1	61.15(±0.50) [a]	59.23(±0.68) [a]	59.29(±1.43) [a]
	4	55.88(±0.24) [b]	57.73(±0.11) [a]	56.96(±0.96) [b]
	8	53.25(±0.28) [b]	53.64(±0.70) [b]	54.77(±0.17) [b]
	12	50.88(±0.82) [c]	50.45(±0.22) [c]	52.49(±0.25) [c]
C*ab	1	20.31(±0.31) [a]	20.97(±0.11) [a]	21.13(±0.81) [a]
	4	22.82(±0.37) [a]	22.80(±0.33) [a]	22.70(±0.28) [a]
	8	24.03(±0.45) [b]	24.44(±0.58) [b]	23.61(±0.54) [b]
	12	25.26(±1.23) [b]	25.21(±0.45) [b]	25.05(±0.06) [b]
	Low temperature			
L	1	34.55(±0.13) [a]	34.67(±0.40) [a]	34.03(±1.15) [a]
	6	35.98(±0.96) [a]	35.26(±0.11) [a]	34.03(±1.15) [a]
	12	35.86(±0.13) [b]	36.73(±0.19) [b]	35.10(±0.37) [a]
	18	37.24(±0.15) [b]	36.81(±0.38) [b]	36.71(±0.36) [a]
	24	38.77(±0.01) [b]	38.26(±0.47) [b]	35.76(±0.11) [a]
a	1	12.30(±0.22) [a]	12.91(±0.44) [a]	13.01[a](±0.35)
	6	13.69(±0.19) [b]	13.42[a](±0.13) [a]	14.40[a](±0.13)
	12	14.60(±0.52) [c]	15.56(±0.22) [b]	14.53(±0.54) [a]
	18	15.97(±1.33) [c]	16.02(±0.86) [b]	15.63(±0.69) [b]
	24	17.22(±1.09) [c]	16.61(±0.03) [b]	15.63(±0.68) [b]
b	1	15.62(±0.25) [a]	16.79(±0.48) [a]	15.25(±0.34) [a]
	6	16.36(±0.37) [a]	16.36(±1.30) [b]	16.05(±0.42) [b]
	12	17.38(±0.88) [b]	18.27(±1.12) [b]	17.69(±0.08) [b]
	18	19.59(±0.57) [b]	19,77(±0.61) [b]	21.82(±0.24) [c]
	24	21.78(±0.12) [c]	20.00(±0.03) [c]	21.82(±0.24) [c]
***h**ab**	1	50.02(±1.06) [a]	48.84(±0.66) [a]	48.81(±0.49) [a]
	6	50.47(±0.59) [a]	51.33(±0.80) [a]	49.40(±0.30) [a]
	12	52.62(±0.94) [b]	51.82(±0.11) [a]	51.61(±0.58) [a]
	18	54.66(±0.43) [b]	55.25(±0.56) [b]	55.61(±0.21) [b]
	24	58.08(±0.93) [c]	57.53(±0.15) [b]	56.55(±0.54) [b]
C*ab	1	21.12(±0.78) [a]	20.87(±0.24) [a]	20.67(±0.30) [a]
	6	21.94(±0.59) [a]	21.78(±0.11) [a]	21.02(±0.11) [a]
	12	22.98(±0.76) [a]	23.22(±0.92) [b]	22.86(±0.61) [a]
	18	23.65(±0.34) [b]	23.95(±0.59) [b]	23.40(±0.27) [b]
	24	26.39(±0.03) [c]	28.75(±0.76) [c]	26.84(±0.20) [c]

control: 0 µL/mL of chitosan and essential oil + 2 g/100 mL of glycerol
CHI 4 + MVEO 2.5: CHI 4 mg/mL + MVEO 2.5 µL/mL; CHI 4 + MVEO 1.25: CHI 4 mg/mL + MVEO 1.25 µL/mL

[a - c] For each trial, different superscript letters in the same column denote differences ($p \leq 0.05$) between values obtained for different days of storage according to Tukey's test.

APÊNDICE B - Artigo 2: Impact of composite coatings containing chitosan and *Mentha* essential oils on mold occurrence and quality of table grapes foi enviado para publicação no periódico **Postharvest Biology and Technology**.

Impact of composite coatings containing chitosan and *Mentha* essential oils on mold occurrence and quality of table grapes

Running-title: Impact of edible coatings on mold occurrence in grapes

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Abstract

In the current study, we evaluated the efficacy of composite coatings that contained shrimp chitosan (CHI) and *Mentha piperita* L. (MPEO) or *Mentha* x *villosa* Huds (MVEO) essential oils to control the occurrence of mold infections in table grapes (*Vitis labrusca* L.) that were caused by *Aspergillus niger*, *Botrytis cinerea*, *Penicillium expansum* and *Rhizopus stolonifer* during storage at room temperature (over 12 days) and low temperature (over 24 days). The impacts of the tested composite coatings on the physical, physicochemical and sensory characteristics of table grapes during storage were also assessed. The composite coatings, including CHI (8 and 4 mg/mL) and MPEO or MVEO (5, 2.5 and 1.25 µL/mL), delayed the appearance of mold infections caused by all test fungi and decreased the incidence of such infections in artificially infected grapes during storage at both room and low temperature. The assayed composite coatings (CHI 4 mg/mL; MPEO and MVEO, 2.5 and 1.25 µL/mL) did not negatively affect the quality of grapes over time during storage, in terms of the physical, physicochemical and sensory attributes. However, the quality parameters of color (h*) and firmness were enhanced in coated fruits. These results indicate that composite coatings that contain CHI and MPEO or MVEO at low doses are potential alternatives to control mold infections and the related post-harvest losses of table grapes.

Keywords: *Mentha*, polymer, edible coatings, grapes, fungi infection, post-harvest treatment

1. Introduction

The consumption of table grapes (*Vitis labrusca* L.) has been associated with several benefits to consumers because grapes are a source of phenolic compounds that possess antioxidant and oxygen radical-scavenging properties (Krikorian et al., 2012), and protective effects against degenerative diseases, particularly cardiovascular diseases (Cadez et al., 2010; Merín et al., 2014). These characteristics have represented added value for fresh table grapes, which increases both the interest in their daily consumption and the demand for high-quality fruits in the marketplace (Duan et al., 2011). However, the table grape is a highly perishable, non-climacteric fruit that experiences severe postharvest problems, such as loss of firmness, berry drop, stem discoloration, desiccation and fungal rot (Meng et al., 2008).

The postharvest fungal decay of table grapes is a major problem that affects fruit quality during storage and frequently results in grapes becoming unmarketable. These harmful effects on table grapes are typically associated with mold infections that are induced by *Aspergillus niger*, *Botrytis cinerea*, *Penicillium expansum* and *Rhizopus stolonifer* (Abdolahi

et al., 2010; Santos et al., 2012; Sousa et al., 2013; Tian et at., 2015). The colonization of table grapes by *A. niger*, *B. cinerea, P. expansum* or *R. stolonifer* can result in the development of a characteristic set of symptoms in infected fruits, which are termed gray, blue, black and soft-rot mold, respectively (Lichter et al., 2002; Bautista-Baños et al., 2014).

The control of mold infections in table grapes has conventionally been achieved through the use of chemical fungicides, primarily SO_2, during both pre and postharvest periods. However, SO_2 application is becoming restrictive in many countries because SO_2 residues are potentially hazardous to human health (Sánchez-González et al., 2011). Additionally, SO_2 frequently has injurious effects on fresh table grapes and can induce bleaching of the berries and browning of the rachis (Smilanick et al., 1990; Sánchez-González, 2011).

As an alternative to the use of synthetic fungicides in table grapes, researchers have considered the use of edible coatings that contain chitosan (CHI) and essential oils (EOs), which could provide environmentally friendly treatments to control mold infections and maintain fruit quality during storage (Liu et al., 2007; Santos et al., 2012; Oliveira et al., 2014). These coatings create a semi-permeable barrier on fruits that can control microbial growth and reduce moisture loss and respiration rate, thereby preventing infection and physical damage and enhancing product appearance (Gao et al., 2013; Fagundes et al., 2014). Because CHI and EOs are generally recognized as safe (GRAS) by the FDA, this facilitates their use in the formulation and application of coatings as preservation techniques for fruits (Fagundes et al., 2014).

CHI is a high molecular weight cationic polysaccharide that is obtained by the deacetylation, in alkaline media, of chitin (β-(1–4)-2-acetamido-D-glucose and β-(1–4)-2-amino-D-glucose units), which are extracted from the exoskeleton of crustaceans, fungi, and insects (Kanatt et al., 2008). Studies have shown that the incorporation of EOs into CHI dispersions can markedly improve their antimicrobial activities and some of their physicochemical properties, such as biodegradability and the ability to form films and water vapor barriers, thereby extending the shelf life of fruits (Kanatt et al., 2008; Santos et al., 2008; Sánchez-González et al., 2011).

In the food industry, the EOs of *Mentha* species, primarily *Mentha piperita* L. essential oil (MPEO), are used as flavoring agents in foods and beverages (Burt, 2004) and are commonly exploited because of their antioxidant, antimicrobial and sensory properties (Tyagi and Malik., 2011; Riahi et al., 2013). *Mentha* × *villosa* Hudson is an aromatic plant hybrid of *Mentha spicata* L. and *Mentha suaveolens* Ehrh. that is commonly used in Brazilian

folk medicine (Lahlou et al., 2002). MPEO and *Mentha* x *villosa* Huds essential oil (MVEO) have shown promising results in the inhibition of food-related fungi, including phytopathogenic fungi (Riahi et al., 2013; Lima et al., 2014). Although the application of CHI and some EOs has proven to be effective in controlling mold infections in table grapes (Santos et al., 2012; Sousa et al., 2013; Oliveira et al., 2014), studies verifying the efficacy of composite coatings containing CHI and MPEO or MVEO to control the occurrence of different mold infections in fruits are scarce or nonexistent.

This study evaluated composite coatings that contained shrimp CHI and reduced doses of MPEO or MVEO as postharvest treatments to prevent the occurrence of mold infections caused by *A. niger, B. cinerea, P. expansum* or *R. stolonifer* in table grapes that had been artificially contaminated with each test fungi. In addition, the effects of the tested composite coatings on some physical, physicochemical and sensory characteristics of table grapes during storage were verified.

2. Materials and methods

2.1 Materials

Commercially mature table grapes (*Vitis labrusca* L.), i.e., grapes with purple color, were obtained from EMPASA (Supplies and Services Company of Paraíba, João Pessoa, Brazil). Grapes with no visible sign of mechanical damage or fungal infection were selected and standardized according to size, appearance, color and shape. Prior to the assays, grapes were surface-disinfected via immersion in a sodium hypochlorite solution (1 mL/100 mL, pH 7.2 adjusted using 1 M NaOH) for 15 min, washed with sterile distilled water and dried for 2 h in a safety cabinet.

Aspergillus niger URM 5162 (*A. niger*), *Botrytis cinerea* URM 2802 (*B. cinerea*), *Penicillium expansum* URM 3396 (*P. expansum*) and *Rhizopus stolonifer* URM 3482 (*R. stolonifer*) were obtained from the University of Recife Mycology Culture Collection (Center for Biological Sciences, Federal University of Pernambuco, Recife, Brazil). The stock cultures were subcultured in Sabouraud agar (Himedia, India) at 25 °C for seven days to facilitate sufficient sporulation. The fungal spores were collected in a sterile saline solution (0.85 g/100 mL NaCl) in Sabouraud broth (Himedia, India), and the resulting suspension was filtered through a triple layer of sterile gauze to retain the hyphal fragments. The number of spores present in the suspension was quantified using a hemocytometer. The spore

concentration was adjusted with sterile saline solution to yield an inoculum of approximately 10^6 spores/mL (Rasooli and Owlia, 2005).

The shrimp shells used for CHI extraction came from a single species of shrimp, *Litopenaeus vannamei* (Boone 1931) (*L. vannamei*), and were provided by Aquamaris Aquaculture S/A (João Pessoa, Brazil).

Mentha piperita L. (*M. piperita*) and *Mentha x villosa* Huds (*M. villosa*) leaves were collected at the Medicinal Plants Garden, Institute for Research in Drugs and Medicines, Federal University of Paraíba (João Pessoa, Brazil) in July 2011 (7°08'29''S, 34°50'48''W). Voucher specimens for *M. piperita* and *M. villosa* were deposited at the Herbarium Prisco Bezerra, Federal University of Ceará (Fortaleza, Brazil) under numbers 14423 and 14996, respectively.

2.2 Extraction and identification of EO constituents

Fresh *M. piperita* (10 kg) and *M. villosa* (10 kg) leaves were each subjected to steam distillation for approximately 8 h. Subsequently, the EOs were dried using anhydrous sodium sulfate, filtered and stored in screw-capped amber flasks (4 °C). The EOs obtained from both plant species presented with a yellowish coloration and a characteristic odor. GC-MS analysis (using analytical conditions as described in Lima et al., 2013) of the EOs revealed that L (-)-menthol (30.31%) and rotundifolone (70.2%) were the major constituents of MPEO and MVEO, respectively (data not shown).

2.3 Extraction of CHI from shrimp shells

The shrimp shells were washed under warm running tap water to remove soluble organics, adherent proteins and other impurities. The shells were collected, boiled in distilled water for 1 h to remove the remaining tissues and dried in an oven (Fanem, model 320, Brazil) at 160 °C for 2 h to make the shells more brittle and to break down the crystalline structure of the chitin. The dried shells were ground into a fine powder using a standard grinder (Model TE-330, Tecnal, São Paulo, Brazil) (Kucukgulmez et al., 2011).

To obtain the CHI, shells were demineralized by immersion in 1.7 N HCl (1:30 w/v) for 6 h at room temperature (25 °C) with stirring (150 rpm); chitin was deproteinized by immersion in 2.5 N NaOH at 65 °C for 6 h, and shells were deacetylated with 2.5 N NaOH (1:50 w/v, 120 ºC, overnight), filtered and washed with deionized water until the filtrate was neutral and dried (90 °C, 2 h) (Kucukgulmez et al., 2011). The resulting CHI, in powder form, was vacuum packaged and stored under refrigeration (7 °C) until it was used in the

subsequent assays. IR spectra and x-ray diffraction of the extracted CHI were used to determine the degree of deacetylation (83%) and the degree of crystallinity (71%), respectively. The identity of shrimp CHI was confirmed based on the diffraction peaks at angles of 10° and 20° 2Θ, relative to the crystallographic letter (ICDD 00-035-1974) (data not shown).

2.4 Production of CHI and EO coatings

CHI concentrations of 4 and 8 mg/mL were used to form the edible coatings because 4 mg/mL was the lowest concentration that was capable of forming a viscous solution with coating features that permitted its application as a coating for grapes. CHI concentrations greater than 8 mg/mL formed highly viscous dispersions that were not capable of forming homogeneous and continuous films when they were applied to fruits. For the tested EOs, the concentrations were set at 5, 2.5 and 1.25 µL/mL because researchers have stated that EOs could be incorporated at low concentrations (< 10 µL/mL) in coating dispersions to minimize their impact on the olfactory perception of consumers (Perdones et al., 2012). Studies assessing the *in vitro* antifungal properties of EOs against common mold-causing fungi have found inhibitory effects when these substances are tested at concentrations ranging from 1 - 5 µL/mL (Yadegarinia et al., 2006; Tyagi et al., 2011; Perdones et al., 2012).

CHI (8.0 and 4.0 mg/mL) was initially diluted in acetic acid (1 mL/100 mL) with constant stirring (120 rpm) for 6 h at room temperature. Subsequently, different quantities of MPEO or MVEO were added (final concentrations of 5, 2.5 or 1.25 µL/mL), followed by stirring for an additional 18 h at room temperature. To assay the application of the solution formed by combining CHI and EOs on table grapes, glycerol was added (2 mL/100 mL) as a plasticizer when the EO was incorporated into the coating dispersions (Ojagh et al., 2010).

2.6 Effect of CHI and EOs on mold infections in grapes

The grapes were first immersed in 500 mL of an inoculum solution (approximately 10^6 spores/mL) of the test fungal strain for 1 min, with gentle stirring using a sterile glass stem, and they were then kept in a biosafety cabinet at room temperature for complete drying (approximately 1 h). The fruits were subsequently immersed for 1 min in 500 mL of the coating solution, which contained different CHI and MPEO or MVEO concentrations, with gentle stirring using a sterile glass stem. The fruits were dried on a nylon filter to drain the excess liquid, packed in a polyethylene container and covered with a lid. One group of fruits was stored at room temperature (25 °C), and the other group was stored at low temperature (12 °C). In addition, a control experiment was performed in which CHI and EO solutions

were replaced with sterile distilled water containing glycerol (2 g/100 mL). Each treatment included 40 fruits. At different storage time intervals (room temperature 1, 4, 8 and 12 days; low temperature 1, 6, 12, 18 and 24 days), the fruits were examined for signs of fungal infection (mold) (Feng and Zeng, 2007; Liu et al., 2007). The results were expressed as the time until the appearance of visible signs of mold infection and the percentage of grapes infected with fungi (incidence) when occurred the appearance of visible signs of mold infection and at the end of each storage period (at low and room temperature).

The test fungi were verified for their ability to cause a specific mold infection in grapes (virulence), with a characteristic set of symptoms. Continuous re-inoculations and re-isolations on grapes were carried out to maintain pathogenicity of the test fungi (Bautista-Baños et al., 2003). The characteristic set of symptoms for black mold (caused by *A. niger*) included darkening and softening of the fruits at the site of infection, followed by breaking of the skin and development of a dark mold. The characteristic signs of infection for gray mold (caused by *B. cinerea*) included circular areas that were darker and softer than the other fruit parts, with subsequent abundant sporification that ranged in color from white to gray. The characteristic signs of infection for blue mold (caused by *P. expansum*) included circular areas where the fruit tissues were darker and softer than the other fruit parts, with subsequent abundant sporification that ranged in color from white to blue or gray. The characteristic infection signs for soft-rot (caused by *R. stolonifer*) included watery areas that were quickly covered by coarse, gray, hairy mycelia to form a mass of black sporangia at their tips (Lichter et al., 2002; Bautista-Baños et al., 2014).

2.7 Physical and physicochemical analyses of grapes

The grapes were evaluated for weight loss and general quality parameters, such as color, firmness, soluble solids (SS) and titratable acidity (TA) at the same time that they were evaluated for mold infection. To determine weight loss during storage, the fruit was weighed at different time intervals. Weight loss was calculated as a percentage of the initial weight (Oliveira et al., 2014). Firmness was determined using a 3-mm-diameter probe (1/8) coupled to a TA-XT2 Texture Analyzer (Stable Micro Systems, Haslemere, UK), and the results were expressed as N/mm (Oliveira et al., 2014). The SS content was determined using a digital refractometer (Model HI 96801, Hanna Instruments, São Paulo, Brazil), and the results were expressed in ºBrix (Oliveira et al., 2014). The TA was determined using phenolphthalein as an indicator with 0.1 N NaOH, and the results were expressed as mmol H^+/100 g of fruit (Oliveira et al., 2014). Skin color was measured at three different equatorial positions on the

fruit using the CIE*Lab* system (L * a * b *). The hue angle (h^*_{ab}) and chroma (C^*_{ab}) were measured in a CR-300 colorimeter (Minolta Co., Osaka, Japan) using a 10 mm quartz cuvette, consistent with the standards of the International Commission on Illumination (CIE, 1986). The CIE*Lab* color scale (L*a*b*) was used with a D^{65} illuminant (standard daylight) at a 10° angle. Using reference plates, the apparatus was calibrated in the reflectance mode, and the specular reflection was excluded.

2.8 Sensory analysis of grapes

Grapes treated with CHI and MVEO or MPEO were subjected to acceptance and preference tests at different time intervals (1, 4, 8 and 12 days) throughout low temperature storage (to ensure the microbiological safety of the samples offered to the panelists). Sixty untrained tasters participated in the acceptance and preference tests. Sensory analyses received prior approval from an Ethics Research Committee (protocol 712.884/2014). The analysis was performed under controlled temperature and lighting conditions in individual booths.

Each panelist received three units of grapes that had been treated with the different CHI-EO coatings and were served on disposable white plates that were coded with a random three-digit number. The three-grape samples were served simultaneously in a blind, random sequence immediately after their removal from cold storage. The tasters were asked to eat a salty biscuit and drink water between samples to avoid aftertaste effects. For the preference test, the tasters were asked to select the most and least appreciated samples based on an overall evaluation. The intent to purchase was assessed on a five-point structured hedonic scale that ranged from one (certainly would not buy) to five (certainly would buy). For the acceptability of appearance, color, flavor, texture, aftertaste and overall assessment, a nine-point structured hedonic scale was used, which ranged from one (dislike very much) to nine (like very much) (Santos et al., 2012).

2.9 Statistical analysis

All analyses were performed in triplicate, and the results were expressed as the mean of the three replicates. Statistical analyses were performed using descriptive statistics (mean and standard deviation) and inferential tests (ANOVA followed by Tukey's test or the Kruskal-Wallis test) to determine statistically significant differences ($p \leq 0.05$) between treatments. For the statistical analyses, the computational Sigma Stat software 2.03 was used.

3. Results

3.1 Effect of CHI and EO coatings on mold infections in grapes

When table grapes were artificially contaminated with *A. niger, B. cinerea, P. expansum* or *R. stolonifer* spores and then coated with composite coatings containing CHI-MPEO or CHI-MVEO, the appearance of visible signs of mold infection was strongly delayed compared with uncoated fruits throughout the assessed storage period and at both room and low temperatures (Tables 1 and 2). The grapes that were not coated with CHI-MPEO or CHI-MVEO but were contaminated with *A. niger* and stored at low temperature exhibited visible signs of mold infection on the 12th day of storage (42% of the fruits were infected), whereas the fruits infected with *B. cinerea, P. expansum* or *R. stolonifer* exhibited visible signs of mold infection on the 6th day of storage (32 - 38 % of the fruits were infected) when they were stored at low temperature. Uncoated grapes that were infected with the test fungi and stored at room temperature exhibited visible signs of mold infection on the 4th day of storage (46 - 67% of the fruits were infected).

Table grapes that were artificially infected with the test fungi and treated with composite coatings of CHI at 8 mg/mL and MPEO or MVEO at 5, 2.5 or 1.25 µL/mL or with CHI at 4 mg/mL and MPEO or MVEO at 5 µL/mL displayed no visible signs of mold infection throughout the 24-day low-temperature storage period. The grapes that were stored at room temperature and treated with composite coatings containing CHI at 8 or 4 mg/mL and MVEO or MPEO at 5 µL/mL displayed no visible signs of mold infections throughout the 12-day storage period. However, grapes that were stored at room temperature and treated with composite coatings of CHI at 8 or 4 mg/mL and MVEO or MPEO at 2.5 or 1.25 µL/mL displayed visible signs of mold infection by *A. niger, B. cinerea, P. expansum* and *R. stolonifer* between the 4th and 8th days of storage (6 - 53% of the fruits were infected). The infection rates of all tested mold-causing fungi in grapes that were treated with composite coatings of CHI-MPEO or CHI-MVEO and stored at room temperature and low temperature was less than 55% and 35% at the end of the storage period, respectively; whereas for uncoated grapes, the infection rates were greater than 80%.and 55%, respectively. The highest infection rates among coated fruits were always detected in table grapes treated with composite coatings that contained CHI at 4 mg/g and MPEO or MVEO at 1.25 µL/mL. In general, the control of mold infection in grapes using CHI and EOs directly varied with the quantity of EO in the test composite coatings and weakened over time. Thus, only the composite coatings containing CHI at 4 mg/mL and MVEO or MPEO at 5 and 2.5 µL/mL were used in subsequent assays of the quality characteristics of table grapes over time.

3.2 Effects of CHI and EOs on the physical, physicochemical and sensory characteristics of grapes

The physical and physicochemical changes in uncoated grapes and grapes coated with CHI and MVEO or MPEO were evaluated during storage at room temperature and low temperature (Tables 3 and 4, respectively). The grapes coated with CHI-MPEO or CHI-MVEO and stored at low temperature exhibited weight losses that were similar ($p > 0.05$) to those of uncoated fruits for up to 6 days of storage. Thereafter, however, the rate of weight loss was lower ($p \leq 0.05$) in coated fruits. Lower ($p \leq 0.05$) weight losses for coated fruits versus uncoated fruits stored at room temperature were observed on the 4th day of storage and persisted throughout the subsequent storage period. Overall, grapes coated with CHI and EOs and stored at room temperature exhibited greater ($p \leq 0.05$) weight loss during storage than did coated fruits that were stored at low temperatures.

Grapes treated with coatings comprising CHI and MPEO or MVEO at both amounts tested showed differences ($p \leq 0.05$) in firmness, color L* and color h* for most storage time periods at both room and low temperature (Tables 3 and 4). No differences ($p > 0.05$) were observed in TA, SS, color a* and b* between coated and uncoated grapes for any time period under storage at either at room or low temperature. Differences ($p \leq 0.05$) in specific quality attribute for the same fruit group (coating/no coating treatment) were observed across different time periods under storage at both room and low temperature.

Differences ($p < 0.05$) in firmness were observed between uncoated grapes and grapes coated with CHI-MPEO or CHI-MVEO throughout the storage period at both room and low temperature. Uncoated grapes exhibited lower values ($p \leq 0.05$) for firmness compared with coated grapes. Both the coated and uncoated grapes exhibited sharp decreases ($p \leq 0.05$) in firmness over the duration of the storage period (Tables 3 and 4). Grapes that were stored at room temperature and low temperature, regardless of treatment, maintained their initial TA and SS values until the 8th and 12th days of storage, respectively; the TA values subsequently decreased ($p \leq 0.05$), whereas the SS values remained stable in later time periods. For most time periods, the SS/TA ratios for coated grapes did not differ ($p > 0.05$) from those observed for uncoated fruits (data not shown), regardless of storage temperature.

Uncoated grapes and grapes coated with CHI-MPEO and CHI MVEO were predominantly purple throughout storage at both room and low temperature, and the a* and b* values (positive values, close to zero) did not differ ($p > 0.05$) among the fruit groups. Furthermore, under all treatment conditions, there was a decrease ($p \leq 0.05$) in the h* value

and a concomitant increase in the C* value that was due to a change in fruit skin color from blue to purple over time. In addition, an increase in the lightness (L* value) was observed when grapes were coated with CHI and EOs at both concentrations. There was no difference ($p > 0.05$) in color between samples stored at room or low temperature.

The grapes, both uncoated and coated with CHI-MPEO or CHI-MVEO, were subjected to acceptance, purchase intention and preference tests following 1, 4, 8 and 12 days of storage at low temperature (Table 5). The uncoated and coated grapes received similar scores ($p > 0.05$) for all assessed sensory attributes (appearance, color, flavor, texture, aftertaste and overall assessment) throughout storage; therefore, only the results for (low temperature) storage on days one and 12 are presented. The coated and uncoated fruit groups received scores in the "liked slightly" or "liked moderately" categories for all of the sensory parameters tested. When the panelists were asked to indicate their intent to purchase the grapes, the responses were generally "possibly purchase" for all groups and storage periods. Similarly, there was no difference ($p > 0.05$) in preference among the treatment groups for the duration of the storage period. Moreover, no difference was observed ($p > 0.05$) in the values for all of the assessed quality parameters between fruits treated with coatings containing the different amounts of each tested EO. Overall, no differences ($p > 0.05$) were observed between the values for all of the assessed quality parameters in the fruits treated with coatings containing the same amount of MPEO or MVEO.

4. Discussion

The control of post-harvest mold infections in table grapes has been a significant concern for producers and retailers because these diseases reduce the quality, shelf-life and safety of fruits on the market (Duan et al., 2011). Moreover, current consumer trends have demanded control of these infections and causative agents through the use of natural bioactive molecules, which could totally or partially replace the use of synthetic fungicides in fruits (Cabral et al., 2013; Fagundes et al., 2014). For these reasons, this study assessed the efficacy of composite coatings containing shrimp CHI (obtained from *L. vanammei* shells) and MPEO or MVEO to control common mold infections as well as their effect on the quality of table grapes during storage at room or low temperature. The composite coatings containing CHI-MPEO and CHI-MVEO strongly delayed the appearance of the mold signs induced by *A. niger*, *B. cynerea*, *P. expansum* and *R. stolonifer* in artificially infected table grapes. The incidence of infected grapes in fruit groups coated with the test composite coatings was consistently decreased and never reached infection rates greater than 45% by the end of the

assessed storage period for both room- and low-temperature treatments. In comparison, uncoated table grapes first showed signs of mold infection as early as the 4th and 12th days of storage for room- and low-temperature storage, respectively, and they reached a 100% infection rate by the end of the storage period for both temperature conditions.

The inhibitory effects of the composite coatings containing CHI-MVEO and CHI-MPEO were enhanced when the fruits were stored at low temperature. The decreased incidence of mold infections in these fruits groups could be related to either the weaker pathogenicity of molds at low temperatures (Oliveira et al., 2014) or the slowing of physiological processes in fruits at low temperatures, which delays their senescence (Oliveira et al., 2014); resistance to mold infections is higher in fruits experiencing delayed senescence (Sánchez-González et al., 2011).

The anti-fungal effects of CHI-based coatings have classically been associated with the effects of CHI on the permeability of fungal cell membranes because of the interactions between the positively charged amino groups of CHI and the negatively charged phospholipids of the plasma membrane (Laflamme et al., 1999; Ait-Ouazzou et al., 2012). Researchers have also proposed that the CHI molecule is able to enter the cytoplasm and translocate to the nucleus of the fungal cell, thereby interfering with RNA and protein synthesis (Palma-Guerrero et al., 2008). The anti-fungal effects of MPEO and MVEO are primarily associated with the effects of their major constituents, menthol (cyclic monoterpene) and roduntifolone (oxygenated monoterpene), respectively. These compounds induce depolarization and physical or chemical alterations in fungal cell membranes, thereby disrupting various metabolic activities (Ait-Ouazzou et al., 2012).

Because the physical, physicochemical and sensory properties of fruits are the characteristics that influence shelf life and consumer acceptance, this study assessed the effects of the test composite coatings on these attributes of table grapes during storage. The CHI-MPEO and CHI-MVEO coatings protected the table grapes from softening, as demonstrated by the reduced weight loss and increased firmness of fruits during storage at both room and low temperature. This is an interesting effect on fruit quality attributes because grapes normally soften during the postharvest period, which reduces their shelf-life, consumer appeal and marketability. Weight loss in fresh fruits is primarily associated with the water loss caused by transpiration and respiration (Amarante et al., 2001; Gao et al., 2013). CHI coatings form a layer on the surface of the pericarp and act as a protective barrier that reduces respiration and transpiration across the fruit surface, thereby delaying senescence (Sánchez-Gonzalez et al., 2011; Hong et al., 2012). Because of their hydrophobic properties, EOs have

been incorporated into CHI-based coatings to improve the preventive effects and reduce moisture loss in fruits (Vargas et al., 2008; Sun et al., 2014). Fruit groups treated with composite coatings of CHI-MPEO or CHI-MVEO showed weight losses of less than 5%, which is the normal acceptable limit for weight loss in table grapes (Deng et al., 2006; Gao et al., 2013).

The mold inhibition induced by CHI-MPEO and CHI-MVEO composite coatings may also help maintain the firmness of fruits over time. Previous studies have found that the water loss (primarily related to weight loss) of fruits coincides with their decreased firmness (Deng et al., 2006). The decreased firmness in grapes is associated with the action of cell-wall degrading enzymes, which hydrolyze starch to sugar and protopectin to water-soluble pectin (Meng et al., 2008; Gao et al., 2013). In addition to the enzymes that normally occur in fruits, cell-wall degrading enzymes are delivered by mold-forming fungi during colonization and infection, which induce the characteristic softening in infected fruits (Sánchez-González et al., 2011; Sun et al., 2014). As a result, the CHI-MPEO and CHI-MVEO composite coatings could positively impact maintenance of firmness in table grapes by both (i) reducing water loss and fruit senescence and (ii) decreasing cell-wall degradation through the deactivation of mold-forming fungi on artificially infected fruits.

Color is one of the most important quality parameters in fruits, and it affects the purchase decision of consumers. In grapes, a decrease in the h* values during storage reflects the development of sample browning. The pattern of decrease in h*values of coated grapes suggests that the composite coatings of CHI-MPEO and CHI-MVEO slow the browning process in table grapes, which is caused by non-enzymatic and enzymatic reactions that result in chlorophyll degradation to form pheophytins (Karadeniz et al., 2000; González-Barrio et al., 2005); this may be partially related to the general slowing of the senescence process in coated grapes. However, this pattern could also be due to the oxygen barrier created by the test coatings because oxygen actively participates in processes related to fruit browning (Pastor et al., 2011). The application of test coatings also resulted in higher L* values in table grapes, which are expected because of the characteristics of high luminosity and transparency presented by CHI-films (Peng and Li, 2013; Oliveira et al., 2014). Although the CHI-MPEO and CHI-MVEO composite coatings may have impacted the metabolic activity of table grapes, the results of the analysis on the physical and physicochemical parameters of the fruits assessed in this study are consistent with the data obtained in previous studies on healthy and marketable table grapes (Satnos et al., 2012; Sousa et al., 2013).

The application of the tested coatings did not change the sensory characteristics of table grapes during storage or the intention of panelists to purchase them. These findings are worthy of note because the possible negative effects of EOs (at doses sufficient to cause microbial inhibition in food matrices) on the sensory characteristics of food, particularly its odor and taste, are commonly described as a limiting factor for the practical application of EOs as antimicrobials in food conservation systems (Melgarejo-Flores et al., 2013). Additionally, researchers have stated that CHI could negatively impact the taste of foods by provoking the development of an acidic or even slightly bitter and astringent taste as a result of an increase in protonated amine groups related to the use of acetic acid (1 g/100 mL) to dissolve CHI (Perdones et al., 2012; Santos et al., 2012). However, early studies have consistently reported no negative impacts of post-harvest treatments with CHI and/or EOs on the sensory characteristics of grapes (Santos et al, 2012; Sousa et al., 2013; Kim et al., 2014; Oliveira et al., 2014).

5. Conclusions

The results of the current study showed that the combined application of CHI from shrimp (*L. vannamei*) and MPEO or MVEO to form composite coatings strongly inhibited the mold infections caused by *A. niger*, *B. cinerea*, *P. expansum* and *R. stolonifer* in table grapes during storage at room and low temperature. These coatings delayed both the appearance of mold infections and the incidence of infected fruits. The tested coatings did not negatively affect the physical, physicochemical or sensory aspects of table grapes during storage. Interestingly, specific quality attributes of color and firmness were improved in CHI-MPEO- and CHI-MVEO-coated fruits. These findings are sufficient to consider the application of composite coatings that contain shrimp CHI and MPEO or MVEO at reduced doses as potential post-harvest treatments to control mold infections and thus extend the shelf-life of table grapes.

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Table 1. Occurrence of visible signs of mold infection in uncoated grapes and grapes coated with chitosan (CHI) and *M. piperita* essential oil (MPEO), followed by storage at room temperature (25 °C, 12 days) and low temperature (12 °C, 24 days).

Treatments	**Days of storage until detection of mold infection (percent of infected fruits)**		**Percent of infected fruits at the end of storage**	
	Room temperature	**Low temperature**	**Room temperature**	**Low temperature**
	A. niger			
Control	4th(46%)	12th(42%)	12th(82%)	24th(64%)
CHI 8 + MPEO 5	nd	nd	nd	nd
CHI 8 + MPEO 2.5	8th(24%)	nd	12th(37%)	nd
CHI 8 + MPEO 1.25	8th(25%)	nd	12th(42%)	nd
CHI 4 + MPEO 5	nd	nd	nd	nd
CHI 4 + MPEO 2.5	8th(36%)	6th(16%)	12th(44%)	24th(29%)
CHI 4 + MPEO 1.25	8th(33%)	6th(26%)	12th(45%)	24th(35%)
	B. cinerea			
Control	4th(57%)	6th(35%)	12th(86%)	24th(59%)
CHI 8 + MPEO 5	nd	nd	nd	nd
CHI 8 + MPEO 2.5	8th(12%)	nd	12th(24%)	nd
CHI 8 + MPEO 1.25	8th(14%)	nd	12th(26%)	nd
CHI 4 + MPEO 5	nd	nd	nd	nd
CHI 4 + MPEO 2.5	8th(46%)	12th(16%)	12th(54%)	24th(31%)
CHI 4 + MPEO 1.25	8th(53%)	12th(26%)	12th(63%)	24th(33%)
	P. expansum			
Control	4th(67%)	6th(32%)	12th(86%)	24th(56%)
CHI 8 + MPEO	nd	nd	nd	nd
CHI 8 + MPEO 2.5	6th(13%)	nd	12th(18%)	nd
CHI 8 + MPEO 1.25	6th(15%)	nd	12th(32%)	nd
CHI 4 + MPEO 5	nd	nd	nd	nd
CHI 4 + MPEO 2.5	8th(48%)	12th(16%)	12th(54%)	24th(31%)
CHI 4 + MPEO 1.25	6th(53%)	12th(21%)	12th(60%)	24th(32%)
	R. stolonifer			
Control	4th(63%)	6th(38%)	12th(88%)	24th(56%)
CHI 8 + MPEO 5	nd	nd	nd	nd
CHI 8 + MPEO 2.5	6th(17%)	nd	12th(24%)	nd
CHI 8 + MPEO 1.25	6th(19%)	nd	12th(26%)	nd
CHI 4 + MPEO 5	nd	nd	nd	nd
CHI 4 + MPEO 2.5	8th(39%)	6th(18%)	12th(48%)	24th(33%)
CHI 4 + MPEO 1.25	6th(48%)	12th(24%)	12th(54%)	24th(34%)

Control: 0 µL/mL of CHI and essential oil + 2 g/100 mL of glycerol
nd: not detected.
CHI 8 + MPEO 5: CHI 8 mg/mL + MPEO 5 µL/mL; CHI 8 + MPEO 2.5: CHI 8 mg/mL + MPEO 2.5 µL/mL; CHI 8 + MPEO 1.25: CHI 8 mg/mL + MPEO 1.25 µL/mL; CHI 4 + MPEO 5: CHI 4 mg/mL + MPEO 5 µL/mL; CHI 4 + MPEO 2.5: CHI 4 mg/mL + MPEO 2.5 µL/mL; CHI 4 mg/mL + MPEO 1.25 µL/mL

Table 2. Results of occurrence of visible signs of mold infection in grapes uncoated and coated with chitosan (CHI) and *M. villosa* essential oil (MVEO), followed by storage at room (25 °C, 12 days) and low temperatures (12 °C, 24 days).

Treatments	**Days of storage for detection of first signs of mold infection (percent of infected fruits)**		**Percent of infected fruits at the end of the storage time**	
	Room temperature	**Low temperature**	**Room temperature**	**Low temperature**
	A. niger			
Control	4th(46%)	12th(42%)	12th(76%)	24th(56%)
CHI 8 + MVEO 5	nd	nd	nd	nd
CHI 8 + MVEO 2.5	8th(6%)	nd	12th(12%)	nd
CHI 8 + MVEO 1.25	8th(8%)	nd	12th(14%)	nd
CHI 4 + MVEO 5	nd	nd	nd	nd
CHI 4 + MVEO 2.5	4th(28%)	6th(13%)	12th(44%)	24th(34%)
CHI 4 + MVEO 1.25	4th(31%)	6th(17%)	12th(45%)	24th(28%)
	B. cinerea			
Control	4th(57%)	6th(35%)	12th(85%)	24th(57%)
CHI 8+ MVEO 5	nd	nd	nd	nd
CHI 8 + MVEO 2.5	8th(12%)	nd	12th(24%)	nd
CHI 8 + MVEO 1.25	8th(14%)	nd	12th(26%)	nd
CHI 4 + MVEO 5	nd	nd	nd	nd
CHI 4 + MVEO 2.5	8th(33%)	12th(16%)	12th(56%)	24th(25%)
CHI 4 + MVEO 1.25	8th(35%)	12th(26%)	12th(61%)	24th(39%)
	P. expansum			
Control	4th(67%)	6th(30%)	12th(85%)	24th(56%)
CHI 8 + MVEO 5	nd	nd	nd	nd
CHI 8 + MVEO 2.5	6th(10%)	nd	12th(20%)	nd
CHI 8 + MVEO 1.25	6th(13%)	nd	12th(18%)	nd
CHI 4 + MVEO 5	nd	nd	nd	nd
CHI 4 + MVEO 2.5	6th(45%)	12th(19%)	12th(55%)	24th(32%)
CHI 4 + MVEO 1.25	8th(53%)	12th(24%)	12th(64%)	24th(30%)
	R. stolonifer			
Control	4th(63%)	6th(41%)	12th(94%)	24th(58%)
CHI 8 + MVEO 5	nd	nd	nd	nd
CHI 8 + MVEO 2.5	6th(21%)	nd	12th(29%)	nd
CHI 8 + MVEO 1.25	6th(23%)	nd	12th(32%)	nd
CHI 4 + MVEO 5	nd	nd	nd	nd
CHI 4 + MVEO 2.5	6th(42%)	6th(22%)	12th(54%)	24th(34%)
CHI 4 + MVEO 1.25	8th(51%)	12th(28%)	12th(62%)	24th(41%)

Control: 0 µL/mL of CHI and essential oil + 2 g/100 mL of glycerol
nd: not detected.
CHI 8 + MVEO 5: CHI 8 mg/mL + MVEO 5 µL/mL; CHI 8 + MVEO 2.5: CHI 8 mg/mL + MVEO 2.5 µL/mL; CHI 8 + MVEO 1.25: CHI 8 mg/mL + MVEO 1.25 µL/mL; CHI 4 + MVEO 5: CHI 4 mg/mL + MVEO 5 µL/mL; CHI 4 + MVEO 2.5: CHI 4 mg/mL + MVEO 2.5 µL/mL; CHI 4 mg/mL + MVEO 1.25 µL/mL

Table 3. Mean values of physical and physicochemical quality parameters for uncoated grapes and grapes coated with chitosan (CHI) and *M. piperita* essential oil (MPEO), followed by storage at room temperature (25 °C, 12 days) and low temperature (12 °C, 24 days).

Treatments	Room temperature (25 °C)				Low temperature (12 °C)				
	Days of storage				Days of storage				
	1	4	8	12	1	6	12	18	24
	Firmness (N/mm)								
Control	6.6^{bA}(±0.54)	4.8^{bB}(±0.07)	4.8^{bB}(±0.37)	4.7^{bC}(±0.60)	7.2^{aA}(±0.76)	6.5^{bB}(±0.50)	7.9^{aA}(±1.36)	5.6^{bC}(±0.30)	5.4^{bC}(±0.42)
CHI 4 + MPEO 5	7.6^{aA}(±0.37)	7.1^{aA}(±0.40)	6.5^{aB}(±0.20)	5.4^{aC}(±0.18)	7.5^{aA}(±0.09)	7.5^{aA}(±0.57)	7.5^{bA}(±0.58)	6.8^{aB}(±0.23)	6.6^{aB}(±0.47)
CHI 4 + MPEO 2.5	7.1^{aA}(±0.28)	6.9^{aA}(±0.10)	6.3^{aB}(±0.15)	5.5^{aC}(±0.29)	7.4^{aA}(±0.37)	7.4^{aA}(±0.31)	7.5^{bA}(±0.39)	6.8^{aB}(±0.30)	6.4^{aB}(±0.16)
	Titrable acidity (mmol H^+/100 g of fruit)								
Control	63.1^{A}(±2.79)	62.0^{A}(±2.20)	54.4^{B}(±2.46)	48.8^{C}(±0.34)	63.6^{A}(±0.67)	62.1^{A}(±0.13)	54.2^{B}(±2.80)	54.3^{B}(±3.19)	45.4^{C}(±1.06)
CHI 4 + MPEO 5	63.2^{A}(±1.63)	60.4^{A}(±0.70)	52.7^{B}(±2.91)	46.5^{C}(±0.33)	63.1^{A}(±2.04)	63.9^{A}(±0.25)	57.1^{B}(±1,97)	53.3^{B}(±1.58)	43.5^{C}(±0.65)
CHI 4 + MPEO 2.5	64.3^{A}(±0.14)	62.6^{A}(±0.83)	50.4^{B}(±0.97)	46.3^{C}(±0.06)	65.6^{A}(±0.13)	64.1^{A}(±0.45)	55.7^{B}(±1.00)	53.1^{B}(±0.32)	44.9^{C}(±2.23)
	Soluble solids (ºBrix)								
Control	14.6^{A}(±0.01)	12.2^{B}(±0.05)	11.2^{C}(±0.06)	11.1^{C}(±0.06)	13.3^{A}(±0.00)	12.1^{B}(±0.05)	11.9^{C}(±0.00)	11.6^{C}(±0.05)	11.3^{C}(±0.00)
CHI 4 + MPEO 5	14.1^{A}(±0.00)	12.2^{B}(±0.01)	11.1^{C}(±0.00)	11.2^{C}(±0.00)	13.1^{A}(±0.05)	12.2^{B}(±0.00)	11.5^{C}(±0.00)	11.3^{C}(±0.05)	11.4^{C}(±0.05)
CHI 4 + MPEO 2.5	14.5^{A}(±0.01)	12.7^{B}(±0.01)	11.1^{C}(±0.00)	11.3^{C}(±0.00)	13.8^{A}(±0.05)	12.5^{B}(±0.05)	11.8^{C}(±0.05)	11.4^{C}(±0.05)	11.9^{C}(±0.05)
	Colour (L*)								
Control	23.6^{bA}(±0.22)	22.9^{bB}(±0.19)	22.6^{bB}(±0.19)	21.9^{bC}(±0.01)	22.7^{bA}(±0.99)	22.7^{bA}(±0.71)	20.2^{cB}(±0.52)	20.6^{cB}(±0.70)	19.5^{cC}(±0.01)
CHI 4 + MPEO 5	25.7^{aA}(±0.31)	23.5^{aB}(±0.41)	23.7^{aB}(±0.41)	23.9^{aB}(±0.39)	23.9^{aA}(±0.49)	23.4^{aA}(±0.51)	23.8^{aA}(±0.40)	23.6^{aA}(±0.34)	22.4^{aB}(±0.79)
CHI 4 + MPEO 2.5	23.8^{b}(±0.16)	23.9^{a}(±0.38)	23.4^{a}(±0.17)	23.4^{a}(±0.40)	23.6^{aA}(±0.94)	23.5^{aA}(±0.18)	21.5^{bB}(±1.17)	21.4^{bB}(±1.37)	21.1^{bB}(±0.26)
	Colour (h*)								
Control	26.6^{aA}(±0.36)	21.9^{aB}(±2.11)	20.5^{aB}(±1.33)	19.7^{aC}(±1.19)	27.5^{aA}(±0.63)	22.9^{aB}(±0.11)	20.1^{aB}(±1.03)	19.9^{aC}(±0.19)	19.2^{aC}(±1.23)
CHI 4 + MPEO 5	25.2^{bA}(±1.27)	19.8^{bB}(±1.61)	18.7^{bB}(±1.82)	19.5^{aB}(±0.42)	26.3^{bA}(±1.72)	20.2^{bB}(±1.23)	19.3^{bB}(±1.52)	18.2^{bB}(±0.24)	19.9^{aB}(±0.49)
CHI 4 + MPEO 2.5	25.2^{bA}(±0.60)	19.8^{bB}(±0.37)	19.1^{bB}(±1.54)	19.4^{aB}(±0.50)	26.1^{bA}(±0.80)	20.7^{bB}(±0.56)	19.8^{bB}(±1.50)	18.8^{bB}(±0.57)	19.8^{aB}(±0.40)

Control: 0 µL/mL of CHI and essential oil + 2 g/100 mL of glycerol

CHI 4 + MPEO 5: CHI 4 mg/mL + MPEO 5.0 µL/mL; CHI 4 + MPEO 2.5: CHI 4 mg/mL + MPEO 2.5 µL/mL

$^{A-C}$ For each trial, different superscript capital letters in the same row denote differences ($p \leq 0.05$) between mean values (for the same treatments) based on Tukey's test.

$^{a-c}$ For each trial, different superscript lowercase letters in the same column denote differences ($p \leq 0.05$) between mean values (for the different treatments) based on Tukey's test.

Table 4. Mean values of physical and physicochemical quality parameters in uncoated grapes and grapes coated with chitosan (CHI) and *Mentha x villosa* Huds essential oil (MVEO) at different concentrations, followed by storage at room temperature (25 °C, 12 days) and low temperature (12 °C, 24 days).

Treatments	Room temperature (25 °C)				Low temperature (12 °C)				
	Days of storage				Days of storage				
	1	4	8	12	1	6	12	18	24
	Firmness (N/mm)								
Control	7.0^{bA}(±0.64)	6.9^{bA}(±0.40)	6.0^{bB}(±0.47)	5.0^{bC}(±0.44)	7.0^{aA}(±0.66)	7.1^{bA}(±0.05)	6.1^{bB}(±1.63)	5.1^{bC}(±0.45)	5.2^{bC}(±0.45)
CHI 4 + MPEO 5	7.4^{aA}(±0.67)	7.2^{aA}(±0.57)	6.8^{aB}(±0.30)	5.7^{aC}(±0.53)	7.7^{aA}(±0.06)	7.5^{aA}(±0.75)	6.8^{aB}(±0.85)	5.6^{aC}(±0.56)	5.7^{aC}(±0.55)
CHI 4 + MPEO 2.5	7.3^{aA}(±0.48)	7.3^{aA}(±0.30)	6.7^{aB}(±0.60)	5.7^{aC}(±0.27)	7.9^{aA}(±0.31)	7.4^{aA}(±0.36)	6.9^{aB}(±0.93)	5.8^{aC}(±0.30)	5.9^{aC}(±0.61)
	Titrable acidity (mmol H^+/100 g of fruit)								
Control	60.0^{A}(±0.20)	58.2^{A}(±2.79)	54.6^{B}(±2.46)	45.8^{C}(±2.34)	62.4^{A}(±0.76)	55.1^{B}(±0.31)	54.6^{B}(±0.55)	53.5^{B}(±0.29)	44.4^{C}(±1.00)
CHI 4 + MPEO 5	61.4^{A}(±0.80)	58.4^{B}(±1.63)	50.7^{B}(±2.91)	46.5^{C}(±2.33)	61.3^{A}(±044)	57.9^{B}(±0.52)	55.3^{B}(±0.95)	54.3^{B}(±0.78)	43.5^{C}(±0.76)
CHI 4 + MPEO 2.5	61.6^{A}(±0.63)	58.7^{B}(±0.14)	54.4^{B}(±0.97)	47.3^{C}(±2.06)	64.2^{A}(±0.51)	54.1^{B}(±0.54)	56.2^{B}(±1.04)	51.5^{B}(±0.88)	42.9^{C}(±0.45)
	Soluble solids (ºBrix)								
Control	14.6^{A}(±0.01)	13.2^{B}(±0.01)	12.9^{C}(±0.00)	12.3^{B}(±0.00)	13.4^{A}(±0.00)	12.7^{B}(±0.05)	11.6^{C}(±0.05)	11.9^{C}(±0.01)	11.2^{C}(±0.01)
CHI 4 + MPEO 5	14.2^{A}(±0.00)	13.2^{B}(±0.01)	12.3^{B}(±0.00)	12.3^{B}(±0.00)	13.5^{A}(±0.05)	12.8^{B}(±0.05)	11.3^{C}(±0.01)	11.7^{C}(±0.00)	11.4^{C}(±0.05)
CHI 4 + MPEO 2.5	14.6^{A}(±0.00)	12.5^{B}(±0.01)	12.7^{B}(±0.00)	12.6^{B}(±0.00)	13.6^{A}(±0.05)	12.6^{B}(±0.05)	11.4^{C}(±0.05)	11.8^{C}(±0.05)	11.6^{C}(±0.05)
	Color (L*)								
Control	23.5^{bA}(±0.15)	22.0^{bB}(±0.76)	22.5^{bB}(±0.45)	21.4^{bC}(±0.44)	23.4^{bA}(±0.56)	22.7^{bA}(±0.54)	21.3^{cB}(±0.43)	20.6^{cB}(±0.12)	19.5^{cC}(±0.21)
CHI 4 + MPEO 5	26.5^{aA}(±0.18)	24.5^{aB}(±0.14)	23.2^{aB}(±0.34)	23.7^{aB}(±0.36)	24.5^{aA}(±0.36)	24.4^{aA}(±0.37)	22.6^{aA}(±0.55)	21.7^{aA}(±0.43)	21.4^{aB}(±0.29)
CHI 4 + MPEO 2.5	24.2^{bA}(±0.23)	24.1^{aA}(±0.45)	23.4^{aB}(±0.56)	23.6^{aB}(±0.56)	24.6^{aA}(±0.76)	24.2^{aA}(±0.26)	22.4^{bB}(±0.56)	21.6^{bB}(±0.73)	21.2^{bB}(±0.26)
	Color (h*)								
Control	26.7^{aA}(±0.56)	21.6^{aB}(±1.11)	20.4^{aB}(±1.56)	19.2^{aC}(±1.49)	26.4^{aA}(±0.56)	21.5^{aB}(±0.43)	20.0^{aB}(±1.00)	19.6^{aC}(±0.23)	19.6^{aC}(±0.55)
CHI 4 + MPEO 5	25.2^{bA}(±1.65)	19.2^{bB}(±0.61)	19.7^{bB}(±1.35)	19.7^{aB}(±0.62)	25.4^{bA}(±1.86)	20.9^{bB}(±1.24)	19.2^{bB}(±0.99)	18.3^{aB}(±0.45)	19.8^{aB}(±0.45)
CHI 4 + MPEO 2.5	25.3^{bA}(±0.80)	19.5^{bB}(±0.47)	19.4^{bB}(±1.47)	19.4^{aB}(±0.30)	25.2^{bA}(±0.40)	20.5^{bB}(±0.78)	19.3^{bB}(±0.78)	18.6^{aB}(±0.67)	19.9^{aB}(±0.52)

Control: 0 µL/mL of CHI and essential oil + 2 g/100 mL of glycerol

CHI 4 + MPEO 5: CHI 4 mg/mL + MPEO 5.0 µL/mL; CHI 4 + MPEO 2.5: CHI 4 mg/mL + MPEO 2.5 µL/mL

$^{A-C}$ For each trial, different superscript capital letters in the same row denote differences ($p \leq 0.05$) between mean values (for the same treatments) based on Tukey's test.

$^{a-c}$ For each trial, different superscript lowercase letters in the same column denote differences ($p \leq 0.05$) between mean values (for the different treatments) based on Tukey's test.

Table 5. Mean values of sensory qualities in uncoated grapes and grapes coated with chitosan (CHI) and *M. piperita* essential oil (MPEO) or *M. villosa* Huds essential oil (MVEO) at different concentrations, followed by storage at low temperature (12 °C, 12 days).

Attributes	**Treatments**	**MPEO**		**MVEO**	
		Days of storage			
		0	**12**	**0**	**12**
Appearance	Control	7.31(±0.82)	7.53(±0.52)	7.48(±0.61)	7.06(±0.96)
	CHI 4 + MPEO 5	7.44(±0.26)	7.00(±0.06)	7.35(±0.58)	7.47(±0.17)
	CHI 4 + MPEO 2.5	7.16(±0.05)	7.07(±0.23)	7.63(±0.33)	7.03(±0.33)
Color	Control	7.34(±0.90)	7.53(±0.52)	7.19(±0.72)	7.60(±0.47)
	CHI 4 + MPEO 5	7.54(±1.06)	7.00(±0.06)	6.39(±0.33)	7.55(±0.29)
	CHI 4 + MPEO 2.5	7.25(±0.92)	7.07(±0.23)	6.68(±0.19)	7.77(±0.41)
Flavor	Control	7.56(±0.10)	7.29(±0.60)	7.48(±0.41)	7.48(±0.65)
	CHI 4 + MPEO 5	7.44(±0.95)	7.06(±0.29)	6.97(±0.63)	7.13(±0.99)
	CHI 4 + MPEO 2.5	7.22(±0.26)	6.97(±0.56)	7.13(±0.19)	7.16(±0.53)
Taste	Control	6.22(±0.62)	6.71(±0.44)	6.48(±0.41)	6.96(±0.65)
	CHI 4 + MPEO 5	6.37(±0.26)	6.64(±0.35)	6.97(±0.57)	6.68(±0.99)
	CHI 4 + MPEO 2.5	6.62(±0.18)	6.97(±0.58)	6.12(±0.33)	6.16(±0.53)
Aftertaste	Control	6.22(±0.62)	6.44(±0.32)	6.59(±0.13)	6.16(±0.32)
	CHI 4 + MPEO 5	6.37(±0.26)	6.66(±0.40)	6.03(±0.47)	6.61(±0.61)
	CHI 4 + MPEO 2.5	6.62(±0.18)	6.53(±0.54)	6.72(±0.28)	6.52(±0.03)
Firmness	Control	6.06(±0.07)	6.03(±0.47)	6.87(±0.33)	6.97(±0.24)
	CHI 4 + MPEO 5	6.94(±0.19)	6.67(±0.31)	6.55(±0.52)	6.97(±0.25)
	CHI 4 + MPEO 2.5	6.16(±0.53)	6.22(±0.22)	6.84(±0.84)	6.68(±0.61)
Overall evaluation	Control	7.56(±0.01)	7.61(±0.34)	7.38(±0.36)	6.81(±0.74)
	CHI 4 + MPEO 5	7.06(±0.22)	7.19(±0.19)	7.16(±0.13)	6.68(±0.94)
	CHI 4 + MPEO 2.5	7.00(±0.24)	7.13(±0.54)	6.97(±0.58)	6.93(±0.29)

Control: 0 µL/mL of CHI and essential oil + 2 g/100 mL of glycerol
CHI 4.0 + MPEO 5: CHI 4 mg/mL + MPEO 5.0 µL/mL; CHI 4 + MPEO 2.5: CHI 4 mg/mL + MPEO 2.5 µL/mL

Fig. 1. Effects of chitosan (CHI) and *M. piperita* L. essential oil (MPEO) or *M. villosa* Huds essential oil (MVEO) on weight loss of table grapes during storage at low temperature and room temperature. A and B: fruits coated with composites containing CHI (4 mg/mL) and MPEO at 5 or 2.5 µL/mL and stored at low temperature and room temperature, respectively; C and D: fruits coated with composites containing CHI (4 mg/mL) and MVEO at 5 or 2.5 µL/mL and stored at low temperature and room temperature, respectively. (♦) CHI at 4 mg/mL + MPEO or MVEO at 5 µL/mL; (▲) CHI at 4 mg/mL + MPEO or MVEO 2.5 µL/mL; (■) Control, CHI at 0 mg/mL + MPEO or MVEO at 0 µL/mL.

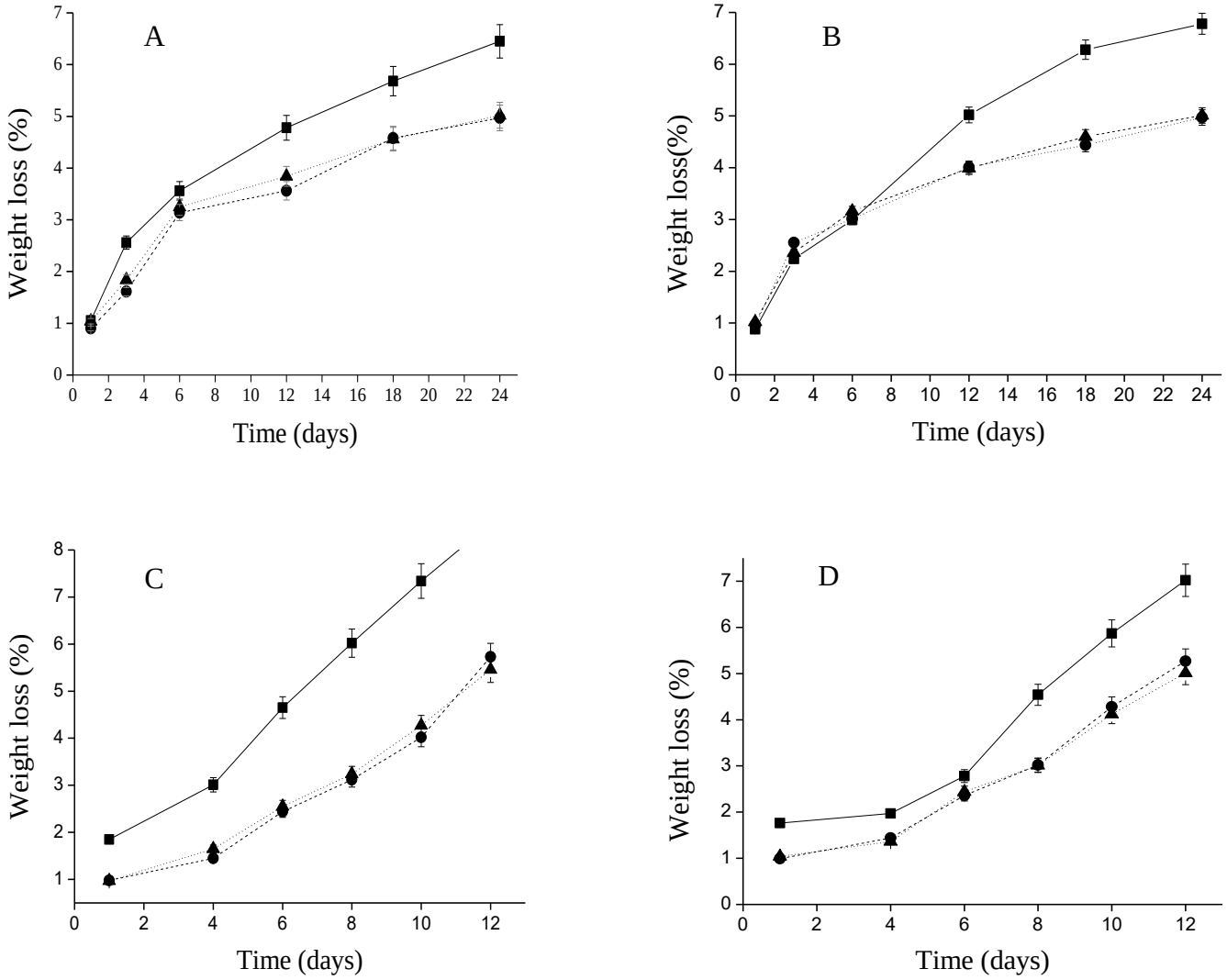

A
Weight loss (%)
Time (days)
B
Weight loss(%)
Time (days)
C
Weight loss (%)
Time (days)
D
Weight loss (%)
Time (days)

Fig. 1

APÊNDICE C - Formulário do teste aceitação sensorial e intenção de compra de tomate cereja ou uva revestidos com quitosana e óleos essenciais de *Mentha*.

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

Nome: _____ Idade: _____ e-mail: _____

Fone: _____ Escolaridade: _____Data:

Você está recebendo 05 amostras codificadas de (Uva 'Isabel' revestidas com quitosana e óleo essencial de Mentha). Prove-as da esquerda para direita, avalie sensorialmente as amostras de acordo com cada atributo nos quadros e escreva o valor da escala que você considera correspondente à amostra (código) no que diz respeito aos atributos avaliados. Antes de cada avaliação, você deverá fazer uso da água e da bolacha.

9 - gostei muitíssimo
8 - gostei muito
7 - gostei moderadamente
6 - gostei ligeiramente
5 - nem gostei/nem desgostei
4 - desgostei ligeiramente
3 - desgostei moderadamente
2 - desgostei muito
1 - desgostei muitíssimo

TRIBUTOS					
Aparência					
Cor					
Aroma					
Sabor					
Sabor residual					
Firmeza					
Avaliação Global					

Agora indique sua atitude ao encontrar estas frutas/vegetais no mercado.

5 - compraria
4 - possivelmente compraria
3 - talvez comprasse/ talvez não comprasse
2 - possivelmente não compraria
1 - jamais compraria

TRIBUTOS	**AMOSTRAS**				
Intenção de compra					

Comentários: _____

APÊNDICE D - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Prezado (a) Senhor (a)

Esta pesquisa é sobre a Incorporação de óleos essenciais de plantas aromáticas do nordeste brasileiro em envoltórios a base de quitosana como alternativa para inibição de fungos patógenos pós-colheita e está sendo desenvolvido como parte do Projeto de Doutorado da aluna Ingrid Conceição Dantas Guerra, sob a orientação do(a) Prof. Dr. Evandro Leite de Souza.

O objetivo do estudo é o de avaliar a aplicabilidade do envoltório de quitosana extraída do resíduo de camarão, incorporada com óleos essenciais de plantas aromáticas cultivadas no nordeste brasileiro, como alternativa para o biocontrole do crescimento de fungos patógenos pós-colheita em frutos tropicais, de modo a aproveitar os resíduos oriundos da indústria de pescado, e reduzir a deterioração e consequentemente o desperdício de frutos.

Solicitamos a sua colaboração para preenchimento dos formulários de aceitabilidade dos produtos elaborados, como também sua autorização para apresentar os resultados deste estudo em eventos da área de saúde, e em revistas científicas. Por ocasião da publicação dos resultados, seu nome será mantido em sigilo. Informamos ainda que essa pesquisa não oferece riscos, previsíveis, para a sua saúde.

Esclarecemos que sua participação no estudo é voluntária e, portanto, o(a) senhor(a) não é obrigado(a) a fornecer as informações e/ou colaborar com as atividades solicitadas pelo Pesquisador(a). Caso decida não participar do estudo, ou resolver a qualquer momento desistir do mesmo, não sofrerá nenhum dano, nem haverá modificação na assistência que vem recebendo na Instituição.

Os pesquisadores estarão a sua disposição para qualquer esclarecimento que considere necessário em qualquer etapa da pesquisa.

Diante do exposto, declaro que fui devidamente esclarecido(a) e dou o meu consentimento para participar da pesquisa e para publicação dos resultados. Estou ciente que receberei uma cópia desse documento.

_____

Assinatura do Participante da Pesquisa

Assinatura do Pesquisador

Contato com o Pesquisador (a) Responsável:

Caso necessite de maiores informações sobre o presente estudo, favor ligar para o (a) pesquisador (a) **INGRID CONCEIÇÃO DANTAS GUERRA.** Endereço (Setor de Trabalho): Universidade Federal da Paraíba Campus IV, Departamento de Hotelaria e Gastronomia**.** Telefone: 32923767/8813549. E-mail: ingridcdantas@hotmail.com

Contato do Comitê de ética em pesquisa: Campus Universitário S/N Bairro: Castelo Branco, João Pessoa, PB, Brasil. CEP: 58051-900.

ANEXOS

ANEXO A - CERTIDÃO DE APROVAÇÃO DO PROJETO NO COMITÊ DE ÉTICA

PARECER CONSUBSTANCIADO DO

CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: INCORPORAÇÃO DE ÓLEOS ESSENCIAIS DE PLANTAS AROMÁTICAS DONORDESTE BRASILEIRO EM ENVOLTÓRIOS A BASE DE QUITOSANA COMOALTERNATIVA PARA INIBIÇÃO DE FUNGOS PATÓGENOS PÓS-COLHEITA

Pesquisador: INGRID CONCEIÇÃO DANTAS GUERRA

Área

Temática:

Versão: 4

CAAE: 13160713.9.0000.5188

Instituição Proponente: Centro de Ciência da Saúde

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 712.884

Data da Relatoria:

26/06/2014

Apresentação do Projeto:

Trata-se do Projeto de pesquisa intitulado: Incorporação de óleos essenciais de plantas aromáticas do Nordeste Brasileiro em envoltórios a base de quitosana como alternativa para inibição de fungos patógenos pós-colheita. Nesse trabalho os voluntários (n=60) irão participar de um teste organoléptico de escolha de frutos. Esse é um projeto de tese apresentado ao Programa de Pós-Graduação em Nutrição do Centro de Ciências da Saúde da Universidade Federal de Paraíba.

Objetivo da Pesquisa:

Objetivo Primário: Avaliar a aplicabilidade do envoltório de quitosana extraída do resíduo de camarão, incorporada com óleos essenciais de plantas aromáticas cultivadas no nordeste brasileiro, como alternativa para o biocontrole do crescimento de fungos patógenos pós-colheita em frutos tropicais. Objetivo Secundário: Produzir e caracterizar a quitosana obtida do resíduo industrial (carapaça) da produção do camarão Litopenaus vannamei; Determinar a Concentração Inibitória Mínima e a Concentração Fungicida Mínima da quitosana e de óleos essenciais de

plantas cultivadas no Nordeste brasileiro e ainda Analisar o efeito da aplicação do envoltório formado pela quitosana e óleos essenciais sobre a microbiota autóctone, crescimento fúngico e parâmetros físico-químicos e sensoriais indicadores de qualidade durante o armazenamento de frutos. Sendo esta ultima etapa a realizada com participação de voluntários.

Avaliação dos Riscos e Benefícios:

Riscos: As quantidades de óleos utilizadas podem alterar negativamente a aceitação sensorial do fruto, podendo vir a prejudicar o uso em larga escala. Em principio, os riscos são mínimos já que a avaliação sensorial de sabor não implica em ingestão do produto.

Benefícios: indiretos já que se obtém maiores conhecimentos sobre as formas de preservação dos frutos.

Comentários e Considerações sobre a Pesquisa:

Trata-se de um projeto de pesquisa com excelente fundamentação teórica. A metodologia da pesquisa está bem detalhada e as etapas de participação dos voluntários também estão claramente explicitadas. Os objetivos são coerentes com a metodologia.

Considerações sobre os Termos de apresentação obrigatória:

O TCLE está adequadamente apresentado.

Recomendações:

nada a declarar

Conclusões ou Pendências e Lista de Inadequações:

nada a declarar

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

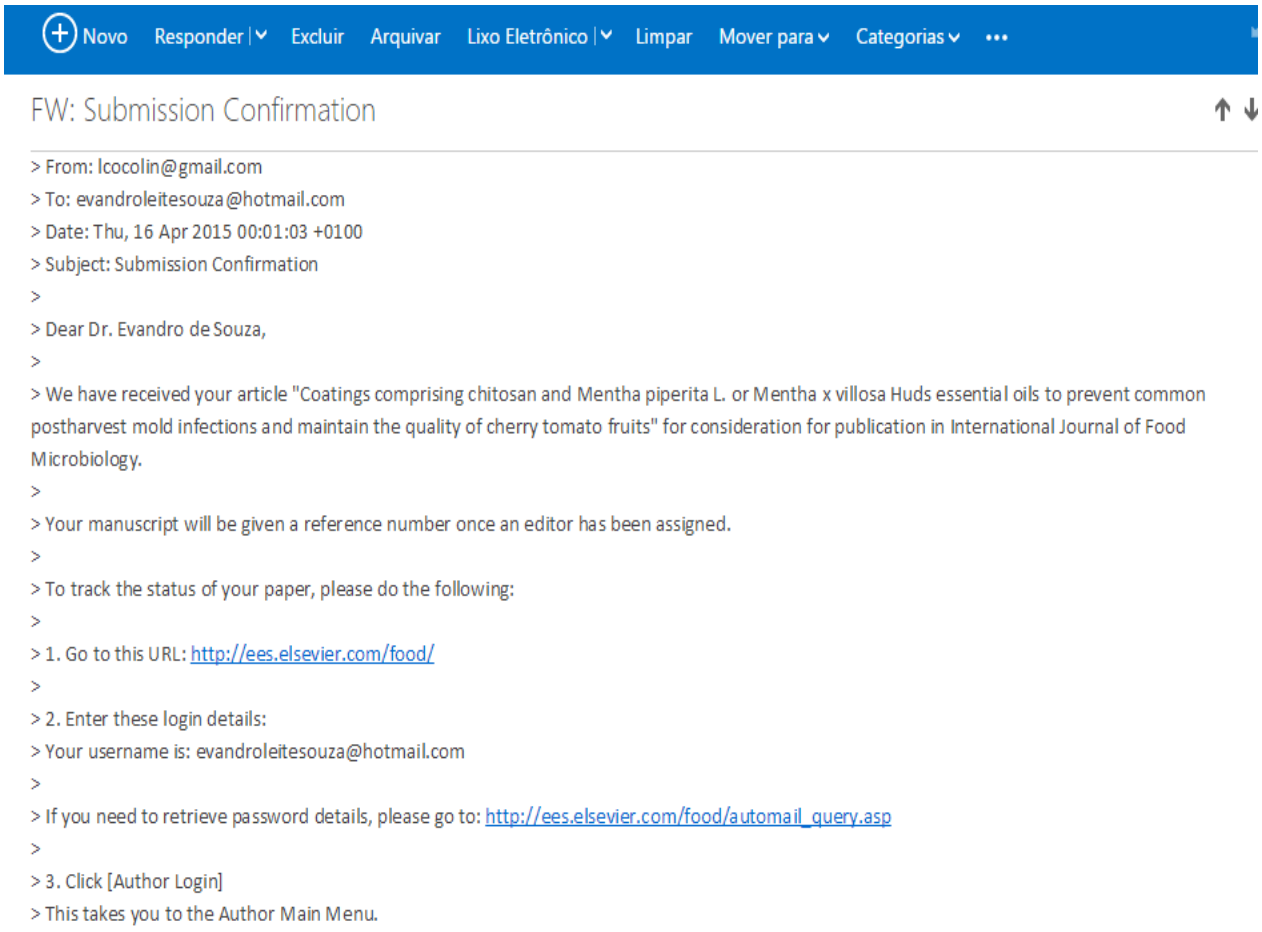
Considerações Finais a critério do CEP:

Endereço: UNIVERSITARIO S/N
Bairro: CASTELO BRANCO **CEP:** 58.051-900
UF: PB **Município:** JOAO PESSOA
Telefone: (83)3216-7791 **Fax:** (83)3216-7791 **E-mail:** eticaccs@ccs.ufpb.br; elianemduarte@hotmail.com

JOAO PESSOA, 09 de Julho de 2014

Assinado por: Eliane Marques Duarte de Sousa (Coordenador)

ANEXO B - COMPROVANTES DE ENVIO DOS ARTIGOS



Novo Responder | Excluir Arquivar Lixo Eletrônico | Limpar Mover para Categorias •••

FW: Submission Confirmation

> From: lcocolin@gmail.com
> To: evandroleitesouza@hotmail.com
> Date: Thu, 16 Apr 2015 00:01:03 +0100
> Subject: Submission Confirmation
>
> Dear Dr. Evandro de Souza,
>
> We have received your article "Coatings comprising chitosan and Mentha piperita L. or Mentha x villosa Huds essential oils to prevent common postharvest mold infections and maintain the quality of cherry tomato fruits" for consideration for publication in International Journal of Food Microbiology.
>
> Your manuscript will be given a reference number once an editor has been assigned.
>
> To track the status of your paper, please do the following:
>
> 1. Go to this URL: http://ees.elsevier.com/food/
>
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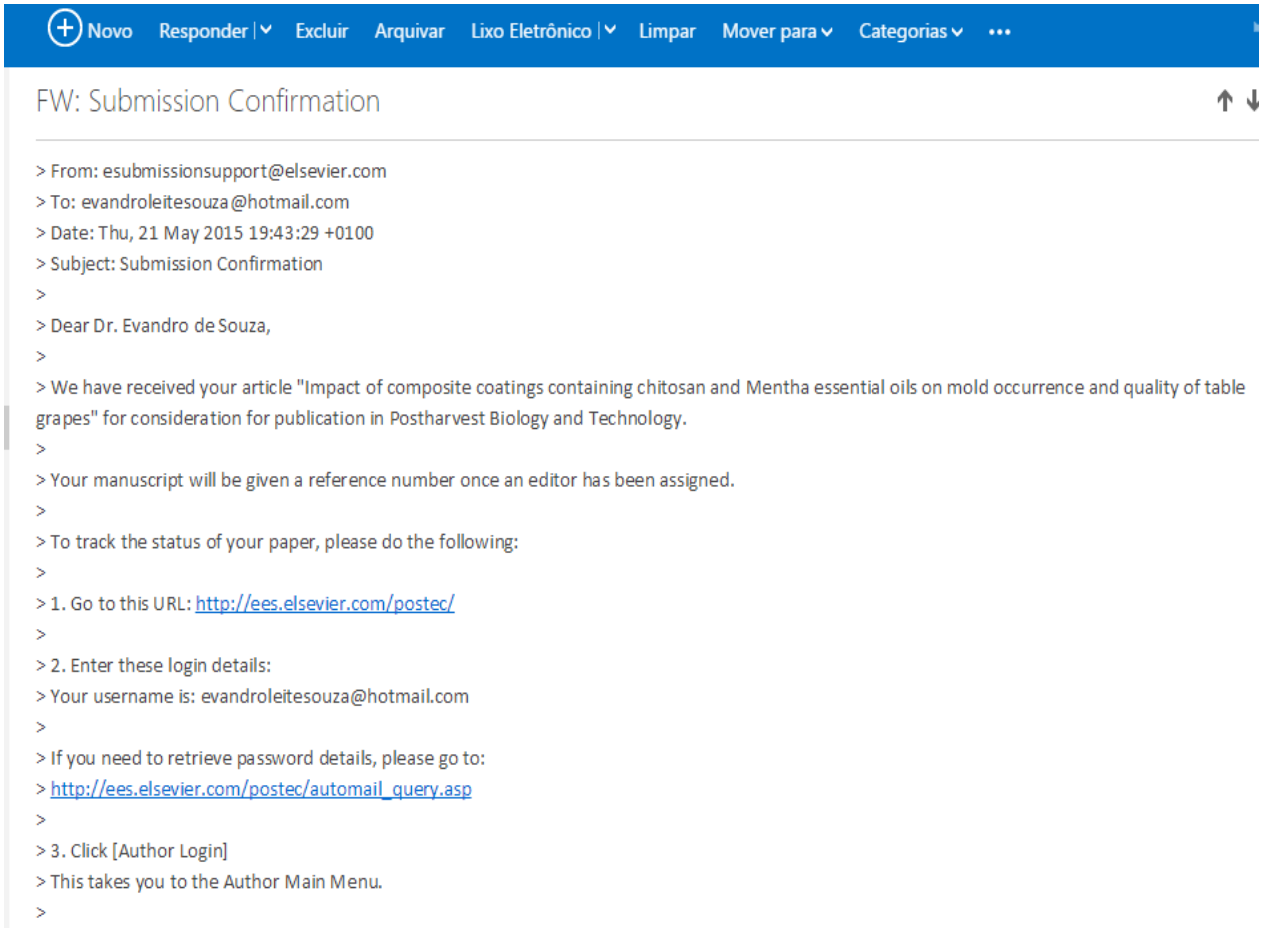
>
> If you need to retrieve password details, please go to: http://ees.elsevier.com/food/automail_query.asp
>
> 3. Click [Author Login]
> This takes you to the Author Main Menu.



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FW: Submission Confirmation

> From: esubmissionsupport@elsevier.com
> To: evandroleitesouza@hotmail.com
> Date: Thu, 21 May 2015 19:43:29 +0100
> Subject: Submission Confirmation
>
> Dear Dr. Evandro de Souza,
>
> We have received your article "Impact of composite coatings containing chitosan and Mentha essential oils on mold occurrence and quality of table grapes" for consideration for publication in Postharvest Biology and Technology.
>
> Your manuscript will be given a reference number once an editor has been assigned.
>
> To track the status of your paper, please do the following:
>
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> 2. Enter these login details:
> Your username is: evandroleitesouza@hotmail.com
>
> If you need to retrieve password details, please go to:
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> 3. Click [Author Login]
> This takes you to the Author Main Menu.
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