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ESTUDO DE COBERTURA COMESTÍVEL COM PROPRIEDADES ANTIFÚNGICAS E ATMOSFERA MODIFICADA NA MANUTENÇÃO DA QUALIDADE DE TOMATE CEREJA (*Lycopersicon esculentum* var. Cerasiforme)

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Florianópolis, Junho de 2013

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RESUMO

O tomate é uma hortaliça de grande produção e consumo no Brasil. No entanto, esse vegetal é susceptível a deterioração durante o período pós-colheita. O objetivo do trabalho foi a aplicação de diferentes tecnologias pós-colheita para aumentar o período de vida útil do tomate cereja cereja **(***Lycopersicon esculentum* var. Cerasiforme). Dentre as alternativas proposta foi avaliar, de forma independente, a influência do uso de atmosfera modificada ativa e aplicação de cobertura comestível, com atividade antifúngica, em tomate cereja. Foram realizados testes preliminares para avaliação da higienização das amostras com ozônio, validação do equipamento para análise de gases e escolha da embalagem mais adequada ao produto. Durante o período de armazenamento foram avaliados a produção e consumo de dióxido de carbono e oxigênio, respectivamente, e consequentemente as taxas de respiração a partir dos dados de concentração de O_2 e CO_2, associados aos parâmetros físico-químicos, químicos e microbiológicos. Para o estudo das coberturas foram realizados testes *in vitro* e *in vivo* para determinar os melhores antifúngicos para eliminação de *Botrytis cinérea* e *Alternaria alternata*. Para avaliar alterações na qualidade das amostras com cobertura comestível foram realizados análise de taxa respiratória, sólidos solúveis, pH, acidez titulável, cor, perda de peso, textura, etanol, acetaldeído e análise sensorial. A capacidade antifúngica da cobertura foi determinada através da análise de incidência e severidade do mofo cinzento e mancha marrom causados por *Botrytis cinérea e Alternaria alternata*, respectivamente. Os resultados dos testes preliminares mostraram que o ozônio, assim como o cloro, foi eficaz na eliminação de microrganismos. O analisador de gases mostrou ser uma alternativa viável, em função dos resultados precisos e exatos obtidos quando comparado com o cromatógrafo gasoso. Dentre as embalagens testadas, a embalagem de PPBO/PEBD foi a mais apropriada para utilização em tomates cereja armazenados sob atmosfera modificada. A atmosfera contendo a concentração gases de 5% O_2 + 5% CO_2 + 90% N_2 mostrou os melhores resultados para as características físico-químicas e taxa respiratória dos tomates armazenados a 5 e 10 °C. Esta mesma atmosfera inibiu processos químicos que alteram compostos como os açúcares e os ácidos orgânicos, em comparação com as amostras controle. Estes resultados sugerem que a combinação desta atmosfera (5 % O_2 + 5% CO_2 + 90 % N_2) com a temperatura de 5 °C é capaz de prolongar a vida útil de tomates cereja por 25 dias. Em geral, os

melhores resultados para a redução do mofo cinzento causado por *Botrytis cinerea* em frutos de tomate cereja foram obtidas com coberturas contendo 2,0% de carbonato de potássio, fosfato de amônio, bicarbonato de potássio, ou carbonato de amónio, enquanto que 2,0% de metilparabeno de sódio, etilparabeno de sódio, propilparabeno de sódio foram os melhores antifúngicos contra a podridão negra causada por *Alternaria alternata*. As duas alternativas aplicadas mostraram respostas efetivas no prolongamento da vida útil de tomates cerejas podendo ser aplicadas separadamente pela indústria do setor. A atmosfera modificada apresentou maior manutenção das características físico-químicas quando comparada com a melhor cobertura comestível.

Palavras chaves: armazenamento, atmosfera modificada, coberturas comestíveis, tomate cereja, pós-colheita.

ABSTRACT

The tomato is a vegetable of great production and consumption in Brazil. However, this plant is susceptible to deterioration during post-harvest. The objective of this work was to apply different postharvest technologies to increase the shelf life of the tomato (*Lycopersicon esculentum* var. Cerasiforme). Among the alternative the proposal was to evaluate independently the influence of the use of active modified atmosphere and application of edible coating with antifungal activity in tomato. Preliminary tests were performed to evaluate the cleaning of samples with ozone, validation of the gas analysis equipment and choice of the most suitable packaging to the product. During the storage were evaluated consumption and production of oxygen and carbon dioxide, respectively, and the respiration rate from the data the concentration of O_2 and CO_2 associated with the physical-chemical and microbiological. To study of edible coartings were performed tests *in vitro* and *in vivo* to determine the best antifungal agents for the elimination of *Botrytis cinerea* and *Alternaria alternata*. To evaluate changes in the quality of the samples with edible coating were performed analysis of respiration rate, soluble solids, pH, titratable acidity, color, weight loss, texture, ethanol, acetaldehyde and sensory analysis. The ability antifungal of coating was determined by analyzing incidence and severity of gray mold and black rot caused by *Botrytis cinerea* and *Alternaria alternata*, respectively. The results of preliminary tests showed that ozone, and chlorine was effective in eliminating microorganisms. The gas analyzer proved to be suitable due the precise and accurate results obtained when compared with the gas chromatograph. Among the packings, packing PPBO / LDPE was most suitable for use in cherry tomatoes stored under modified atmosphere. The atmosphere containing gases concentration of 5% O_2 + 5% CO_2 + 90% N_2 showed the best results for the physico-chemical and respiratory rate of tomatoes stored at 5 and 10 °C. This same atmosphere inhibited chemical processes that alter compounds such as sugars and organic acids, compared with the control samples. These results suggest that the combination of this atmosphere (5% O_2 + 5% CO_2 + 90% N_2) at the temperature of 5 °C is able to prolong the life of cherry tomatoes for 25 days. Overall, the best results for reduction of gray mold on cherry tomato fruit were obtained with coatings containing 2.0% of potassium carbonate, ammonium phosphate, potassium bicarbonate, or ammonium carbonate, while 2.0% sodium

methylparaben, sodium ethylparaben, and sodium propylparaben were the best ingredients for coatings against black rot.

Keywords: storage, modified atmosphere, edible coatings, cherry tomatoes, post-harvest.

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1 INTRODUÇÃO

O tomate (*Lycopersicon esculentum* L.) é uma das hortaliças mais produzidas e consumidas no mundo, para ambos os mercados: produtos frescos e indústrias de alimentos processados (FENG et al., 2011). Porém, é um produto perecível pela fragilidade dos seus tecidos, e pela manutenção de sua atividade metabólica após a colheita (VIEITES, 1998).

O desperdício de frutas e hortaliças para o consumo *in natura* durante o processo de armazenamento é uma preocupação constante no setor alimentício do país. Segundo Henz e Moretti (2005), as perdas da produção de tomate podem chegar a 86% dependendo do cultivar, modo de beneficiamento e armazenamento. O período pós-colheita em que as perdas são mais evidentes é a venda no varejo, chegando a 32%. Entre as causas de perdas está o aparecimento de doenças pós-colheita que atacam os frutos de tomate, causando pequenas manchas até a deterioração completa (SIMÃO e RODRÌGUEZ, 2009).

A atmosfera modificada é uma técnica que pode ser empregada para proteger o produto de contaminações, assim como retardar o processo de senescência do vegetal através da redução da taxa respiratória (SANDHYA, 2010). Esta técnica envolve alteração da atmosfera gasosa que envolve o produto através da redução da concentração de O_2 e aumento do conteúdo de CO_2. A composição gasosa dentro da embalagem é alterada ao longo do período de armazenamento do produto devido a fatores como respiração e difusão de gases através da embalagem. Portanto, a composição gasosa dentro da embalagem dependerá do balanço entre as reações metabólicas do produto e as características de permeabilidade do filme. Entre os fatores que devem ser observados para aplicação da atmosfera modificada esta a concentração de O_2 e CO_2 a ser utilizada, a permeabilidade da embalagem a gases e vapor de água, assim como temperatura de armazenamento do produto (KADER et al., 1989; GONTARD et al.,1995; FONSECA et al., 2002;)

A mistura de gases utilizada depende do tipo de produto, material de embalagem e temperatura de armazenamento. A permeabilidade do material da embalagem deve ser adequada para a taxa respiratória do produto (SANDHYA, 2010). Baixas concentrações de O_2 juntamente com altas concentrações de CO_2 podem reduzir a taxa respiratória, retardar o amadurecimento, diminuir a produção de etileno, e causar alterações na textura, resultando na extensão da vida de

prateleira do produto (DAS et al., 2006). Hong e Gross (2001) verificaram que o uso de atmosfera modificada manteve a qualidade de tomates minimamente processados armazenados durante 15 dias a 5°C.

O uso de coberturas comestíveis com intuito de reduzir a contaminação por fungos e as alterações das características físico-químicas em vegetais é uma tendência nacional e internacional. O tomate é susceptível à doenças pós-colheita causadas por vários fungos patogênicos. *Botrytis cinerea* e *Alternaria alternata* estão entre os fungos mais comuns responsáveis por podridões em frutos de tomate (WANG et al., 2009).

Fungicidas químicos sintéticos têm sido usados para reduzir a deterioração pós-colheita de fungos, mas devido ao mau uso destes produtos tem ocorrido problemas relacionados com a toxicidade, gerando impacto negativo sobre o meio ambiente e a saúde humana, medidas alternativas para o controle de doenças são cada vez mais exigidas (SPADARO e GULLINO, 2004). A utilização de biofilmes comestíveis é um método químico alternativo para preservar a qualidade pós-colheita de frutos e vegetais O interesse do consumidor em produtos naturais levou os pesquisadores a desenvolver novos biofilmes comestíveis que possam melhorar a segurança alimentar e manter as características físico-químicas durante a pós-colheita. Estes biofilmes fornecem uma barreira semipermeável a troca de água e gases entre o alimento e o ambiente circundante, reduzindo a taxa respiratória e a perda de umidade durante o armazenamento (PEREZ-GAGO et al., 2002; NAVARRO-TARAZAGA et al., 2007).

Vários trabalhos têm incidido sobre o desenvolvimento de biofilmes à base de polissacarídeos ou proteínas com aditivos naturais para controlar o crescimento microbiano em vegetais. Os principais polissacarídeos incluídos em formulações de biofilmes comestíveis são o amido e derivados, derivados de celulose, quitosana, pectina, gomas de alginato e outros (TZOUMAKI et al., 2009). Filmes contendo polissacarídeos apresentam uma boa barreira a gases, mas pobre barreira à umidade. No entanto, biofilmes contendo lipídeos são utilizados para fornecer barreira ao vapor de água. Alguns biofilmes podem conter componentes lipídicos e polissacarídeos, melhorando as características de barreira do composto filmogênico (VALENCIA-CHAMORRO et al. 2011). Os agentes antimicrobianos vêm sendo adicionados aos biofilmes comestíveis para retardar o crescimento de bactérias, leveduras e bolores durante o armazenamento e distribuição de produtos frescos ou

minimamente processados (ZHAO et al., 2010; ALI et al., 2010; VALENCIA-CHAMORRO et al., 2011; PANE et al., 2012).

Alguns trabalhos mostram que as coberturas comestíveis à base de hidroxipropil metilcelulose (HPMC) e lipídeos, como cera de abelha, cera de carnaúba ou resina preservaram a qualidade pós-colheita de frutos, reduzindo a perda de peso e mantendo a firmeza e qualidade sensorial dos produtos revestidos (PEREZ-GAGO et al., 2002; PEREZ-GAGO et al., 2003; PEREZ-GAGO et al., 2005; NAVARRO-TARAZAGA et al., 2007; VALENCIA-CHAMORRO et al., 2009; VALENCIA-CHAMORRO et al., 2010).

As informações sobre a atmosfera mais adequada para manutenção das características físico-químicas de tomate cereja é limitada. Assim como poucos estudos tem avaliado aplicação de coberturas comestíveis, com propriedades antifúngicas neste fruto. Com intuito de desenvolver uma tecnologia capaz de reduzir as perdas que ocorrem durante a pós-colheita do tomate cereja, o objetivo deste estudo foi determinar a melhor atmosfera gasosa e temperatura a serem aplicadas para dimunuir as alterações físicas, químicas e microbiológicas de tomates cereja durante o armazenamento, e consequentemente aumentar vida útil do produto. Além do desenvolvimento e aplicação de coberturas comestíveis a base de hidroxipropilmetilcelulose (HPMC), com adição de diferentes antifúngicos para impedir o crescimento de *Botrytis cinerea* e *Alternaria alternata*, e logo reduzir a deterioração que ocorre durante o armazenamento do produto causada por fungos, e também diminuir as alterações nas características físico-químicas e sensoriais de tomate cereja contribuindo para manutenção da qualidade do produto por um período maior de tempo.

2 OBJETIVOS

2.1 Objetivo Geral

O presente trabalho teve por objetivo avaliar diferentes metodologias visando aumentar o período de conservação de tomate cereja: atmosfera modificada ativa e coberturas comestíveis, com propriedades antifúngicas.

Os objetivos específicos foram:

-Avaliar qual o material de embalagem é mais adequado para o armazenamento dos tomates, associado às condições de estocagem e taxa respiratória do produto;

-Determinar a melhor atmosfera gasosa e temperatura a ser aplicada na conservação do fruto, através da taxa respiratória e análises físico-químicas;

- Avaliar a atividade *in vitro* dos aditivos alimentares com propriedades antifúngicas contra *B. cinerea* e *A. alternata*;

-Elaborar uma cobertura comestível a base de hidroxipropilmetilcelulose (HPMC) estável, contendo aditivos alimentares antifúngicos selecionados *in vitro*;

-Determinar a atividade curativa destas coberturas no controle de mofo cinzento e podridão negra em tomates cereja, inoculados artificialmente com *B. cinerea* e *A. Alternata*.

- Avaliar qual melhor metodologia para manutenção da qualidade e prolongamento da vida útil de tomate cereja.

3 ESTRUTURA DO TRABALHO

Para estudar os conteúdos e apresentar os temas tratados em uma sequência lógica, este trabalho foi estruturado da seguinte forma:

Capítulo 1 – *Revisão da Literatura*. Neste capítulo é apresentado o *Estado da Arte* que sustenta este trabalho, trata dos diversos aspectos referentes à matéria-prima utilizada tomate cereja (L. *esculentum* var. Cerasiforme.), as alterações físico-químicas decorrentes do processo de maturação e técnicas de conservação que foram utilizadas; como atmosfera modificada e coberturas comestíveis.

Capítulo 2 - *Uso de ozônio na sanitização de Tomate cereja.* Neste capítulo é apresentado o efeito do uso de ozônio e cloro na higienização de tomate cerja, o resultado foi avaliado através da contagem microbiana dos frutos.

Capítulo 3 – *Avaliação de um método experimental para determinar a concentração de O_2 e CO_2 em produtos acondicionados sob atmosfera modificada.* Neste capitulo é apresentada uma comparação entre dois métodos para determinação da concentração de gases. A confiabilidade dos dados obtidos em analisador automático de gases foi avaliada mediante a comparação dos dados obtidos através de um método convencional (cromatografia gasosa).

Capítulo 4 – *Efeito da atmosfera modificada ativa na manutenção da qualidade de tomate cereja***.** Neste capítulo são apresentados os resultados de taxa respiratória e alterações físico-quimicas de tomates cereja armazenados em três diferentes atmosferas, armazenados a temperatura de 5°C.

Capítulo 5 – *Avaliação de diferentes embalagens e atmosfera modificada para conservação de tomate cereja***.** Neste capítulo é apresentado um estudo a respeito da taxa respiratória e alterações físico-químicas de tomate cereja, armazenadas em diferentes embalagens plásticas e temperatura de 10°C.

Capítulo 6 – *Aplicação de atmosfera modificada e baixa temperatura na conservação de tomate cereja*. Neste capítulo, a taxa respiratória,

etileno, carotenoides totais, açúcares, ácidos orgânicos e firmeza de frutos de tomate cereja armazenados em atmosfera modificada foram apresentados.

Capítulo 7 – *Atividade antifúngica de aditivos de alimentos in vitro e como ingredientes de cobertura a base de hidroxipopilmetilcelulose contra Botrytis cinerea e Alternaria Alternata em tomate cereja.* Neste capítulo são apresentados a avaliação de diferentes antifúngicos *in vitro* e como ingredientes de cobertura comestível contra os dois principais fungos causadores de doenças em tomates.

Capítulo 8 – *Efeito antifungico de cobertura comestível a base de hidroxipropilmetilcelulose contra Botrytis cinerea e atributos de qualidade de tomate cereja armazenado sob refrigeração.*
Neste capítulo são apresentadas a avaliação do efeito antifúngico de compostos selecionados in vitro. Foram verificados também a capacidade da cobertura de manter em manter as características fisíco-químicas de tomate cereja armazenado sob refrigeração.

Capítulo 9 – *Efeito antifungico de cobertura comestível a base de hidroxipropilmetilcelulose contra Alternaria alternata e atributos de qualidade de tomate cereja armazenado sob refrigeração.* Neste capítulo são apresentadas a avaliação do efeito antifúngico de compostos selecionados *in vitro*. Foram verificados também a capacidade da cobertura de manter em manter as características fisíco-químicas de tomate cereja armazenado sob refrigeração.

CAPÍTULO 1:
REVISÃO LITERÁRIA

1. REVISÃO LITERÁRIA

1.1 Tomate

1.1.1 Origem do tomate

O tomateiro é originário da América do Sul, mais especificamente da região localizada entre o Equador e o norte do Chile, onde podem ser encontradas muitas espécies desde o litoral do Pacífico até uma altitude de 2000 metros, na região dos Andes. É uma planta que se adapta a quase todos os tipos de clima, não tolerando, porém, temperaturas extremas (LOPES e STRIPARI, 1998).

Provavelmente a domesticação do tomate ocorreu no México, por tribos indígenas primitivas que lá habitavam e então foi levado para outras partes do mundo, por viajantes europeus na primeira metade do século XVI. Tudo indica que o tomateiro foi introduzido no Brasil por imigrantes europeus no fim do século XIX, mas a difusão e o incremento do consumo começaram a ocorrer depois da primeira Guerra Mundial, por volta de 1930 (ALVARENGA, 2004).

Desde a sua domesticação no México, até sua aceitação e cultivo na Europa e Estados Unidos em meados do século XIX, o tomateiro vem sofrendo seleções, com consequente melhoria na qualidade dos frutos. Após sua introdução no Brasil, supostamente pela imigração européia, iniciaram-se também as atividades de melhoramento. O surgimento do tomate 'Santa Cruz' no Rio de Janeiro, por volta de 1940, assinala um importante marco na trajetória dessa espécie no Brasil (NAGAI, 1989). A identificação de sua notável riqueza nutricional, especialmente quanto à presença de vitaminas, aliado ao seu agradável sabor e cor, contribuíram para a rápida popularização de seu consumo no país (ESPINOZA, 1991).

1.1.2 Tomate Cereja

Há muitas espécies de tomates cultivados e consumidos no Brasil, destacando-se o tomate cereja (L. *esculentum* var. Cerasiforme), considerado uma forma ancestral de tomate, devido ao tamanho e a forma destes frutos (2 a 2,5 cm de diâmetro) serem intermediários entre o tomate selvagem e o cultivado (WARNOK, 1988). Segundo Taylor (1986) a espécie cultivada *Lycopersicum solanum* originou-se da espécie andina *Lycopersicon esculentum* var. cerasiforme. O tomate

cereja (*Lycopersicon esculentum* var. cerasiforme), contribuiu sobremaneira para o desenvolvimento de cultivares mais resistentes a pragas e a doenças (FILGUEIRA, 2000).

Este tipo de tomate pertence a um grupo de cultivares para mesa, tendo crescido em importância nos mercados das grandes cidades (final da década de 90). Sua forma pode ser redonda, periforme ou ovalada, a coloração varia do amarelo ao vermelho, e a massa varia de 5 a 30g. Na maioria das vezes, apresentam frutos biloculares e suas pencas podem apresentar de 6 a 18 frutos (ALVARENGA, 2004).

O tomate do tipo cereja é considerado como uma hortaliça exótica, incorporada em cardápios de restaurantes por serem pequenos e delicados, trazendo novos sabores e enfeites aos pratos e aperitivos, com vantagem de ter tamanho reduzido evitando desperdício (MACHADO et al., 2003).

1.1.3 Caracterização dos cultivares

Sendo um fruto de origem tropical, o tomate (*Lycopersicon esculentum)*, é uma planta dicotiledônea pertencente à família das solanáceas. Compreende esta família 85 gêneros distribuídos em todo o mundo, sendo especialmente abundante nas Américas (JOLY, 1979).

1.1.4 Coloração e Formato

Normalmente os consumidores relacionam tomates com a coloração vermelha, mas com o aparecimento de novos cultivares, hoje existe a disposição dos consumidores um grupo de coloração de tomates, que é determinada pela cor final (FEAGRI/UNICAMP, 2004):

Figura 1.1: Coloração de tomates


Vermelho


Rosado


Amarelo

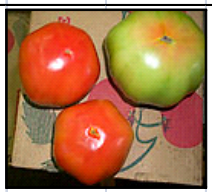

Laranja

Fonte: Feagri/Unicamp, 2004.

Os tomates de coloração laranja e amarelo não são comuns no Brasil. Tomates vermelhos quando submetidos a altas temperaturas podem apresentar coloração amarelada, característica encontrada facilmente nos meses mais quentes do ano, principalmente no Norte e Nordeste (FEAGRI/UNICAMP, 2004).

Os frutos de tomate podem ser identificados, primeiramente, pelo formato, o qual pode estar relacionado à sua finalidade de uso. Nos últimos anos tem aumentado a diversidade dos produtos oferecidos, sendo ainda mais comum o formato oblongo e redondo.

Figura 1.2: Características do tomate.

Grupo	Foto	Utilização	Formato
Santa Cruz		Salada e mollho	Oblondo
Caqui		Saladas e lanches	Redondo
Saladete		Saladas	Redondo
Italiano		Saladas e molho	Oblongo Alongado
Cereja		Aperitivos e saladas	Oblongo e Redondo

Fonte: Feagri/Unicamp, 2004.

1.1.5 Durabilidade

A durabilidade está relacionada à vida pós-colheita ou vida útil do produto em condições normais de conservação.

Longa vida: esta é uma denominação utilizada para os tomates de cultivares que possuem uma vida pós-colheita mais prolongada,

permanecendo firmes por um período maior de tempo. Muitas vezes é utilizado para transporte em longas distâncias.
Normal: os tomates que possuem esta característica têm menor vida útil, duram menos, mas, em geral, são mais saborosos que os tomates longa vida (FEAGRI/UNICAMP, 2004)

1.1.6 Composição dos tomates

O teor de cada componente presente no tomate depende da variedade, nutrição e condições de cultivo, o que dificulta a apresentação de valores precisos. O fruto fresco do tomateiro é rico em vitamina C e seu conteúdo calórico é baixo, devido a sua escassez em matéria seca e gordura. É também uma excelente fonte de vitaminas A e ferro (GOULD, 1991).

Os tomates verdes têm concentrações relativamente elevadas de amido, que podem superar 1% do peso fresco (BORGUINI, 2002). Os compostos nitrogenados diminuem desde a formação do fruto até o início da maturação. Durante a maturação, os aminoácidos livres totais permanecem relativamente constantes, mas a concentração em ácido glutâmico aumenta com o amadurecimento do produto (LAPUERTA, 1995).

A vitamina C está presente no tomate este vegetal apresenta também quantidades de ácido cítrico e ácido málico. A acidez máxima durante a maturação coincide com a aparição da cor rosada, decrescendo progressivamente, dependendo da variedade (BALDWIN et al., 1998).

O sabor dos tomates resulta de uma interação complexa entre açúcares, ácidos orgânicos, minerais e componentes do aroma. A fração volátil do tomate está constituída por mais de 400 substâncias, entre as quais se encontram hidrocarbonetos, éteres, aminas e uma ampla gama de moléculas heterocíclicas (BALDWIN et al., 1998).

O tomate possui de 7 a 8,5% de sólidos onde 1% corresponde a pele e a semente. Os açúcares constituem a maioria dos sólidos solúveis nas variedades comerciais de tomate, com valores de 1,5 a 4,0% do peso seco, o que equivale a 65% dos sólidos solúveis totais. Os açúcares livres mais abundantes são a glicose e a frutose, que se encontram em proporções similares, o teor de sacarose esta em torno de 0,1%. No tomate cereja o açúcar em maior quantidade é a frutose (PICHA 1987; GOULD, 1991; LAPUERTA, 1995). O teor de açúcares aumenta significativamente quando o fruto alcança uma cor amarelo-rosada e aumenta paulatinamente durante a maturação (LAPUERTA, 1995).

A concentração de lipídeos no tomate é baixa, variando de 10 a 20 mg de lipídios insaponificáveis por grama de matéria seca (BORGUINI, 2002). O potássio é o mineral mais abundante e o que tem maior influência na qualidade do fruto e, junto com nitratos e fosfatos, constitui 93% das substâncias minerais do tomate. O cálcio esta presente em torno de 0,12% (BALDWIN et al., 1998).

A cor verde dos tomates não maduros se deve à presença de clorofila *a* e *b*. No início a cor muda gradualmente de verde-escuro para verde-claro e em seguida ocorre o surgimento de pigmentos amarelos, alaranjados e vermelhos. A perda da cor verde resulta da quebra da estrutura de clorofila causada, principalmente, pelas mudanças de pH, pela presença de sistemas oxidantes e pela atividade de clorofilases (AWAD, 1993).

A coloração do fruto maduro é causada pela presença de carotenóides, particularmente licopeno (vermelho) e caroteno (amarelo). A proporção em que se encontram determina a intensidade de cor dos frutos. A distribuição dos pigmentos é diferente na pele e na polpa e pode ser influenciada pela intensidade e qualidade da luz. Os carotenóides presentes no tomate apresentam importante papel na prevenção de doenças como câncer, catarata, e doenças do coração (AGARWAL; RAO, 2000; SESSO et al., 2003).

Como o licopeno é o principal pigmento do tomate, a degradação deste tem sido objeto de várias investigações. Rodriguez-Amaya et al. (1997), estudaram a degradação do licopeno em solução de hexano ou éter de petróleo e na presença de oxigênio, e demostraram a importância da temperatura neste processo. Perdas de 15 e 25 % foram observadas durante 3 horas a 65 e 100 °C, respectivamente.

1.1.7 Importância Sócio-Econômica do Tomate

A safra mundial de tomates 2007/08 dimensionada pela FAO situou-se em 129,64 milhões de toneladas, produção 2,7% superior à da safra imediatamente anterior e 2,1% maior que a apurada em 2005/06, quando foram obtidas 126,99 milhões de toneladas (ICEPA, 2010)

A produção de tomates na América do Sul, embora difundida em todos os países, tem apresentado pouca evolução nas últimas safras, tanto em relação à área plantada quanto em relação à produção. Segundo a FAO, foram plantados 142.936 hectares em 2009, área 2,7% maior que a cultivada na safra anterior e praticamente idêntica à safra de 2007. Os países de maior área plantada são Brasil, com 67.605 hectares,

Argentina, com 17.369 hectares, Colômbia, com 15.293 hectares e Chile, com 13.000 hectares. Vale ressaltar que o Chile apresentou uma redução de 13,4% na área de plantio em relação à safra 2008 e de 33,4% em relação à safra 2007 (ICEPA, 2011)

A produção nacional foi diretamente afetada por este cenário de depressão na área de cultivo do tomate. As 3.667 mil toneladas apuradas na safra 2010/11 representam retração de 1% do volume produzido na safra 2009 e de 15% sobre a safra de 2008. A Região Sudeste permanece como maior produtora, com 39% da produção nacional, seguida do Centro-Oeste, que representa 28%. O rendimento médio das lavouras brasileiras na safra 2010/11 ficou em 59.327 quilos por hectare, 2,2% menor que a safra anterior e 6,9% inferior ao obtido na safra 2008/09 (ICEPA, 2011)

Para o estado de Santa Catarina cultivo de tomates na safra 2009/10 foi dimensionado pelo IBGE em 2.693 hectares. Comparado à safra anterior, a área encolheu 1,6%; em relação à safra 2007/08, apresenta incremento de 21%. Nacionalmente esta área coloca Santa Catarina como sexto maior plantador. A produção de 186.802 toneladas mostra-se 2,4% maior que a obtida no ano anterior e 58,4% superior ao volume atingido na safra 2007/08. Esse montante quando comparado aos outros estadosda Federação, posicionam Santa Catarina como sétimo maior produtor nacional (ICEPA, 2011).

1.1.8 Qualidade pós-colheita do tomate

A qualidade pós-colheita dos frutos relaciona-se com o conjunto de atributos ou propriedades que, entre outros, os tornam apreciáveis como alimento. De modo abrangente, a qualidade pode ser definida como um conjunto de características que permitem diferenciar um produto de outro e que tem influência na determinação do grau de aceitação pelo consumidor. Dentre estes componentes, devem ser considerados os atributos físicos, sensoriais e a composição química (CHITARRA; CHITARRA, 2005).

Os principais fatores que afetam a qualidade pós-colheita dos frutos incluem o cultivar (KIM, et al. 1993), crescimento na pré-colheita, maturidade do fruto na colheita, estado fisiológico do produto, manuseio durante a pós-colheita e o armazenamento. No periodo pós-colheita os frutos são submetidos a modificações físico-químicas e bioquímicas que afetam principalmente a cor, sabor e textura reduzindo a qualidade do produto (GORNY et al., 1998).

O tomate é um fruto perecível que necessita de alguns cuidados para sua conservação por um período de tempo maior. As alterações fisiológicas que ocorrem no período pós-colheita podem estar relacionadas a fatores como respiração, produção de etileno e crescimento microbiano. Métodos de conservação como: higienização, baixas temperaturas, atmosfera modificada e aplicação de coberturas comestíveis podem ser utilizados com intuito de aumentar a vida de prateleira deste produto.

1.2 Fatores que Afetam Qualidade Pós-Colheita do Produto

1.2.1 Respiração

A respiração é um processo metabólico que fornece energia para os processos bioquímicos da planta. Este processo corresponde às reações oxidativas de compostos orgânicos, que são transformados em água e dióxido de carbono com produção de energia química, utilizada para biossíntese de novos compostos indispensáveis ao perfeito funcionamento e manutenção da planta como um todo (FONSECA et al., 2002).

A produção de energia pela fotossíntese e sua utilização pelo processo respiratório são os eventos primordiais do metabolismo vegetal. Na fase pós-colheita, a fotossíntese torna-se limitada e os órgãos de armazenamento, se maturos, utilizam suas reservas metabólicas para reações de síntese (CHITARRA; CHITARRA, 2005).

O processo respiratório é um bom indicador das taxas metabólicas de vegetais, seu controle pode ser um efetivo meio de regular todo o metabolismo vegetal e estender a pós-colheita destes produtos (MATHOOKO, 1996).

A taxa respiratória depende da temperatura e da composição da atmosfera (O_2, CO_2 e etileno) que envolve o produto (MAHAJAN e GOSWAML, 2001). De acordo com Gürakan e Bayindirh (2005), a atmosfera modificada pode reduzir a atividade respiratória dos frutos armazenados. Isto ocorre pela diminuição da atividade de enzimas envolvidas na respiração, devido à utilização de baixas concentrações de O_2 e altas concentrações de CO_2, fatores que reduzem, em geral, a taxa de utilização de substratos de reserva (MAHAJAN e GOSWAML, 2001). Portanto, frutos armazenados sob refrigeração e em atmosfera modificada ou controlada geralmente apresentam maior vida pós-colheita (PEPPELENBOS, 1996; LIU et al., 2004)

A respiração aeróbica apresenta papel fundamental na manutenção da integridade e funcionamento celular, através do fornecimento de energia SAQUET e STREIF (2000). Segundo KADER, (1986) a diminuição do teor de O_2 disponível para frutas e vegetais reduz a taxa respiratória (produção de CO_2/consumo de O_2), que geralmente requer no mínimo de 1 a 3% de oxigênio, dependendo do produto, para evitar a mudança de respiração aeróbia para anaeróbia. De acordo com Siriphanich e Kader (1986), altas concentrações de CO_2 podem limitar o suprimento de energia necessário para sobrevivência dos tecidos. Assim, quando a respiração aeróbia é reduzida drasticamente, o tecido vegetal aumenta a respiração anaeróbia para aumentar o nível de energia disponível (PEPPELENBOS, 1996).

A glicólise pode funcionar bem sem oxigênio, porém o processo posterior no qual ocorre a redução do piruvato e oxidação do NADH (nicotinamida adenina dinucleotídeo**)** necessita deste gás (PEPPELENBOS, 1996). Taiz e Zeiger (2004) citam que, em situações de deficiência de oxigênio, o piruvato produzido na glicólise é descarboxilado, pela enzima descarboxilase, gerando acetaldeído, e o NADH reduz este acetaldeído em NAD^+ e etanol. Segundo Mathooro (1996) durante a fermentação, principalmente em períodos prolongados de deficiência de O_2, pode ocorrer o acúmulo de etanol, acetaldeído e lactato (MATHOOKO, 1996; TAIZ e ZEIGER, 2004), os quais podem favorecer o desenvolvimento de distúrbios fisiológicos e a formação de sabor e aroma alcoólico (WATKINS et al., 1997), o que muitas vezes impossibilita a comercialização do produto.

Nos frutos, a atividade respiratória é influenciada, pelo menos em parte, pela sua composição, quando completamente formados e, pelas alterações químicas que ocorrem durante a fase da maturação. As substâncias que possivelmente tomam parte ativa nessas alterações são proteínas, glicídeos, lipídeos, ácidos orgânicos, vitaminas, minerais e alguns componentes da parede celular, como hemiceluloses e pectinas. A respiração resulta em modificações profundas desses constituintes, que podem ser altamente indesejáveis sob ponto de vista da qualidade (CHITARRA; CHITARRA, 2005).

Injúrias mecânicas podem estimular o aumento da taxa respiratória, induzir a síntese do etileno, oxidação de compostos fenólicos, aumentar a atividade enzimática e o desenvolvimento microbiológico acelerando assim a perda de qualidade especialmente de atributos como cor e firmeza. Portanto, o controle da respiração é

condição essencial para manutenção da qualidade e para o prolongamento da vida pós-colheita dos produtos horticolas (ROCHA; MORAIS, 2003).

Krammes et al. (2003), avaliando a taxa de produção de CO_2 de tomates da cultivar Santa Clara, acondicionados em jarras suprimidas com ar durante 18 dias, verificaram redução da taxa respiratória com o tempo de armazenamento.

Rocculi et al. (2006) estudando o consumo de oxigênio de maçãs armazenadas por 4 dias a temperatura de 4°C, verificaram que o uso de atmosfera modificada ativa reduziu o consumo de oxigênio quando comparado com atmosfera modificada passiva.

Pesquisas realizadas com cenoura *baby* mostraram que cenouras armazenadas em atmosfera com menor concentração de O_2 apresentaram menores taxas respiratórias, alterações no conteúdo de vitamina C e carotenóides (SIMÕES et al., 2011).

O uso de coberturas comestíveis também pode reduzir a taxa respiratória de vegetais. Segundo Hernandez-Munoz et al., (2006) atualmente, tem se dispensado grande atenção ao potencial de aplicação de polímeros naturais, tais como proteínas e polissacarídeos, como coberturas de frutos e hortaliças, com o objetivo de reduzir as taxas de respiração e transpiração. Isso decorre do alto coeficiente de permeabilidade seletiva (CO_2/O_2) conferido por tais substâncias e ao incremento das propriedades mecânicas, de forma a auxiliar na manutenção da integridade estrutural do tecido vegetal.

Segundo Lee et al. (2003) a atividade respiratória de maçãs minimamente processadas da variedade Fuji, tratadas com coberturas comestíveis a base de proteína concentrada do soro do leite, glicerol e cloreto de cálcio, armazenadas 4ºC durante duas semanas, apresentou uma redução de 20%.

1.2.2 Produção de Etileno

O etileno é um hidrocarboneto (C_2H_4), que atua como fitormônio, desempenhando um papel importante na regulação do processo deteriorativo intrínseco da planta. Ele controla muitos estádios do desenvolvimento, tais como, maturação de frutos climatéricos, senescência de folhas e flores. Sua síntese autocatalítica é fortemente estimulada por fatores exógenos, como infecções fúngicas e/ou bacterianas, injúrias mecânicas, estresses hídrico, térmico e salino, e

também por outros fitormônios (THEOLOGIS et al., 1992; BOUZAYEN et al.,1997).

A via de biossíntese do etileno foi descrita por Yang e Hoffman (1984). O aminoácido metionina é o precursor biológico do etileno em todas as plantas superiores, e é convertido em etileno pela via de biossíntese que compreende dois passos com reações enzimáticas. Na primeira reação, o S-adenosil-metionina (SAM) é convertido em ácido 1-carboxílico-1-aminociclopropano (ACC) pela ação da enzima ACC sintetase (ACCS). O ACC é então metabolizado pela enzima ACC oxidase (ACCO), por uma reação de oxidação que necessita de O_2 e ferro, e que é ativada pelo CO_2 para produzir etileno (THEOLOGIS et al., 1992; GRIERSON, 1998)

Os frutos são classificados em dois grupos: climatéricos e não-climatéricos. No processo de maturação dos frutos climatéricos, ocorre um aumento significativo na taxa respiratória e na produção de etileno (KADER et al., 1989). Em produtos climatéricos, tais como a maçã, o pêssego, a pera, o melão, o tomate, e o kiwi, o processo de maturação/senescência é acompanhado de um incremento da síntese de etileno e da intensidade respiratória (SLATER et al., 1985).

Em frutos climatéricos como o tomate, a senescência e o armazenamento podem ser governadas por mutantes de amadurecimento que controlam a síntese do etileno, o desenvolvimento da cor, a perda de firmeza e vários outros padrões fisiológicos (FIGUEIRAS, 1996).

A utilização de atmosfera modificada com concentrações de O_2 em torno de 2,5% pode reduzir a produção de etileno pela metade, retardando o amadurecimento dos frutos (ESCALONA et al., 2006). O uso de coberturas comestíveis também pode reduzir a produção de etileno em vegetais. Fontes et al. (2008) verificaram redução da taxa de produção de etileno de maçãs minimamente processadas com coberturas comestíveis a base de alginato e fécula de mandioca, armazenadas durante 12 dias a 2°C.

1.2.3 Alterações nos Atributos Físicos de Qualidade

1.2.3.1 Textura

A textura encontra-se entre os mais importantes atributos da qualidade de frutas e vegetais. E pode ser definida como um grupo de características físicas que surge dos elementos estruturais dos alimentos. A textura é percebida pelo sentido do tato e relacionada com a

deformação, desintegração e fluxo do alimento submetido à determinada força, que podem ser medidas objetivamente por funções de massa, tempo e distância (RIZVI; TONG, 1997; VU et al., 2004).

A aceitação de vegetais depende de inúmeros fatores, incluindo aparência, textura, sabor e valor nutricional. Vegetais que mantêm a firmeza e a crocância são altamente desejáveis porque os consumidores associam esses atributos de textura ao frescor do vegetal *in natura* (FILLION; KILCAST, 2002; NI et al., 2005).

Os componentes pécticos estão diretamente envolvidos na fase de amadurecimento, e, por conseguinte, no processo de amaciamento dos frutos. Substâncias pécticas são macromoléculas glicosídicas de alta massa molecular que formam o maior componente da lamela média (ALKORTA et al.,1998; ALMEIDA et al., 2005) . Segundo Vu et al. (2004), alterações na textura de frutas e vegetais durante o processamento podem estar relacionadas com mudanças enzimáticas e não-enzimáticas da pectina. (ASSIS et al., 2000; REN; KERMONDE, 2000).

Os métodos de conservação como atmosfera modificada e coberturas comestíveis, podem auxiliar na manutenção da textura durante o armazenamento. As alterações nos atributos de qualidade são diretamente influenciadas pela composição gasosa em que o produto é submetido. No entanto, poucos estudos quantificam os efeitos da atmosfera modificada no amolecimento dos frutos (HERTOG, 2001). Segundo SOUZA et al. (2002) a atmosfera modificada obtida através do uso de filmes de polietileno reduziu as perdas na firmeza da polpa de Manga Tomy, armazenadas a temperatura de 11°C, possibilitando uma vida útil pós-colheita de 42 dias. Ali et al. (2010) verificou manutenção da textura de tomates recobertos com goma arábica, e armazenados durante 20 dias a temperatura de 20°C.

1.2.3.2 Perda de Massa

A perda de água pode ser uma das principais causas de deterioração de vegetais, já que resulta em perdas quantitativas, perdas na aparência (murchamento), na textura (amolecimento) e na qualidade nutricional (KADER, 1986). O processo de respiração está associado ao da transpiração, principal fator responsável pela perda de peso de vegetais. Estes dois processos são considerados vitais para as frutas e hortaliças. A perda de peso, associada diretamente a perda de água é prejudicial principalmente nos casos em que é suficientemente alta para

afetar a aparência e a aceitabilidade do produto. Os produtos perecíveis, mesmo quando colocados em condições ideais, sofrem alguma perda de peso durante o armazenamento devido ao efeito combinado da respiração e da transpiração (SONG et al., 2002; CHITARRA ; CHITARRA, 2005;).

A redução da temperatura permite que a pressão de vapor da água presente nos tecidos diminua evitando sua evaporação, consequentemente murchamento, enrugamento e perda de turgescência, impedindo assim, a perda da qualidade sensorial do produto (CHITARRA; CHITARRA, 2005).

O uso de técnicas de conservação como atmosfera modificada e coberturas comestíveis, pode auxiliar na redução da perda de umidade do produto. O envolvimento de frutos em coberturas comestíveis vem sendo amplamente utilizada na preservação da qualidade de vegetais, contribuindo de forma significativa para o decréscimo de perdas pós-colheita, através da redução da atividade metabólica e da perda de água, melhorando seu aspecto comercial, oque reflete no aumento do período de comercialização (VILA, 2004).

Segundo Kader (1986), a perda de água pelos vegetais pode ser minimizada pela atmosfera modificada ou controlada, devido à elevada umidade relativa propiciada por esta técnica. Rocculi et al. (2004) avaliando maçãs armazenadas em 4 diferentes atmosferas, não verificaram perda de massa notável em nenhuma das amostras, segundo o autor, durante a estocagem ocorreu pequena perda de água devido ao processo de transpiração e respiração.

1.2.3.3 Cor

A mudança de cor ocorre durante a maturação de muitos frutos, e compõe um dos critérios mais importantes utilizado pelo consumidor para julgar sua maturidade. A mudança mais comum consiste no desaparecimento da cor verde, seguido do aparecimento de várias cores que variam do amarelo ao vermelho (AWAD, 1993).

Essas alterações na coloração do produto são freqüentemente acompanhadas de mudanças indesejáveis na aparência e nas propriedades sensoriais do produto, ocasionando a diminuição da vida de prateleira e do valor de mercado (ARAÚJO, 1995).

A cor vermelha é o atributo de qualidade mais visível e importante de alguns frutos maduros, para consumo fresco e processado. Para o mercado de tomates frescos, a cor do fruto tem efeito

significativo em sua comercialização. Essa coloração é o resultado da combinação de pigmentos carotenóides, entre os quais o licopeno é o mais abundante, seguido de carotenos e xantofilas (LÓPEZ et al., 2001). Em tomates, há uma intensa degradação de clorofila durante o amadurecimento, com síntese gradual de licopeno que além de exercer a função de ingrediente colorante, tem mostrado potencial na redução de risco de doenças, sendo um eficiente antioxidante (BARRET; ANTHON, 2001; LÓPEZ et al., 2001).

O uso de atmosfera modificada em vegetais pode contribuir no atraso do desenvolvimento da cor. Hobson (1980) observou redução do amadurecimento e aumento da vida pós-colheita em tomates acondicionados em filmes de polietileno sob atmosfera modificada Ali et al. (2004), em estudo realizado com tomates cereja sob atmosfera modificada passiva, armazenados durante 15 dias a 15 °C, verificaram que o O_2 é o principal componente reponsável pela alteração da cor, sendo que o CO_2 não teve efeito sobre o desenvolvimento da coloração.

Injúrias mecânicas pós-colheita permitem o acesso do O_2 aos tecidos do vegetal e o contato da enzima polifenoloxidases (PPO) com o substrato. A principal consequência é a formação de melaninas, pigmentos escuros que prejudicam a aceitação destes vegetais. Como o O_2 é requerido para iniciar a reação, a utilização de filmes comestíveis pode ser útil para reduzir as taxas de escurecimento (MARTINEZ; WHITAKER, 1995). Perez-Gago et al. (2006) verificaram redução no escurecimento de maçãs tratadas com coberturas compostas por concentrado proteico de soro e ácido ascórbico, armazenadas durante 12 dias, na temperatura de 5 °C.

1.2.4 Alterações nos Atributos Químicos de Qualidade

1.2.4.1 pH e SST

A composição química dos alimentos varia naturalmente, devido ao grau de maturação e também aos fatores ambientais (MERCADANTE et al.,1997). Indicadores de qualidade, tais como cor, pH, acidez titulável e teor de sólidos solúveis, são empregados para avaliar a qualidade dos alimentos, no período pós-colheita.

O tomate apresenta as principais características nutricionais da maioria dos vegetais de sua classe: possui baixo valor calórico e gorduras, sendo composto basicamente de água, de açúcares e de ácidos. Por ser um fruto climatérico, a taxa de respiração do tomate se eleva no

início do amadurecimento, resultando em uma série de transformações físico-químicas (KLUGE; MINAMI, 1997), caracterizadas por alterações fisiológicas e bioquímicas no fruto (FACHIN, 2003).

O teor de sólidos totais (°Brix) é usado como indicador de açúcares solúveis totais em frutas e indica o seu grau de amadurecimento (CECCHI, 1999). Em condições controladas, o sistema de produção influencia os teores de sólidos totais, sendo 4,7 °Brix em sistema orgânico, enquanto esse teor é de 4,2 °Brix para tomates cultivados em sistema convencional, respectivamente (FERREIRA, 2004; BORGUINI; SILVA, 2007).

Acidez titulável total é um importante parâmetro na apreciação do estado de conservação de um produto alimentício, já que, na maioria das vezes, a decomposição do alimento quase sempre altera a concentração de íons de hidrogênio (IAL, 1985), além de influenciar as características organolépticas dos alimentos. O seu teor nos frutos pode variar em função do grau de maturação e das condições de crescimento (CECCHI, 1999), podendo variar de 0,33% a 0,41% no tomate cultivado em sistemas de produção orgânico e convencional (RESENDE et al., 1997; FERREIRA, 2004).

1.2.4.2 Sabor e Aroma

A qualidade do tomate baseia-se, entre outros aspectos, nas suas características físico-químicas que tornam o produto um vegetal bastante consumido, além de sua qualidade nutricional, caracterizada por vários compostos que podem auxiliar na manutenção da saúde (ANZA et al., 2006).

O sabor e o aroma são apreciados em conjunto e essas características correlacionam-se e são consideradas como atributo de qualidade único. O amadurecimento de frutas, em geral conduz a um aumento na doçura devido ao aumento no teor de açúcares, e ao decréscimo da acidez pela redução nos teores de compostos ácidos e fenólicos e aumento nas características do sabor e aroma principalmente pela emanação dos compostos voláteis. Em frutas climatéricas, o pico da evolução dos componentes coincide grosseiramente com o pico da atividade respiratória (CHITARRA e CHITARRA, 2005)

O sabor do tomate é geralmente determinado pelo conteúdo de sólidos solúveis, ácidos e presença de vários compostos voláteis. Segundo estudo de Jones e Scott (1984), a maior contribuição para o sabor e consequente aceitabilidade, é dada pelos valores totais de

açúcares e ácidos encontrados nos frutos. O sabor do tomate melhora concomitantemente com a acidez e os açúcares presentes no fruto, e também pode ser afetado por outros fatores, como a variedade, o cultivo, a maturação e o armazenamento (OEY et al., 2008).

O aroma é o conjunto das sensações do olfato, estimuladas pelos componentes voláteis que em conjunto, conferem características especificas a cada produto. O aroma característico de determinado alimento resulta da interação de vários compostos voláteis (CHITARA e CHITARRA, 2005). Assim, a qualidade do produto torna-se comprometida se houver oxidação de compostos de aroma ou perdas por migração através da embalagem. Portanto, o uso de flmes ou películas comestíveis com boas propriedades de barreira ao oxigênio e aromas pode aumentar a estabilidade sensorial do alimento.

O conteúdo de voláteis é um dos atributos de qualidade mais importante do tomate fresco, juntamente com a cor, odor e textura. Mais de 400 compostos voláteis foram identificados em frutos de tomate, estes compostos voláteis incluem, por exemplo, aldeídos diferentes, cetonas, álcoois, furanos e terpenos (KAARINA et al., 2011). Baldwin et al. (1998) sugeriram que 15 a 20 diferentes compostos voláteis presentes em tomate têm impacto sobre a percepção humana.

Métodos de conservação como atmosfera modificada e a aplicação de coberturas comestíveis são alternativas para redução nas alterações de compostos químicos que compõem o tomate cereja. Artés et al. (1999) verificaram alterações não significativas nos conteúdos de sólidos solúveis totais, pH e acidez titulável de tomates inteiros e minimamente processados armazenados sob atmosfera modificada passiva e ativa (7,5 % O_2 e 0 % CO_2), durante 10 dias nas temperaturas de 2 e 10 °C. Estes autores observaram que o uso destas atmosferas manteve o aroma dos frutos durante o período de avaliação.

1.2.4.3 Antioxidantes

A constatação de que os vegetais possuem substâncias biologicamente ativas que trazem benefícios à saúde ou efeitos fisiológicos desejáveis, tem impulsionado estudos sobre a sua propriedade antioxidante, e que a eficácia depende da estrutura química e da concentração desses fitoquímicos no alimento (FRANKEL, 1993; MADSEN; BERTELSEN, 1995).

Os antioxidantes podem ser definidos como qualquer substância, que, presente em baixas concentrações, quando comparada a

um substrato oxidável, atrasa ou inibe a oxidação desse substrato de maneira eficaz (SIES; STAHL, 1995; AUST et al., 2001; HANDELMAN, 2001). O teor desses compostos em vegetais é amplamente influenciado por fatores genéticos, por condições ambientais, além do grau de maturação e variedade da planta, entre outros. Constata-se, ainda, que a atividade antioxidante é influenciada pelo substrato lipídico utilizado no ensaio, pelo solvente e pela técnica de extração empregada (FRANKEL, 1993, MADSEN; BERTELSEN, 1995). No que concerne aos solventes orgânicos, o metanol, por conseguir extrair elevada quantidade de compostos bioativos, tem sido considerado como o mais efetivo (ECONOMOU et al., 1991).

Os carotenóides, compostos antioxidantes, são corantes naturais presentes nos vegetais (cenouras, tomates, espinafre, laranjas, pêssegos, entre outros), sendo que a sua estrutura química é composta por ligações duplas conjugadas, que são responsáveis por sua cor e por algumas de suas funções biológicas (STAHL; SIES, 1999). Constituem cerca de 700 compostos lipossolúveis encontrados nas plantas, responsáveis pelas cores das folhas e dos frutos. Dentre esses, aqueles mais abundantes nas plantas e também presentes no plasma sanguíneo são: α- caroteno, β-caroteno, β-criptoxantina, luteína, licopeno e zeaxantina (RODRIGUEZ-AMAYA, 2001).

Resultados de estudos têm permitido estabelecer relação entre o aumento no consumo de alimentos ricos em carotenóides com a diminuição no risco de várias doenças (GIOVANNUCCI, 1999; TAPIERO et al., 2004). Isso porque os carotenóides, em função de sua estrutura altamente insaturada, tornam sequestradores de oxigênio e dos radicais peróxidos, além de modularem o metabolismo carcinogênico, inibirem a proliferação celular, estimularem a comunicação entre células e elevarem a resposta imune no organismo (DI MASCIO et al., 1989; SIES; STAHL, 1998; OLSON, 1999).

O tomate apresenta carotenóides, como licopeno e β-caroteno em quantidades nutricionalmente significativas, em média 2573 e 449 μg / 100 g de fruto, respectivamente (GOULD, 1992). Esse teor varia conforme o tipo e o grau de amadurecimento dos frutos. Segundo Giovannucci (1999), o tomate vermelho maduro contém maior quantidade de licopeno do que β-caroteno, sendo responsável pela cor vermelha, predominante nos frutos. As cores das espécies de tomate diferem do amarelo para o vermelho alaranjado, dependendo da razão licopeno/ β-caroteno da fruta, que também está associada com a

presença da enzima beta-ciclase, a qual participa da transformação do licopeno em β-caroteno.

Estudos recentes evidenciam que esses compostos podem reduzir significativamente o risco de desenvolvimento de câncer, como o de próstata, de garganta, de pulmão e de intestino e doenças cardiovasculares (GIOVANNUCCI, 1999; TAPIERO et al., 2004). Entretanto, diferentes tipos de fertilizantes influenciam nos principais componentes antioxidantes de tomates, podendo ter um efeito negativo sobre a concentração desses compostos (TOOR et al., 2006), embora os autores afirmem que são necessários estudos em escala comercial, para que seja possível a confirmação de tais resultados.

1.2.5 Crescimento Microbiano

A qualidade microbiológica de alimentos processados está relacionada com a presença de microrganismos deteriorantes e patogênicos que irão interferir nas características sensoriais do produto, tais como cor, odor, textura e aparência durante o período de vida útil (VANETTI, 2004). Alguns dados registram que, simultaneamente ao aumento do consumo de frutas e hortaliças frescas é observada também uma tendência de aumento do envolvimento desses produtos em surtos de infecções alimentares (BEUCHAT, 2002).

A matériaprima de origem vegetal está sujeita às diversas fontes de contaminação microbiana ao longo do seu cultivo e processamento, como água de irrigação, solo, equipamentos, manipuladores, utensílios e água de lavagem. Assim, o processamento de vegetais exige a implementação de um sistema de garantia de qualidade por unidades que processam esse tipo de produto (CRUZ et al., 2006).

Para Alzamora et al. (2000), as condições do processo de higienização e conservação, seja de forma isolada ou combinada, podem permitir a contaminação, a sobrevivência e/ou a multiplicação de microrganismos, inclusive dos patogênicos. Para os vegetais frescos em geral, a contaminação por microrganismos patogênicos pode ocorrer em diferentes fases, desde a sua produção até o consumo. Dentre os microrganismos encontrados em vegetais, podem ser destacados os bolores e leveduras, coliformes totais e psicrotróficos (NEGUYEN; CARLIN, 1994).

Os fungos particularmente leveduras, fazem parte da microbiota natural de frutas, sendo detectados com frequência em vegetais minimamente processados e, se presentes em grande quantidade, podem

provocar alterações nos produtos embalados, como a fermentação, que altera as características sensoriais dos mesmos (BEUCHAT, 2002).

É fundamental a análise de amostras de vegetais para coliformes, pois esse microrganismo pode contaminar o produto tanto no plantio e colheita quanto no processamento (ORDÓÑEZ, 2005). As bactérias do grupo coliforme são bastante comuns, pois se originam do próprio solo de cultivo, porém, as linhagens de *Escherichia coli* não devem fazer parte da microbiota normal, se os produtos forem cultivados em solo livre de contaminação fecal, irrigados com água de boa qualidade e manipulados sob condições de boas práticas. Sob esse aspecto, essas bactérias são indicadoras da qualidade higiênico sanitária dos produtos. A presença de patógenos como *Salmonella, Shigella, Y. enterocolitica, E. coli* enteropatogênica, enterotoxigênica ou enterohemorrágica em frutas e hortaliças pode levar a ocorrência de surtos de infecção alimentar (BEUCHAT, 2002).

Os microrganismos psicrotróficos têm a temperatura ótima de crescimento entre 25 e 35 °C, mas podem multiplicar-se também a 5 °C ou temperaturas inferiores. Ou seja, eles são predominantes e os principais causadores de alterações nos alimentos refrigerados (ORDÓÑEZ, 2005).

Muitos estudos têm sido realizados para verificar a sobrevivência de microrganismos, como por exemplo, a *Salmonella*, em produtos frescos durante o período pré-colheita (GUO et al., 2002), estas pesquisas verificam a capacidade de microrganismos sobreviverem e se desenvolverem em produtos com baixo pH, como tomate (TASSOU e BOZIARIS, 2002).

A Resolução Brasileira -RDC nº12, de 2 de janeiro de 2001, para frutas frescas, "*in natura*", preparadas (descascadas, selecionadas ou fracionadas) sanificadas, refrigeradas ou congeladas, para consumo direto, determina que os valores de coliformes não devem ultrapassar $5x10^2$ UFC/g, e estabelece que deve ocorrer ausência de Salmonela em 25 g de amostra para este tipo de produto.

Estudos com vegetais frescos devem abranger realização de algumas análises microbiológicas, tais como: contagem de Coliformes, Psicrotróficos, Salmonella e fungos para verificar se o método de conservação aplicado reduz o crescimento microbiano e garante a qualidade higiênico-sanitária destes produtos.

1.3 Métodos de sanitização: Hipoclorito de sódio x ozônio

A sanitização é de extrema importância para a segurança dos produtos vegetais, uma vez que deve promover a redução a níveis aceitáveis pela legislação ou inativação dos microrganismos patogênicos. Pode ser realizada por métodos químicos ou físicos. Na sanitização por métodos físicos emprega-se calor (vapor, água quente), e radiação ultravioleta, enquanto que na sanitização por meio de métodos químicos são utilizados agentes químicos como hipoclorito de sódio, dióxido de cloro, ácido peracético, ozônio entre outros (OLMEZ; KRETSCHMAR, 2009).

Pesquisas têm discutido a eficiência das técnicas de higiene aplicadas em frutas e vegetais que utilizam substâncias como cloro, peróxidos e ozônio (PIROVANI et al., 2000; WEISSINGER, 2000). Alguns testes têm sido realizados sobre a aplicação de ozônio em vegetais como: maçãs, alface, cenoura para avaliar seu efeito em bolores, leveduras e bactérias (HAN et al., 2002).

1.3.1 Hipoclorito de Sódio

Os compostos clorados têm sido utilizados como sanitizantes no processamento de alimentos por várias décadas, assim como na desinfecção de produtos e superfícies nas empresas de processamento e na redução da população microbiana da água utilizada durante as operações de higienização e embalagem (PARISH et al., 2001). Nas suas diversas formas químicas, o cloro é o agente sanitizante adotado em maior escala, sendo um germicida de amplo espectro de ação e muito utilizado no Brasil (VANETTI, 2004). Porém, nos últimos anos, tem aumentado a preocupação quanto à produção de compostos orgânicos clorados e seus impactos sobre a saúde humana. Ainda assim, em virtude de sua conveniência e baixo custo, os compostos clorados continuam sendo utilizados nas indústrias processadoras de alimentos, nos serviços de alimentação e a nível doméstico (PARISH et al., 2001).

A concentração de cloro na água para sanitizar frutas e hortaliças frescas e minimamente processadas em escala comercial está na faixa de 50 mg / L a 200 mg / L. Estudos relatam que concentrações de cloro livre de 50 a 200 mg / L podem inativar células vegetativas de bactérias e fungos (SIMONS; SANGUANSRI, 1997). Entretanto, a atividade do cloro depende de condições tais como carga inicial de microrganismos, pH da água, tipo de produto, presença de matéria orgânica e concentração da forma ativa (BASTOS, 2006).

Todavia, concentrações elevadas de cloro podem causar problemas como descoloração, perda de qualidade e aumento na corrosão de equipamentos. Outro ponto importante diz respeito à formação de trihalometanos e cloraminas que ocorrem pela combinação de cloro com a matéria orgânica e que é bastante prejudicial para a saúde humana pelo seu potencial carcinogênico (KIM et al., 1999; VANETTI, 2004).

Tendo em vista os efeitos prejudiciais que os compostos clorados podem trazer para a saúde humana, novas tecnologias para higienizar e estender a vida pós-colheita de frutas e hortaliças tem sido aplicadas. Entre elas, destaca-se a água ozonizada que se mostra eficiente em alguns estudos realizados e, ao contrário do hipoclorito de sódio não produzem subprodutos prejudiciais à saúde (LAMIKANRA et al., 2005).

Fantuzzi et al. (2004), avaliando a microbiota bacteriana de repolho minimamente processado após etapa de sanitização com três sanitizantes (solução de hipoclorito de sódio a 200 mg/L, solução comercial à base de composto orgânico clorado para verduras e frutas na concentração de 0,66% e solução de ácido acético 1 %) por 10 minutos e estocagem sob refrigeração a 1 °C e 5 °C e sob abuso de temperatura (12 °C), observaram que a sanitização com hipoclorito de sódio reduziu em até 1,8 ciclos logarítmicos a população de microrganismos aeróbios mesófilos.

1.3.2 Ozônio

A indústria alimentícia está pesquisando desinfetantes que sejam efetivos contra patógenos e sejam seguros para o uso em alimentos. Um dos candidatos é o ozônio que está sendo utilizado como sanitizante no tratamento de águas na Europa desde o início do século XX (KIM et al., 1999).

Em 1785, Van Marum, filósofo alemão, observou as características eletrostáticas do ar devido ao ozônio. Schombein, em 1801, reportou o odor característico como sendo uma nova substância, de nome ozônio, e sugeriu que o gás ocorreria naturalmente na atmosfera. Na Alemanha, em 1875, Siemens criou o primeiro gerador de ozônio – ozonizador. O primeiro experimento utilizando ozônio no tratamento da água foi realizado em 1893 em Leyde, na Holanda, no tratamento das águas do rio Reno. Em 1906, em Nice, na França,

realizou-se o primeiro tratamento de vegetais com água ozonizada, em escala industrial (YANG; CHEN, 1979).

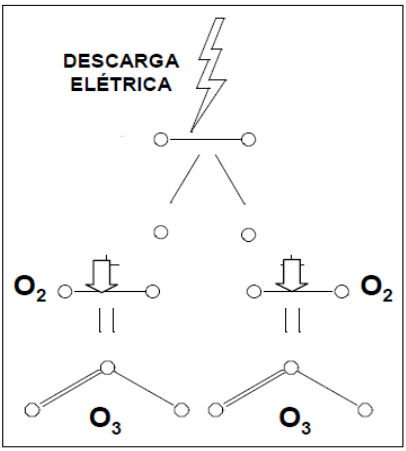
O ozônio é um sanitizante alternativo ao cloro e tem se mostrado muito eficaz na inativação de bactérias, vírus e cistos de *Giardia* e *Criptosporidium*, ambos protozoários resistentes ao cloro (CAVALCANTE, 2007). Além de não deixar resíduos no alimento, por se decompor rapidamente em oxigênio molecular atóxico, o ozônio usado em baixas concentrações e durante pouco tempo de contato com o produto, pode ter eficiência semelhante ou melhor que o hipoclorito de sódio na redução da contaminação microbiológica em operações de sanitização (PRESTES, 2007).

1.3.2.1 Geração do ozônio

A formação do ozônio ocorre naturalmente na estratosfera em pequenas quantidades (0,005 mg / L) pela ação da irradiação solar ultravioleta no oxigênio (KIM e YOUSEF, 2000). Quando utilizado na indústria é geralmente gerado em sistemas fechados, sendo produzido em baixas concentrações pelo oxigênio atmosférico ou pela radiação de 185 nm de comprimento de onda emitida por lâmpadas UV (KIM et al., 1999). Porém o método de descarga eletroquímica, conhecido como efeito corona, é o mais utilizado, pois gera uma quantidade maior de ozônio com menor custo.

Guzel-seydim et al. (2004) explicam que para gerar ozônio, pelo método de descarga corona, é necessário que ocorra uma ruptura da molécula do oxigênio diatômico, formando dois fragmentos de oxigênio que podem reagir com outras moléculas também de oxigênio e formar a molécula de O_3. No método de descarga corona há dois eletrodos, um de alta e outro de baixa tensão, formando um vão entre eles. Segundo Kim et al. (1999), uma corrente alternada com alta voltagem é aplicada através deste vão, na presença de ar atmosférico ou oxigênio, ocorre a excitação dos elétrons de oxigênio induzindo a quebra das moléculas de O_2. Os átomos quebrados combinam-se com outras moléculas de oxigênio diatômico e formam o ozônio. A produção de ozônio varia, dependendo da voltagem, frequência da corrente, do vão de descarga elétrica e da pressão absoluta no interior do vão. A Figura 1.2 apresenta um esquema da produção de ozônio pelo método de descarga corona.

Figura 1. 3: Produção de O_3 pelo método de descarga corona



1.3.2.2 Efeitos antimicrobianos do ozônio

A inativação de bactérias pelo ozônio é um processo complexo, pois o ozônio ataca vários constituintes celulares como proteínas, lipídios insaturados e enzimas da membrana celular, peptoglicanas da parede celular, enzimas e ácidos nucléicos do citoplasma; além de proteínas e peptoglicanas da capa dos esporos bacterianos e capsídeos virais (KHADRE et al., 2001)

Dessa maneira, o que basicamente diferencia o ozônio de outros agentes desinfetantes é seu mecanismo de destruição dos microrganismos. O cloro, especificamente, atua por difusão através da parede celular, agindo sobre os elementos vitais localizados no interior da célula, como enzimas, proteínas, DNA e RNA. O ozônio, por apresentar uma capacidade de oxidação superior, age diretamente na parede da célula, causando sua ruptura e morte em menor tempo de contato, inviabilizando a recuperação dos microrganismos após o ataque (PEZZI, 2009)

O ozônio é um dos mais fortes agentes oxidantes comumente disponíveis. É instável à pressão e temperatura ambiente com uma meia vida de 15 minutos e é decomposto a O_2 a temperaturas superiores a 35 °C (ADASKAVEG et al., 2002).

Segundo Zhang et al. (2006), o tratamento com água ozonizada (4,3 µg / L por 1 minuto) em morangos armazenados sob atmosfera modificada ativa (2,5% O_2 e 10% CO_2) à temperatura de 4 °C prolongou a vida útil deste produto por de 8 dias. Estudos realizados por

Cavalcante (2007), com alface americana inoculada com *E. coli* O157:H7 e esporos de *Bacillus subtilis*, demonstraram que 1,0 mg / L de água ozonizada por minuto, na ausência de matéria orgânica, reduziu no mínimo 6,57 e 5,27 ciclos log destes microrganismos, respectivamente.

1.4 Métodos de Conservação para Manutenção da Qualidade de Tomate

1.4.1 Atmosfera modificada (AM)

As tecnologias disponíveis para o transporte e armazenamento de vegetais são diversas. Dentre os métodos disponíveis, aqueles com maior capacidade de conservação e utilização comercial são o armazenamento em atmosfera controlada ou atmosfera modificada (GÜRAKAN e BAYINDIRH, 2005). Segundo Brackmann et al. (2004) a atmosfera modificada é uma alternativa que visa incrementar o efeito do frio no armazenamento de frutos.

O uso da atmosfera artificial teve início com os egípcios, que já armazenavam alimentos em recipientes hermeticamente fechados. Com os frutos, os primeiros experimentos foram realizados na França, em 1821, por Jacquet Beard, mas, o grande avanço tecnológico da atmosfera controlada deve-se a Kidd e West, que iniciaram seus estudos em 1918, na Inglaterra (BRACKAMNN 2002). Essa técnica tem sido aplicada com considerado sucesso na Europa, desde a metade do século, e nos Estados Unidos vem ganhando espaço desde 1980 (SOUZA et al., 2001).

O armazenamento em atmosfera controlada (AC) consiste no prolongamento da vida pós-colheita de produtos, por meio da modificação e controle dos níveis dos gases durante o armazenamento. Já a atmosfera modificada trata do envolvimento do produto em uma embalagem polimérica, a qual é posteriormente fechada para que ocorra a modificação das pressões parciais dos gases em seu interior (THOMPSOM, 2002). Esta modificação da atmosfera ocorre devido ao balanço entre o consumo de O_2 e a liberação de CO_2, ambos decorrentes do processo respiratório dos frutos, e a permeabilidade do filme polimérico a estes gases. Assim, a intensidade da modificação da atmosfera depende da atividade respiratória do produto armazenado e da permeabilidade do filme polimérico (FONSECA et al., 2002)

A atmosfera modificada utilizada em alimentos pode ser passiva, utilizando a propriedade de permeabilidade do material da embalagem,

ou ativa onde se emprega uma mistura de gases específicos juntamente com o material permeável da embalagem. O objetivo de ambas as atmosferas é balancear o gás dentro da embalagem, onde a atividade respiratória do produto deve ser a menor possível. Em geral a composição dentro da embalagem deve estar em torno de 2-5% O_2, 2-5% CO_2 (ALZAMORA et al., 2000). Moleyar e Narasimham (1994) estudaram o comportamento de tomates armazenados em temperaturas de 10 a 15 °C, e verificaram que as condições ótimas para o armazenamento deste vegetal variam de 3 a 5 % de O_2 e CO_2.

A dificuldade no desenvolvimento de novos produtos utilizando vegetais frescos, em contraste com outros tipos de alimentos, está no fato de frutas e hortaliças continuarem seus processos fisiológicos, consumindo oxigênio e liberando dióxido de carbono e vapor de água depois de embaladas. Embalagens com atmosfera modificada têm sido desenvolvidas nas últimas décadas como uma técnica para reduzir esses processos fisiológicos e manter a qualidade de vegetais, este método de conservação é aplicado com sucesso no aumento de vida de prateleira de alguns produtos (SCIFÒ et al., 2009; SANDHYA, 2010). No entanto, apesar de ser utilizada com bons resultados para alguns vegetais, a adoção desta técnica restringe-se a um número limitado de frutos (KADER; WARTINS, 2000). Segundo estes autores, a falta e informações sobre a taxa respiratória de frutos em condições de atmosfera modificada e sobre a permeabilidade de filmes constituem alguns dos fatores responsáveis pelo uso limitado desta técnica. Muitas vezes o produto é embalado em filmes de permeabilidade insuficiente resultando em desenvolvimento de reações de fermentação indesejáveis (JACXSENS et al., 2000).

Existem muitos fatores que afetam a atmosfera modificada de produtos frescos, entre eles a difusão de gases e a produção de etileno do produto. O movimento de gases (O2, CO2 e C2H4) nos tecidos é realizado pela difusão das moléculas sob um gradiente de concentração. Vegetais têm diferentes quantidades de vazios internos com ar (batatas 1-2%, tomate 15-20%, maçãs 25-30%). Uma quantidade limitada de espaços vazios leva ao aumento da resistência à difusão gasosa (SANDHYA, 2010). O etileno (C2H4) é um hormônio vegetal natural e desempenha um papel central no amadurecimento dos vegetais. A produção de C2H4 é reduzida pela metade em atmosferas com níveis de O2 em torno de 2,5%. Esta redução da concentração de O2 retarda o

amadurecimento, inibindo tanto a produção quanto a ação do C2H4. O etileno está diretamente relacionado ao desenvolvimento da coloração em vegetais. Segundo Ali et al. (2004), o uso de atmosfera modificada com baixas concentrações de O2, atrasaram o desenvolvimento da coloração de tomate cereja armazenado a 15 °C durante 15 dias.

Para que o uso de atmosfera modificada seja eficiente, é necessário o monitoramento de alguns parâmetros, tais como: análises da composição gasosa no interior da embalagem, físico-químicas e microbiológicas durante a vida útil do produto (SOUZA et al., 2001).

A idéia de modificar a atmosfera ao redor de um produto alimentício, com o fim de aumentar a vida útil, se transformou em tecnologia aplicada comercialmente na conservação diversos produtos: carnes, produtos lácteos, aves, pescado, produtos de confeitaria, frutas e hortaliças (SARANTÓPOULOS; SOLER, 1994; SANDHYA, 2010**).**

Cocci et al., (2006) estudaram a utilização de atmosfera modificada ativa e passiva em maçãs minimamente processadas armazenadas a temperatura de 4 °C durante 8 dias, tratadas com solução de ácido ascórbico e ácido cítrico, embaladas em filmes de polipropileno, tanto a atmosfera modificada passiva quanto a ativa apresentaram efeito preservativo para cor do produto, no entanto, a atmosfera modificada ativa mostrou resultados melhores.

1.4.1.1 Gases utilizados na embalagem com atmosfera modificada

Os três principais gases usados em embalagem com atmosfera modificada são CO_2**,** O_2 e N_2. Utilizados isoladamente ou em combinação, esses gases são comumente aplicados para reduzir as alterações fisiológicas durante o armazenamento (SANDHYA, 2010). A escolha da mistura gasosa é influenciada por fatores como: sensibilidade do produto ao O_2 e CO_2 e microbiota capaz de crescer no produto (CHURCH e PARSON, 1995).

1.4.1.1.1 Oxigênio

O oxigênio é um gás incolor e inodoro que é altamente reativo a combustão. Este gás possui baixa solubilidade em água (0,040 g/kg em100 kPa, 20 °C) e promove vários tipos de reações de deterioração em alimentos, incluindo a oxidação das gorduras, reações de escurecimento e oxidação de pigmentos. A maioria das bactérias e fungos necessitam de oxigênio para o crescimento. Portanto, para

aumentar à vida útil dos alimentos, a atmosfera dentro da embalagem deve conter baixa concentração de oxigênio (SANDHYA, 2010). Segundo Farber, (1991) atmosferas contendo 2-5% de oxigênio são recomendadas para o uso em embalagens com atmosfera modificada.

1.4.1.1.2 Gás carbônico

O dióxido de carbono é um gás que se dissolve facilmente na água (1,57 g / kg a 100 kPa, 20 °C) para produzir o ácido carbônico (H_2CO_3) que aumenta a acidez da solução e reduz o pH, fator com implicações significativas para a atmosfera modificada (AM) de alimentos. (SANDHYA, 2010). O CO_2 é solúvel tanto em meio aquoso como lipídico e possui efeito bacteriostático e fungistático (SARANTÓPOULOS; SOLER, 1994).

Organismos como *Salmonella*, *Shigella* e *E.coli*, têm sido implicados em surtos com diversos produtos e, portanto, há uma preocupação sobre seu comportamento sob condições de atmosfera modificada (AMANATIDOU et al., 1999). Dos três principais gases usados em AM o CO_2 é o mais importante para redução do crescimento microbiano, pois possui uma atividade antimicrobiana significativa e direta, atua na alteração da membrana celular do microrganismo, prejudicando absorção de nutrientes, e inibindo as reações enzimáticas levando a mudanças de pH intracelular e alterações nas propriedades físico-químicas das proteínas (FARBER, 1991). O CO_2 também previne ou retarda os efeitos prejudiciais do etileno em frutas e hortaliças frescas, tais como a perda de firmeza e a incidência de desordens fisiológicas (KADER, 1986).

1.4.1.1.3 Nitrogênio

O nitrogênio é um gás relativamente não-reativo, sem odor, gosto ou cor, este gás possui baixa solubilidade tanto em meio aquoso como lipídico (0,018 g / kg a 100 kPa, 20 °C)**.** Por ser um gás quimicamente inerte, o N_2 é usado para substituir o O_2, retardar a rancidez oxidativa e inibir o crescimento de microrganismos aeróbios. Devido à sua baixa solubilidade e menor permeabilidade através da embalagem em relação ao O_2 e CO_2, é usado como um gás de enchimento para prevenir o colapso da embalagem, que pode ser um problema em atmosferas contendo altas concentrações de CO_2 (CHURCH, 1993).

1.4.1.2 Embalagem

As embalagens empregadas para as frutas e hortaliças frescas e minimamente processadas têm o objetivo de criar uma barreira que possa retardar a perda do sabor e aroma desejável e do vapor de água, enquanto restringe a troca de CO_2 e O_2, modificando a atmosfera em que o produto se encontra (FONSECA et al., 2000). O conhecimento das taxas gasosas no interior das embalagens, que acondicionam vegetais intactos e minimamente processados, é de grande importância para a manutenção da vida pós-colheita dos mesmos (PADULA, 2006).

O mercado Brasileiro de embalagens com atmosfera modificada tem crescido significativamente nos últimos anos tanto em variedade, quanto em diversidade de produtos embalados com essa tecnologia. Os avanços mundialmente observados no desenvolvimento das embalagens flexíveis para o acondicionamento de alimentos, muito tem contribuído para aumentar o interesse por esse mercado (JUNQUEIRA; LUENGO, 1999).

No entanto, ainda é difícil alcançar o objetivo de produzir frutas e hortaliças frescas prontas para o consumo de boa qualidade e com vida de prateleira prolongada. O principal problema é que existem poucos materiais de embalagem que são permeáveis o suficiente para regular a respiração desses alimentos. Entre as limitações apresentadas pelos filmes poliméricos estão: não manter uma boa concentração de O_2 e CO_2 no interior da embalagem, principalmente se o produto apresenta alta taxa respiratória (ALZAMORA et al., 2000), e alta barreira ao vapor de água causando condensação dentro da embalagem e consequentemente aumento no crescimento microbiano (FONSECA et al., 2000).

Materiais de embalagem como polipropileno biorientado (PPBO) e polietileno de baixa densidade (PEBD) têm sido bastante utilizados em estudos realizados com frutas e hortaliças frescas e minimamente processados.

Pilon (2003) encontrou bons resultados para as propriedades físico-químicas, análise microbiológica e sensorial de hortaliças minimamente processadas embaladas com filme multicamadas laminado do tipo PPBO/PEBD (polipropileno biorientado e polietileno de baixa densidade).

Os filmes laminados de polipropileno biorientado com polietileno de baixa densidade (PPBO/PEBD) foram uma boa opção de material de embalagem, para comercialização de hortaliças folhosas armazenadas a

temperatura de 5,5 °C, quando utilizadas misturas gasosas com teor de oxigênio maior que 2%, evitando assim a respiração anaeróbia (SARANTÓPOULOS; OLIVEIRA, 2002).

Brackmann et al. (2006) avaliaram embalagens de PEBD com espessuras 40 μm, 60 μm e 90 μm e embalagens de PEMD (polietileno de média densidade) com espessuras de 40 μm e 60μm, empregadas na conservação de melões híbridos minimamente processados armazenados a temperatura de 4 °C durante 25 dias. Os filmes avaliados mantiveram de modo semelhante à qualidade dos melões. Porém, o filme de PEBD com espessura de 40μm apresentou maior concentração de O_2 e menor de etileno.

Alguns trabalhos mostram a importância do uso de embalagens de diferentes polímeros para minimizar as taxas respiratórias de vegetais. Por exemplo, Souza et al. (2007) ao usar embalagens rígidas de polietileno (PE) e polipropileno (PP) seladas passiva e ativamente, verificaram que estas embalagens não são recomendadas para acondicionar pequi minimamente processado, em razão do nível de O_2 estar próximo a 0% no terceiro dia de armazenamento, o que compromete a sua qualidade, devido à respiração anaeróbia.

Rodrigues et al. (2008) mostraram que a utilização de filmes de PEBD proporcionou a modificação passiva da atmosfera ao redor de fatias de manga, o equilíbrio foi atingido após 8 dias de armazenamento. Os autores concluíram que o acondicionamento das frutas com atmosfera modificada influiu positivamente na manutenção das características sensoriais e qualidade microbiológica das fatias de manga.

1.4.1.3 Temperatura

a temperatura é um dos fatores de maior influência na respiração, havendo um valor ideal para manutenção de cada tipo de vegetal, para que alcance um máximo de qualidade. Dentro de uma variação fisiológica própria de cada espécie, a taxa de respiração aumenta geralmente com a temperatura (CHITARRA; CHITARRA, 2005).

Segundo Wiley (1997) a maioria das reações metabólicas que ocorrem em frutas e hortaliças é catalisada por enzimas. Para o controle da atividade enzimática de frutas e hortaliças processadas é necessário a utilização de baixas temperaturas desde o processamento até a distribuição. Processos metabólicos, tais como a respiração e as taxas de

maturação são sensíveis à temperatura. Reações biológicas em geral aumentam 2-3 vezes para cada 10°C de aumento na temperatura. Portanto, o controle da temperatura é de vital importância para que um sistema de atmosfera modificada seja eficaz.

Utilizada juntamente com a refrigeração a atmosfera controlada ou modificada, pode reduzir a respiração em até 50%, quando comparada com a taxa respiratória do produto armazenado apenas sob refrigeração. O armazenamento em atmosfera modificada permite maior tempo de conservação, porque combina alta umidade e controle das pressões parciais de O2 e CO2 no interior das embalagens, o que diminui a atividade respiratória (CHITARRA e CHITARRA, 2005). Estudos realizados por Donadon et al. (2004) mostram que a intensidade respiratória de laranjas pêra, estocadas a 5, 10 e 21 °C, foi tanto mais elevada quanto maior a temperatura de armazenamento. Bhande et al. (2008) comparam o efeito da temperatura na taxa respiratória de bananas armazenadas a temperatura de 10 e 30 °C. Os autores encontraram valores maiores para taxa respiratória de bananas armazenadas a 30 °C. Este resultado era esperado, visto que a temperatura tem sido identificada como fator de maior influência na taxa respiratória de frutas.

A baixa temperatura também é um dos fatores mais importantes para evitar o crescimento microbiano. Os microrganismos capazes de crescer na temperatura de refrigeração se denominam microrganismos psicrotróficos. Os produtos minimamente processados são manipulados e armazenados sob refrigeração, assim os microrganismos psicrotróficos são especialmente importantes para este tipo de produto (WILEY, 1997). Laranjas pêra armazenadas a 5 °C e 10 °C, apresentaram baixa contagem de mesófilos e psicrotróficos, atestando a eficiência das práticas sanitizantes adotadas durante a preparação destes produtos e a importância do armazenamento a baixas temperaturas (DONADON et al., 2004).

Ainda que a refrigeração prolongue a vida útil da maior parte dos alimentos, algumas frutas e hortaliças procedentes de países tropicais e subtropicais sofrem alterações fisiológicas quando expostas a temperaturas inferiores às requeridas para seu armazenamento ótimo (ORDÓÑEZ, 2005). Por exemplo, a banana, abóbora e pepino sofrem desordem pelo frio sob temperaturas inferiores a aproximadamente 11 °C, ao passo que certas cultivares de pêra e maçã podem suportar longos períodos de armazenamento a 0 °C (CHITARRA e CHITARRA, 2005).

Van Dijk et al. (2006), avaliando tomates armazenados em 4 diferentes temperaturas (3, 12, 20 e 25 °C), verificaram que a perda de massa das amostras depende da temperatura, estes autores observaram perda de massa em torno de 6 % para as amostras armazenadas a 25 °C, durante 30 dias.

Oliveira et al. (2007) avaliaram a qualidade de mamão 'Golden' minimamente processado armazenado a temperaturas de 5 e 10 °C. Os autores concluíram que na temperatura de 5 °C, o produto pode ser armazenado durante 8 dias, sem risco de contaminação e sem alterações significativas nas características físico-químicas.

1.4.2 Coberturas Comestíveis

1.4.2.1 Formação de Coberturas Comestíveis

A intensa produção e comercialização de materiais plásticos, derivados de polímeros sintéticos, utilizados principalmente em embalagens têm provocado sérios problemas ambientais em função da alta durabilidade que apresentam. Em média levam cerca de 100 anos para se decompor totalmente no meio ambiente (ROSA et al., 2001)

Atualmente a maior conscientização ambiental é uma grande aliada no avanço das pesquisas e desenvolvimento de novas tecnologias que visam diminuir o impacto ambiental causado por materiais fabricados a partir de polímeros sintéticos (MARQUES, 2005). Filmes comestíveis e revestimentos são alternativas não poluentes desenvolvidas para ampliar a vida de prateleira de produtos (RHIM; SHELLHAMMER, 2005).

Varias técnicas têm sido desenvolvidas para a formação de filmes (KROCHTA et al., 1994), podendo a solução filmogênica ser aplicada diretamente sobre o material a ser recoberto, ou em um suporte para posterior utilização do filme formado. Gennadio e Weller (1990) afirmaram que não existe uma distinção clara entre filmes e coberturas e que estes dois termos são utilizados. No entanto, em geral, as coberturas são aplicadas e formadas diretamente na superfície do produto, enquanto os filmes são formados separadamente como folhas finas e então aplicados aos produtos.

Embora muitas funções dos filmes e coberturas comestíveis sejam semelhantes, existem requerimentos adicionais para que as coberturas possam ser aplicadas em alimentos, como possuir características sensoriais aceitáveis, ter propriedades de barreira,

mecânicas e de adesão adequadas. Além de apresentar estabilidade microbiológica e físico-química, ser segura para saúde, não contendo componentes tóxicos e serem produzidas a partir de materiais renováveis e de baixo custo (DIAB et al., 2001).

Na formação de biofilmes podem ser empregadas diversas classes de compostos naturais. Kester e Fennema, (1986), considerando a natureza do material utilizado, classificaram os biofilmes como: à base de proteínas, polissacarídeos, lipídeos e blendas poliméricas (misturas de componentes). A mistura de diferentes polímeros leva a obtenção de novos materiais, com propriedades intermediárias aos componentes puros utilizados. A nova propriedade obtida a partir da mistura de biopolímeros dependerá da natureza do polímero original, da forma de processamento da mistura e das interações entre estes componentes (ELIAS, 1984)

1.4.2.1.1 Polissacarídeos

Os polissacarídeos são os hidrocolóides mais utilizados em frutas e hortaliças (KESTER; FENNEMA, 1986; KROCHTA; JOHNSTON, 1997) e formam parte da maioria das formulações que existem atualmente no mercado.

Biofilmes desenvolvidos a partir de polissacarídeos apresentam boas propriedades mecânicas e organolépticas e são barreiras efetivas de aromas e gases de baixa massa molar como o oxigênio e dióxido de carbono (DEBEAUFORT et al., 2000). No entanto, a maior limitação para a aplicação destes filmes é a permeabilidade ao vapor de água, devido a sua hidrofílicidade (YANG; PAULSON, 2000). Para melhorar essa característica dos filmes compostos por polissacarídeos, componentes como lipídeos são acrescentados a formulação dos biofilmes.

São muitos os materiais que vêm recebendo atenção pela sua capacidade inerente de se decompor no meio ambiente. Dentre os polissacarídeos mais utilizados na formação de filmes ou blendas poliméricas estão os derivados hidrossolúveis de celulose, hidroxipropilmetilcelulose (ZACCARON, et al., 2005), amido (SOARES et al., 2005), quitosana (REMUÑAN-LOPES; BUDMEIER, 1997), entre outros.

Entre os polissacarídeos mais utilizados nas formulações de coberturas comestíveis encontram-se os derivados de celulose (poli-β-(1→4)-D-glucopiranose). Devido a disposição de grupos hidroximetil

na cadeia polimérica, a celulose apresenta uma estrutura cristalina compacta que impede sua solubilidade em sistemas aquosos. No entanto, sua solubilidade pode aumentar mediante a inclusão de grupos funcionais na cadeia, através de reações de esterificação interferindo na formação da estrutura cristalina. Quando se trata de celulose alcalina seguida de ácido cloroacético, cloreto de metila ou de propileno se obtem a carboximetilcelulose (CMC), metilcelulose (MC) e Hidroxipropilmetilcelulose (HPMC), respectivamente (KESTER; FENEMA, 1986). O aumento da solubilidade destes compostos tem impulsionado o desenvolvimento de recobrimentos comestíveis utilizando estes derivados de celulose.

1.4.2.1.1 Hidroxipropilmetilceluloses (HPMC)

A celulose é formada por unidades D-glicopiranosídeos unidas por ligações 1→4 numacadeia longa e não ramificada. As ligações na celulose são do tipo β-glicosídicas, as quais levama formação de uma cadeia linear (SOLOMONS, 2000). Os três grupos hidroxilas da celulose formam fortes ligações secundárias entre as cadeias, impedindo sua fusão [1]. Devido à sua Os três grupos hidroxilas da celulose infusibilidade e insolubilidade, a celulose é geralmente convertida em derivados para torná-se mais processável.

O hidroxipropilmetilcelulose (HPMC) é um éter de celulose onde os hidrogênios e grupos hidroxilas da celulose foram parcialmente substituídos por alquil ou por grupos alquil substituídos para modificar as características da celulose nativa (PEKEL et al., 2004). Na bibliografia encontram-se numerosos trabalhos sobre coberturas comestíveis aplicadas em frutas. Em cítricos, a aplicação de cobertura composta por HPMC resultou em efetiva redução da perda de peso e manutenção da firmeza das mexericas cv. 'Fortune' (PÉREZ-GAGO et al., 2002), 'Clemenules' (PÉREZ-GAGO, 2006) e 'Ortanique' (NAVARRO-TARAZAGA et al., 2008). Nestes trabalhos, a efetividade dos recobrimentos dependeu da composição e das condições de armazenamento.

1.4.2.1.2 Lipídeos

Os lipídeos são utilizados na formulação de coberturas como coadjuvante com o objetivo de reduzir a permeabilidade ao vapor de água do revestimento, já que estes componentes possuem natureza não-polar ou hidrofóbica, e, assim, proporcionam uma boa barreira contra a

migração de umidade. Além disso, lipídeos podem conferir brilho e melhorar a aparência visual dos produtos alimentares (GONTARD et al., 1995).

A permeabilidade ao vapor de água depende da relação hidrofóbica/hidrofílica proporcionada pelos componentes, da polaridade, grau de insaturação e ramificação dos lipídeos presentes no filme (GONTARD et. al., 1994). Ayranci e Tunc (2001) ao adicionarem os ácidos esteárico, palmítico e láurico em filmes de metilcelulose, observaram uma redução da permeabilidade ao vapor de água.

Quando se produzem biofilmes emulsionados é importante o controle de fatores como: velocidade de agitação, temperatura e formação de espuma, uma vez que estes parâmetros exercem grande influência nas propriedades mecânicas e de barreira dos biofilmes obtidos. Quanto menor for o diâmetro dos glóbulos de lipídeo obtidos e quanto mais homogênea for a sua distribuição, melhores serão as propriedades de barreira ao vapor de água e aos gases (BALDWIN, et al., 1997; GALLO et al., 2000).

Ácidos graxos, como o ácido oleico (AO), estão entre os derivados de lipídeos que pode, potencialmente, melhorar as propriedades de barreira a umidade de filmes hidrofílicos. O ácido oleico é líquido à temperatura ambiente, portanto, é facilmente miscível com biopolímeros, sem necessidade de aquecimento. Além disso, não é muito sensível à oxidação, o que aumenta a segurança alimentar e evita alterações que comprometem as características sensoriais do produto (GHANBARZADEH; ALMASI, 2011).

1.4.2.1.3 Agente Plastificante

Após o processo de secagem alguns filmes podem apresentar aspecto quebradiço tornando o mesmo inviável para utilização. Assim, torna-se necessário o uso de agentes plastificantes, definidos por KESTER e FENNEMA (1986) como compostos de baixa volatilidade e alto ponto de fusão, capazes de reduzir as forças intermoleculares e aumentar a mobilidade das cadeias poliméricas. Com isso há uma melhora da flexibilidade e da extensibilidade do filme, evitando assim a ruptura do mesmo durante o manuseio e armazenagem (MCHUGH; KROCHTA, 1994; LIN et al., 2000).

A formação da cobertura é dependente de dois tipos de interação; coesão (forças atrativas entre as moléculas do filme) e adesão (forças de atração entre o filme e o substrato). Quando o plastificante é

incorporado aos filmes poliméricos podem ocorrer mudanças nas propriedades de adesão, permeabilidade ao vapor de água (LIN et al., 2000), ao oxigênio (IRISSIN-MANGATA et al., 2001), e propriedades mecânicas e térmicas como resistência a tração e transição vítrea, respectivamente. O plastificante também deve ser compatível com o polímero de modo que o mesmo apresente-se totalmente disperso na solução filmogênica (IRISSIN-MANGATA et. al., 2001), evitando assim, que haja a formação de um filme com camadas distintas.

Filmes comestíveis preparados a partir de proteínas (zeína do milho e glúten de trigo) e celuloses (metilcelulose e hidroxipropil celulose) foram estudados em relação à permeabilidade a gases (PARK; CHINNAN, 1995). Os autores observaram um aumento da permeabilidade a O_2, CO_2 e vapor de água com o aumento da concentração de plastificante, nos filmes de celulose. Por outro lado à adição de lipídeos no filme de hidroxipropil celulose diminuiu a permeabilidade a gases.

Sorbitol e glicerol são plastificantes comumente utilizados em diversos processos de elaboração de filmes, sendo o sorbitol cristalino a temperatura ambiente e o glicerol líquido (ANKER et al., 2002).

1.4.2.1.4 Aditivos antimicrobianos para alimentos

Os aditivos utilizados para evitar a deterioração biológica são denominadas
antimicrobianos ou conservantes. Esta categoria inclui compostos naturais ou sintéticos com efeitos toxicológicos conhecidos em mamíferos e no ambiente. Compostos antimicrobianos mais utilizados incluem os ácidos orgânicos e os seus sais, carbonatos e bicarbonatos, parabenos, quitosana, enzimas, bacteriocinas, polipeptídeos, extractos naturais, ou óleos essenciais.

Uma grande variedade de agentes antimicrobianos é adicionada em filmes e revestimentos comestíveis para controlar o crescimento microbiológico e prolongar a vida de prateleira. Agentes antimicrobianos utilizados para a formulação de películas comestíveis e revestimentos devem ser classificados como aditivos de grau alimentício ou compostos geralmente reconhecido como seguro (GRAS) pelos regulamentos apropriados (PALOU et al., 2002)

Agências reguladoras Internacionais são responsáveis pela aprovação de antimicrobianos para o uso em alimentos. Na Europa, os

compostos são regulados pela da União Europeia (UE, 1989) e nos Estados Unidos pela *Food and Drug Administration* (FDA, 2008).

1.4.2.1.5 Agentes antimicrobianos químicos

Os ácidos orgânicos são os antimicrobianos químicos sintéticos mais comuns e incluem os ácidos acéticos, benzóico, cítrico, fumárico, láctico, málico, propiónico, sórbico, succínico, tartárico entre outros. Estes ácidos inibem o crescimento de células bacterianas e fúngicas. Sorbato de potássio (SP) e benzoato de sódio (BS) são os dois sais de ácidos orgânicos mais amplamente utilizados como aditivo antimicrobiano alimentar. O ácido benzóico é também chamado ácido fenilfórmico ou benzeno-carboxílico. A atividade antimicrobiana do ácido benzóico e BS está relacionada com o pH, e os mais eficazes são as formas não dissociadas (CHIPLEY, 2005). O ácido sórbico é um ácido graxo insaturado. O grupo carboxil do ácido sórbico é altamente reativo com o sódio, cálcio ou potássio, e resultam na formação de vários sais e ésteres (STOPFORTH et al., 2005).

O SP a forma mais solúvel do sorbato é bem conhecida pela sua potente atividade anti-fúngica. A ação antimicrobiana do sorbato também é dependente do pH. Em geral, a atividade do SP é maior a valores de pH baixos, porém também podem ser eficazes a valores de pH até 7. No entanto, antimicrobianos orgânicos à base de ácido, como propionatos ou benzoatos, só mostram uma considerável atividade antimicrobiana em valores baixos de pH, como 5-5,5 e 4-4,5, respectivamente (STOPFORTH et al., 2005). As espécies bacterianas e fungicas inibidas por sorbatos pertencem ao género *Alternaria*, *Penicillium*, e outros. Vários estudos também indicaram um aumento de efeitos antimicrobianos do sorbato quando combinado com vários fosfatos. Combinações de sorbato ou benzoato com propianato podem inibir os microrganismos com concentrações reduzidas de cada conservante (STOPFORTH et al., 2005).

O ácido propiónico é um ácido monocarboxílico de ocorrência natural, a atividade antimicrobiana de sais de propionato é dependente do pH, sendo também mais eficazes na sua forma não dissociada, a baixo pH. O ácido propiónico é essencialmente inibidor de fungos, no entanto, algumas leveduras e bactérias também podem ser inibidas (DOORES, 2005).

Os parabenos são os ésteres alquílicos do ácido para-hidroxibenzóico. O comprimento de cadeia de parabenos determina a

sua solubilidade na água, quanto menor comprimento de cadeia, maior a solubilidade em água dos parabenos. Parabenos são inibitórios para bactérias gram-positivas e gram-negativas ou fungos, porém fungos geralmente são mais suscetíveis a parabenos que bactérias (DAVIDSON, 2005). O pH óptimo para a atividade antimicrobiana dos parabenos está na faixa de 3,0-8,0.

1.4.2.1.6 Agentes antimicrobianos naturais

Agentes antimicrobianos naturais incluem quitosana, polipeptideos, óleos essenciais de plantas, especiarías e extratos. A quitosana é um polissacarídeo preparado por desacetilação da quitina: componente abundante de casca de crustáceos (COMA et al, 2002; NO et al., 2007), este polissacarideo inibe o crescimento de uma grande variedade de fungos, leveduras e bactérias. A nisina, uma proteína hidrófoba, é um polipéptido de baixa massa molecular, produzido pela bactéria *Lactococcus lactis*, esta proteina tem um largo espectro de atividade contra bactérias gram-positivas, mas não inibe significativamente a bactérias gram-negativas, leveduras ou bolores (THOMAS; BROUGHTON, 2005). A nisina foi provada ser não tóxica e reconhecida como GRAS pelo FDA dos EUA em 1969. Desde então, tem sido amplamente utilizado na indústria alimentar como um conservante seguro e natural (SEBTI et al.,2007).

A natamicina é um antifúngico natural produzido por *Streptomyces natelensis*. A natamicina não tem nenhum efeito sobre bactérias, mas é ativa contra quase todos os fungos e leveduras. A natamicina é normalmente aplicada como um tratamento de superfície dos queijos duros, secos ou curados (TURÉ et al., 2009).

A lisozima é uma enzima que compreende 129 aminoácidos com ligações dissulfureto (CAGRI et al., 2004), apresenta atividade antimicrobiana contra células vegetativas de uma grande variedade de organismos, incluindo inúmeros organismos patogênicos e deteriorantes. As bactérias gram-negativas são geralmente menos sensíveis a lisozima do que as bactérias gram-positivas, devido a proteção da parede celular pela membrana exterior que estas bactéria possuem (JOHNSON; LARSON, 2005).

Plantas, ervas, especiarias e seus derivados como os óleos essenciais podem conter um grande número de substâncias que são conhecidos por inibir várias atividades metabólicas de bactérias, leveduras, e fungos (LÓPEZ-MALO et al., 2005). Óleos essenciais de

angélica, anis, cenoura, cardamomo, canela, cravo, coentro, endro, erva-doce, alho, noz-moscada, orégano, salsa, alecrim, sálvia ou timol são inibidoras de várias bactérias deteriorantes ou patogênicas, e também fungos e leveduras (CAGRI et al., 2004).

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CAPÍTULO 2:
USO DE OZÔNIO NA SANITIZAÇÃO DE TOMATE CEREJA

2.1. Introdução

Os compostos clorados têm sido utilizados como sanitizantes no processamento de alimentos por várias décadas, assim como na desinfecção de produtos e superfícies nas empresas de processamento e na redução da população microbiana da água utilizada durante as operações de higienização e embalagem (PARISH et al., 2001).

Todavia, concentrações elevadas de cloro podem causar problemas como descoloração, perda de qualidade e aumento na corrosão de equipamentos. Outro ponto importante diz respeito à formação de trihalometanos e cloraminas que ocorrem pela combinação de cloro com a matéria orgânica e que é prejudicial para a saúde humana pelo seu potencial carcinogênico (KIM et al., 1999; VANETTI, 2004).

O ozônio é um sanitizante alternativo ao cloro e tem se mostrado muito eficaz na inativação de bactérias, vírus e cistos de *Giardia* e *Criptosporidium*, ambos protozoários resistentes ao cloro (CAVALCANTE, 2007). Este composto é um gás relativamente instável, parcialmente solúvel em água e apresenta meia-vida que varia de 20 a 30 min em água destilada a 20 °C (KHADRE et al., 2001). Na década de 90, os Estados Unidos reconheceram o ozônio como uma substância GRAS (*General Recognized as Safe*) para aplicação direta em produtos alimentícios. A partir disso, houve um crescente interesse na aplicação de ozônio no processamento de alimentos (GRAHAM, 1997).

O ozônio é um agente antimicrobiano eficiente que tem grande potencial de uso na indústria de alimentos e sua aplicação durante o processamento e/ou estocagem aumenta a vida de prateleira dos produtos (SILVA et al., 2011). A inativação de microrganismos pelo ozônio é menos efetiva quando aplicada diretamente sobre a superfície do alimento do que o ozônio em meio líquido. A inativação da microbiota em alimentos por ação do ozônio depende muito da natureza e da composição da superfície dos alimentos, do tipo de contaminação microbiana, bem como o grau de associação dos microrganismos com os alimentos (KIM et al., 1999). Pérez et al. (1999) afirmam que alguns resultados contraditórios reportados sobre os efeitos do ozônio sugerem que a eficácia deste gás deve ser avaliada individualmente para cada tipo de produto.

Um dos usos importantes de O_3 é o tratamento pós-colheita das culturas.

Pode ser aplicado a alimentos, como um gás ou como uma forma

dissolvida em água. Os principais objetivos da aplicação do O_3 na fase de pós-colheita são as seguintes: inativação de crescimento bacteriano (XU, 1999; SHARMA et al., 2002); prevenção do crescimento de fungos (PEREZ et al., 1999; PALOU et al., 2002) e controle de pragas no armazenamento (KELLS et al., 2001; MENDEZ et al., 2002). Na pós-colheita de morango, Pérez et al. (1999) observaram degradação por fungos após 4 dias de armazenamento sob a ozonização.

O objetivo deste trabalho foi avaliar o efeito do uso de ozônio e cloro, aplicados na higienização de tomate cereja, sobre as características microbiológicas do fruto.

2.2. Materiais e métodos

2.2.1 Matéria prima

Os tomates cerejas utilizados nos experimentos foram cultivados e colhidos na região de Florianópolis-SC. Após a colheita os frutos foram acondicionados em ambiente refrigerado até o transporte para o laboratório. As amostras foram armazenadas a 7ºC e 80% de umidade relativa por 24 horas até a realização das análises. Os tomates cereja foram selecionadas pelo tamanho, grau de maturação e integridade física.

2.2.2 Quantificação de ozônio em água

A quantificação de ozônio em água foi realizada de acordo com a metodologia descrita por Rakness et al. (2010). Foram preparadas duas soluções estoque de triossulfonato índigo de potássio, a solução estoque primária (RI) e a solução estoque secundária (RII). A solução primária foi obtida a partir da mistura de 1 L de água e 770 mg de triossulfonato índigo de potássio ($1{,}248 \times 10^{-3}$ M). A solução estoque secundária foi obtida pela diluição 1:10 da solução RI ($1{,}248 \times 10^{-4}$ M). A amostra de água ozonizada (90 mL) foi adicionada a 10 mL da solução RII para posterior leitura em espectrofotômetro (800XI, Femto) a 600 nm. A absorbância da amostra foi comparada com o "branco", que foi preparado a partir da diluição de 10 mL de solução RII ($1{,}248 \times 10^{-5}$ M) e 90 mL de água destilada, a leitura do branco foi realizada a 600 nm. A equação 1 foi utilizada para o cálculo do ozônio em água:

$$mg/L = \frac{100\ x\ \Delta Abs}{f\ x\ SV\ x\ b} \qquad \text{(Eq. 1)}$$

Onde:

ΔAbs = diferença entre a absorbância da amostra e absorbância do branco a 600 nm

b = tamanho da célula do espectrofotômetro (cm)

SV = Volume da amostra (mL)

f = 0,42 L $mg^{-1}cm^{-1}$ (constante de proporcionalidade)

O valor da constante de proporcionalidade (f) foi determinado no trabalho original de Bader e Hoigné (1980).

2.2.3. Sanitização dos Tomates Cereja

Os tomates cereja foram divididos em três lotes para lavagem com água destilada (controle), sanitização em ozônio e cloro, separadamente. Um dos lotes de tomate foi imerso em água ozonizada em diferentes concentrações e tempos de contato com ozônio, conforme planejamento fatorial 2^2 com três repetições no ponto central (Tabela 2.1). As variáveis independentes foram: concentração de ozônio ($\mu g.mL^{-1}$) e tempo de contato do produto com água ozonizada (min). Os experimentos com ozônio foram comparados às amostras tratadas com água clorada (100 $\mu g.mL^{-1}$ por 15 min) e imersão em água destilada por 15 min (controle). Todos os tratamentos foram realizados na proporção 1:2 (massa de fruta: volume de água). Após a higienização, as amostras dos diferentes tratamentos foram centrifugadas utilizando uma centrifuga manual higienizada, durante 1 min para retirada do excesso de água.

Figura 2.1: Gerador de Ozônio
(http://www.geradoresdeozonio.com.br/produtos.htm)

2.2.4 Análises microbiológicas

Os frutos foram acondicionados em embalagens estéreis de polipropileno biorientado e polietileno de baixa densidade (PPBO/PEBD) e armazenados a 8 ºC por 4 h, até o início das análises microbiológicas. Foram analisados como resposta, os coliformes totais e termotolerantes, *Escherichia coli*, *Listeria* spp, *Salmonella* spp, contagem de psicrotróficos, bolores e leveduras para os diferentes tratamentos aplicados. As análises microbiológicas foram realizadas de acordo com metodologia descrita no Compêndio de Métodos para Análises Microbiológicas de Alimentos (APHA, 1992), na central de análises do Departamento de Ciência de Alimentos da UFSC. A Tabela 2.1 apresenta a matriz completa 2^2 com repetição no ponto central.

Tabela 2. 1: Matriz do planejamento fatorial 2^2 para o processo de sanitização com ozônio.

Ensaio [a]	*Valores codificados*		*Valores reais*	
	X_1	X_2	X_1 *(ppm)*	X_2 *(min)*
1	-1	-1	0,5	1
2	+1	-1	1,5	1
3	-1	+1	0,5	10
4	+1	+1	1,5	10
5	0	0	1,0	5
6	0	0	1,0	5
7	0	0	1,0	5

[a] Experimentos realizados em ordem aleatória; X_1 = concentração de ozônio; X_2= tempo de contato do ozônio com o tomate

2. 3. Resultados e discussão

2.3.1. Concentração de ozônio em água

Foram realizados vários testes com diferentes concentrações de O_3 gasoso e tempos de produção de ozônio, com objetivo de alcançar concentrações em torno de 0,50, 1,0 e 1,50 ppm de ozônio em água. A Tabela 2.2 apresenta a concentração O_3 gasoso (ppm), tempo de produção de O_3 (min) e a concentração de O_3 na água (ppm).

Tabela 2. 2: Concentração de ozônio em água

Concentração O_3 gasoso (ppm)	**Tempo produção de O_3 (min)**	**Concentração O_3 na H_2O (ppm)**
34	3	0,58 ± 0,07
34	4	0,98 ± 0,05
34	6	1,55 ± 0,05

*****Volume de água no tanque = 25 L.

2.3.2. Contagens microbianas após sanitização com ozônio

Devido os baixos valores de pH da maioria dos frutos, a microbiota típica destes produtos é composta de bolores e leveduras. Entre as espécies frequentemente encontradas estão: *Botrytis cinerea* e *Aspergillus niger*, *Candida, Cryptococcus, Fabospora, Kluyveromyces, Pichia, Saccharomyces, e Zygosaccharomyces* (CHEN, 2002). Grande variedade de bactérias patogênicas como *Listeria monocytogenes, Salmonella spp., Shigella spp., Aeromonas hydrophila, Yersinia enterocolitica e Staphylococcus aureus*, bem como algumas cepas patogênicas de Escherichia coli podem estar presentes em vegetais frescos (BREIDT; FLEMING, 1997). Portanto, a aplicação de métodos de sanitização nestes produtos implica na realização de análises microbiológicas para verificar a eficiência do método utilizado na eliminação destes microrganismos.

A Tabela 2.3 apresenta as contagens microbianas de amostras de tomate cereja sanitizados com ozônio em sete diferentes ensaios. Através dos resultados verifica-se que a amostra não apresentava contagens significativas de coliformes a 35 e 45°C, *Escherichia coli, Listeria e Salmonela*. Observando os valores obtidos para bolores, leveduras e contagem de psicrotrófilos, percebe-se que houve pequena diferença entre os ensaios realizados com ozônio, porém todos apresentaram boa inativação dos microrganismos testados. Diante do exposto, optou-se pela utilização da menor concentração de ozônio aliado ao menor tempo de contato com o produto.

Segundo a Resolução - RDC nº 12, de 2 de janeiro de 2001, para frutas frescas, "*in natura*", preparadas (descascadas, selecionadas ou fracionadas) sanificadas, refrigeradas ou congeladas, a contagem de coliformes não devem ultrapassar $5x10^2$ UFC/g. A legislação estabelece também que deve ocorrer ausência de Salmonella em 25 g de amostra para este tipo de produto. Porém esta resolução não estabelece padrões para bolores e leveduras. A ANVISA, através da Instrução Normativa nº 12 de 10 de setembro de 1999, regulamentou os padrões de identidade e as características mínimas de qualidade para polpas de frutas, estabelecendo valores máximos de $5x10^3$ UFC/g para bolores e leveduras. Os resultados obtidos neste estudo estão de acordo com os critérios estabelecidos pela legislação brasileira, para presença de microrganismos em produtos vegetais. Lee et al. (2003) relatam que substâncias tóxicas podem ser produzidas quando contagens microbiológicas excedem 10^6 UFC/g, neste trabalho nenhum microrganismo ultrapassou crescimento de 10^2 UFC/g, quando tratados

com ozônio.

Comparando os resultados obtidos para sanitização com ozônio e cloro observa-se que os mesmos são efetivos na eliminação de microrganismos, quando comparados ao controle (higienização com água) tornando o produto próprio para o consumo. Apesar de estes sanitizantes possuírem potencial antimicrobiano similar, optou-se pelo uso do ozônio por ser um produto que não deixa resíduos no alimento sanitizado.

Agentes à base de cloro são freqüentemente utilizados para desinfetar superfícies e produtos alimenticios, bem como reduzir as populações microbianas da água aplicada na limpeza e operações de embalagem (DELAQUIS et al., 2004). Porém, devido aos riscos ambientais e á saúde (DYCHDALA,1991), o uso de cloro é proibido na produção orgânica na Europa. A produção de compostos orgânicos clorados, como os trihalometanos, que são potenciais carcinógenos (FAWELL, 2000), criou a necessidade de investigar a eficiência de desinfetantes não tradicionais e outras tecnologias alternativas. O estudo realizado neste trabalho mostra que o ozônio pode substituir o cloro na higiene de vegetais, e evitar o problema causado pela presença de resíduos clorados no produto.

O ozônio tem sido utilizado na indústria de processamento de alimentos como gás e dissolvido em água. Ambos têm sido usados como bactericida sobre uma vasta gama de produtos alimentares incluindo carnes, aves, ovos, frutas, hortaliças, sucos e frutos do mar, bem como no saneamento das superfícies de contato com o produto (GUZEL-SEYDIM et al., 2003) A água ozonizada reduz populações microbianas e estende a vida útil de frutas frescas e produtos hortícolas (KIM et al., 1999). Das et al. (2006) verificaram que o uso de 10 mg/L de ozônio pode ser utilizado para eliminar a *Salmonella enteridis* da superficie de tomates cereja.

O ozônio é uma alternativa ao cloro no processamento tradicional de alfaces frescas sem afetar negativamente a qualidade sensorial do produto (BELTRAN, 2005). Segundo Williams et al. (2005) o ozônio pode substituir a pasteurização térmica no processamento de suco de maçã e suco de laranja e produzir reduções da ordem de 10^5 em populações de *Escherichia coli* O157:H7 e *Salmonella*. O ozônio é capaz de reduzir efetivamente as populações inoculadas de *Listeria monocytogenes* e *Escherichia coli* em agrião, espinafre, coentro, alface e aipo (WARRINER et al., 2005). Amoras (Barth et al., 1995), alface

(Kim e Yousef, 1998), cenoura (Liew e Prange, 1994) foram tratados com ozônio gasoso e apresentaram resultados para bolores e bactérias, incluindo a Salmonella.

Tabela 2. 3: Planejamento fatorial 2^2 para o processo de sanitização de tomate com ozônio.

	Variáveis independentes		***Variáveis dependentes***						
Ensaio*	**Concentração de ozônio (ppm)**	**Tempo de contato (min)**	**Bolores e leveduras (UFC/g)**	**Coliformes a 35°C (NMP/g)**	**Coliformes a 45°C (NMP/g)**	**Contagem de psicrotrófilos a 22°C (UFC/g)**	**Escherichia coli (NMP/g)**	**Listeria spp**	**Salmonella spp**
1	0,5	1	$4,0 \times 10^2$	< 3	< 3	$1,9 \times 10^2$	< 3	Ausência em 25 g	Ausência em 25 g
2	1,5	1	$1,1 \times 10^2$	< 3	< 3	$1,4 \times 10^2$	< 3	Ausência em 25 g	Ausência em 25 g
3	0,5	10	$1,1 \times 10^2$	< 3	< 3	$3,4 \times 10^2$	< 3	Ausência em 25 g	Ausência em 25 g
4	1,5	10	$4,3 \times 10^2$	< 3	< 3	$3,7 \times 10^2$	< 3	Ausência em 25 g	Ausência em 25 g
5	1,0	5	$6,0 \times 10^2$	< 3	< 3	$1,3 \times 10^2$	< 3	Ausência em 25 g	Ausência em 25 g
6	1,0	5	$7,0 \times 10^2$	< 3	< 3	$3,8 \times 10^2$	< 3	Ausência em 25 g	Ausência em 25 g
7	1,0	5	$7,0 \times 10^2$	< 3	< 3	$3,8 \times 10^2$	< 3	Ausência em 25 g	Ausência em 25 g

Tabela 2. 4: Limpeza de tomate com cloro e água destilada.

Limpeza*	Concentração (ppm)	Tempo de contato (min)	Bolores e leveduras	Coliformes a 35°C	Coliformes a 45°C	Contagem de psicrotrófilos (22°C)	Escherichia coli	Listeria spp	Salmonella spp
Cloro	100	15	$1,0 \times 10^2$	< 3	< 3	$4,0 \times 10^2$	< 3	Ausência em 25 g	Ausência em 25 g
Controle	-	15	$7,1 \times 10^3$	$9,3 \times 10^1$	< 3	$8,4 \times 10^4$	< 3	Ausência em 25 g	Ausência em 25 g

*Limpeza realizada com 100 g de amostra em 1L de água destilada.

2.4. Conclusões

Os resultados obtidos para as amostras higienizadas com hipoclorito de sódio foram semelhantes aos encontrados para amostras tratadas com ozônio. Ambos os tratamentos reduziram a contagem microbiana quando comparados com o controle. Sendo assim, é possível realizar a higienização de tomate cereja reduzindo o crescimento microbiano optando por tratamentos com menor concentração de ozônio (0,5 µg.mL-1) e menor tempo de contato do produto com a água ozonizada (1 min).

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CAPÍTULO 3:
EVALUATION OF AN EXPERIMENTAL METHOD FOR DETERMINING O_2 AND CO_2 CONCENTRATION: APPLICATION TO PHYSALIS FRUIT AND CHERRY TOMATO PACKAGING UNDER MODIFIED ATMOSPHERE

Evaluation of an experimental method for determining O_2 and CO_2 concentration:
Application to physalis fruit and cherry tomato packaging under modified atmosphere

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Abstract

The aim of this study was to evaluate the reliability of an automatic gas analyzer for O_2 and CO_2 analysis in physalis fruit and tomato packaging under modified atmosphere. The results of the analyzer were contrasted with results obtained by gas chromatography. The fruits were packed in multilayer bags under modified atmosphere. The results obtained for O_2 and CO_2 with the Dansensor analyzer were similar to those found by the gas chromatograph. The coefficients of variation of the results obtained in the Dansensor analyzer were low, indicating that the readings of this equipment are accurate and have good reproducibility.

Keywords: gas concentration, Dansensor analyzer, gas chromatograph.

3.1. Introduction

Tomato and physalis both are climacteric fruits. However, there present different metabolic activity metabolic as consequence of the storage conditions. The respiratory rate depends on the vegetable studied, temperature and atmospheric composition (O_2, CO_2 and ethylene) surrounding the product (MAHAJAN; GOSWAML, 2001). The respiratory process is a good indicator of the metabolic rates of plants, their control can be an effective means of regulating the whole plant metabolism and extend the postharvest of the fruits and vegetables (MATHOORO, 1996). Modified atmosphere is a technology that can be applied to control or to reduce the respiration rate of these products and to extend their shelf life

The basic principle underlying storage under modified and controlled atmospheres is modifying the gas composition in the microenvironment to minimize the respiratory rate and other biochemical processes of the fruits and vegetables (BARBOSA, 2011). This modification is based on the alteration of O_2, CO_2, and N_2 levels in packages. The usage of modified atmosphere associated to packaging in low temperatures also helps product conservation (LANCHERO et al., 2007)

The usage of low O_2 concentrations and high CO_2 concentrations balanced with N_2 levels is proposed by many researchers as the ideal packaging conditions for some selected fresh fruits and minimally-processed vegetables (JACKXSENS et al., 2004) One of the important steps in the use of modified atmosphere is the monitoring of the gas concentration in the package, physical-chemical, sensorial, and microbiological properties evaluated during the storage period or shelf life.

In order to assess the quality of fruits and vegetables, reliable methods which can be compared and tracked are needed. Unreliable analytical data can lead to disastrous decisions and irreparable financial damage. To ensure that a new analytical method can generate reliable and interpretable information about the sample, it must undergo an assessment called validation (MATHOORO, 1996).

Calibration is one of the key stages in chemical analysis. The analytical curve is the most-often used tool to quantification and consists of determining the response of a particular instrument to various concentrations of test substance (MAHAJAN; GOSWAML, 2001; RIBANI et al., 2004)

Factors such as speed, sensitivity, and specificity can contribute to the analysis of gases used in food packaging. Through the use of gas analysis equipment, it is possible to carry out experiments with these characteristics, which is a differential of chromatographic methods for gas analysis.

Based on these principles, the aim of this study was to evaluate the reliability of O_2 and CO_2 concentration results in an automatic gas analyzer (PBI Dansensor, CheckMate II) when compared to a gas chromatograph (Cromacon, CG35) in physalis fruit and tomato packaging under modified atmosphere.

3.2. Material and Methods

Samples of physalis (*Physalis angulata* L.) and cherry tomatoes (*Lycopersicon esculentum* L. var. cerasiform) were put into bio-oriented polypropylene and low density polyethylene packages (PPBO/PEBD) with dimensions of 17.5x24.0 cm, thickness of 75 μm, water vapor permeability of 7 $g/m^2/day$, oxygen permeability of 2,000 $cm^3/m^2/day$ and CO_2 permeability of 5,469 $cm^3/m^2/day$, each pack containing 100 g of one of the samples.

A gas mixture containing 5% of O_2, 10% of CO_2, and 85% of N_2 was injected into the packages using a vacuum sealer (Selovac, 200B). The samples were stored under refrigeration at 15 °C and relative humidity of 68% for 30 hours. The gas concentration readings (O_2 and CO_2) were made for both samples during 30 hours of storage in a gas analyzer (PBI Dansensor, CheckMate II) with a zircon detector and detection limit from 0 to 100% of O_2 and CO_2. A 3-mL gas aliquot was removed from inside the bags using the syringe attached to the equipment.

The gas analyses by chromatography were made in a gas chromatograph (Cromacon, CG35) with a thermal conductivity detector, PM5 column (O_2 and N_2), 1.8 m, molecular sieve of 5 Å, and Porapak-Q column (CO_2), 1.8 m, using Helium as carrier gas and flow rate of 30 mL/min, column and injector temperatures of 65 °C, and detector temperature of 220 °C. The chromatograph was calibrated with a mixture of synthetic air and a volume of 1 mL was injected.

The collection of gas samples from the package for gas chromatography was carried out by connecting a rubber septum outside the package and subsequently removing the gases with a glass syringe

with a volume of 1 mL. The gas readings were performed in triplicate for each packaging, using destructive samples.

The results were treated statistically for the standard deviation, variation coefficient, and Spearman correlation test for nonparametric data in the statistical package of Statistica 8.0 (Statsoft Inc, USA), considering $p<0.05$ as the minimum level of significance. The results were expressed as absolute values of correlation (r), where "0" indicates a total absence of linear correlation and "1" a perfect linear relationship.

3.3. Results and Discussion

The data for the O_2 and CO_2 concentrations during storage period obtained by gas chromatography and the gas analyzer are shown in Tables 3.1 and 3.2, respectively. According to Table 3.1, it can be seen that the readings of O_2 concentration in both devices were statistically identical ($p\geq0.05$). It was observed that the gas analyzer can be considered an accurate equipment to read the concentration of O_2, since it showed variation coefficients lower than those obtained with the gas chromatograph.

Table 3.1: Oxygen concentration measurements in gas chromatograph (GC) and in gas analyzer (GA).

Time (hours)	Oxygen (%)							
	Physalis				Cherry tomato			
	GC	VC*	GA	VC*	GC	VC*	GA	VC*
1	-	-	-	-	4.80 ± 0.26^{a}	5.71	4.85 ± 0.02^{a}	0.36
2	4.33 ± 0.26^{a}	5.99	4.39 ± 0.00^{a}	0.00	4.75 ± 0.11^{a}	2.25	4.80 ± 0.00^{a}	0.00
3	4.15 ± 0.23^{a}	5.54	4.23 ± 0.01^{a}	0.25	4.64 ± 0.45^{a}	9.69	4.75 ± 0.03^{a}	0.59
4	3.31 ± 0.24^{a}	7.37	3.22 ± 0.01^{a}	0.18	4.43 ± 0.18^{a}	4.14	4.55 ± 0.01^{a}	0.13
5	3.21 ± 0.19^{a}	5.87	3.28 ± 0.01^{a}	0.18	4.38 ± 0.14^{a}	3.12	4.50 ± 0.01^{a}	0.12
6	3.36 ± 0.24^{a}	7.19	3.28 ± 0.01^{a}	0.30	4.36 ± 0.17^{a}	3.80	4.46 ± 0.00^{a}	0.00
7	2.55 ± 0.15^{a}	5.83	2.47 ± 0.06^{a}	2.23	4.24 ± 0.21^{a}	4.96	4.31 ± 0.01^{a}	0.13
8	3.21 ± 0.11^{a}	3.57	3.10 ± 0.01^{a}	0.19	-	-	-	-
9	2.74 ± 0.11^{a}	3.88	2.66 ± 0.01^{a}	0.38	4.14 ± 0.03^{a}	0.72	4.23 ± 0.01^{b}	0.14
10	2.96 ± 0.19^{a}	6.39	2.94 ± 0.07^{a}	2.26	-	-	-	-
11	3.09 ± 0.17^{a}	5.57	2.88 ± 0.01^{a}	0.20	4.04 ± 0.04^{a}	1.06	4.15 ± 0.01^{b}	0.14
12	2.28 ± 0.14^{a}	6.18	2.17 ± 0.00^{a}	0.00	-	-	-	-
13	-	-	-	-	4.20 ± 0.22^{a}	5.00	4.31 ± 0.00^{a}	0.00
30	2.34 ± 0.22^{a}	9.18	2.21 ± 0.01^{a}	0.45	4.40 ± 0.13^{a}	2.80	4.40 ± 0.00^{a}	0.00

* VC - variation coefficient (%). Equal lower-case letters in the lines indicate that the samples do not differ significantly ($p \geq 0.05$).

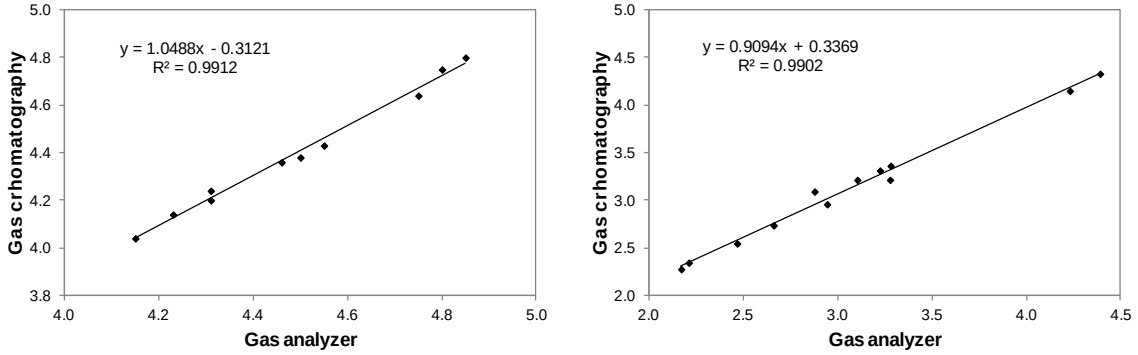
Table 3.2: Carbon dioxide concentration measurements in gas chromatograph (GC) and in gas analyzer (GA).

Time (hours)	Carbon dioxide (%)							
	Physalis				Cherry tomato			
	GC	VC*	GA	VC*	GC	VC*	GA	VC*
1	8.46 ± 0.01^{a}	0.10	7.87 ± 0.06^{b}	0.73	8.16 ± 0.03^{a}	0.41	8.25 ± 0.17^{a}	2.09
2	9.20 ± 0.04^{a}	0.43	9.17 ± 0.06^{a}	0.63	8.23 ± 0.04^{a}	0.50	8.27 ± 0.12^{a}	1.43
3	9.46 ± 0.02^{b}	0.23	9.57 ± 0.06^{a}	0.60	8.32 ± 0.00^{a}	0.05	8.32 ± 0.00^{b}	0.00
4	11.94 ± 0.10^{a}	0.87	11.60 ± 0.10^{b}	0.86	9.20 ± 0.11^{a}	1.20	8.93 ± 0.06^{b}	0.65
5	12.03 ± 0.08^{a}	0.70	11.80 ± 0.00^{b}	0.00	9.03 ± 0.09^{a}	1.00	8.90 ± 0.00^{b}	0.00
6	11.55 ± 0.09^{a}	0.81	11.37 ± 0.06^{b}	0.51	9.36 ± 0.04^{a}	0.40	9.10 ± 0.00^{b}	0.00
7	13.43 ± 0.06^{a}	0.41	13.33 ± 0.06^{a}	0.43	9.27 ± 0.07^{a}	0.73	9.13 ± 0.06^{a}	0.63
8	13.02 ± 0.05^{a}	0.41	12.67 ± 0.06^{b}	0.46	-	-	-	-
9	13.69 ± 0.23^{a}	1.67	13.13 ± 0.06^{b}	0.44	9.72 ± 0.05^{a}	0.56	9.43 ± 0.06^{b}	0.61
10	13.03 ± 0.04^{a}	0.34	12.63 ± 0.06^{b}	0.46	-	-	-	-
11	15.39 ± 0.04^{a}	0.24	15.13 ± 0.06^{b}	0.38	9.97 ± 0.04^{a}	0.36	9.70 ± 0.10^{b}	1.03
12	14.46 ± 0.08^{a}	0.53	14.40 ± 0.00^{a}	0.00	-	-	-	-
13	-	-	-	-	9.64 ± 0.06^{a}	0.65	9.37 ± 0.06^{b}	0.62
30	14.40 ± 0.08^{a}	0.56	14.23 ± 0.06^{b}	0.41	9.58 ± 0.02^{a}	0.16	9.43 ± 0.00^{b}	0.00

* VC - variation coefficient (%). Equal lower-case letters in the lines indicate that the samples do not differ significantly ($p \geq 0.05$).

The readings of CO_2 concentration by gas chromatography were significantly different from those obtained by the gas analyzer ($p \leq 0.05$). This result could be due to the low variability of the data and is directly related to the accuracy of these methods for measurement the carbon dioxide concentration. The variation coefficients of the values obtained in the Dansensor analyzer were lower, indicating that the readings of this equipment are accurate and have good reproducibility. However, the lower variation of the Dansensor analyzer is also due to the fact that this equipment is less sensitive than the gas chromatograph. This was observed in both products (physalis and cherry tomatoes) stored under modified atmosphere.

The results obtained with each device showed a good linearity between the methods (Figures 3.1 and 3.2). For O_2 and CO_2 concentration, the correlation coefficients obtained were higher than 0.99. This way, the Dansensor equipment presented high accuracy through the repeatability, intermediate precision, and reproducibility of the data measurements from samples. According Ribanni et al.[6] the accuracy of the analytical method provides information on the similarity of results, that is, it measures the method's repeatability.



(a) (b)

Figure 3.1: Relation between O_2 concentration measurements by gas chromatography and gas analyzer. The ratio for physalis is shown in (a) and for cherry tomato in (b).

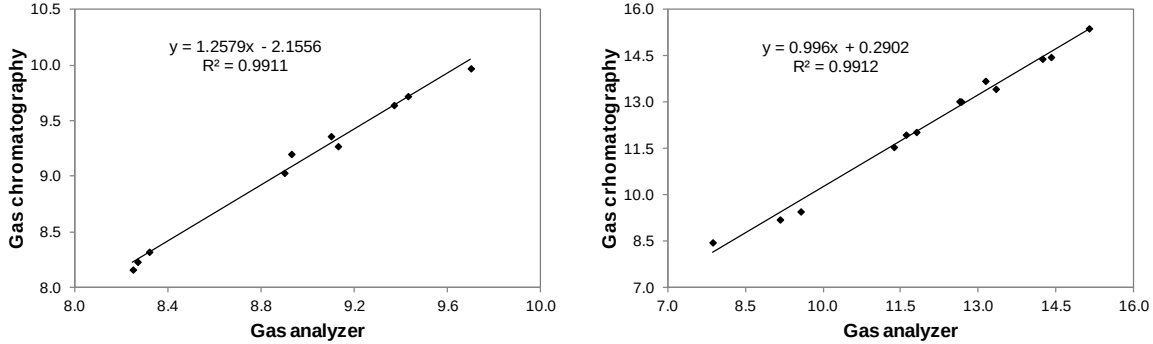


Figure 3.2: Relation between CO_2 concentration measurements by gas chromatography and gas analyzer. The ratio for physalis is shown in (a) and for cherry tomato in (b

Table 3.3 shows the values of Spearman correlation for O_2 and CO_2 concentration obtained by CG and Dansensor equipament for samples of physalis and tomatoes stored under modified atmosphere. A correlation coefficient higher than 98% was obtained, indicating that the use of the gas analyzer provides reliable results for the measurement of the concentration of oxygen and carbon dioxide inside the packaging.

Table 3.3: Sperman correlation analysis for O_2 e CO_2 in modified atmosphere packages.

Parameter	Sperman correlation	
	Physalis	Cherry tomato
O_2	0.9860	0.9969
CO_2	0.9890	0.9878

3.4. Conclusion

The results obtained in this study showed that the Dansensor gas analyzer is a reliable tool when compared to gas chromatography. The results obtained with the two methods showed good linearity and correlation index higher than 98%, confirming that the use of the gas analyzer provides reliable results for oxygen and carbon dioxide measurement. The Dansensor gas analyzer can be applied perfectly in the laboratory routine as a fast, safe, and low cost tool for O_2 and CO_2 analyses in food packaging.

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CAPÍTULO 4:
EFFECT OF ACTIVE MODIFIED ATMOSPHERE ON THE MAINTAINING POSTHARVEST OF PHYSICO-CHEMICAL CHARACTERISTICS OF CHERRY TOMATO

Effect of active modified atmosphere on the maintaining postharvest of physico-chemical characteristics of cherry tomato

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Abstract

The effects of active modified atmosphere packaging (MAP) on the postharvest quality of cherry tomatoes stored at cold temperature (5 °C) and bioriented polypropylene/low-density polyethylene (BOPP/LDPE) were investigated. Four different atmospheres were tested: synthetic air (control), 5% O_2 + 95% N_2 (MAP 1), 5% O_2 + 5% CO_2 + 90% N_2 (MAP 2) and 5% O_2 + 10% CO_2 + 85% N_2 (MAP 3). MAP 1 showed growth of fungi preventing the analysis from 20 days storage. The control and MAP 3 showed similar results to the increased mass loss and texture of the samples. MAP 2 inhibited the increase of respiration rate, delayed the weight loss, and the formation of red color. Through this gas concentration it was possible to maintain the firmness and delay changes in pH and soluble solids content. Therefore, the combination of MAP 2 treatment and low temperature was effective with regard to delaying the maturity and the quality of the fruit during the storage of cherry tomatoes. The results showed that active MAP could extend the shelf-life of cherry tomatoes for 25 days and the gas concentration could influence the physic-chemical characteristics of cherry tomatoes.

Keywords: cherry tomatoes, modified atmosphere package (MAP), postharvest quality

4.1. Introduction

The tomato is one of the most popular vegetables worldwide. The demand for fresh market tomatoes has led to procedures that prolong storage of tomato fruit, allowing long-distance shipping (BOUKOBZA; TAYLOR, 2002). Tomato fruit has been a relatively short postharvest life and, during fruit ripening, many processes that affect quality takes place (HOEBERICHTS et al., 2002).

Prolonging the freshness or shelf life of fruits and vegetables after harvest is important for ensuring a safe and nutritional diet at an affordable cost. Low temperature storage is a major means of preserving freshness of post-harvest produce. However, storage under reduced O_2 and elevated CO_2 partial pressures can provide an additional means of reducing metabolic activity and increasing shelf life (ZAGORY; KADER, 1988; LEE et al., 1995). Modified-atmosphere packaging consists in altering the normal composition of air to provide an atmosphere for decreasing the respiration rate of the product, preserving its quality and increasing its shelf-life (FARBER et al., 2003). This can be achieved by using active or passive modified-atmosphere packaging by the interaction between two processes; the respiration rate of the produce and the transfer of gases through the packaging material, with no further control exerted over the initial gas composition (KADER; WATKINS 2000; FARBER et al., 2003; MAHAJAN et al., 2007). However, in MAP, these two processes are dependent on many other factors such as film thickness and surface area, product weight, free space within the pack, and temperature (CHARLES et al., 2003; SANDHYA, 2010). For instance, a limited volume of headspace in the package could lead to an increase in resistance to gas diffusion. Also, metabolic processes such as respiration rate and various endogenous enzymatic and film permeability increases with the increase in temperature (SANDHYA, 2010). Active modification occurs by the displacement of gases in the package, which are then replaced by a desired mixture of gases. This involves the addition of active agents into the packaged food product, such as oxygen and carbon dioxide scavengers, carbon dioxide, ethylene and water vapor removals and aroma releasing compounds (CHURCH, 1994; PHILLIPS, 1996; SANDHYA, 2010). On the other hand, passive modification occurs when the product is packaged using a selected film type, and a desired atmosphere develops naturally as a consequence of product respiration and diffusion of gases through the film (MOLEYAR; NARASIMHAM,

1994). Respiration is a metabolic process that provides the energy for the biochemical processes of fruits and vegetables. Aerobic respiration consists of oxidative breakdown of organic reserves (including carbohydrates, lipids and organic acids) to simpler molecules, including CO_2 and water, with release of energy; consuming O_2 in a series of enzymatic reactions. Senescence begins as the stored starch and sugar is consumed; the rate of substrate consumption is simply determined by the rate of respiration. Consequently, shelf life is inversely related to the respiration rate (FARBER et al., 2003). Two of the most important factors in determining respiration rate during postharvest storage are temperature and gas composition. Respiration is widely assumed to be slowed down by decreasing available O_2 as a consequence of reduction of overall metabolic activity (KADER, 1986; SOLOMOS; KANELLIS, 1989). Normally, in modified-atmosphere packages the concentration of O_2 is kept low (1–5%) to reduce the respiration rate of fruits and vegetables, which prolongs the shelf-life of the products (FONSECA et al., 2002). However, at excessively low O_2 levels <1% anaerobic respiration may occur, resulting in tissue deterioration and production of off-flavors and off-odors (LEE et al., 1995; AUSTIN et al., 1998; ARES et al., 2007). Carbon dioxide is the only gas used in MAP that confers a significant level of antimicrobial influence on the product. Microbial growth is reduced when there is a high concentration of carbon dioxide in the products due to an increased lag phase and generation time during the log phase of microbial growth (PHILLIPS, 1996).

A major problem with storage and marketing of cherry tomatoes is their relatively fast deterioration in quality and short shelf-life. Many studies have been developed in order to find technology that reduces the respiratory rate and the process of senescence of tomatoes. Sabir and Agar (2011) studied the effects of 1-methylcyclopropene (1-MCP), modified atmosphere packaging and the combination of the two used to store and maintain quality of tomatoes. These authors verified that MAP with and without 1-MCP, reduced weight loss and maintained elasticity compared to the control and 1-MCP alone. Artés et al. (1999) evaluated the effects of calcium chloride washings and passive or active modified atmosphere packaging (MAP) on maintenance of quality of fresh-cut tomatoes and verified that active MAP should be used for maintaining fresh-cut tomatoes when stored at 10 ℃. Akbudak et al. (2012) evaluated the effect of antifungal and passive modified atmosphere packaging on the quality of cherry tomatoes and found that

these treatments were effective with regard to fruit quality in cherry tomatoes. Odriozola-Serrano et al. (2008) studied the feasibility of minimal processing and modified atmosphere packaging (5% O_2 + 5% CO_2) to preserve color attributes and bioactive compounds of fresh-cut tomatoes and observed that fresh-cut tomatoes maintained the main antioxidant compounds and color parameters for 21 days at 4 °C.

However, there has been limited information available on the use of active modified atmosphere in respiration rate and delaying ripening processes, including influence of firmness, loss weight and color during storage of cherry tomatoes. Therefore, the aim of this study was to determine the most suitable active modified atmosphere which is also more efficient in reducing respiratory rate and senescence processes that occur during the post-harvest storage of cherry tomatoes.

4.2. Materials and methods

4.2.1. Plant material and storage conditions

Cherry tomatoes (*Lycopersicon esculentum* L.) used in the experiments were commercially grown and collected in the Florianópolis area (Brazil) and stored for up to 24 hours at 5 °C until use. Before each experiment, the cherry tomatoes were selected by uniform size, color and physical integrity. Samples were washed in running water and sanitized in a 0.5 ppm ozonized solution for 1 min, then air-dried at room temperature. Afterwards, 100 g of the fruit was placed into the bags of bi-oriented polypropylene/low-density polyethylene (BOPP/LDPE). Packages containing the fruit were divided into four batches. Each batch was filled with a different gas composition: synthetic air (control), 5% O_2 + 95% N_2 (MAP 1), 5% O_2 + 5% CO_2 + 90% N_2 (MAP 2) and 5% O_2 + 10% CO_2 + 85% N_2 (MAP 3). The gas composition was injected using a vacuum sealer (200B, Selovac, São Paulo, Brazil), pressure and injection time were 1.1 bar for 12 seconds. Cherry tomatoes inside the bags were stored in temperature-controlled chambers (model ECB-EX, ExpectronTecnologia Industrial Ltda, São José, SC, Brazil) at 5 °C. The samples were assessed at 6, 12, 20 and 25 days. During the storage period, the relative humidity (RH) of the atmosphere ranged from 80 to 85%. Each experiment used three different packages and all experiments were carried out in triplicate. Package characteristics: thickness 75 μm, dimensions 175 x 240 mm,

permeability O_2: 2000 (cm^3/m^2.day), permeability CO_2: 5469 (cm^3/m^2.day) permeability stream: 7 (g/m^2.day).

4.2.2. pH

The pH determination was performed according to AOAC (2005). Cherry tomatoes juice was obtained by compressing the fruit pulp to obtain 30 mL of juice and pH was determined using a digital pH meter (Q400MT, Quimis).

4.2.3 Total soluble solids (TSS)

The total soluble solids content of the samples was determined in the juice of the cherry tomatoes using a digital refractometer (AR 200, Reichert Analytical Instruments), which provides direct measurements in ° Brix, with a resolution of 0.1.

4.2.4. Weight loss

The difference between initial and final fruit weight was considered as total weight loss during each storage interval and calculated as percentages on a fresh weight basis by the standard AOAC (2005) method. The results were expressed in %.

4.2.5. Firmness

The compression force was determined using a digital texture analyzer TAXT2i (Stable Micro System, Surrey, UK) with a 50-N load cell. The experiment was conducted with a 45 mm diameter cylindrical probe and test speed, pre-test and post-test were 1 mm/s, 2 mm/s and 5 mm/s, respectively, the strain used was 10% of tomato. Fifteen fruit for each treatment were randomly selected the results were expressed in N.

4.2.6. Color

The color of the skin cherry tomatoes was measured with a Minolta (Miniscan EZ, Hunterlab, Reston, USA) on 20 fruits per treatment, using the Hunter color parameters, L*, a*, b*, chroma (C) and hue angle (h). Each measurement was taken at three locations for each cherry tomato. A standard white calibration plate was employed to calibrate the spectrophotometer.

4.2.7. Gas concentration in the head-space of the bags and respiration rate

Head-space gases CO_2 and O_2 in the bags were measured using a PBI Dansensor CO_2/O_2 gas analyzer (Checkmate 9900, Ringsted, Denmark) after removal from cold storage. Gas samples were analyzed from three replicates of each sample. The respiration rates of O_2 consumption were obtained from O_2 concentrate. A computer program was developed using the MATLAB® software (Mathworks Inc., USA) to determine the respiration rates using the model proposed by Lee, et al. (1996), according to Equation 1:

$$r_{O2} = -\frac{d[O_2]}{100dt}\left(\frac{V}{m}\right) + \frac{SP_{O2}(0{,}21 - [O_2]/100)p}{mL}$$

where rO_2 is the respiratory rates for O_2 consumption expressed as mL kg^{-1} h^{-1}; $[O_2]$ is the concentrations of O_2, respectively, expressed as %; *L* is the thickness of the film in m; *S* is the area of the bag (m^2); PO_2 is the permeability of the film for O_2, respectively (mL m^{-2} h^{-1} atm^{-1}); t is the time in h; *Vm* is the free volume in the bag (mL); and m is the mass of product in the bag (kg).

4.2.8. Statistical analysis

Statistical analysis was performed using Statgraphics 5.1. (Manugistics Inc., Rockville, MD, USA). Specific differences between means were determined by Fisher's protected least significant difference test (LSD, $P < 0.05$) applied after an analysis of variance (ANOVA).

4.3. Results and Discussion

4.3.1. Gas evolution

Figure 4.1 shows the gas evolution of O_2 and CO_2 inside the package containing cherry tomatoes stored at four different MAPs (control: synthetic air; MAP 1: 5% O_2 + 95% N_2; MAP 2: 5% O_2 + 5% CO_2 + 90% N_2; MAP 3: 5% O_2 + 10% CO_2 + 85% N_2) at 5 °C for 25 days. There was an increase in the CO_2 concentration and reduction in the O_2 concentration for all the atmospheres, due to the respiratory rate of the product. The control showed greater fluctuation of the values of O_2 and CO_2 (19 - 5.4% and 1.9 - 14.0%), possibly due to the greatest amount of O_2 available for the respiration of the tomatoes. Despite the

high respiratory rate values, the final gas concentration in the package did not allow anaerobic respiration to take place. MAP 1 showed a large variation of the concentrations of O_2 and CO_2 (4.6 - 0.69 and 0.22 - 9.2%), the final gas concentration within the package probably allowed the anaerobic respiration and fungal growth. According to KADER (1986) the decrease in O_2 content available for fruits and vegetables reduces the respiratory rate (production CO_2 / consumption O_2), which generally requires at least 1-3% oxygen, depending on the product, to avoid the change from aerobic respiration to anaerobic. When O_2 levels are excessively low (<1%), anaerobic respiration may occur, resulting in tissue deterioration and the production of off-flavors and off-odors (LEE et al., 1995; AUSTIN et al., 1998; ARES et al., 2007). MAP 2 showed the lowest average values of concentration of O_2 and CO_2 (5.0 – 4.33 and 5.0% - 5.76%). There was rapid equilibrium between the respiration product and permeability of the packaging. O_2 and CO_2 concentration at the end of the storage period maintained aerobic respiration. MAP 3 showed average values of O_2 and CO_2 concentration from 5.0 % to 2.5% and from 9.6 % to 11.4%, respectively. Moleyar and Narasimham (1994) studied the behavior of tomatoes stored at temperatures of 10 to 15 °C and found that the optimal conditions for storage of the fruit range from 3 to 5% of O_2 and CO_2.

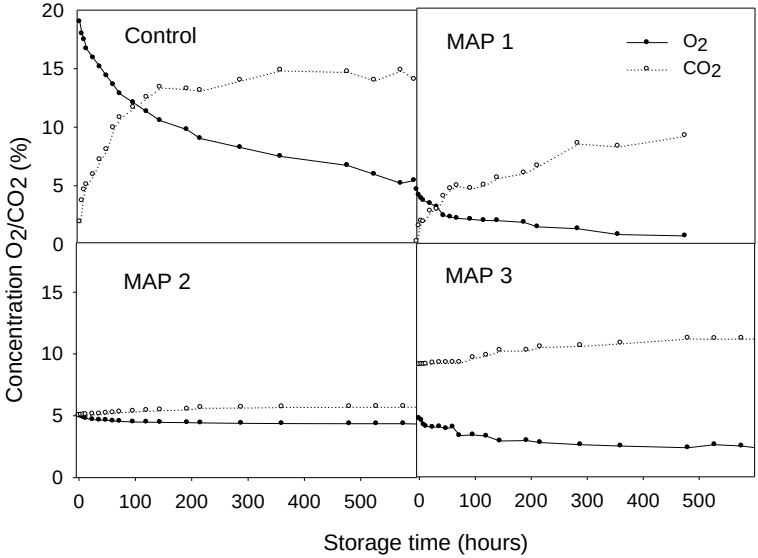

Figure 4.1. Gas evolution of O_2 and CO_2 inside the package containing cherry tomatoes stored four different MAPs (control: synthetic air; MAP 1: 5% O_2 + 95% N_2; MAP 2: 5% O_2 + 5% CO_2 + 90% N_2; MAP 3: 5% O_2 + 10% CO_2 + 85% N_2) at 5 °C by 25 days.

4.3.2. Respiration rate

Fig. 4.2 shows respiration rate based on O_2 consumption of cherry tomatoes stored in four different MAPs at 5 °C for 25 days. Initially, the respiration rate is high for all studied conditions, after a few hours of storage there is a decrease in the available oxygen and a reduction in respiratory rate. The biggest change in the respiratory rate was observed in the samples stored in MAP 1 (10-6.4 mLO_2 / Kg.h), probably due to the lack of CO_2 becoming more favorable to the development of fungi which resulted in increased respiratory rate. The second major change in respiratory rate was observed for samples packaged in the control from 8.4 to 5.3 mLO_2 / Kg.h, possibly due to the amount of O_2 available for respiration of the tomatoes. MAP 2 presented the lowest respiration rate between the atmospheres tested (6.6 - 5.8 mLO_2/ Kg.h). For this atmosphere the equilibrium between the respiratory rate and permeability of the package was reached earlier. MAP 3 showed intermediate respiratory rate (7.5 -6.3 mLO_2 / Kg.h),

lower than MAP 1 but higher than MAP 2. Similar values (8.5-5.5 mL O_2/kg.h) were found by Goyette et al. (2012) evaluating the respiratory rate of tomatoes stored in chambers for 450 hours at 13 °C.

The results of this study for the respiration rate are consistent with behavior found for weight loss, gas evolution and texture. During fruit ripening, depolymerization or shortening of the chain length of pectin occurs with increased activity of pectinesterase and poligalactronase. Low concentrations of oxygen and high concentrations of carbon dioxide reduce the activities of these enzymes and allows the retention of firmness of vegetables during storage (SALUNKHE et al.,1991). Krammes et al. (2003) evaluated the rate of CO_2 production for the tomato cultivar Santa Clara, packed in jars, removed from air for 18 days, observing the reduction in respiratory rate with storage time.

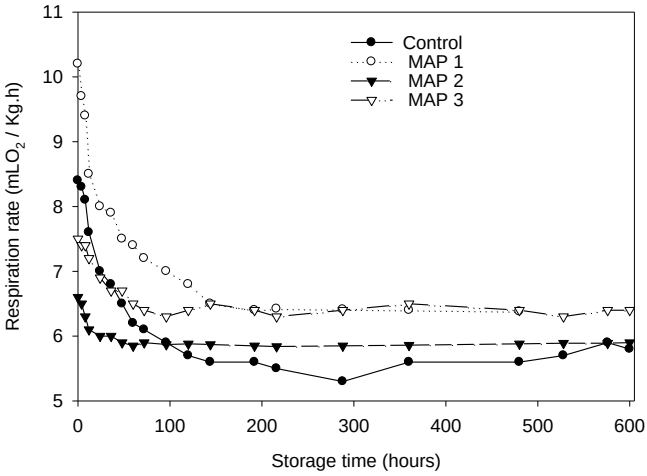

Figure 4.2. Respiration rate based in O_2 consumption of cherry tomatoes stored in four different MAPs (control: synthetic air; MAP 1: 5% O_2 + 95% N_2; MAP 2: 5% O_2 + 5% CO_2 + 90% N_2; MAP 3: 5% O_2 + 10% CO_2 + 85% N_2) at 5 °C by 25 days.

4.3.3. pH

Table 4.1 shows the results of the pH of the cherry tomatoes in the four different MAPs. The results showed the same behavior for all the studied atmospheres. However, for MAP 1, the gas composition 5% O_2 + 95% N_2 presented fungal growth on the 20 day of storage, probably

due to the absence of CO_2 in this atmosphere. These samples were considered until 20 days of storage.

The pH values from the first to the last day of storage ranged from 0.17, 1.07, and 0.11 to control, MAP 1, and MAP 3 respectively, while MAP 2 had varied 0.06. MAP 2 showed less change during storage, compared to the other atmospheres. In the control, composed by 21% O_2, it is possible that the tomatoes respired more because of the amount of O_2 available, leading to larger changes in the pH of the product. Also, the amount of CO_2 (10%) present in MAP 3 could have contributed to the significant changes in pH, because some products are sensitive to CO_2.

According to Sandhya (2010) some products are sensitive to CO_2, these include the tomato. According to Siriphanich and Kader (1986), high concentrations of CO_2 may limit the power supply required for tissue survival. Thus, where aerobic respiration is dramatically reduced, the plant tissue anaerobic respiration increases to increase the level of available energy (PEPPELENBOS, 1996). It is desirable for vegetables that the pH remains below 4.5 to prevent the growth of microorganisms in the product. Typically tomatoes have sufficient acidity to maintain a pH below 4.6 and, accordingly, are not classified as a low acid food. Because of this, the tomatoes do not require the more drastic treatments required of low acid foods classified for the destruction of spoilage microorganisms, to ensure food safety (ANTHON and BARRETT, 2012). The decrease in pH that occur with maturity and over-maturity are due to the loss of citric acid (ANTHON et al., 2011).

4.3.4. Total soluble solids

Table 4.1 shows the results of total soluble solids (TSS) of cherry tomatoes stored in four different MAPs at 5 °C for 25 days. The results show an increase with further reduction in values of TSS during the storage of the cherry tomatoes, for all atmospheres. Samples stored in MAP 1 showed higher variation in values, probably due to the proliferation of fungi (range 1.97 Brix).

Among the gases used in modified atmosphere, CO_2 is the most important gas for reducing microbial growth because it has a direct and significant antimicrobial activity, acts in altering the cell membrane of the microorganism, hinders the absorption of nutrients, and inhibits the enzymatic reactions leading to intracellular pH changes and changes in

physico-chemical properties of proteins (FARBER, 1991). The CO_2 also prevents or delays the damaging effects of ethylene on fresh fruits and vegetables, such as loss of firmness and incidence of physiological disorders (KADER, 1986). However, some products may be more sensitive to higher concentrations of CO_2: MAP 3 containing 10% CO_2 showed results near to those obtained for the control. MAP 2 showed the smallest changes when compared to the other atmospheres (0.67 °Brix). These results indicate that modified atmosphere can reduce the respiratory rate, slowing the metabolic processes that alter physical and chemical parameters such as TSS. Similar results have been reported by Guillén et al. (2006) who evaluated cherry tomatoes stored at 10 °C for 28 days. Arts et al. (1999) evaluated the chemical qualities such as pH, TSS and concluded that the use of passive and active atmosphere processes delayed ripening of tomatoes stored at 2 and 10 °C for 10 days.

Table 4.1: pH and TSS values of storage cherry tomato in four different MAPs (Control: synthetic air; MAP 1: 5% O_2 + 95% N_2; MAP 2: 5% O_2 + 5% CO_2 + 90% N_2; MAP 3: 5% O_2 + 10% CO_2 + 85% N_2) at 5 °C by 25 days

Storage time (days)	Control	MAP 1	MAP 2	MAP 3
		pH		
0	3.55±0.02 a	3.51±0.03 a	3.55±0.02 a	3.59 ±0.01 a
6	3.58±0.01 b	3.90±0.00 b	3.55±0.01 a	3.68 ±0.01 b
12	3.66±0.02 c	4.09±0.02 c	3.50±0.01 a	3.69 ±0.01 bc
20	3.72±0.02 d	4.98±0.01 d	3.61±0.01 b	3.70 ±0.00 c
25	3.62±0.03 c		3.58±0.02 ab	3.67 ±0.01 b
		TSS		
0	4.93±0.06 a	4.00±0.17 a	4.93±0.06 a	5.83±0.15 a
6	5.87±0.06 b	5.97±0.12 c	5.60±0.17 b	6.87±0.06 b
12	5.77±0.06 c	5.80±0.00 c	5.40±0.30 c	6.83±0.15 b
20	4.73 ±0.15 d	4.60±0.26 b	4.80±0.10 d	5.70±0.10 c
25	4.37±0.06 e		4.70±0.10 e	4.82±0.08 d

Control = synthetic air; MAP 1= 5% O_2 + 95% N_2; MAP 2: 5% O_2 + 5% CO_2 + 90% N_2; MAP 3: 5% O_2 + 10% CO_2 + 85% N_2. [z] Means in columns with different letters are significantly different according to Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

4.3.5. Color

Table 4.2 shows the color parameters of the cherry tomatoes under different modified atmospheres and stored at 5 °C for 25 days. The Lightness (L) and a* parameter showed decreased and increased differences respectively, these changes indicate a significant increase in red color of tomatoes in all MAPs during the storage period.

MAP 1 samples showed fungal growth making it impossible to continue with the analysis after 20 days of storage. These samples showed pronounced and significant changes in the parameters L and a*. MAP 2 showed less variation in values between the first and last day of

analysis (L: 5.8 and a*: 2.30), while MAP 3 (L: 7.03 and a*: 3.61) showed changes similar to the control (L: 8.70 and a*: 4.30). The difference between the chroma (C) values during storage of samples can indicate slight saturation of red color of samples.

Ali et al. (2004) found increased values of chroma (C) 27.87 to 46.67 of cherry tomatoes stored without packaging for 15 days at 15 °C. In this study increase in chroma was lower, indicating that MAP can delay changes in color of tomatoes by reducing the respiration rate and ethylene production. Ethylene acts on chlorophyll degradation that causes discoloration of the green color of the fruits and vegetables. In this study, the low temperatures reduced the ethylene production and consequently the formation of the red color of the tomato stored at 5 °C. The maintenance of greener skin color on fruit can be explained by the reduction of ethylene action on the process of chlorophyll degradation (JIANG et al., 1999). Martínez-Romero et al. (2009) evaluated tomatoes stored at 8 °C for 28 days. They observed an increase in red color in both peel and pulp samples. Odriozola-Serrano et al. (2008) evaluated tomatoes stored for 14 days at 5 °C under a modified atmosphere and verified that no significant changes occurred for the values L and h.

Table 4.2: Color values of storage cherry tomato in four different MAPs (control = synthetic air; MAP 1= 5% O_2 + 90% N_2; MAP 2: 5% O_2 + 10% CO_2 + 90% N_2; MAP 3: 5% O_2 + 10% CO_2 + 90% N_2) at 5 °C by 25 days.

Storage Time (days)	Control					MAP1				
	L	a*	b*	C	h	L*	a*	b*	C	h
0	30.9±2.0a	19.0±2.1a	19.6±0.4a	27.4±1.0a	45.8±2.2a	25.9±3.3a	23.0±1.3a	26.1±3.5a	30.5±2.4a	45.6±3.3a
6	27.3±1.8ab	18.1±1.0a	19.3±1.9a	28.8±1.2ab	41.9±3.1a	23.4±2.0ab	25.3±3.9ab	23.9±1.6b	34.0±3.8b	44.9±3.0a
12	26.2±1.7b	22.7±0.2b	21.3±0.5a	28.8±0.8ab	45.4±0.9a	21.7±0.5ab	29.5±0.6ab	22.6±0.6b	43.8±0.8c	45.8±2.0a
20	25.0±1.7b	21.9±1.1ab	22.2±1.6ab	30.0±3.9ab	48.1±2.4ab	18.3±1.4c	33.3±2.1b	33.3±2.6c	54.6±3.3d	44.5±0.8a
25	22.2±1.1c	23.3±0.4b	23.5±1.0b	31.7±3.0b	50.6±1.8b					
	MAP 2					MAP 3				
0	27.8±2.0a	19.1±1.6a	19.7±2.4a	23.4±1.9a	43.6±2.1a	30.2±2.9a	17.6±0.2ab	29.5±2.1a	34.4±2.2a	44.5±0.4a
6	26.8±2.1ab	17.1±2.2a	18.9±1.9a	25.7±1.7a	48.0±2.6a	27.7±1.6b	16.2±0.8b	27.2±2.0b	33.4±2.9a	47.9±0.5bc
12	24.9±2.4ab	19.0±3.2a	17.3±1.3a	25.8±1.3a	42.5±3.6a	24.6±3.3c	17.1±2.9ab	32.6±2.3c	38.3±0.5b	46.4±0.5b
20	23.6±1.1b	20.5±1.6ab	21.9±3.1a	27.4±2.1a	48.4±2.1a	23.9±2.2c	19.7±1.8bc	29.4±2.5a	34.1±0.7a	47.8±1.4bc
25	22.0±2.3b	21.4±1.4b	21.6±2.7a	27.8±1.8a	46.1±2.5a	23.2±0.9c	21.2±0.1c	32.1±1.5c	37.4±1.1b	49.1±0.5c

Control = synthetic air; MAP 1= 5% O_2 + 90% N_2; MAP 2: 5% O_2 + 5% CO_2 + 90% N_2; MAP 3: 5% O_2 + 10% CO_2 + 90% N_2. [z] Means in columns with different letters are significantly different according to Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA

4.3.6. Weight loss

Figure 4.3 shows the loss weight (%) of cherry tomatoes stored under different MAPs. Weight loss increased with storage time for the four atmospheres tested. However, in all modified atmospheres the weight loss was very small. The low water vapor transmission rate of LDPE / BOPP films (7g/m^2.dia), combined with the transpiration rate of cherry tomatoes, can have developed the saturated condition in the packages, which was responsible for the small weight loss. So, the positive effects of storage of fresh pre-climacteric fruits in sealed plastic films may be, in certain cases, the combination of its effects on the O_2 and CO_2 content within the fruit and the maintenance of high moisture content.

MAP 2 had the lowest mass loss during storage (0.18%), indicating that this modified atmosphere is suitable for reducing the mass loss of the cherry tomatoes. MAP 3 showed weight loss values very close to the control 0.28% and 0.26% respectively, showing that modified atmosphere containing 10% CO_2 does not reduce water loss when compared to tomato storage in synthetic air. Sandhya (2010) states that the most appropriate atmosphere for the storage of tomatoes is 5 % O_2 and 0 % CO_2, but the results this study showed was that 0% CO_2 can cause fungal growth due to the fungicidal property of CO_2.

MAP 1 showed the greatest weight loss (0.26% in 20 days) when compared to other atmospheres probably due to fungal growth. Van Dijk et al. (2006) evaluated tomatoes stored at different temperatures and noted that the mass loss of the samples depends on temperature, these authors observed a weight loss of about 6% for samples stored at 25 °C for 30 days. Akbudak et al. (2012) studied the effects of pre-harvest harpin (H) and modified atmosphere packaging (MAP) on the storage and fruit quality of cherry tomatoes and observed that atmospheres surrounding the fruits were a good barrier for moisture transfer.

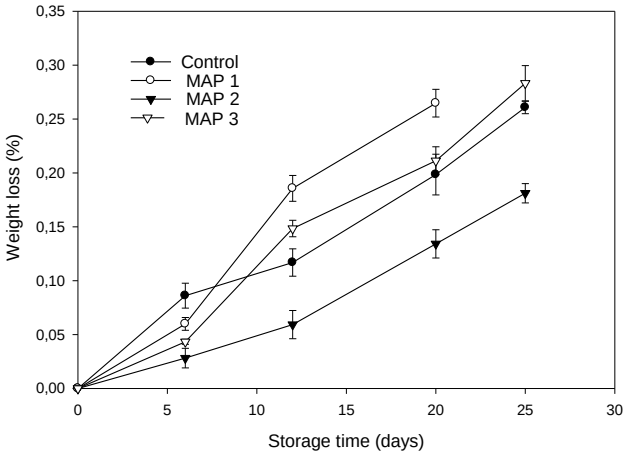

Figure 4.3. Loss weight (%) of cherry tomato in four different MAPs (control: synthetic air; MAP 1: 5% O_2 + 90% N_2, MAP 2: 5% O_2 + 10% CO_2 + 90% N_2; MAP 3: 5% O_2 + 10% CO_2 + 90% N_2) storage at 5 °C by 25 days.

4.3.7. Texture

Figure 4.4 shows the compression force (N) of cherry tomatoes in four different MAPs stored at 5 °C for 25 days. Compressive force values of the fruits decreased with the storage time for all atmospheres tested. MAP 1 had the highest average values of compressive force (6.11-2.59 N in 20 days of storage), this marked variation was possibly due to the growth of fungi in samples conditioned in this modified atmosphere. The control and MAP 3 presented close variations: 6.71-4.61 N and 6.58-4.71 N, respectively, indicating that both an atmosphere containing atmospheric air or an atmosphere with 10% CO_2 act similarly in reducing the loss of texture of the product. The samples stored in MAP 2 showed the smallest reduction of compressive force during storage (6.05-4.86 N) indicating that this is the best atmosphere to maintain the texture of cherry tomatoes at 5 °C. These results are consistent with the results for weight loss from the product, where MAP 1 presented highest weight loss and MAP 2 the lowest weight loss among the studied atmospheres.

Thompson (1998) and Kuenwoo et al. (2000) showed in their studies for tomatoes storage under MAP, that the lower the concentration of CO_2 inside the package, the better the fruit firmness. The fruit softening occurs due to deterioration of the cell wall and intracellular material (SEYMOUR et al., 1993). According to Errington et al. (1997), the ripening of tomatoes is accompanied by significant degradation pectins of the cell wall. This degradation is partly due to the action of hydrolytic enzymes such as polygalacturonase. Tomatoes are very prone to water loss, leading to softening of the product during ripening (MENCARELLI and SALTVEIT, 1988). However, the use of a modified atmosphere can extend the shelf life of fresh produce, by reducing water loss, metabolic activities and browning of the surface of the product (GORNY, 1997).

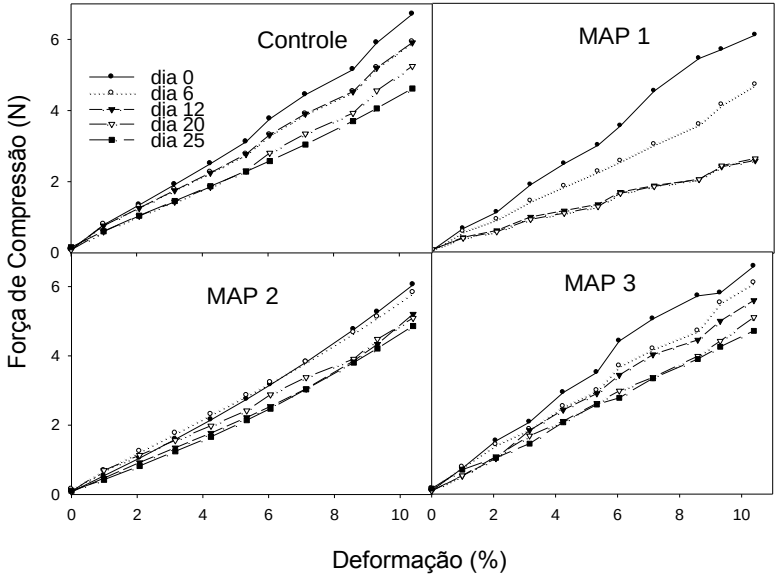

Figure 4.4. Compression force (N) of cherry tomato in four different MAPs (control: synthetic air; MAP 1: 5% O_2 + 95% N_2; MAP 2: 5% O_2 + 5% CO_2 + 90% N_2; MAP 3: 5% O_2 + 10% CO_2 + 85% N_2) storage at 5 °C by 25 days.

4.4. Conclusions

This study showed senescence inhibition of cherry tomatoes by effect of different MAPs (control: synthetic air; MAP 1: 5% O_2 + 95% N_2; MAP 2: 5% O_2 + 5% CO_2 + 90% N_2; MAP 3: 5% O_2 + 10% CO_2 + 85% N_2). MAP 2 showed minor changes in the pH and TSS indicating that this atmosphere may be able to reduce changes in chemical properties which can alter the taste of the product. This same atmosphere reduces changes in physical parameters such as weight loss and texture. Consequently the cherry tomatoes stored under MAP 2 showed the lowest respiration rate. Thus, it is concluded that for storage at 5 °C the best gas composition for cherry tomatoes comprised 5% O_2 + 5% CO_2 balanced with N_2,. MAP 2 could also be applied to other commercially important tomato cultivars to evaluate the performance of this MAP. Moreover, new research should focus on the evaluation of MAP in combination with other alternative methods as part of an integrated strategy for commercial control of senescence of tomatoes during storage.

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CAPÍTULO 5:
EVALUATION OF DIFFERENT PACKAGES AND ACTIVE MODIFIED ATMOSPHERE IN THE QUALITY POSTHARVEST OF THE CHERRY TOMATO

Evaluation of different packages and active modified atmosphere in the quality postharvest of the cherry tomato

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Abstract

Fresh food products are more susceptible to decay because of increase in the respiration rate after harvesting. The respiration of fresh fruits and vegetables can be reduced by many preservation techniques. Modified atmosphere packaging (MAP) technology is largely used for vegetable raw materials. The aim of this work was to evaluate the influence of the packaging and the active modified atmosphere (MAP) on the cherry tomatoes stored at 10 °C. The tomatoes were packed under synthetic air (control) and in the modified atmosphere: 5% O_2 + 5% CO_2 + 90% N_2 (MAP 1) and 5% O_2 + 10% CO_2 + 85% N_2 (MAP 2). Four types of packaging were tested and the best packaging was bi-oriented polypropylene/low-density polyethylene (BOPP/LDPE). The MAP 1 presented the lower respiration rate, lower loss weight, and delayed the formation of red color. Through this gas concentration was possible keep the firmness, and delayed changes in pH and soluble solids content. Therefore, the combination of the modified atmosphere 5% O_2 + 5% CO_2 + 90% N_2 and low temperature showed more effective with regard to delaying the maturity along the storage and fruit quality in cherry tomatoes.

Keywords: cherry tomatoes, package, modified atmosphere package (MAP)

5.1. Introduction

The use of modified atmosphere packaging (MAP) in postharvest preservation of fruits and vegetables is one important technique to reduce losses and maintain quality, considerably extending the effect of low temperature storage. High relative humidity in the atmosphere surrounding the product, low O_2, and high CO_2 concentrations in the package can potentially reduce respiration rate, ethylene sensitivity, and production, as well as decay and physiological changes such as oxidation, with the resulting benefit of extending product life (SALVADOR et al., 2002). Optimum MAP can be achieved by using polymeric films where the gas transmission rate through the surface area of the film, product respiration and CO_2 and O_2 levels within package at optimum temperature are related by a simple material balance (ESCALONA et al., 2007).

MAP is a passive or active dynamic process of altering gaseous composition within a package. This is achieved by the interaction between two processes; the respiration rate of the product and the transfer of gases through the packaging material, with no further control exerted over the initial gas composition (KADER; WATKINS, 2000; FARBER et al., 2003; MAHAJAN et al., 2007). However, in MAP, these two processes are dependent on many other factors such as film thickness and surface area, product weight, free space within the package and temperature (CHARLES et al., 2003; SANDHYA, 2010). For instance, a limited volume of headspace in the package could lead to an increase in resistance to gas diffusion. Also, metabolic processes such as respiration rate and various endogenous enzymatic, and film permeability increases with increase in temperature.

Active packaging involves the interaction between the package and food product in order to extend the shelf life of it (SANDHYA, 2010). This involves the addition of active agents into the packaged food product, such as oxygen and carbon dioxide scavengers, carbon dioxide, ethylene and water vapor removals and aroma releasing compounds (CHURCH, 1994; PHILLIPS, 1996; SANDHYA, 2010). When selecting an optimum modified atmosphere, information on the respiration rates at different gas compositions is required. A major problem is that the consumption of O_2 and production of CO_2 by the commodities are metabolically interrelated making it difficult to study the effect of one gas independent of the other (SALTVEIT, 2003). Active MAP involves a quick process of gas flushing or gas replacement

or the use of gas-scavenging agents to establish a desired gas mixture within the package (KADER; WATKINS, 2000; CHARLES et al., 2003; FARBER et al., 2003), while avoiding a build up of unsuitable gases. Hence, a decrease in respiration rate delays enzymatic degradation of complex substrates, thereby extending the shelf life of the product. However, at excessively low O_2 levels <1% anaerobic respiration may occur, resulting in tissue deterioration and production of off-flavors and off-odors (LEE et al., 1995; AUSTIN et al., 1998; ARES et al., 2007). Carbon dioxide is the only gas used in MAP that confers a significant level of antimicrobial influence on the product. Microbial growth is retarded at high concentration of carbon dioxide in various products, due to an increased lag phase and generation time during the log phase of microbial growth (PHILLIPS, 1996).

For fresh agricultural products firmness is a quality attribute of prime importance. During growth, ripening and senescence this fruit is like all other plants, continuously subjected to enzymatic modifications (STOLLE-SMITS et al., 1999). During the post-harvest period the expressions of these modifications reflect themselves in changes in fruit firmness, in its chemical composition and in its color (VAN DIJK et al., 2006). The use of atmosphere suitable process can reduce the softening of vegetables. Akbudak et al. (2007) evaluated the effects of hot water treatment (HWT) and modified atmosphere packaging (MAP) on the storage and fruit quality of cherry tomatoes and found that softening proceeded more rapidly, especially for tomatoes stored without modified atmosphere.

A major problem with storage and marketing of cherry tomato is its relatively fast deterioration in quality and short shelf-life. Many studies have been developed in order to find a technology that reduces the respiratory rate and the process of senescence of tomatoes. Ali et al. (2010) showed that using 10% gum arabic as an edible coating, delay the ripening process of tomatoes stored at 20 °C, and at shelf life can be extended up to 20 days without any spoilage and off-flavor. Sabir and Agar (2011), studying the effects of 1-methylcyclopropene (1-MCP), modified atmosphere packaging and their combination, on storage and quality maintenance of tomatoes, verified that MAP with and without1-MCP reduced weight loss and maintained to elasticity compared with control and 1-MCP alone. Akbudak et al. (2012) evaluated the effect of pre-harvest plant bioactivator and passive modified atmosphere

packaging on quality of cherry tomato, and found that these treatments were effective with regard to fruit quality in this product.

However, there has been limited information available on the use of active modified atmosphere in respiration rate and delaying ripening processes, including the influence of this on the firmness, weight loss and color during storage of cherry tomatoes. Therefore, the aim of this study was to determine the most suitable packaging for respiration of cherry tomato and active modified atmosphere for maintenance of physicochemical characteristics of cherry tomatoes during the post-harvest storage during 20 days at 10 °C.

5.2. Materials and Methods

5.2.1. Definition of the best package

To determine the most appropriate packing for cherry tomatoes four types of packaging were testes, as shown in Table 5.1. Samples of 100 g of tomato were packed in vacuum sealer (200B, Selovac). The tests were performed in modified atmosphere (5% O_2, 10% CO_2 and 85% N_2) and stored at 10 °C. To determine the best packing the samples were analyzed overtime to reach equilibrium in hours and the concentration of gases in equilibrium was determined. The equilibrium concentration in the gas was determined by the reading of three consecutive points without varying concentrations of gases (O_2 and CO_2) using a gas analyzer (PBI Dansensor, CheckMate II) with a zircon detector and detection limit from 0 to 100% of O_2 and CO_2. A 1 mL gas aliquot was removed from inside the bags using the syringe attached to the equipment.

Table 5.1: Specifications of the package.

Package	Thickness (μm)	Dimensions (mm)	Permeability		
			O_2 (cm^3/m^2.day)	CO_2 (cm^3/m^2.day)	Stream (g/m^2.day)
BOPP/LDPE [1]	75	175 x 240	2000	5469	7
PE/PA [2]	90	175 x 240	< 65	-	< 5
PE/PA/EVOH [3]	90	175 x 240	< 3	-	< 5
EVA [4]	56	175 x 240	4500	-	45

[1] BOPP – bi-oriented polypropylene and LDPE - low density polyethylene;

[2] PE - packing up to 7 layers extruded polyethylene-based and PA - copolymer of polyamide (PA);
[3] Film extruded in 7 layers based on polyethylene (PE), polyamide copolymer (PA) and ethylene vinyl alcohol (EVOH).
[4] EVA - ethylene and vinyl acetate.

5.2.2. Raw material and storage under modified atmosphere

Cherry tomatoes (*Lycopersicon esculentum* L.) used in the experiments were commercially grown and collected in the Florianópolis area (Brazil) and stored up to 24 h at 5 °C until use. Before each experiment, cherry tomatoes were selected of uniform size, color and physical integrity. Samples were washed in running water and sanitized in a 0.5 ppm ozonized solution during 1 min, then the fruits were air-dry at room temperature. After determining the best packaging for the storage of the tomatoes, 100 grams of cherry tomatoes were place into the bags. The packages containing the fruit were divided into three batches, the batch was filled with synthetic air (Control), 5% O_2 + 5% CO_2 + 90% N_2 (MAP 1) and 5% O_2 + 10% CO_2 + 85% N_2 (MAP 2). The gas composition was injected using a vacuum sealer (200B, Selovac, São Paulo, Brazil) equipment, pressure and injection time were 1.1 bar for 12 seconds. Cherry tomatoes inside the bags were stored in temperature-controlled chambers (model ECB-EX, ExpectronTecnologia Industrial Ltda, São José, SC, Brazil) at 10 °C. The samples were assessed in 6, 12 and 20 days. During the storage period, the relative humidity (RH) of the atmosphere ranged from 80 to 85%. Each experiment used three different packages and all experiments were carried out in triplicate.

5.2.3. Physical-chemical parameters

5.2.3.1. pH

The pH determination was performed according to AOAC (2005). Cherry tomatoes juice was obtained by compressing the fruit pulp to obtain 30 mL of juice and pH was determined using a digital pH meter (Q400MT, Quimis).

5.2.3.2.Total soluble solids (TSS)

The total soluble solids content of the samples was determined in the juice of the cherry tomatoes using a digital refractometer (AR

200, Reichert Analytical Instruments), which provides direct measurements in ° Brix, with a resolution of 0.1.

5.2.3.3. Weight loss

The difference between initial and final fruit weight was considered as total weight loss during each storage interval and calculated as percentages on a fresh weight basis by the standard AOAC (2005) method. The results were expressed in %.

5.2.3.4. Firmness

The compression force was determined using a digital texture analyzer TAXT2i (Stable Micro System, Surrey, UK) with a 50-N load cell. The experiment was conducted with a 45 mm diameter cylindrical probe and test speed, pre-test and post-test were 1, 2 and 5 mm/s, respectively, the strain used was 10% of tomato. Fifteen fruit for each treatment were randomly selected and the results were expressed in grams.

5.2.3.5. Color

The color of the skin cherry tomatoes was measured with a Minolta (Miniscan EZ, Hunterlab, Reston, USA) on 20 fruits per treatment, using the Hunter color parameters, L*, a*, b*, chroma (C) and hue angle (h). Each measurement was taken at three locations for each cherry tomato. A standard white calibration plate was employed to calibrate the spectrophotometer.

5.2.3.6. Head-space gas and respiration rate

Head-space gases CO_2 and O_2 were measured using a PBI Dansensor CO_2/O_2 gas analyzer (Checkmate 9900, Ringsted, Denmark) after removal from cold storage. Gas samples were analyzed from three replicates of each sample. The respiration rates of O_2 consumption and CO_2 evolution were obtained from a CO_2 and O_2 concentrate. A computer program was developed using the MATLAB® software (Mathworks Inc., USA) to determine the respiration rates using the model proposed by Lee et al. (1996), according to Equation 1:

$$r_{O2} = -\frac{d[O_2]}{100dt}\left(\frac{V}{m}\right) + \frac{SP_{O2}(0{,}21 - [O_2]/100)p}{mL} \quad (1)$$

Where: rO_2 is the respiratory rates for O_2 consumption expressed as mL kg^{-1} h^{-1}; $[O_2]$ is the concentrations of O_2, expressed as %; *L* is the thickness of the film in m; *S* is the area of the bag (m^2); PO_2 is the permeability of the film for O_2 and CO_2, respectively (mL m^{-2} h^{-1} atm^{-1}); t is the time in h; *Vm* is the free volume in the bag (mL); and m is the mass of product in the bag (kg).

5.2.4. Statistical analysis

Statistical analysis was performed using Statgraphics 5.1. (Manugistics Inc., Rockville, MD, USA). Specific differences between means were determined by Fisher's protected least significant difference test (LSD, $P < 0.05$) applied after an analysis of variance (ANOVA).

5.3. Results and Discussion

5.3.1. Package for cherry tomatoes

Figure 5.1 shows the behavior of the gaseous atmosphere inside the package containing cherry tomatoes. The samples were stored at 10 °C for 48 hours in packaging ethylene and vinyl acetate (EVA), bi-oriented polypropylene /low density polyethylene BOPP/LDPE, polyethylene (PE)/polyamide copolymer (PA) and PE/PA/ethylene vinyl alcohol (EVOH), under an atmosphere of 5% O_2, 10% CO_2 and 85% N_2. After the application of the gas inside the package containing the vegetable, it is expected to occur little reduction in oxygen levels and a slight increase in the concentration of carbon dioxide, due to the respiration of the fruit and the film permeability to gases. For the EVA bags it was observed that there was an increasing on the O_2 concentration and a reduction of the CO_2 concentration. This result indicates that EVA material is more permeable to O_2 and less permeable to CO_2. After 48 hours of storage, the O_2 concentration increased from 5 to 9.9 % and CO_2 concentration reduced from 10 to 2.1 %, which directly influenced on the increased respiratory rate of tomatoes.

Samples packaged in PE/PA/EVOH and PE/PA/PE showed a reduction in the O_2 concentration from 4.93 to 1.56% and from 4.93 to 0.83% and increased amounts of CO_2 from 9.1 to 14.4 and from 9.1 to 15.37%, respectively, indicating that the packages has low O_2 permeability and high permeability to CO_2, may bringing the samples to anaerobiosis. Carbon dioxide can inhibit respiration of the fruit, but at

high concentrations can cause injuries on plants and, depending on the product and the oxygen concentration, can result in the accumulation of ethanol and acetaldehyde in the tissues (KADER, 1986). Sandhya (2010) showed that 2% O_2 level anaerobic respiration can result in the development of off-flavors and off-odors. The storage of fruit and vegetables in concentrations of 5 to 20% CO_2 can cause changes in the activity of specific enzymes in respiratory metabolism, with the effect of uncoupling oxidative phosphorylation. Studies on the effects of concentration of CO_2 in the Krebs cycle intermediates and enzymes, showed that there was accumulation of succinic acid, due to inhibition of succinic dehydrogenase by excess CO_2 (MONNING, 1983). Thus, elevated levels of CO_2 inhibit enzymes in the Krebs cycle inducing the plant to anaerobic respiration.

For samples packed in BOPP/LDPE bags, the gas concentration showed less change during the 48 hours of storage (O_2: 4.93-3.33% and CO_2: 9.1-10.1%), indicating a balance between the permeability of the film and the respiration of the fruit. The concentrations of O_2 and CO_2 were maintained at levels that reduce the respiration rate and prevent anaerobic respiration. Comparing the results for four different types of packaging, in this case, the best packaging suitable for tomatoes is the packaging of BOPP/LDPE. This polymer is present in films and packaging films for different uses, and has great application in food industry. The successful application of the MAP depends on the characteristics of permeability of the package material used. So many studies have been conducted in order to assess the best packing to be used in accordance with the respiratory rate of the product to be conserved. Pretel et al. (2000) evaluated the O_2, CO_2 and C_2H_4 concentrations of three varieties of apricots stored at 10 °C under four plastic films of different permeabilities. The influence of MAP on the sensory characteristics and shelf life of shiitake mushrooms (*Lentinula edodes*) was also studied using LDPE, PP and macro perforated film (ARES et al., 2006). Akbudak, et al. (2007) studied the effects of hot water treatment (HWT) and modified atmosphere packaging (MAP) using two different types of packaging material (50 micropolyethylene (µPE) and 100 µPE), and verified that 50 micropolyethylene (µPE) treatment produced the best result, for storage of cherry tomatoes.

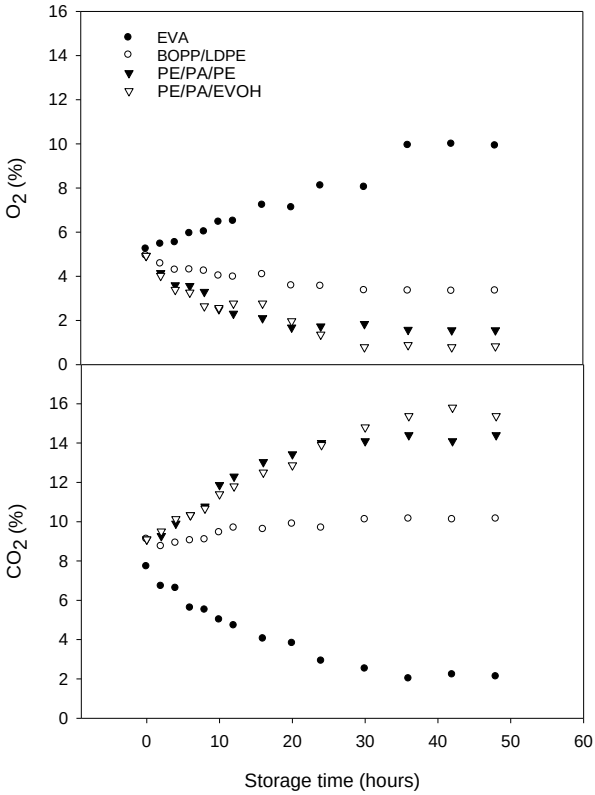

Figure 5.1: Evolution of O_2 and CO_2 concentration for cherry tomatoes in packs of EVA, BOPP/LDPE, PE/PA/PE and PE/PA/EVOH bags, under an atmosphere of 5% O_2 + 10% CO_2 + 85% N_2 for 48 hours at 10 °C.

5.3.2. Physical-chemical parameters of cherry tomatoes packaged in bags of BOPP/LDPE

5.3.2.1. pH

Table 5.2 shows the results of pH for cherry tomato in three different MAPs stored at 10 °C during 20 days. The atmosphere used in the MAP 1 decreased the changes in the pH of cherry tomato as compared with control and MAP 2. The pH values ranged from 0.54 to 0.48 for the control and MAP 2, respectively, while the MAP 1 showed variation of 0.22. As the control is composed by 21% O_2 this possibly increased the respiration rate because the amount of O_2 available, leading to larger changes in the pH of the product, as well as the amount of CO_2 (10%) present in MAP 2 could contributed to the significant changes in pH values. Some plants may be sensitive to higher concentrations of CO_2, accelerating the process of senescence (SANDHYA et al., 2010). Two important quality attributes of processing tomatoes are pH and titratable acidity (TA). The decrease in TA and rise in pH that occurs with maturity and over-maturity are due to a loss of citric acid (ANTHON et al., 2011). Tomatoes typically have sufficient acidity to maintain a pH below 4.6 and, accordingly, are not classified as a low acid food. Because of this, tomatoes do not require drastic treatments as required for foods classified as low acids for the destruction of spoilage microorganisms, to ensure food safety (ANTHON and BARRET, 2012).

Table 5.2: pH and TSS values of storage cherry tomato in three different MAPs (Control: synthetic air; MAP 1: 5% O_2 + 5% CO_2 + 90% N_2; MAP 2: 5% O_2 + 10% CO_2 + 85% N_2) at 10 °C by 20 days.

Storage Time	Control	MAP 1	MAP 2
		pH	
0	3.90±0.01a[z]	3.32±0.04a	3.39±0.00a
6	4.10±0.01b	3.55±0.05b	3.77±0.04b
12	4.24±0.05c	3.49±0.01b	3.81±0.01c
20	4.44±0.01d	3.54±0.01b	3.87±0.02d
		TSS	
0	5.60±0.00a	5.33±0.15a	4.93±0.06a
6	6.53±0.12b	5.60±0.26b	5.80±0.06b
12	5.80±0.00c	4.83±0.12c	4.91±0.02a
20	4.93±0.12d	4.67±0.06c	4.20±0.06c

Control = synthetic air; MAP 1= 5% O_2 + 5% CO_2 + 90% N_2; MAP 2: 5% O_2 + 10% CO_2 + 90% N_2. [z] Means in columns with different letters are significantly different according to Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

5.3.2.2. Total soluble solids

Table 5.2 shows the results of total soluble solids (TSS) of stored cherry tomatoes in the three different MAPs at 10 °C during 20 days. The results show significant reduction of TSS in the during the storage time. The concentrations of soluble solids increased with further reduction during storage. Samples stored in the MAP 1 showed less variation in values. These results can indicate that this modified atmosphere reduced the respiratory rate, slowing the metabolic processes that alter physical and chemical parameters such as SST. According to Buta and Moline (1999), during post-harvest and storage, organic acid content decreases due to its use as a substrate in the respiration or transformation into sugars. In general, there is an increase

in sugar content after harvesting and a decrease in the end of the storage period due to the use of sugar in fruit respiration as an energy source.

Similar results were described by Guillén et al. (2006) to TSS, which evaluated cherry tomatoes stored at 10 °C for 28 days. Artes et al. (1999), evaluating chemical attributes of quality, such as pH and TSS, concluded that the use of passive and active atmospheres delayed the ripening process of tomatoes stored at 2 and 10 °C during 10 days of storage. Ali et al. (2010) observed that the lowest TSS at the end of the storage period was recorded in tomatoes coated with 20% gum arabic. Decreased respiration rates also slow down the synthesis and use of metabolites resulting in lower TSS (YAMAN and BAYOINDIRLI, 2002).

5.3.2.3 Color

Table 5.3 shows the results of color during the storage of cherry tomato in three different MAPs at 10 °C for 20 days. The lightness (L) of samples stored under all MAPs reduced significantly during the storage indicating browning of samples. Samples of control and MAP 2 showed significant change in L parameter starting from the 6 day of storage, while gas composition of MAP 1 showed significant effect on the 20 days of analysis.

The same behavior occurred with a* parameter, however the values increased. L and a* parameters showed decrease and increase respectively, these behavior indicates a significant increase in red color of tomatoes stored in all MAPs during storage period. Comparing the modified atmospheres, the effect was more pronounced for the gas composition of the control and MAP 2 packages.

The difference between the chroma (C) values during storage of samples can indicate slight saturation of red color of samples. The color result is an important indication of the shelf life of fruits and vegetables. The maturation stage can be characterized subjectively by the level of skin color, which is an important parameter to predict the shelf life of fruits. In the case of tomato ripening, different colors are present simultaneously since chlorophyll is degraded from green to colorless compounds at the same time that carotenoids are synthesized from colorless precursor (phytoene) to carotene (paleyellow), lycopene (red), β-carotene (orange) xanthophylls and hydroxylated carotenoids (yellow) (GIULIANO et al., 1993). Ali et al. (2004) showed that individually both the hot water dip treatment (HWT) and low O_2 modified

atmosphere in films delay color development. Kantola and Helen (2001) reported that there were increases in red color values (a) during storage in tomatoes stored under NA or MAP conditions, however, these increases occurred at desired levels. Akbudak et al. (2012), evaluating cherry tomatoes, verified a retarding effect on color alteration in fruits in both cultivars and this effect was accelerated especially with the inclusion of passive MAP treatment.

Table 5.3: Color values of storage cherry tomato in three different MAPs (Control: synthetic air; MAP 1: 5% O_2 + 5% CO_2 + 90% N_2; MAP 2: 5% O_2 + 10% CO_2 + 85% N_2) at 10 °C by 20 days.

Storage time (days)	L	a*	b*	C	h
			Control		
0	32.1±2.4a[z]	16.7±1.6a	21.9±1.1a	32.1±1.7a	38.8±1.4a
6	26.6±1.0b	23.4±1.4b	21.9±1.6a	32.4±2.5a	39.8±1.8a
12	25.8±1.5b	26.3±2.2b	25.1±1.2b	34.2±1.9ab	43.0±0.3b
20	22.0±2.0c	26.3±1.5b	33.4±3.0c	37.4±2.4b	53.5±0.2c
			MAP 1		
0	30.3±3.0a	17.4±0.1a	18.1±1.1a	26.0±1.0a	44.1±1.9a
6	28.1±1.5a	17.9±1.3a	21.5±1.5b	25.5±1.1a	42.8±2.1a
12	28.7±1.3a	18.4±1.0a	21.2±1.1b	27.8±1.6ab	49.8±2.2b
20	25.5±0.9b	21.3±1.1b	22.5±0.6b	28.1±0.9b	49.2±2.2b
			MAP 2		
0	31.7±2.1a	17.0±1.3a	25.7±1.4a	33.2±2.0a	41.9±0.5a
6	27.1±1.2b	23.0±1.2b	24.4±1.7a	32.3±0.7a	44.5±0.8b
12	26.0±1.8bc	24.4±1.5bc	23.9±0.7a	30.4±1.6a	46.6±0.5c
20	22.5±2.1c	26.8±1.3c	20.5±1.8b	30.7±1.4a	50.6±1.9d

Control: synthetic air; MAP 1: 5% O_2 + 5% CO_2 + 90% N_2; MAP 2: 5% O_2 + 10% CO_2 + 85% N_2. [z] Means in columns with different letters are significantly different according to Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

5.3.2.4. Weight loss

Figure 5.2 shows the weight loss (%) of stored cherry tomato in three different MAPs at 10 °C during 20 days. Weight loss increased

with storage time, for the three atmospheres, however in both modified atmospheres weight loss was very small. The low water vapor transmission rate of LDPE/BOPP films, combined with the transpiration rate of cherry tomatoes, developed a nearly saturated condition in the packages, which was responsible for the small weight loss. So, the positive effects of storage of fresh pre-climacteric fruits in sealed plastic films may be, in certain cases, a combination of the effects on the O_2 and CO_2 contents within the fruit and the maintenance of high moisture content. The effect of moisture content is more likely a reduction in stress of the fruit, which may be caused by a rapid rate of water loss in unwrapped fruit (THOMPSON, 1998). The samples stored in MAP 1 showed less weight loss when compared to the control and MAP 2. At the end the control, MAP 1 and MAP 2 storage period, weight loss were around 0.38, 031 and 0.37%, respectively. Unpackaged cherry tomatoes showed a weight loss of 10% after 25 of storage at 5 °C (data not shown), suggesting that dehydration is an important process in the loss of quality of cherry tomatoes during postharvest storage. This could be attributed to the fact that tomatoes are only protected by a thin and porous epidermal structure, which does not prevent a quick superficial dehydration (SINGER, 1986).

Akbudak et al. (2012) evaluated weight loss of cherry tomatoes in passive MAP using plastic film materials of the different O_2 and CO_2 permeabilities and found that weight loss was significantly higher in tomatoes stored under normal atmosphere (NA) compared to MAP. In a similar study, Aguayo et al. (2004) determined, in their study using tomatoes, that the weight loss in the fruits wrapped with plastic film material and hence whose atmospheric combinations were altered and lower, when compared with those stored unwrapped. Kuenwoo et al. (2000), studying ripe tomato (cv. Pinky World) fruits, packaged in low density PE and stored for 28 days at 4 or 10 °C, found that fresh weight was maintained better in 4 °C and 40 μm PE. Guillén et al. (2006), evaluating cherry tomatoes stored at 10 °C for 28 days, verified a weight loss of approximately 12%.

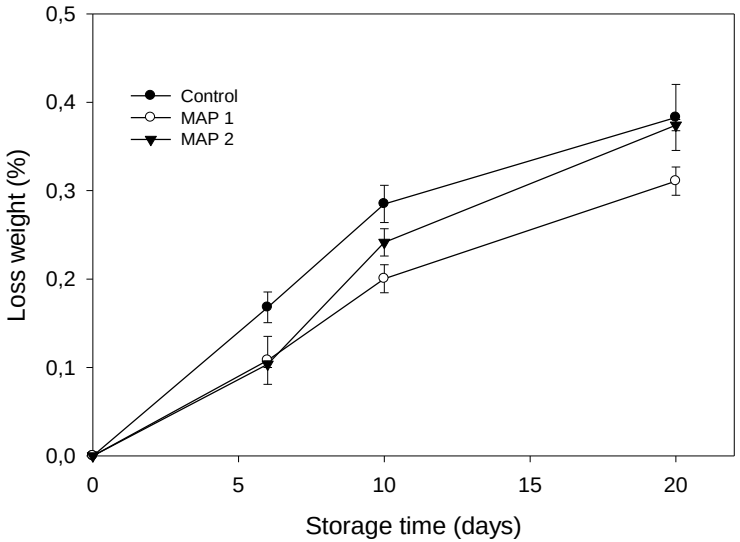

Figure 5.2. Weight loss (%) of storage cherry tomato in three different MAPs (Control = synthetic air; MAP 1= 5% O_2 + 5% CO_2 + 90% N_2; MAP 2: 5% O_2 + 10% CO_2 + 85% N_2) at 10 °C by 20 days.

5.3.2.5. Texture

Figure 5.3 shows the compression force (N) of cherry tomato packed into three different MAPs at 10 °C during 20 days. There was a decrease of compression force during storage for all atmospheres tested. For the control, occurs greater reduction in compressive force (4.67-2.31 N), and therefore larger loss of firmness, at 10% deformation. The samples stored under MAP 2 showed reduction of 6.01-4.00 N in 10% deformation, the next change occurred for the samples stored in synthetic air (control).

Samples stored under conditions of MAP 1 presented the lowest reduction in compressive force (4.86-3.37 N), compared to control and MAP 2. This result showed that the gas concentration of MAP 1 can maintain the firmness of cherry tomato during storage of 20 days at 10 °C. These results are in agreement with the weight loss of the product, the MAP1 and MAP 2 had the highest and lowest water loss, respectively.

According to Vu et al. (2004), alterations in the texture of fruits and vegetables during processing may be related to changes in enzymatic and non-enzymatic pectin. The enzymatic degradation of pectin is catalyzed by different enzymes. The activity of these enzymes can be detected mainly in the early stages of ripening, and is involved in fruit softening during ripening. Low respiration rate can limit the activities of these enzymes and allow retention of the firmness during storage (SALUNKHE et al., 1991). According to Errington et al. (1997), the ripening of tomatoes is accompanied by significant degradation of pectin present in the cell wall. This degradation is partly due to the action of hydrolytic enzymes such as polygalacturonase. Tomatoes are very prone to water loss, leading to softening of the product during ripening (MENCARELLI and SALTVEIT, 1988). Akbudak et al. (2012) evaluated the use of modified atmospheres and cherry tomatoes and found that softening was faster for the samples that were not placed under MAP.

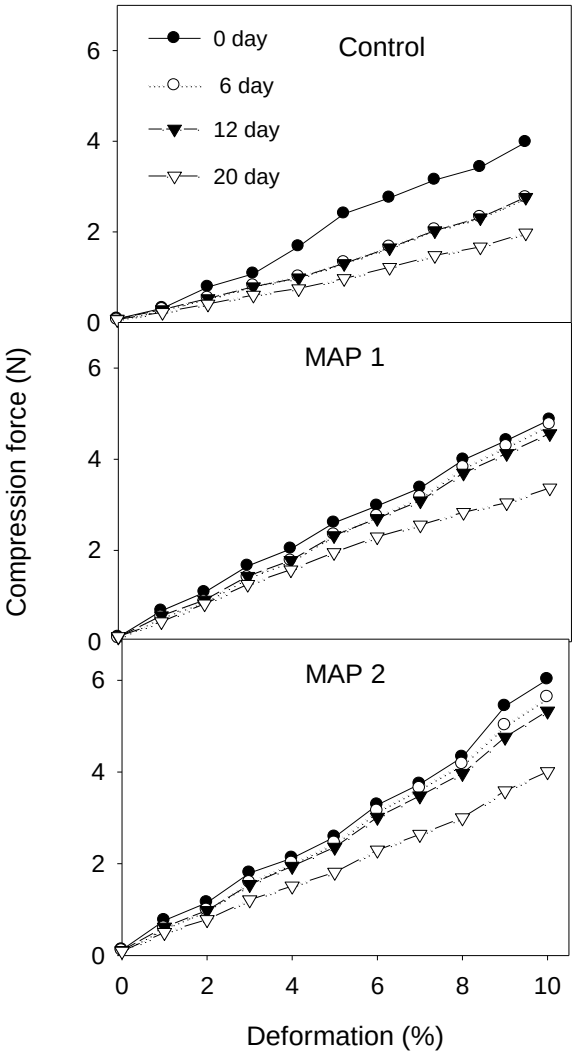

Figure 5.3. Compression force (g) of cherry tomato in three different MAPs (Control: synthetic air; MAP 1= 5% O_2 + 5% CO_2 + 90% N_2; MAP 2: 5% O_2 + 10% CO_2 + 85% N_2) at 10 °C by 20 days.

5.3.2.6. Gas evolution

Figure 5.4 shows the gas evolution of O_2 and CO_2 inside the package containing cherry tomatoes stored in three different MAPs at 10 °C by 20 days. A classical modification of the internal atmosphere was observed: a decrease in O_2 and an increase in CO_2 concentration during the transient period and a steady state (FISHMAN et al., 1995). This increase in the amount of CO_2 is probably because the production of CO_2 is higher than the permeation for packaging was already observed by Salvador et al. (2002).

Control sample showed higher fluctuation of the values of O_2 and CO_2 (19-1.8% and 0.8-13.9%, respectively), possibly due to the greatest amount of O_2 available for respiration of tomatoes, despite the high respiratory rate values, the final gas concentration in the package do not allow that anaerobic respiration takes place. The MAP 1 showed the lowest average values of concentration of O_2 and CO_2 (5.0-3.8 and 5.0%-5.3%). There was observed a rapid equilibrium between the processes of product respiration and permeability of the packaging.

The MAP 2 showed changes in the concentrations of O_2 and CO_2 (5.0-1.9% and 9.0-13.1%, respectively), final values within the packaging does not allow the anaerobic respiration. According to Kader (1986), the decrease in O_2 content available for vegetables reduces the respiratory rate (production CO_2 / consumption O_2), which generally requires at least 1-3% oxygen, depending on the product, to avoid the change in aerobic to anaerobic respiration. Excessively low O_2 levels <1% anaerobic respiration may occur, resulting in tissue deterioration and production of off-flavors and off-odors (LEE et al., 1995; AUSTIN et al., 1998; ARES et al., 2007). Moleyar and Narasimham (1994) studied the behavior of tomatoes stored at temperatures from 10 to 15 °C and found that the optimal conditions for storage of the vegetable ranged from 3 to 5% of O_2 and CO_2. Krammes et al. (2003) evaluated the rate of CO_2 production in tomato cultivar Santa Clara, packed in jars removed by air for 18 days observing similar behavior to that found in this work, namely to increase the production and subsequent stabilization of CO_2.

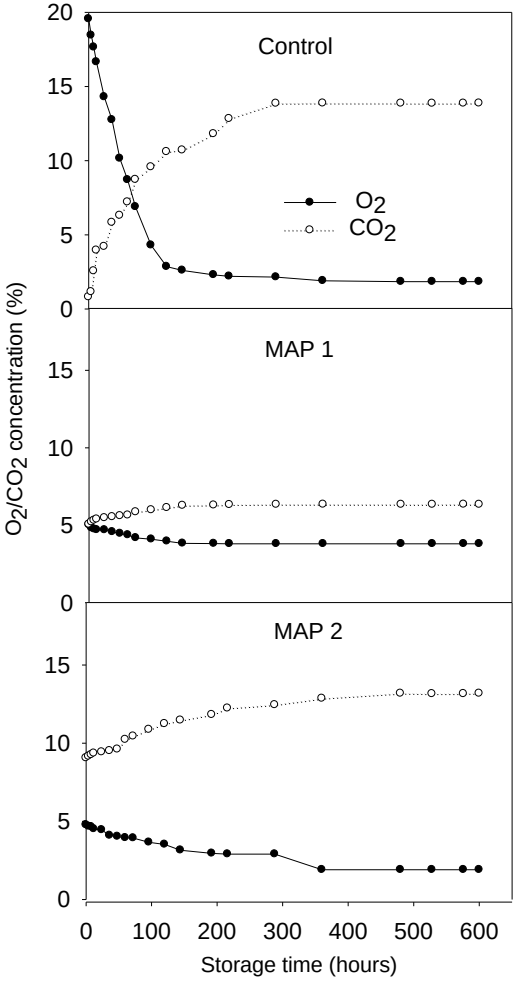

Figure 5.4. Gas evolution of O_2 and CO_2 inside the package containing cherry tomatoes stored in three different MAPs (Control: synthetic air; MAP 1: 5% O_2 + 5% CO_2 + 90% N_2, MAP 2: 5% O_2 + 10% CO_2 + 90% N_2) at 10 °C by 20 days.

5.3.2.7. Respiration rate

Figure 5.5 shows the respiration rate based in O_2 consumption of cherry tomatoes stored under modified atmosphere at 10 °C during 20

days. Initially, the respiration rate is high in all atmospheres, and then this rate is decreased due to the smallest amount of O_2 available for respiration of the product. The highest change in respiratory rate was observed in the samples stored in control (23.0-7.0 mLO_2 / Kg.h), possibly by the amount of O_2 available for respiration of tomatoes. The second major decrease in respiratory rate was observed for samples packed in MAP 2 (14.0-6.0 mLO_2 / Kg.h). The MAP 1 presented the lowest respiration rate between the atmospheres tested (8.1-6.2 mLO_2/Kg.h) and, in the early hours, occurred the equilibrium between the respiration of the product and the permeability of the package. Similar values (8.5-5.5 mLO_2/kg.h) were found by Goyette et al. (2012), evaluating the respiratory rate of tomatoes stored in chambers for 450 hours at 13 °C. The results for the respiration rate are consistent with behavior previously found for weight loss, gas evolution and firmness.

During fruit ripening, depolymerization or shortening of the chain length of pectin occurs with increased activities of pectinesterase and polygalacturonase. Low concentrations of oxygen and high concentrations of carbon dioxide reduce the activities of these enzymes and allow the retention of firmness of vegetables during storage (SALUNKHE et al., 1991). Odriozola-Serrano et al. (2008) reported a significantly reduction in O_2 concentration in the package headspace was observed over time when fresh-cut tomatoes were preserved at 5 °C. Charles et al. (2003) evaluated gas exchange dynamics in the LDPE filled with tomatoes, variety "Grace" and one oxygen absorber, sealed under air at 20 °C, and verified that it was necessary less than 50 h to reach equilibrium atmosphere.

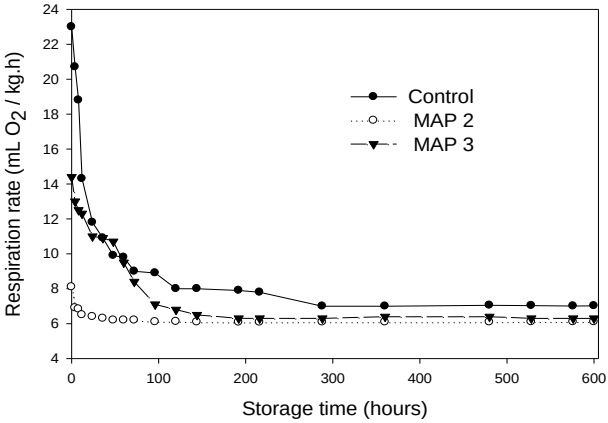

Figure 5.5. Respiration rate based in O_2 consumption of cherry tomatoes stored in three different MAPs (Control = synthetic air; MAP 1= 5% O_2 + 5% CO_2 + 90% N_2; MAP 2: 5% O_2 + 10% CO_2 + 90% N_2) at 10 °C by 20 days.

5.4 Conclusions

The present study showed that BOPP/LDPE bags, on the conditions studied, showed effective to package cherry tomatoes under modified atmosphere. The samples stored in MAP 1 had the lowest respiration rate between the atmospheres evaluated, and consequently showed the lowest physicochemical changes during the period evaluated. Therefore, modified atmosphere content 5% O_2, 5% CO_2 and N_2 balance presented the best results to maintenance the quality of the cherry tomatoes, stored at 10 °C for 20 days.

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CAPÍTULO 6:
APPLICATION OF MODIFIED ATMOSPHERE AND LOW TEMPERATURE TO PRESERVATION POSTHARVEST OF CHERRY TOMATOES

Application of modified atmosphere and low temperature to preservation postharvest of cherry tomatoes

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Abstract

The effects of active modified atmosphere packaging (MAP) on the postharvest quality of cherry tomatoes stored at cold temperature (5°C) and bioriented polypropylene /low density polyethylene BOPP/LDPE bags were investigated. The atmosphere composition used in the packaging was 5% O_2 + 5% CO_2 (MAP), synthetic air (control). The variables measured weight loss, firmness, sugar, organic acids, color, lycopene, respiration rate, and ethylene biosynthesis during 25 d. The results showed that active MAP could extend the shelf-life of cherry tomatoes to 25 d and the gas concentration could influence the postharvest quality of cherry tomatoes. MAP treatment decreased the respiration rate and ethylene contents, reduced weight loss, lycopene biosynthesis and the formation of red color. Through the use of MAP it was possible to maintain the firmness, and delay changes in sugar and organic acids contents. Therefore, the combination of MAP and low temperature treaments was effective with regard to delaying the maturity along the storage period, preserving the fruit quality of cherry tomatoes.

Keywords: cherry tomatoes, modified atmosphere package (MAP), postharvest quality

6.1. Introduction

The increasing growth in the consumption of fresh fruits and vegetables over the last century has driven commercial demand for improving the storage/transit conditions to manage postharvest disease proliferation and also maintain the quality (i.e., flavour, colour, nutritional aspects, firmness, 'shelf-life', and processing attributes) of fresh produces (TZORTZAKIS, et al., 2007).

The tomato is important worldwide, both for the fresh and the processing markets. This vegetable is available all year round and is rich in compounds including vitamin C, flavonoids, and carotenoids, which are believed to be beneficial to human health (WOLD et al., 2004). Tomatoes have been ranked as a first source of lycopene (71.6%), as well as an important source of vitamin C (12.0%), pro-vitamin A carotenoids (14.6%), beta-carotene (17.2%), and vitamin E (6.0%) (RAFFO et al., 2006). However, this vegetable has a relatively short postharvest life and during fruit ripening many processes affecting quality takes place (HOEBERICHTS et al., 2002).

Vegetable are living organisms which continue to respire after harvesting. Shelf-life can be extended by reducing gases transfer rates and by controlling factors such as the gas composition (O_2, CO_2 and ethylene), surrounding the fruit, water vapor permeability, temperature, relative humidity, and light. Respiration is a metabolic process that provides the energy for the biochemical processes of plant cells. Various substrates used in important biosynthetic pathways in the plant are formed during respiration. Aerobic respiration consists of oxidative breakdown of organic reserves to simpler molecules, including CO_2 and water, with release of energy. The organic substrates broken down in this process may include carbohydrates, lipids, and organic acids (FONSECA et al. 2002).

Tomato fruit characteristically follow a climacteric ripening pattern which is controlled by ethylene (CARRARI and FERNIE, 2006), involving a wide range of physical, chemical, biochemical. and physiological changes. Thus, most of the tomato postharvest storage technologies are focused on controlling the respiration and action of ethylene in order to gain a delay of these changes (MARTÍNEZ-ROMERO et al., 2007; SERRANO et al., 2008). Tomatoes and derived products are major sources of lycopene and contribute significantly to carotenoid intake for humans. However, processing and storage

conditions of tomato products may cause lycopene degradation as precisely reviewed by Nguyen and Schwartz (1999).

An important strategy to control some of these transformations and degradations is the use of modified atmospheres (LIN AND ZHAO, 2007). Modified atmosphere packaging (MAP) storage and Controlled atmosphere (CA) storage are used to increase the shelf-life of fruit and vegetables. MAP is the alteration of the gaseous environment produced as a result of respiration (passive MAP) or by the addition and removal of gases from food packages (active MAP) to manipulate the levels of O_2 and CO_2. Depleted O_2 and/or enriched CO_2 levels can reduce respiration, delay ripening, decrease ethylene production, retard textural softening, slow down compositional changes associated with ripening, thereby resulting an extension in shelf life (DAS et al., 2006).

Generally, 3–8% CO_2 and 2–5% O_2 are recommended for fruits and vegetables for MAP storage (FARBER, 1991). Storage of pink 'Buffalo' tomatoes (*Lycopersicon esculentum* Mill.) in 4% O_2+ 2% CO_2 at 12 °C contributed to extended their shelf-life (NUNES et al., 1996). In contrast, Ratanachinakorn et al. (1997) found that pink 'Bermuda' tomatoes were not injured by exposure to 0.5% O_2 for 1 day or 80% CO_2 for 2 days at 22 °C. Rocculi et al. (2006) evaluating the metabolism of Golden Deliciousapples verified that active modified atmosphere decreased the rate of oxygen consumption compared with passive atmosphere. Akbudak et al. (2012) studied the effect of pre-harvest harpin and passive modified atmosphere packaging on quality of cherry tomato and found that these treatments were effective with regard to the fruit quality. For fresh tomatoes, texture, flavour and colour are the most important quality attributes, which directly relate to their marketing value (LIU et al., 2009).

However, there has been limited information available on the use of active modified atmosphere in respiration rate and delaying ripening processes, including influence on texture, colour, lycopene, sugars, and organic acids, during storage of cherry tomatoes. Therefore, the aim of this study was to determine whether the gas composition 5% O_2 + 5% CO_2 + balance N_2 has the potential to be used as an modified atmosphere for delaying ripening of cherry tomatoes during storage whilst maintaining their physical-chemical and antioxidant properties.

6.2. Material and methods

6.2.1. Plant material and storage conditions

Cherry tomatoes (*Lycopersicon esculentum* L.) used in the experiments were commercially grown and collected in Florianópolis city (Santa Catarina State, southern Brazil) and stored up to 24 h at 5 °C until use. Fruit were free from previous postharvest treatments. Before each experiment, cherry tomatoes were selected according to uniform size, color, and physical integrity. Samples were washed in running water and sanitized in a 0.5 ppm ozonized solution during 1 min, then allowed to air-dry at room temperature. One hundred grams of cherry tomatoes were place into multilayer plastic bags (low density polyethylene [LDPE] and biorientated polypropylene [BOPP]); 175-mm wide x 240-mm long, 75-mm thick and permeability of O_2 2.000 cm^3/m^2 per day and CO_2 5.469 cm^3/m^2 per day (Lamine Cia Package, SP, Brazil). The packages containing the fruit were divided into two batches, the first batch was filled with a gas composition of 5% O_2 + 5% CO_2 + 90% N_2 and the second one was filled with synthetic air (control samples). The gas composition into the multilayer plastic bags was achieved by injecting the gases using a vacuum sealer (200B, Selovac, São Paulo, Brazil) apparatus (pressure and injection time at 1.1 bar and 12 s). Cherry tomatoes inside the bags were stored in temperature-controlled chambers (model ECB-EX, Expectron Tecnologia Industrial Ltda, São José, SC, Brazil) at 5 °C by 25 days. The samples were assessed in 6, 12, 20 and 25 days. During the storage period, the relative humidity (RH) of the atmosphere ranged from 80 to 85%. Each experiment used three different packages and all experiments were carried out in triplicate.

6.2.2. Weight loss

Tomato samples were weighed at day 0, 6, 12, 20 and 25. The difference between initial and final fruit weight was considered as total weight loss during each storage interval and calculated as percentages on a fresh weight basis by the standard AOAC (2005) method.

6.2.3. Firmness

The compression force was determined using a digital texture analyzer TAXT2i (Stable Micro System, Surrey, UK) with a 50-N load cell. The experiment was conducted with a 45 mm diameter cylindrical

probe and test speed, pre-test and post-test were 1 mm/s, 2 mm/s and 5 mm/s, respectively, the strain used was 10% of tomato. Fifteen fruit for each treatment were randomly selected, the results were expressed in newton.

6.2.4 Organic Acids

The analysis of organic acids in fruit samples was performed by high performance liquid chromatography (HPLC) in liquid chromatograph (Series 200, PerkinElmer) equipped with a vacuum degasser, binary pump, manual injector (microsyringe 100 µL), loop de 20 µL and UV-VIS detector, the wavelength range of 250 nm to ascorbic acid and 210 nm for other acids according Facco (2006) modified. For the chromatographic separation used an reverse phase C18 column C18 (ODS-II, 4.6 x 250 mm ID, 3 µm). The mobile phase used for separation of acidic aqueous solution was 0.01 M KH_2PO_4, at a flow rate of 0.7 mL / min, pH adjusted to 2.6 with phosphoric acid and run time of 15 min. The quantification of organic acids was carried out by external standard curve with 6 points for each organic acid (citric, malic, ascorbic, tartaric acid). All samples and the mobile phase were filtered on regenerated cellulose membrane with a diameter of 47 mm and a pore size of 0.45 µm. The same chromatographic conditions were kept for standards and samples. The samples fruit were pressed and juice obtained was diluted with mobile phase (1/9), previously filtered through regenerated cellulose membrane. The juice was filtered through filter paper and Minisart (CR 4, Sartorius). The identification of organic acids in fruit samples was performed by comparison of its retention time with the respective standard. The analyzes were performed in duplicate.

6.2.5. Sugar extractions

Analysis of sugars in the fruit samples was performed by high performance liquid chromatography (HPLC) on liquid chromatograph HPLC (Series 200, PerkinElmer) equipped with a vacuum degasser, binary pump, manual injector (microsyringe 100 µL), loop de 20 µL, refractive index detector, column temperature 50 °C and oven temperature 65 °C. For the chromatographic separation was used the column Lichrospher 100 NH_2 5 µm (250 x 4 mm). The mobile phase used for separation of the sugars was an aqueous solution of 75% acetonitrile at a flow rate of 0.8 mL / min run time 15 min according to Macrae (1998). The quantification of sugar was performed by external

standard curve with 6 points for each pattern (sucrose, glucose, fructose). Were kept the same chromatographic conditions for standards and samples. The samples were pressed fruit juice and 1 g obtained was homogenized in aqueous 75% acetonitrile and transferred to a flask supplementing the volume to 50 mL. The solution was subjected to an ultrasonic bath for 10 min and filtered on filter paper and Minisart (CR 4, Sartorius) for injection into the chromatograph. The identification of the sugars in the fruit samples was performed by comparison of its retention time with the respective standard. All analyzes were performed in duplicate.

6.2.6. Color

The color of the skin cherry tomatoes was measured with a spectrophotometer Hunterlab (Miniscan EZ, Reston, USA) using the Hunter color parameters, L*, a*, b*, chroma (C) and hue angle (h). Each measurement was taken at three locations for each cherry tomato and20 fruits per treatment were used. A standard white calibration plate was employed to calibrate the spectrophotometer.

6.2.7. Extraction and identification of lycopene

The process of extraction of carotenoids was carried out by weighing 2.5 g of product grinded in 20 mL of acetone. The extraction was performed on a magnetic stirrer for 1 h at room temperature, keeping the samples protected from light. The extracts were filtered through cellulose membrane under vacuum, filtered, transferred to centrifuge tubes and added to 20 mL of petroleum ether and 10 mL of distilled deionized water. Centrifugation was performed at 3000 rpm for 10 min. Subsequently the solution of the pigments in petroleum ether was transferred to a flask supplementing the volume to 50 mL with petroleum ether and transferred to the rota-evaporator. The dried residue was dissolved in 3 mL of hexane and 10 uL were injected into a liquid chromatograph (LC-10A, Shimadzu) equipped with a C18 reverse phase column (Vydac 218TP54, 250 x 4.6 mm, internal diameter 5 mm, 30° C) and a UV-visible detector operating at 470 nm. Methanol: acetonitrile (90: 10, v/v) was used as mobile phase at a flow rate of 1 mL/min. The identification of lycopene was done by comparing its retention time with that of the corresponding standard (Sigma-Aldrich Chemie, Steinheim, Germany). For purpose of lycopene quantification in samples, an external standard curve was built taking into account the

values of the peak area of the analyte. The standard curve showed equation: y= 4096.94 x, R^2: 0.984 and concentration range of lycopene: 0-80 µg/mL. The results were expressed in µg/mL of lycopene, resulting from the average calculation of three consecutive injections.

6.2.8. Head-space gas and respiration rate

Head-space gases CO_2 and O_2 were measured using a PBI Dansensor CO_2/O_2 gas analyzer (Checkmate 9900, Ringsted, Denmark) after removal from cold storage. Gas samples were analyzed from three replicates of each sample. The respiration rates of O_2 consumption and CO_2 evolution were obtained from a CO_2 and O_2 concentrate. A computer program was developed using the MATLAB® software (Mathworks Inc., USA) to determine the respiration rates using the model proposed by Lee, et al. (1996), according to Equation 1:

$$r_{O2} = -\frac{d[O_2]}{100dt}\left(\frac{V}{m}\right) + \frac{SP_{O2}(0{,}21-[O_2]/100)p}{mL}$$

where rO_2 is the respiratory rates for O_2 consumption expressed as mL kg^{-1} h^{-1}; $[O_2]$ is the concentrations of O_2 expressed as %; L is the thickness of the film in m; S is the area of the bag (m^2); PO_2 is the permeability of the film for O_2, respectively (mL m^{-2} h^{-1} atm^{-1}); t is the time in h; Vm is the free volume in the bag (mL); and m is the mass of product in the bag (kg).

6.2.8. Ethylene

Concentrations of ethylene (C_2H_4) in the package were accomplished in a gas chromatograph (CG35, Cromacon) column Porapak-Q, 1.8 m, coupled to a thermal conductivity detector. Chromatographic conditions were as follow: column temperature 70 °C and detector 100 °C, argon as the carrier gas with a flow rate at 30 mL/min. The gas sample was collected from the package with the aid of an adapted rubber septum on the outside of the packaging and the gas was withdrawn with a 1 mL syringe and injected into the chromatograph. For ethylene quantification an external standard curve was built . The standard curve was obtained from some mix of N_2 and C_2H_4, and the injected volume of 50 µL. The standard curve showed equation: y= 0.0002x + 10.260, R^2: 0.9948 and concentration range of 0

- 45000 nL. The results were expressed in nL/g, from the average calculation of three consecutive injections.

6.2.9. Statistical analysis

Statistical analysis was performed using the Statgraphics 5.1.package (Manugistics Inc., Rockville, MD, USA). Specific differences between means were determined by Fisher's protected least significant difference test (LSD, $P < 0.05$) applied after an analysis of variance (ANOVA).

6.3. Results and discussion

6.3.1. Weight loss

Figure 6.1 shows the weight loss cherry tomatoes stored in modified atmosphere package (MAP) containing 5% O_2, 5% CO_2, 90% N_2 and atmosphere containing synthetic air (control) by 25 d at 5 ºC. Unpackaged cherry tomatoes showed a weight loss of 10% after 25 of storage at 5°C (data not shown), suggesting that dehydration is an important process in cherry tomatoes quality loss during postharvest storage. This could be attributed to the fact that tomatoes are only protected by a thin and porous epidermal structure, which does not prevent a quick superficial dehydration (SINGER, 1986). At the end the MAP and control samples storage period, weight loss were around 0.26% and 0.18% respectively. Weight loss increased with storage time, for 2 atmospheres, however in both atmospheres, weight loss was very small. The samples stored in packages containing 5% O_2, 5% CO_2, 90% N_2 showed less weight loss when compared to the control.

The low water vapor transmission rate of LDPE/BOPP films, combined with the transpiration rate of cherry tomatoes, developed a nearly saturated condition in the packages, which was responsible for the small weight loss. Therefore, the low weight loss trend in the low O_2 and high CO_2 may be related to water vapor accumulation within plastic film packages during the storage. These effects are especially important, since they confirmed the idea that the softening would commence later in the fruits since the weight loss of fruits subjected to low O_2 and high CO_2. So the positive effects of storage of fresh pre-climacteric fruits in sealed plastic films may be, in certain cases, a combination of its effects on the O_2 and CO_2 content within the fruit and the maintenance of high moisture content. The effect of moisture content is more likely a

reduction in stress of the fruit, which may be caused by a rapid rate of water loss in unwrapped fruit (THOMPSON, 1998).

Akbudak et al. (2012) evaluated weight loss of cherry tomatoes in passive MAP using plastic film materials with various O_2 and CO_2 permeability and found that weight loss was significantly higher in tomatoes stored under normal atmosphere (NA) compared to MAP. In a similar study, Aguayo et al. (2004), determined in their study with tomatoes that the weight loss in the fruits wrapped with plastic film material and hence whose atmospheric combinations were altered was less, compared with those stored unwrapped. Kuenwoo et al. 2000 studying ripe tomato (cv. Pinky World) fruits packaged in low density PE and stored for 28 days at 4 or 10°C found that fresh weight was maintained better in the 4°C and 40µ PE. Guillén et al. (2006), evaluating cherry tomatoes stored at 10 °C for 28 days, verified a weight loss of approximately 12%.

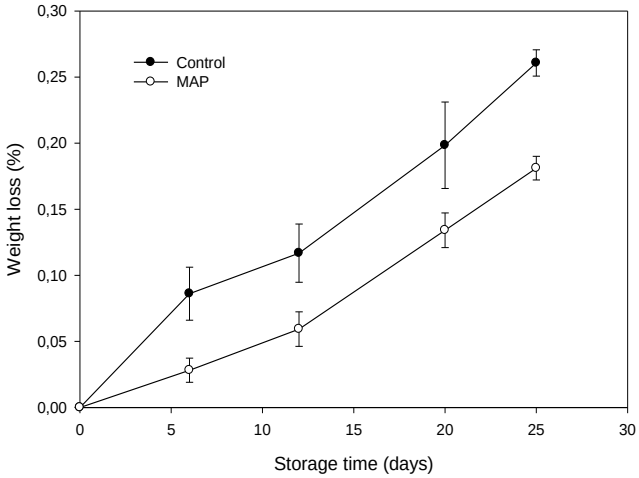

Figure 6.1. Weight loss of cherry tomatoes stored in modified atmosphere package (MAP) containing 5% O_2, 5% CO_2, 90% N_2 and atmosphere containing synthetic air (control) by 25 d at 5 ºC

6.3.2. Firmness

Firmness of cherry tomatoes stored in modified atmosphere package (MAP) containing 5% O_2, 5% CO_2, 90% N_2 and atmosphere

containing synthetic air (control) by 25 d at 5 ºC is showed Figure 6.2. The compressive force decreased during storage for samples stored under MAP and control. For the 10% of deformation, there was a reduction of 1.19 N in compressive force for samples stored in packs containing 5% O_2, 5% CO_2, 90% N_2 during 25 d. While the control samples showed a reduction of 2.09 N in compression force during storage period. These results indicate that the atmosphere used inhibited fruit softening and maintained the firmness throughout the storage compared to control.

The effect of modified atmosphere on the maintenance of fruit firmness is usually related to their control of weight loss. In this work, the samples with highest weight loss (control) showed a greater reduction of texture. Softening of fruit is due to deterioration in the cell structure, cell wall composition and intracellular materials (SEYMOUR et al., 1993). These biochemical process involving the direct suppression of the activities of pectin esterase and polygalacturonase enzymes leading to post-harvest softening of fruit structure or blockage of the synthesis of ethylene which controls the activities of these enzymes especially with MAP treatment (AKBUDAK et al., 2012). Low respiration rate can limit the activities of these enzymes and allow retention of the firmness during storage (SALUNKHE et al., 1991). Kuenwoo et al. (2000) determined in their study on the MAP storage of tomatoes that 50µ PE treatment give better results with respect to firmness values. Akbudak et al. (2012) evaluating of cherry tomatoes verified that generally, firmness values were higher especially for samples stored in passive MAP.

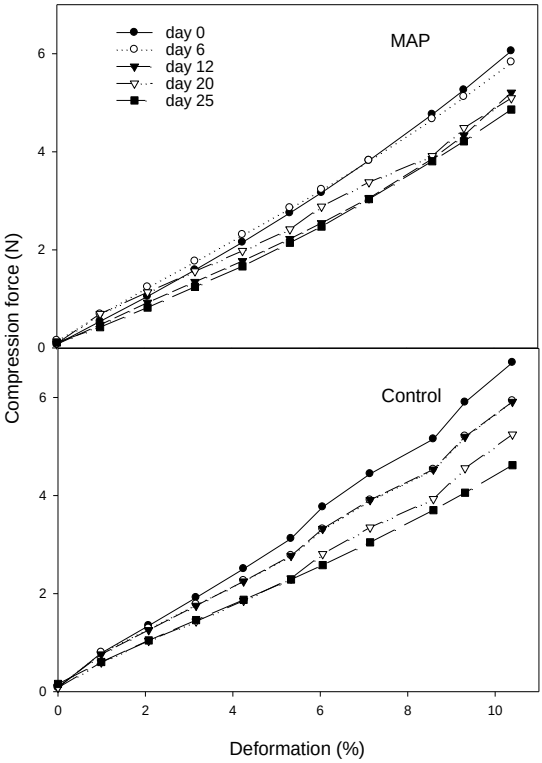

Figure 6.2. Firmness of cherry tomatoes stored in modified atmosphere package (MAP) containing 5% O_2, 5% CO_2, 90% N_2 and atmosphere containing synthetic air (control) by 25 d at 5 ºC.

6.3.3. Sugar

Figure 6.3 present the sugars of cherry tomatoes stored in modified atmosphere package (MAP) containing 5% O_2, 5% CO_2, 90% N_2 and atmosphere containing synthetic air (control) by 25 d at 5 ºC. It is possible to visualize increase with further reduction of the sugar content for samples stored in atmosphere of 5% O_2, 5% CO_2, 90% N_2

and samples stored in synthetic air (control). Fructose showed a variation of 0.07 mg/mL for the control and 0.02 mg/mL for the samples packaged in ATM, while glucose presented range of 0.13 and 0.05 mg/mL to the control and ATM, respectively. No significant change in the content of fructose and glucose for samples stored in MAP was observed. The control samples showed variation ($P < 0.05$) during storage, for both sugars.

Changes in the sugar content can indicate senescence of the product. According to Buta and Moline (1999), during post-harvest and storage, organic acid content decreases due to its use as a substrate in the respiration or transformation into sugars. In general, there is an increase in sugar content after harvesting and a decrease in the end of the storage period due to the use of sugar in fruit respiration as an energy source. In this work, the amounts of fructose and glucose in tomatoes cherry were very close. Some tomatoes storage methods, shown lower sucrose concentration, and accumulate glucose and fructose in an approximate 1:1 ratio (SCHAFFER et al. 1999). The climacteric rise of ethylene and respiration is coincident with the initiation of high sugar import (in the form of glucose and fructose) and rapid starch degradation (LUENGWILAI and BECKLES, 2009, 2010). In this work, up to 15 days of storage there was increased production of ethylene and respiratory rate, as well as increased concentrations of fructose and glucose.

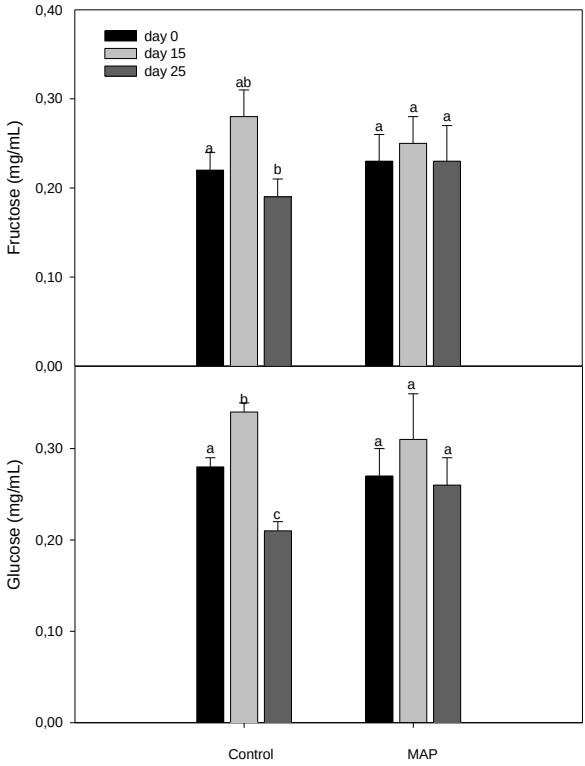

Figure 6.3. Sugars of cherry tomatoes stored in modified atmosphere package (MAP) containing 5% O_2, 5% CO_2, 90% N_2 and atmosphere containing synthetic air (control) by 25 d at 5 ºC.
For each storage atmosphere, columns with different letters are different by Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

6.3.4. Organic acids

Organic acids of cherry tomatoes stored in modified atmosphere package (MAP) containing 5% O_2, 5% CO_2, 90% N_2 and atmosphere containing synthetic air (control), by 25 d at 5 ºC, is showed in Figure 6.4. The analyzed organics acids showed reduction of the values during storage for samples stored under MAP as for the control. However, except for tartaric acids, samples stored under a atmosphere of 5% O_2,

5% CO_2, 90% N_2 decreased ($P < 0.05$) did not significantly during storage. These results are consistent with the contents of sugars.

The malic acid presented reduction of 0.60 mg/mL for the control and 0.10 mg/mL for ATM, tartaric acid showed a reduction of 0.24 for control and 0.16 mg/mL for ATM. Citric and ascorbic acid decreased and 0.35 0.02 mg/mL, respectively for the control, while for ATM reducing showed 0.07 and 0.01 mg/mL, respectively. The combination of MAP and low temperature may reduce the degradation of ascorbic acid in fruits (LEE and KADER, 2000). Reductions observed in organic acids values in relation to ripening resulted from the utilization of acids in respiration and other physiological processes together with carbohydrates (KADER and BEN-YEHOSHUA, 2000).

According to Sadler and Murphy (1998), during post-harvest and storage periods, the concentration of organic acids decreases due to their use as a substrate in the respiration or their transformation into sugars. In study conducted by Akbudak et al. (2012) no significant reductions were observed in ascorbic acid values of cherry tomato cultivars during storage. Also, in some other studies, insignificant reductions were determined in the ascorbic acid contents of cherry tomatoes, similar to our results (MORETTI et al., 2002; RAFFO et al., 2002). Consistently, Odriozola-Serrano et al. (2008) observed no significant loss of ascorbic acid in fresh-cut tomatoes stored under MAP conditions (5 kPa O_2 + 5kPaCO_2) for 21 days at 4 ◦C.

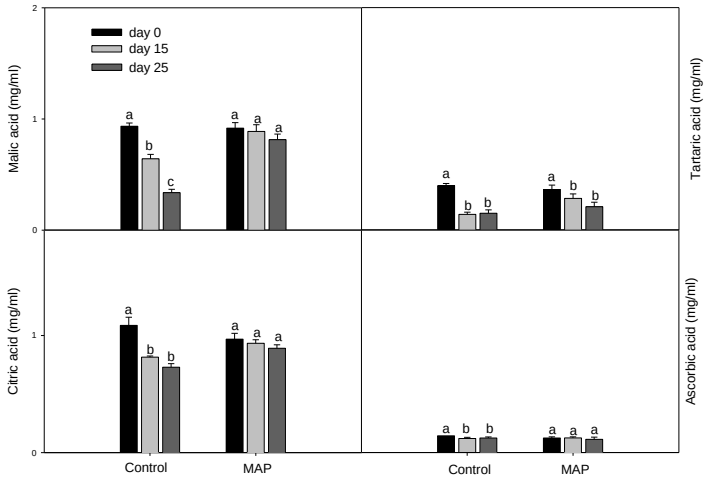

Figure 6.4. Organic acids of cherry tomatoes stored in modified atmosphere package (MAP) containing 5% O_2, 5% CO_2, 90% N_2 and atmosphere containing synthetic air (control), by 25 d at 5 ºC. For each storage atmosphere, columns with different letters are different by Fisher's protected LSD test ($p < 0.05$) applied after an ANOVA.

6.3.5. Color

Table 6.1 shows the results obtained for the color of cherry tomatoes. The color result is an important indication of the shelf life of fruits and vegetables. The maturation stage can be characterized subjectively by the level of skin color, which is an important parameter to predict the shelf life of fruits. The Lightness (L) of samples stored under MAP change significantly during the storage (27.8 - 22.0) indicating little browning of samples.

Changes in the parameter a* (19.0 – 21.4) occurred after 12 days of storage indicating increased red coloration of this period up to 25 days. Control samples showed significant changes ($P < 0.05$) in all parameters. L* and a* parameters showed decreased and increased respectively, showing variation of L: 30.9 - 22.2 and a*:19.6 - 23.5, these changes indicate a significant increase in red color of tomatoes stored in this condition (control) during all storage period. Comparing the two atmospheres changes to the control were more pronounced.

The difference between the crhoma (C) values during storage for control samples can indicate slight saturation of red color of samples, although the chroma not a good indicator of tomato ripening because it essentially is an expression of the purity or saturation of a single color (different colors may have the same chroma values). In the case of tomato ripening, different colors are present simultaneously since chlorophyll is degraded from green to colorless compounds at the same time that carotenoids are synthesized from colorless precursor (phytoene) to carotene (paleyellow), lycopene (red), β-carotene (orange) xanthophylls and hydroxylated carotenoids (yellow) (GIULIANO et al., 1993).

Kantola and Helen (2001) reported that there were increases in red color values (a) during storage in tomatoes stored under NA or MAP conditions, however, these increases occurred at desired levels. Akbudak et al. (2012) evaluating cherry tomatoes, verified a retarding effect on color alteration in fruits in both cultivars and this effect was accelerated especially with the inclusion of passive MAP treatment. Kader and Ben-Yehoshua (2000) determined in their studies that color change proceeded more rapidly in tomatoes subjected to MAP, due to high O_2 concentration especially in the early stages of storage. Ali et al. (2004) showed that individually both the hot water dip treatment (HWT) and low O_2 modified atmosphere in films delay color development.

Table 6.1. Color index of cherry tomatoes stored in modified atmosphere package (MAP) containing 5% O_2, 5% CO_2, 90% N_2 and atmosphere containing synthetic air (control), by 25 d at 5 ºC.

Storage Time (days)	Control[v]					ATM				
	L*	a*	b*	C	h	L	a*	b*	C	h
0	30. 9[a]	19.0[a]	19.6[ab]	27.4 [a]	45.8[ab]	27.8[a]	19.1[a]	19.7[a]	23.4[a]	43.6[a]
6	27.3[a]	18.1[a]	19.3[a]	28.8[ab]	41.9[a]	26.8[ab]	17.1[a]	18.9[a]	25.7[a]	48.0[a]
12	26.2[a]	22.7[b]	21.3[ab]	28.8 [ab]	45.4[ab]	24.9[ab]	19.0[a]	17.3[a]	25.8[a]	42.5[a]
20	25.0 [ab]	21.9[ab]	22.2[ab]	30.0 [ab]	48.1[b]	23.6[b]	20.5[ab]	21.9[a]	27.4[a]	48.4[a]
25	22.2 [b]	23.3[b]	23.5[b]	31.7 [b]	50.6[b]	22.0 [b]	21.4 [b]	21.6[a]	27.8[a]	46.1[a]

[v] Control = samples storage in air. L*= lightness; a*= red/green; b*= yellow/blue. [z] Means in columns with different letters are significantly

different according to Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

6.3.6. Lycopene

Figure 6.5 show the lycopene content of cherry tomatoes stored in modified atmosphere package (MAP) containing 5% O_2, 5% CO_2, 90% N_2 and atmosphere containing synthetic air (control), by 25 d at 5 ºC. The pigments of tomatoes include mostly the green pigments chlorophylls *a* and *b*, the yellow pigment beta-carotene and the red pigment lycopene (FRIEDMAN and LEVIN, 1998), which are metabolized during the ripening of tomatoes.

Lycopene content increased over the period of storage for both samples (MAP and Control). The control samples showed an increase of 27.58 µg/mL in lycopene content. The cherry tomatoes stored under an atmosphere of 5% O_2, 5% CO_2, 90% N_2 showed a lower pronounced increase of lycopene contents (8.36 µg/mL). No significant difference was observed between 15 and 25 days in this condition, a result in accordance with the findings from the color analysis of the fruits.

The storage conditions can alter the biosynthesis of lycopene. Optimum temperature range for lycopene synthesis is between 12 and 32 °C. Temperatures below 12 °C inhibit the biosynthesis and above 32 °C obstruct the process altogether (DUMAS, et al. 2003). No significant reduction were detectedin the lycopene contents of cherry tomato cultivars during normal atmosphere and passive MAP storage (AKBUDAK et al. 2012).

Salunkhe and Wu (1973) found that the chlorophyll catabolism and the biosynthesis of lycopene of mature green tomatoes were completely inhibited for 1 month under 1.2 kPa and 10 days under 10.2 kPa O_2 atmospheres. Odriozola-Serrano et al. (2008) reported no significant changes in lycopene content over a storage time of 21 days at 5 °C in fresh-cut tomatoes packaged under similar MAP (5 kPa O_2 + 5kPa CO_2) conditions to those herein used. Rodriguez-Amaya (1993) found that the stability of lycopene in foods depends greatly on the oxygen availability and the packaging conditions.

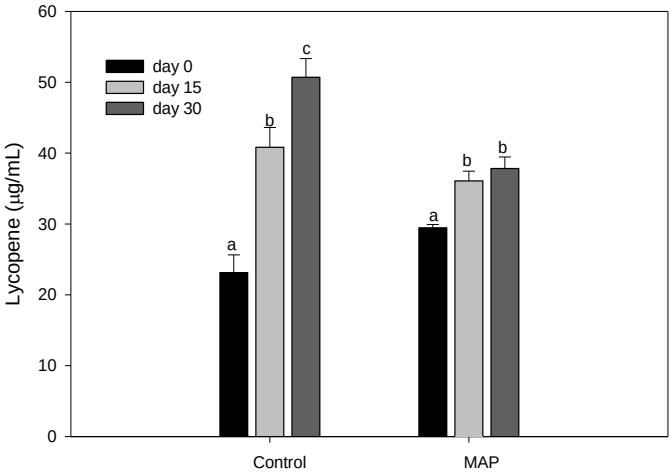

Figure 6.5. Lycopene content of cherry tomatoes stored in modified atmosphere package (MAP) containing 5% O_2, 5% CO_2, 90% N_2 and atmosphere containing synthetic air (control), for 25 d at 5 ºC. For each storage atmosphere, columns with different letters are different by Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

6.3.7. Respiration rate

The values for respiration rate of cherry tomatoes cherry tomatoes stored in modified atmosphere package (MAP) containing 5% O_2, 5% CO_2, 90% N_2 and atmosphere containing synthetic air (control), by 25 d at 5 ºC are shown in Figure 6.6. In the first hours of product storage the respiratory rate is high due to the greater amount of O_2 available. The hours following, respiratory rate decreases due to the reduction of the amount of O_2 available for consumption by fruit. After 100 h of storage occurred equilibrium of respiration rate product. Similar behavior was found by Batu and Thompson, (1998) after a state of equilibrium was reached between respiration of the produce and the diffusion of gases in package, no further changes in the gas concentration within the packs occurred with fruit kept at constant temperature.

The respiratory rate ranged from 6.60 to 5.90 and 8.4 to 6.0 (mL O_2/kg. h) for samples stored in ATM and control, respectively.

Similar values (8.5- 5.5 mL O_2/kg. h) were found by Goyette et al. (2012) evaluating the respiratory rate of tomatoes stored in chambers for 450 hours at 13 °C. In this study, samples stored under ATM showed lower respiration rate compared to the control samples. The higher respiration rate of control samples, according to this larger change in quality parameters evaluated in this work. Probably, respiratory rate of the samples stored in MAP, was not enough sufficient for alter most quality parameters evaluated. During storage, the concentrations of O_2 and CO_2 showed an increase and reduction respectively, with subsequent stabilization of values (data not shown).

Odriozola-Serrano et al. (2008) reported a significantly reduction in O_2 concentration in the package headspace was observed over time when fresh-cut tomatoes were preserved at 5 °C compared with those stored under elevated temperatures. Charles et al. (2003) evaluated gas exchange dynamics in the LDPE filled with tomatoes, variety "Grace and one oxygen absorber, sealed under air at 20 °C, and verified that it was necessary less than 50 h to reach equilibrium atmosphere.

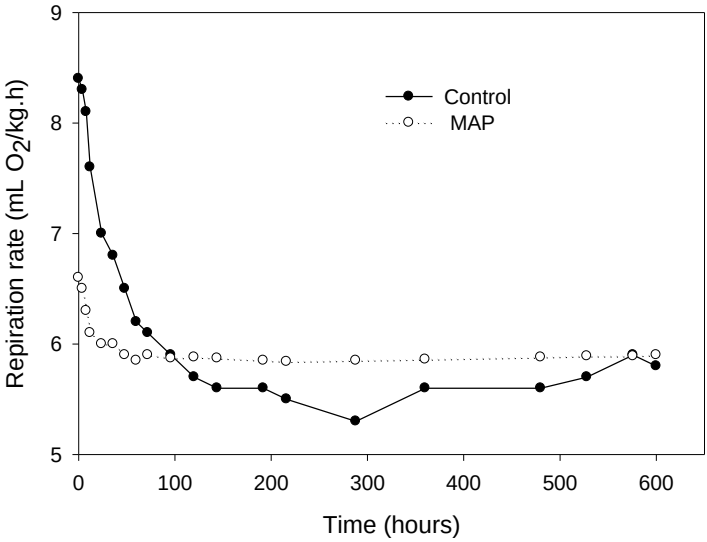

Figure 6.6. Respiration rate based in O_2 consumption of cherry tomatoes stored in modified atmosphere package (MAP) containing 5% O_2, 5% CO_2, 90% N_2 and atmosphere containing synthetic air (control),

by 25 d at 5 ºC. For each storage atmosphere, columns with different letters are different by Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

6.3.8. Ethylene

Ethylene results for cherry tomatoes cherry tomatoes stored in modified atmosphere package (MAP) and synthetic air (control), by 25 d at 5 ºC are shown in Fig. 6.7. There were increases in the concentrations of ethylene during storage of 37 the control and 16.78 nL/g for the ATM. Probably there was a peak in ethylene production during the first 15 days of storage. After this period, it is likely that ethylene production for the product is small, but the amount of ethylene in the package remains constant for both storage conditions. Results showed that MAP reduced ethylene production of cherry tomatoes stored at 5 °C. Similar results were found by Hong and Gross (2001) that evaluated the quality of fresh-cut tomato slices during cold storage under various modified atmosphere packaging conditions. These authors verified increase in CO_2 and ethylene production after 6 d, with subsequent stabilization after 9 d of storage. Low O_2 and elevated CO_2 can significantly reduce the rates of ripening and senescence primarily by reducing the synthesis and perception of ethylene changes in respiration and starch, sugars, chlorophyll, and cell wall constituents during ripening and/or senescence can be reduced by eliminating ethylene action through the use of low O_2/high CO_2 atmospheres (ABELES et al., 1992). According to Hong and Gross (2001) it seems that O_2 may be more important for ethylene production from tomato slices under MAP at 5 °C than CO_2. However, the mechanism(s) by which the change in atmospheric composition in containers responsible for affecting ethylene production of slices under MA at 5 °C is not clear. For Odriozola-Serrano et al. (2008), tomato slices stored in MAP (5 kPa O_2 + 5kPa CO_2) at 5 °C exhibited a gradual increase in ethylene production during the first 7 days, after which the concentration remained unchanged.

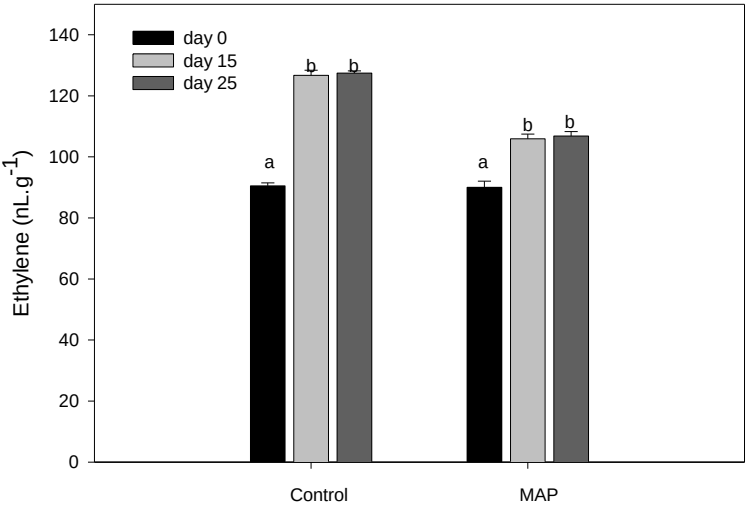

Figure 6.7 Ethylene of cherry tomatoes stored in modified atmosphere package (MAP) containing 5% O_2, 5% CO_2, 90% N_2 and atmosphere containing synthetic air (control), by 25 d at 5 ºC.

6.4. Conclusions

The present investigation showed senescence inhibition of cherry tomatoes by the effect of MAP, resulting in reduction of respiration rate and production of ethylene with consequently maintenance of tissue firmness, inhibition chemical processes that alter compounds such as sugars and organic acids, as compared to the control samples. The conditions applied reduced the changes in lycopene content and color of the product. These results suggest that a combination of MAP and low temperature maintained the cherry tomatoes quality and extending their postharvest life up to 25 d when stored at 5 °C. Parameters such as weight loss and firmness could improve, further with the use of other technology associated with MAP, a suggestion would be the use of edible coating which could assign a protective layer for water loss product.

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CAPÍTULO 7:
ANTIFUNGAL ACTIVITY OF FOOD ADDITIVES *IN VITRO* AND AS INGREDIENTS OF HYDROXYPROPYL METHYLCELLULOSE-LIPID EDIBLE COATINGS AGAINST *BOTRYTIS CINEREA* AND *ALTERNARIA ALTERNATA* ON CHERRY TOMATO FRUIT

Antifungal activity of foods additives *in vitro* and as ingredients of hydroxypropyl methylcellulose-lipid edible coatings against *Botrytis cinerea* and *Alternaria Alternata* on cherry tomato fruit

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Abstract

The antifungal activity of food additives or 'generally recognized as safe' (GRAS) compounds was tested *in vitro* against *Botrytis cinerea* and *Alternaria alternata*. Radial mycelial growth of each pathogen was measured in PDA petri dishes amended with food preservatives at 0.2, 1.0, or 2.0% (v/v) after 3, 5, and 7 days of incubation at 25 °C. Selected additives and concentrations were tested as antifungal ingredients of hydroxypropyl methylcellulose (HPMC)-lipid edible coatings. The curative activity of stable coatings was tested *in vivo* experiments. Cherry tomatoes were artificially inoculated with the pathogens, coated by immersion about 24 h later, and incubated at 20 °C and 90% RH. Disease incidence and severity (lesion diameter) were determined after 6, 10, and 15 days of incubation and the 'area under the disease progress stairs' (AUDPS) was calculated. In general, HPMC-lipid antifungal coatings controlled black spot caused by *A. alternata* more effectively than gray mold caused by *B. cinerea*. Overall, the best results for reduction of gray mold on cherry tomato fruit were obtained with coatings containing 2.0% of potassium carbonate, ammonium phosphate, potassium bicarbonate, or ammonium carbonate, while 2.0% sodium methylparaben, sodium ethylparaben, and sodium propylparaben were the best ingredients for coatings against black rot.

Keywords*: Lycopersicon esculentum*, postharvest disease, gray mold, black rot, food antimicrobials

7.1. Introduction

Cherry tomato (*Lycopersicon esculentum* L.) is one of the most widely produced and consumed horticultural crops in the world, for both fresh produce markets and processed food industries (Feng et al., 2011). This fruit is susceptible to postharvest disease caused by various pathogenic fungi. *Botrytis cinerea* Pers.: Fr. and *Alternaria alternata* (Fr.) Keissl., causing gray mold and black spot, respectively, are among the most common fungal pathogens responsible for postharvest decay on cherry tomato fruit (Wang et al., 2009).

Synthetic chemical fungicides have been used to reduce postharvest fungal spoilage, but because of problems regarding toxicity, fungicide resistance, and negative impact on both the environment and human health, alternative measures for disease control are increasingly demanded (Spadaro and Gullino, 2004). In general, decay control methods that are alternatives to conventional synthetic fungicides can be classified as physical, chemical, or biological (Palou et al., 2008). Several alternative methods of different nature have been assayed against both *B. cinerea* and *A. alternata*, including cold storage in conventional controlled atmospheres, application of heat treatments (Zhong et al., 2010), use of ionizing radiations (Charles et al., 2009), biological control (Wang et al., 2008, 2010), or dips in aqueous solutions of food additives or other chemical compounds. Alternative chemical control methods comprise the use of natural or synthetic compounds with known and low toxicity, usually classified as food additives or 'generally recognized as safe' (GRAS) substances by most of food and drug Administrations worldwide (Larrigaudière et al., 2002; Palou et al., 2002).

The use of edible films and coatings is an alternative chemical method to preserve the postharvest quality of fruits and vegetables (Debeaufort et al., 1998). Consumer interest towards natural healthy products has led researchers to develop new edible films and coatings as an environmentally-friendly technology that may enhance food quality, safety, stability, and the mechanical handling properties by providing a semi-permeable barrier to water vapor, oxygen, and carbon dioxide between the food and the surrounding atmosphere (Greener and Fennema, 1994). In the last decade, several works have focused on the development of coatings based on proteins or polysaccharides with natural food preservatives to control microbial growth on fruits and

vegetables. Antimicrobials can be added to edible coatings to retard the growth of bacteria, yeasts, and molds during storage and distribution of fresh or minimally processed products (Valencia-Chamorro et al., 2011). Coatings containing antimicrobials, such as some organic acids and their salts (Franssen et al., 2004) parabens and other food additives (Valencia-Chamorro et al., 2009b; Yildirim and Yapici, 2007), chitosan, essential oils, or natural plant extracts (Falguera et al., 2011; Sánchez-González et al., 2011) have been effective in delaying the growth of contaminating microorganisms and maintaining the quality during storage and distribution of fresh and fresh-cut horticultural products.

Different treatments have been evaluated for the control of postharvest decay of tomatoes. Essential oils like those from thyme, sage, cassia, or dill have showed significant inhibitory activity against fungal pathogens such as *A. alternata* or *Aspergillus* spp. (Feng and Zheng, 2007; Feng et al., 2011; Tian et al., 2011). Treatments with chitosan provided an effective control of tomato diseases caused by *B. cinerea* and *Penicillium expansum* (Liu et al., 2007). According to Pane et al. (2012), compost teas showed high biological control ability, both *in vitro* and *in vivo* on tomato, against *A. alternata, B. cinerea* and *Pyrenochaeta lycopersici.* Wang et al. (2009) studied the control of postharvest decay on cherry tomatoes by the marine yeast *Rhodosporidium paludigenum* and calcium chloride and verified that the combined treatments showed high activities to reduce black rot caused by *A. alternata*. A combination of heat treatment at 38 ºC and the biocontrol agent *Pichia guilliermondii* prevented cherry tomato spoilage caused by the pathogens *B. cinerea, A. alternata, and Rhizopus stolonifer* (Zhao et al., 2010). However, available information on the development of new edible composite coatings with the addition of antifungal compounds as a new technique to control major fungal postharvest diseases of cherry tomatoes is very limited.

The objectives of this study were to: evaluate the *in vitro* activity of food additives with antifungal properties against *B. cinerea* and *A. alternata*; formulate stable hydroxypropyl methylcellulose (HPMC)-lipid edible composite coatings containing selected antifungal food preservatives; and determine the curative activity of these coatings for the control of gray mold and black rot on artificially inoculated cherry tomatoes.

7.2. Materials and methods

7.2.1. *Pathogens and fungal inoculum*

The strains TAA-1 of *B. cinerea* and TAV-6 of *A. alternata*, obtained from decayed tomatoes in Valencia packinghouses, were isolated, identified, and maintained in the IVIA culture collection of postharvest pathogens. Prior to each experiment, the isolates were grown on potato dextrose agar (PDA; Sigma-Aldrich Chemie, Steinheim, Germany) in petri dishes at 25 ºC for 7-14 days. Depending on the experiment, mycelial plugs from these cultures were used or high-density conidial suspensions were prepared in Tween 80 (0.05%, w/v; Panreac-Química S.A., Barcelona, Spain) in sterile water, passed through two layers of cheesecloth, measured with a haemacytometer, and diluted with sterile water to achieve an inoculum density of 1 x 10^6 spores/mL of *B. cinerea* or *A. alternata.*

7.2.2 Food preservatives

Food preservatives used in this work, molecular formulas, and the corresponding E- code list for food additives in the European Union (EU) are shown in Table 7.1. Most of them are likewise classified as food additives or GRAS compounds by the United States Food and Drug Administration (US FDA). Laboratory reagent grade preservatives (99% minimum purity) were purchased from Sigma-Aldrich Chemie, Fluka Chemie AG (Buchs, Switzerland), Panreac Química S.L.U., or Merck KGaA (Darmstadt, Germany). Potassium silicate (PSi) was purchased from Certis USA L.L.C. (Columbia, MD, USA) as the commercial product Sil-Matrix® (29% PSi).

Table 7.1 Characteristics of antifungal food preservatives tested *in vitro* or *in vivo* for inhibition of *Botrytis cinerea* and *Alternaria alternata.*

Food preservative	Acronym	Molecular formula	E-code[a]	MW[b]
Potassium sorbate	PS	$C_6H_7O_2K$	E-202	150.22
Sodium acetate	SA	CH_3COONa	E-262(i)	82.03
Sodium methylparaben	SMP	$C_8H_7NaO_3$	E-219	174.13
Sodium propylparaben	SPP	$C_{10}H_{11}NaO_3$	E-217	202.19
Sodium ethylparaben	SEP	$C_9H_9NaO_3$	E-215	188.16
Sodium propionate	SP	CH_3CH_2COONa	E-281	96.06
Sodium benzoate	SB	$C_7H_5O_2Na$	E-211	144.11
Potassium carbonate	PC	K_2CO_3	E-501(i)	138.21
Ammonium phosphate	APh	$NH_4H_2PO_4$	E-342(i)	132.07
Ammonium carbonate	AC	$(NH_4)_2CO_3$	E-503(i)	114.1
Potassium silicate	PSi	K_2SiO_3	E-560	154.26
Sodium formate	SF	$HCOONa$	E-237	68.01
Sodium bicarbonate	SBC	$NaHCO_3$	E-500(ii)	84.01
Potassium bicarbonate	PBC	$KHCO_3$	E-501(ii)	100.12
Ammonium bicarbonate	ABC	NH_4HCO_3	E-503(ii)	79.06

[a] E-code = code number for food additives approved by the European Union.

[b] Molecular weight.

7.2.3. Fruit

Cherry tomatoes (*Lycopersicon esculentum* L.) used in the experiments were commercially grown and collected in the Valencia area (Spain) and stored up to 24 h at 5 ºC until use. Fruit were free from previous postharvest treatments or coatings. Before each experiment, fruit were selected, randomized, washed with fruit biodegradable detergent (Essasol V., Didsa, Potries, Valencia), rinsed with tap water, and allowed to air-dry at room temperature.

7.2.4. Determination of in vitro *antifungal activity of food preservatives*

The effect of potassium sorbate (PS), sodium benzoate (SB), sodium acetate (SA), sodium propionate (SP), sodium formate (SF), sodium methylparaben (SMP), sodium ethylparaben (SEP), sodium propylparaben (SPP), and PSi (Table 1) on mycelial growth of *B. cinerea* and *A. alternata* was evaluated on PDA medium amended at 45-55 ºC with sterile aqueous solutions of food additives. Stock solutions of 20% of each salt were used to achieve final salt concentrations of 0.2, 1.0 and 2.0% (v/v). PDA without salts served as control. The amended PDA medium was poured in sterile conditions into 90 mm sterile plastic petri dishes to a thickness of 5 mm, and left to dry at room temperature. The next day, the center of each test plate was inoculated with a 5-mm diameter plug of 7-15 day-old cultures of *B. cinerea* or *A. alternata* and incubated for 7 days at 25 ºC in the dark in a growth cabinet. Radial mycelial growth was determined in each plate after 3, 5 and 7 days of incubation by calculating the mean of two perpendicular fungal colony diameters. For each pathogen, salt, and salt concentration, four replicate plates were used. The results were expressed as percentage of mycelial growth inhibition according to the formula: (dc-dt)/dc×100, where dc = average diameter of the fungal colony on control plates and dt = average diameter of the fungal colony on salt-amended plates.

Other food preservatives (Table 7.1) used in this research in *in vivo* trials, but that were not tested *in vitro* because their *in vitro* antifungal activity against *B. cinerea* and *A. alternata* has already been reported in the literature include: potassium carbonate (PC; Nigro et al., 2006), ammonium phosphate (APh; Nigro et al., 2006), ammonium carbonate (AC; Palmer et al., 1997), sodium bicarbonate (SBC; Mills et al., 2004; Nigro et al., 2006; Palmer et al., 1997), potassium bicarbonate (PBC;

Palmer et al., 1997), and ammonium bicarbonate (ABC; Nigro et al., 2006; Palmer et al., 1997).

7.2.5. Formulation and preparation of antifungal coatings

HPMC (Methocel E15) was purchased from Dow Chemical Co. (Midland, MI, USA) and beeswax (BW) (grade 1) was supplied by Fomesa Fruitech S.L. (Valencia, Spain). Oleic acid and glycerol were from Panreac Química S.L.U. HPMC-lipid edible composite emulsions were prepared combining the hydrophilic phase (HPMC) and the hydrophobic phase (BW) suspended in water. Glycerol and oleic acid were used as plasticizer and emulsifier, respectively. Ratios of HPMC-glycerol (3:1) (dry basis, db) and BW-oleic acid (5:1) (db) were kept constant throughout the study. Tween 80 was also added to the formulations at a concentration of 1.5% (w/w) to improve wetting of the coating and adherence to the tomato fruit. All formulations contained 2% (w/w) of food preservative. Emulsions were prepared as described by Valencia-Chamorro et al. (2008). Briefly, an aqueous solution of HPMC (5% w/w) was prepared by dispersing the HPMC in hot water at 90 ºC and later hydration at 20 ºC. The corresponding food preservative, BW, glycerol, oleic acid, and water were added to the HPMC solution and heated at 98°C to melt the lipids. Samples were homogenized with a high-shear probe mixer (Ultra-Turrax model T25, IKA-Werke, Steufen, Germany) for 1 min at 12,000 and 3 min at 22,000 rpm. Emulsions were cooled under agitation to a temperature lower than 25 ºC by placing them in a water bath and agitation was continued during 25 min to ensure complete hydration of the HPMC. The final solid concentration of the emulsions were optimized to obtain formulations with a viscosity range of 100-150 cP. Emulsions were kept 1 day at 5 ºC before use. The formulations were tested for stability and phase separation.

7.2.6. Curative activity of antifungal coatings

Cherry tomatoes were superficially wounded once in the equator with a stainless steel rod with a probe tip 1 mm wide and 2 mm in length. This wound was inoculated with the corresponding pathogen by placing 10 µl of a spore suspension containing 1 x 10^6 spores/mL of *B. cinerea* or *A. alternata*. Different lots of fruit were used for each pathogen.

After incubation at 20 ºC for 24 h, inoculated fruit were coated by immersion for 30 s in the selected HPMC-lipid edible composite emulsions, drained, and allowed to air-dry at 20 ºC. Inoculated but

uncoated fruit were used as controls. Coated fruit were placed on plastic trays on corrugated cartons and then incubated up to 15 days at 20 ºC and 85-90% RH. In every experiment, each treatment was applied to 3 replicates of 10 fruit each. The experiments were repeated twice.
The incidence of gray mold or black spot was assessed as the number of infected fruit and reported as the percentage of incidence reduction with respect to the control treatments. Disease severity was determined as the diameter of the lesion (mm) and the results were reported as the percentage of severity reduction with respect to the control treatments. Disease development data were used to calculate the area under the disease progress stairs (AUDPS; Simko and Piepho, 2012). Disease incidence and severity were assessed after 6, 10 and 15 days of incubation at 20 ºC.

7.2.7. Statistical analysis

Statistical analyses were performed using the software Statgraphics 5.1 (Manugistics, Inc., Rockville, MD, USA). For both *in vitro* and *in vivo* data, mean differences were determined by Fisher´s protected least significant difference test (LSD, $P<0.05$) applied after an analysis of variance (ANOVA). For disease incidence data, the ANOVA was applied to the arcsine of the square root of the percentage of infected fruit in order to assure the homogeneity of variances. Non-transformed means are shown.

7.3. Results

7.3.1. In vitro *activity of food preservatives*

The determination of antifungal activity in this study was based on the reduction of radial growth of fungal colonies as compared to that on control plates without food preservatives. Significant interactions were found in the ANOVA between the factors food preservative and preservative concentration for the *in vitro* inhibition of both *B. cinerea* and *A. alternata* after 5 and 7 days of incubation at 25 ºC (Table 7.2). These are the incubation periods after which each pathogen covered entirely the control plates (PDA with no addition of preservatives). Therefore, the effect of each preservative was significantly dependent on the concentration at which it was applied.

Table 7.2 Two-way analysis of variance of *in vitro* inhibition in PDA plates (percentage of colony diameter reduction) of *Botrytis cinerea* and *Alternaria alternata* after 5 and 7 days, respectively, of incubation at 25 °C.

	SS	df	MS	*F*-ratio	*P*-value
Botrytis cinerea					
F: Food	61492.4	8	7686.55	677.71	0.0000
preservative	14264.1	2	7082.07	624.42	0.0000
C: Concentration					
F x C	13737.4	16	858.59	75.70	0.0000
Error	918.693	81	11.3419		
Total	90312.7	107			
Alternaria alternata					
F: Food	12590.0	8	15740.10	614.14	0.0000
preservative	13421.5	2	6710.75	261.84	0.0000
C: Concentration					
F x C	18002.1	16	1125.13	43.90	0.0000
Error	2075.99	81	25.6295		
Total	159420.0	107			

Parabens and PSi were the most effective antifungals against both pathogens, with no significant differences among concentrations of 0.2, 1.0 and 2.0% (Table 7.3). In contrast, the food preservatives SF, SA, PS, and SB were significantly more effective at the concentration of 2%. The least effective antifungals in inhibiting *B. cinerea* and *A. alternata* were SF and SA, respectively.

Table 7.3 *In vitro* antifungal activity of food preservatives amended at different concentrations to PDA plates against *Botrytis cinerea* and *Alternaria alternata* after 5 and 7 days, respectively, of incubation at 25 ºC.

Food preservative	Inhibition of *B. cinerea* (%)[a]			Inhibition of *A. alternata* (%)[a]		
	Preservative concentration (%)			Preservative concentration (%)		
	0.2	1.0	2.0	0.2	1.0	2.0
Sodium formate	0.00 cE	28.27 bC	55.26 aC	0.00 bD	0.00 bE	40.00 aE
Potassium silicate	94.32 aA	94.32 aA	94.32 aA	92.38 aA	92.38 aA	92.38 aA
Sodium acetate	7.81 cD	39.63 bB	67.90 aB	0.00 bD	0.00 bE	25.29 aF
Sodium propionate	57.39 bB	87.07 aA	94.18 aA	13.33 cB	52.64 bC	63.83 aC
Sodium methylparaben	94.32 aA	94.32 aA	94.32 aA	93.24 aA	93.24 aA	93.24 aA
Sodium ethylparaben	94.32 aA	94.32 aA	94.32 aA	93.24 aA	93.24 aA	93.24 aA
Sodium propylparaben	94.32 aA	94.32 aA	94.32 aA	93.24 aA	93.24 aA	93.24 aA
Potassium sorbate	58.52 bB	94.32 aA	94.32 aA	4.80 bC	69.33 aB	88.27 aB
Sodium benzoate	39.20 bC	94.32 aA	94.32 aA	0.80 cA	34.93 bD	47.20 aD

[a] Colony diameter reduction with respect to control treatments (non-amended PDA plates).

For each pathogen, means in lines with different lowercase letters and means in columns with different capital letters are significantly different by Fisher´s protected LSD test ($P<0.05$) applied after an ANOVA.

The radial mycelial growth rate of both *B. cinerea* and *A. alternata* during the 7-day incubation period at 25 ºC is shown in Fig. 7.1. This evolution of the mycelial growth is important to assess the temporal pattern for the inhibitory activity of the salts. The colonies of *B. cinerea* and *A. alternata* completely covered control plates (9 mm diameter) after 5 and 7 days of incubation, respectively. The results indicated that both colony size and mycelial growth rate were reduced with increasing concentrations of the food preservatives, with the exception of sodium parabens and PSi that completely inhibited the radial growth of both pathogens at all tested concentrations. The use of SP, PS, and SB effectively reduced the colony diameter of *B. cinerea* and *A. alternata*,

especially at concentrations of 1.0 and 2.0%, while SF and SA did not inhibit the growth of the pathogens regardless the preservative concentration. The growth rate and final colony diameter, however, were significantly lower when these two salts were used at 2.0%. Moreover, in the case of *A. alternata*, radial growth was even greater in plates amended with these two salts at concentrations below 2.0% than in control plates, showing that these substances somehow stimulated fungal growth. According to these results, the salts SF and SA were discarded and the preservatives SMP, SEP, SPP, PSi, SP, PS, and SB, were all selected for use in further *in vivo* tests. As the efficacy of food preservatives may differ in *in vitro* and *in vivo* experiments, the highest salt concentration of 2.0% was chosen to formulate emulsions and check if stable antifungal coatings were obtained. Additionally, the following salts were also tested as emulsion ingredients at this concentration of 2.0%: PC, APh, AC, SBC, PBC, and ABC.

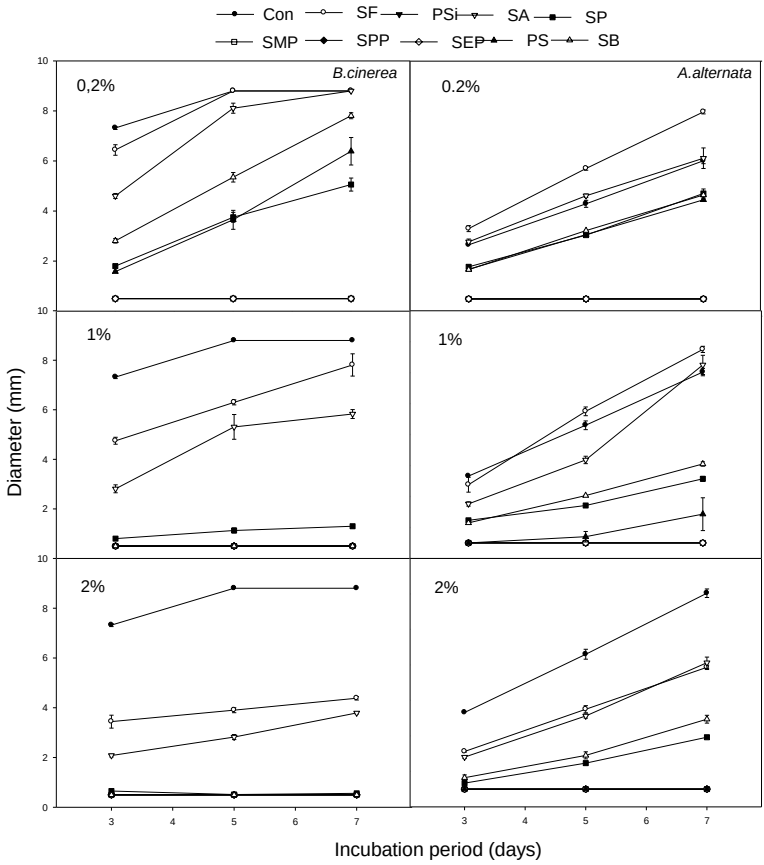

Fig. 7.1. *In vitro* colony diameter of *Botrytis cinerea* and *Alternaria alternata* inoculated in plates containing PDA amended with sodium formate (SF), potassium silicate (PSi), sodium acetate (SA), sodium propionate (SP), potassium sorbate (PS), sodium benzoate (SB), sodium methylparaben (SMP) sodium ethylparaben (SEP) and sodium propylparaben (SPP) at concentrations of 0.2, 1.0, or 2.0% and incubated at 25 °C for 7 days. Controls (Con) were non-amended PDA plates.

7.3.2. Formulation and curative activity of the coatings

Table 7.4 shows the different food preservatives used to prepare the antifungal coatings and the properties of the emulsion formulations. All these emulsions were stable and no phase separation was observed. Therefore, all of them were used for *in vivo* experiments with cherry tomatoes.

Table 7.4 Solid concentration, viscosity and pH of selected HPMC-lipid edible composite emulsions containing antifungal food preservatives.

HPMC-lipid edible coatings with food preservative	Solid concentration (%)	Viscosity (cp)	pH
Potassium sorbate	10.0	151.6	6.73
Sodium methylparaben	10.0	140.4	9.60
Sodium propylparaben	9.0	116.5	10.12
Sodium ethylparaben	10.0	157.0	9.70
Sodium propionate	8.0	103.3	6.68
Sodium benzoate	10.0	142.3	6.39
Potassium carbonate	10.0	123.8	10.98
Ammonium phosphate	6.5	123.1	7.87
Ammonium carbonate	10.0	147.5	9.40
Potassium silicate	10.0	115.1	11.55
Sodium bicarbonate	10.0	118.7	8.22
Potassium bicarbonate	10.0	134.6	8.59
Ammonium bicarbonate	7.0	98.0	8.41

The effect of different edible coatings containing food preservatives on gray mold and black rot development on cherry tomato artificially inoculated with *B. cinerea* and *A. alternata*, respectively, and incubated for 6 days at 20 ºC is shown in Fig. 7.2. Although decay incidence reduction was generally low for both diseases, especially for gray mold, it was significantly (P<0.05) increased by some treatments. Coatings containing SMP, PC, APh, and ABC, and coatings containing SMP, SPP, SB, PSi, and SEP were the most effective in reducing the incidence of gray mold (up to 20%) and black rot (up to 40%), respectively. Similarly, significant differences were observed among the values of severity reduction for both gray mold and black rot. In general, the reduction of disease severity was considerably higher than the reduction of disease incidence, and the reduction of black rot severity was higher

than that of gray mold. The most effective coating antifungal ingredients in reducing severity after 6 days of incubation at 20 ºC were SMP, SP, SB, PC, APh, AC, and PBC for gray mold, with values around 40%, and SMP, SPP, SB, and SEP for black rot, with values of 60-70%. SPP and PSi for gray mold, and ABC for black rot were the worst coating ingredients for severity reduction.

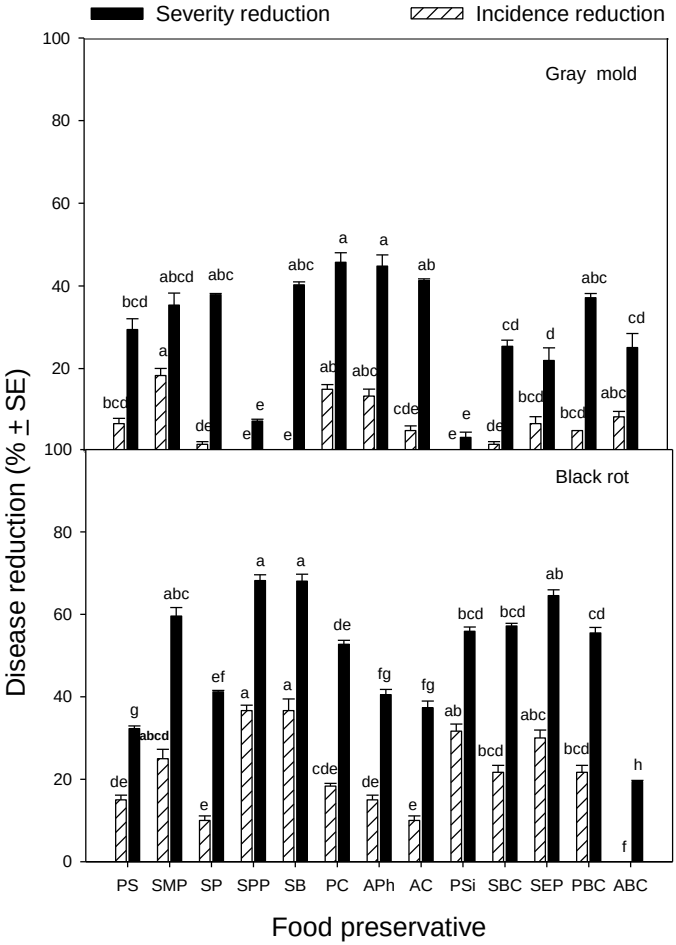

Fig. 7.2. Reductions of the incidence and severity of gray mold and black rot on cherry tomatoes artificially inoculated with *Botrytis cinerea* and *Alternaria alternata*, respectively.

Fig. 7.2. Reductions of the incidence and severity of gray mold and black rot on cherry tomatoes artificially inoculated with *Botrytis cinerea* and *Alternaria alternata*, respectively, coated 24 h later with HPMC-lipid edible composite coatings containing 2% of the following

preservatives as antifungal ingredients and incubated for 6 days at 20 ºC and 90% RH: potassium sorbate (PS), sodium methylparaben (SMP), sodium propionate (SP), sodium propylparaben (SPP), sodium benzoate (SB), potassium carbonate (PC), ammonium phosphate (APh), ammonium carbonate (AC), potassium silicate (PSi), sodium bicarbonate (SBC), sodium ethylparaben (SEP), potassium bicarbonate (PBC), and ammonium bicarbonate (ABC). For each mold, incidence and severity reductions were determined with respect to control fruit (inoculated but uncoated). Disease incidence and severity in control treatments were 100% and 90-110 mm, and 100% and 80-89 mm for gray mold and black rot, respectively. For disease incidence reduction, the ANOVA was applied to arcsine-transformed values. Non-transformed means are shown. For each mold, columns with different letters are significantly different according to Fisher´s protected LSD test ($P<0.05$) applied after the ANOVA.

Mean values for the area under the disease progress stairs (AUDPS) on cherry tomatoes artificially inoculated with *B. cinerea* and *A. alternata* and incubated at 20 ºC and 90% RH for 15 days are shown in Fig. 7.3. There were significant differences ($P<0.05$) between the antifungal treatments and the uncoated controls, except in the case of gray mold and the coating containing SPP. According to AUDPS values, the most effective coatings in reducing disease caused by *B. cinerea* were those containing the food preservatives PC, APh, PBC, and AC. Contrarily, the least effective antifungals against gray mold were SPP and PSi. In the case of disease caused by *A. alternata*, the lowest and the highest AUDPS values in cherry tomato were obtained with the antifungals SEP, SBC, PSi, SMP, and SPP, and APh, ABC, AC, and PS, respectively.

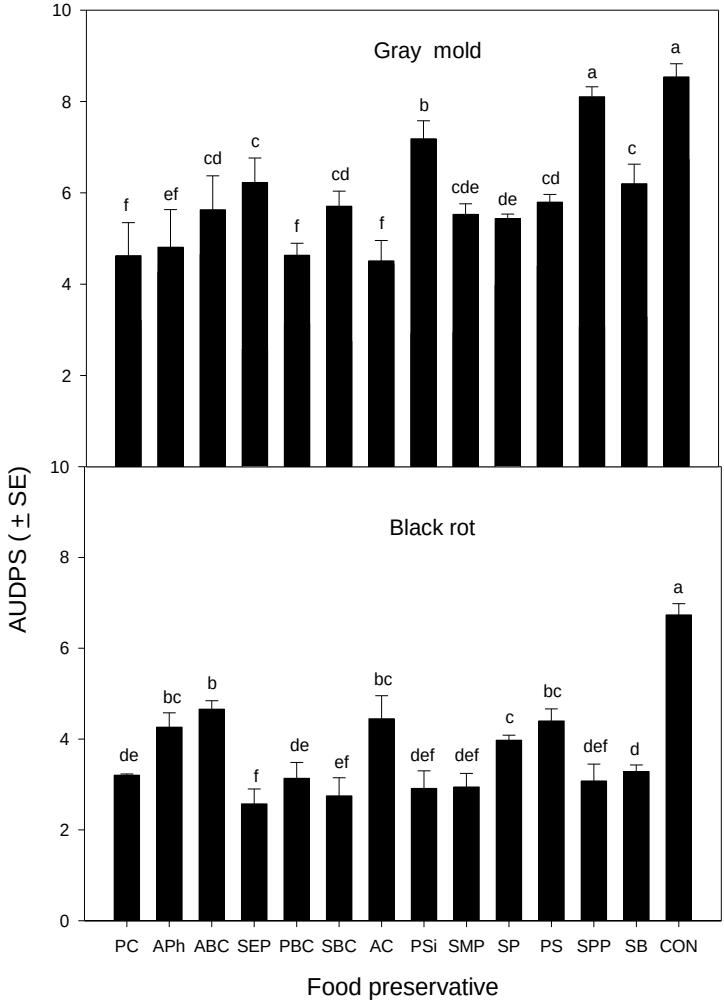

Fig. 7.3. Area under the disease progress stairs (AUDPS) for gray mold and black rot on cherry tomatoes artificially inoculated with *Botrytis cinerea* and *Alternaria alternata*, respectively, coated 24 h later with HPMC-lipid edible composite coatings containing 2% of the following preservatives as antifungal ingredients and incubated for 15 days at 20

ºC and 90% RH: potassium sorbate (PS), sodium methylparaben (SMP), sodium propionate (SP), sodium propylparaben (SPP), sodium benzoate (SB), potassium carbonate (PC), ammonium phosphate (APh), ammonium carbonate (AC), potassium silicate (PSi), sodium bicarbonate (SBC), sodium ethylparaben (SEP), potassium bicarbonate (PBC), and ammonium bicarbonate (ABC). Control fruit (CON) were inoculated but uncoated. For each mold, columns with different letters are significantly different according to Fisher´s protected LSD test ($P< 0.05$) applied after an ANOVA.

7.4. Discussion

This study highlights the antifungal properties of different food preservatives against *B. cinerea* and *A. alternata* and their potential use as ingredients in antifungal edible coatings applied for postharvest disease control of fresh cherry tomatoes. Results showed that most of the tested food preservatives exerted good antifungal activities *in vitro.* The percentage of fungal inhibition in amended PDA medium was dependent on concentration, and the most significant reduction in mycelial growth was obtained with the highest concentration of food preservative. In the case of substances such as SA and SF at low concentrations, the fungus grew even faster that in control plates and final colony diameters were higher. As reported in other cases (Palou et al., 2002), these salts presumably provided additional nutrients and/or enhanced environmental conditions for the development of the pathogen. Nigro et al. (2006) found that SA and SF had a minimum inhibitory concentration (MIC) greater than 2.0% for *B. cinerea* in a colony growth assay. In our study, the use of sodium paraben salts, PSi, SP, PS, and SB at 2% resulted in complete *in vitro* growth inhibition of this fungus. Similarly, sodium parabens, PSi, and PS applied at the concentration of 2% completely inhibited the growth of *A. alternata*. Therefore, the effect of these salts on conidia germination of *B. cinerea* and *A. alternata* was fungicidal under our experimental conditions. These results are in agreement with those from Yapici and Yildirim (2007) who found 100% inhibition of conidia germination of *B. cinerea* in an *in vitro* assay with methylparaben and propylparaben. Karabulut et al. (2005), using PS solutions at 0.5 and 1.0% achieved reductions of 37.7 and 24.4%, respectively, in the growth of *B. cinerea*. Furthermore, Mills et al. (2004) found *in vitro* reductions of the mycelial growth of *B.*

cinerea of 78, 61, and 77% after application of SBC, SB, and PS, respectively. For *A. alternata*, the same authors found reductions of 100 and 66% with the application of propylparaben and PS, respectively.

According to the results from the *in vitro* tests, the antifungals, with the exception of SF and SA, were tested at a concentration of 2% as potential ingredients of HPMC-lipid coatings with antifungal activity against both gray mold and black rot on cherry tomatoes. Since all emulsions prepared with this concentration of antifungal ingredient were stable and showed good physical characteristics, no emulsions were formulated with lower antifungal concentrations. The *in vivo* efficacy of the coatings was evaluated according to the reduction of disease incidence and severity on coated tomatoes previously inoculated with the pathogens. This methodology allowed the assessment of the curative activity of the antifungal coatings. This was necessary because both gray mold and black spot are postharvest diseases caused to a great extent by latent field infections (Barkai-Golan, 2001). The data obtained showed that the antifungal emulsions did not prevent the onset of fungal diseases, since the values of disease incidence reduction were not 100% in any case, and they were generally low. This result might have been influenced by the high concentration of fungal inoculum that was used in these trials (10^6 spores/mL). This high inoculum density of *B. cinerea* and *A. alternata* was used in our *in vivo* screening of antifungal coatings to obtain high percentages of decay on control fruit and to conservatively select only those formulations with higher potential for effective commercial usage. In these tests, however, severity results showed that many of the antifungals incorporated to coatings effectively retarded disease development. From this point of view, it was clear that in general gray mold was less affected than black rot by the antifungal products present in the coatings.

PC, APh, PBC, AC, SMP, SB, and SP were the best food preservatives for coatings against gray mold. Carbonates have been used as natural means to reduce fungal growth in different pathosystems. Nigro et al. (2006) evaluated the activity of 19 inorganic and organic salts to control table grape decay during storage and found that PC significantly reduced the incidence of gray mold caused by *B. cinerea* in *in vivo* tests with small table grape clusters. The activity of carbonates in inhibiting spore germination, germ tube elongation, and production of pectinolytic enzymes in several pathogens is well recognized (Hervieux et al., 2002; Mills et al., 2004; Punja and Grogan, 1982). These salts strongly

inhibited mycelial growth and spore germination of *B. cinerea* as well as polygalacturonase activity. Considering that the proportion of CO_3^{2-} ion is elevated at high pH (>11), the CO_3^{2-} form has been suggested to be responsible in aqueous solutions of the inhibitory activity that leads to reductions of mycelial growth and spore germination (Palmer et al., 1997). According to these workers, the main mode of action of the bicarbonate ion is through its buffering capacity, whereby an alkaline environment is sustained. When this happens, organisms such as *B. cinerea*, which require an acidic environment, expend more energy on fungal acid production than hyphal extension and therefore growth may be inhibited. These same authors verified in *in vitro* tests that dibasic and tribasic phosphates would decrease colony diameters of *B. cinerea* at rates similar to bicarbonates. The mode of action of most salts is postulated to consist in a reduction of fungal turgor pressure that results in the collapse and shrinkage of fungal hyphae causing subsequent inhibition of mycelial growth and sporulation (Fallik et al., 1997). In the case of black rot, coatings formulated with SMP, SPP, and SEP showed the best behavior in reducing disease severity after 6 days of incubation at 20 ºC. Paraben sodium salts were also the best antifungals in *in vitro* tests against *A. alternata*. Methylparaben, ethylparaben, propylparaben, and their sodium salts are GRAS compounds of increasing interest as means to control postharvest decay in fresh horticultural products (Moscoso-Ramírez et al., 2013; Valencia-Chamorro et al., 2011). Parabens are in the undissociated form at pH values of most foods (p*K*a = 8.5) and are effective over a wide pH range of 4–8 (Thompson, 1994). Paraben salts like SMP, SEP, or SPP are more soluble in water than their correspondent parabens and they might interfere on both the germinative and vegetative phases of microbial development, but it has been reported that in fungi spore germination is much more susceptible than vegetative growth (Watanabe and Takesue, 1976). It has been suggested that the general mode of action of these salts is through an uncoupling of oxidative phosphorylation, inhibition of NAD+ and FAD-linked mitochondrial respiration, or the reduction of mitochondrial membrane potential (Soni et al., 2001).

As expected, differences in effectiveness of food preservatives between *in vitro* and *in vivo* trials have been observed in this research. The direct inhibitory effect of the salts amended in PDA on the growth of *B. cinerea* or *A. alternata* in petri dishes can considerably differ from the inhibitory ability of these salts as ingredients of edible coatings applied

to cherry tomatoes. While substances such as paraben salts, PSi, SP, PS, and SB were the most effective for *in vitro* growth inhibition of *B. cinerea*, others like PC, APh, PBC, and AC were the food preservatives that showed the best results for *in vivo* inhibition of gray mold. In the case of *A. alternata*, while salts such as SEP, SMP, and SPP showed similar good behavior in both *in vitro* and *in vivo* assays, others like SB showed dissimilar behavior. Therefore, the present study confirms that *in vitro* tests alone are inappropriate to predict the potential of an antifungal agent to control postharvest diseases of fresh fruits or vegetables, although they can be very useful to select the most suitable agents to be tested in subsequent *in vivo* trials. Similar results were reported by Valencia-Chamorro et al. (2009a) after evaluating salts as ingredients of HPMC-lipid edible coatings for the control of citrus green and blue molds, or by Nigro et al. (2006) in their study on the performance of different salts for the control of gray mold in grapes. The interaction between the salt and the agar medium (Biggs et al., 1997), as well as the interaction between the salt and the environmental conditions (Punja and Grogan, 1982), may play an important role on the *in vitro* inhibition ability; whereas the complex interactions between host, pathogen, and environment that occur during disease development determine the *in vivo* inhibition ability. In some cases, specific interactions between the applied salt and the tissues of the fruit host may involve biochemical reactions that lead to the induction of defense mechanisms that contribute to disease control (Dore et al., 2010; Hervieux et al., 2002). When the antifungal agent is an ingredient of a coating, additional factors involved in the release of the agent to the fruit peel may have also great influence on the antifungal performance. In general, there are three steps that determine the release of antimicrobial agents from polymer matrices: diffusion within the polymer matrices, mass transfer across the interface, and dispersion into the bulk food (Limm and Hollifield, 1995). In this research work, the limited inhibitory activity against gray mold of HPMC-lipid edible composite coatings containing sodium parabens when applied *in vivo* to cherry tomatoes in comparison to the activity *in vitro* against *B. cinerea*, could be likely attributed to a limited chemical release from the coating matrix to the fruit surface. In a study simulating the release of propylparaben from a polymer coating (styrene-acrylate copolymer), Chung et al. (2001) found that the release of the chemical from the coating into water and food-simulating solvents depended on the interactions among

propylparaben, the polymer coating, and the solvents. Similar considerations might apply for the coatings containing PSi as antifungal ingredient. In addition to the release ability of the antimicrobial from the polymer matrix, each type of fruit may considerably differ in skin resistance to the diffusion of the antimicrobial agent, gas diffusion, and fruit respiration rate, among other attributes. Therefore, coatings developed for one fruit species or cultivar may not be suitable for another (Park, 1999).

Additional information about the temporal potential of the edible coatings to reduce fungal growth on cherry tomatoes was obtained with the values of AUDPS. Traditionally, the area under the disease progress curve (AUDPC) was frequently used to combine multiple observations of disease progress into a single value (Shaner and Finney, 1977). However, AUDPS has been recently developed as a new formula to get a better estimate of disease progress (Simko and Piepho, 2012). This approach improves the estimation of disease progress by giving a weight closer to optimal to the first and last observations. In this research, AUDPS results were obtained on artificially inoculated cherry tomatoes through an incubation period of 15 days at 20 ºC and showed that all coatings containing food preservatives significantly reduced the progression of gray mold caused by *B. cinerea* in cherry tomatoes compared with the control treatment, except for those containing SPP. Similarly, all edible coatings formulated with food preservatives reduced the development of black rot caused by *A. alternata*. When AUDPS values are compared to disease reductions after 6 days of incubation at 20 ºC, it appears that some antifungal coatings lacked persistence and reduced fungal growth for only a certain period of time. In the case of gray mold, the higher reductions in disease severity after 6 days were caused by the coatings containing PC, APh, PBC, AC, SMP, SB, and SP, while AUDPS values indicated that only the coatings with PC, APh, PBC, and AC were the most effective along the entire incubation period. In the case of black rot, the best coatings for severity reduction after 6 days were those formulated with the three paraben sodium salts and SB. However, AUDPS values showed that coatings containing the paraben salts, PSi, and SBC were superior through the entire storage period at 20 ºC. According to these results, all antifungal HPMC-lipid edible composite coatings, but especially those containing the salts SB and SP, provided a fungistatic rather than fungicidal effect, and they were not very persistent.

In conclusion, it was observed in this study that the application of HPMC-lipid edible composite coatings containing common food preservatives as antifungal ingredients showed promise as a non-polluting method to reduce losses caused by major tomato postharvest diseases. Further research with selected antifungal edible coatings is needed to define the impact of the application of these treatments on cherry tomato fruit quality and storability. Among the wide range of food preservatives tested as ingredients, the most promising compounds to control decay of cherry tomatoes caused by *B. cinerea* were PC, APh, PBC, and AC, while sodium parabens were the most appropriate against decay caused by *A. alternata*. If the physiological responses of coated fruit are positive, these coatings could be used in postharvest management programs as commercial alternative tools for decay control and shelf life extension, especially in the case of production areas with high incidence of black rot caused by *A. alternata*.

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CAPÍTULO 8:
EFFECT OF ANTIFUNGAL OF HYDROXYPROPYL METHYLCELLULOSE-LIPID EDIBLE COATINGS AGAINST *BOTRYTIS CINEREA* AND QUALITY ATTRIBUTES OF COLD-STORED CHERRY TOMATO FRUIT

Effect of antifungal of hydroxypropyl methylcellulose-lipid edible coatings against *Botrytis cinerea* and quality attributes of cold-stored cherry tomato fruit

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Abstract

Edible composite coatings based on hydroxypropyl methylcellulose (HPMC), hydrophobic components (beeswax), and food preservatives with antifungal properties were evaluated on cherry tomatoes during cold storage. Selected food preservatives included sodium propionate (SP), potassium carbonate (PC), ammonium phosfate (APh) and ammonium carbonate (AC). Cherry tomatoes artificially inoculated with *Botrytis cinerea* (BC) were coated and stored up to 14 d at 5 °C followed by 7 d of shelf life at 20 °C. All antifungal HPMC-lipid coatings reduced the incidence and severity of gray molds on inoculated and cold-stored cherry tomatoes and PC-based coating was the most effective. Analytical and sensory fruit quality was evaluated on intact tomatoes. AC-based coatings was effective to control weight loss and maintain the firmness of coated cherry tomatoes. Respiration rate, firmness, color, sensory flavor, off-flavor, and fruit appearance were not adversely affected by the application of the antifungal coatings. Further studies should focus on the modification of some physical characteristics of the coatings in order to provide better water loss control and higher gloss on coated cherry tomatoes.

Keywords: cherry tomatoes, coating, antifungical, cold storage, sensory quality.

8.1. Introduction

During the last decades, there has been an increasing demand for fresh fruits and vegetables forcing the food industry to develop new and better methods for maintaining food quality and extend shelf life. Furthermore, consumers around the world demand food of high quality, without chemical preservatives and with extended shelf life. Tomato (*Solanum lycopersicum* L.), being a climacteric fruit, has a relatively short postharvest life (ZAPATA et al., 2008), storage is limited by several factors including transpiration, postharvest diseases, increased ripening and senescence.

The most economically important plant diseases are caused by the action of fungi (ZIANI et al., 2009). According to Wang et al. (2009) one of the most common diseases in tomatoes is caused by *Botrytis cinerea* Pers. Several methods and techniques have been developed to extend the shelf-life of fruits and vegetables damaged by the fungus action (SEBTI et al., 2005).

One of these techniques is the release of biocides molecules of antimicrobial agents previously fixed in a biodegradable or edible film and coating (WENG; CHEN, 1997; PALOU et al., 2002; PARK et al., 2004; PALOU et al., 2008). Edible films and coatings are alternative and non-polluting methods that have been developed to extend product shelf life (GREENER; FENNEMA, 1994; PARK, 1999; RHIM; SHELLHAMMER, 2005), and are generally based on biological materials such as proteins, lipids and polysaccharides. The main polysaccharides that can be included in edible coating formulations are starch and starch derivatives, cellulose derivatives, chitosan, pectin, alginate and other gums (TZOUMAKI et al., 2009). Films containing proteins and polysaccharides present a good barrier to gases, but a poor moisture barrier. On the contrary, lipid films are used as an adequate barrier to water vapor. Composite films comprise hydrocolloid components and lipids, thus enhancing the advantages and lower the disadvantages of each (VALENCIA-CHAMORRO et al., 2011).

Edible coatings are used commercially to reduce moisture loss, prevent physical damage, improve product appearance and carry food ingredients including antibrowning agents, colorants, flavours, nutrients, spices and antimicrobials (FRANSSEN; KROCHTA, 2003; MARTÍN-BELLOSO et al., 2005). The functionality of edible coatings can be expanded by incorporating antimicrobials to protect food products from microbial spoilage, extend their shelf-life and enhance their safety

(FRANSSEN; KROCHTA, 2003). Alternative methods of different nature have been assayed against several fungus including *B. cinerea*. Alternative chemical control methods comprise the use of natural or synthetic compounds with known and low toxicity, usually classified as food additives or 'generally recognized as safe' (GRAS) substances by most of food and drug Administrations worldwide (LARRIGAUDIÈRE et al., 2002; PALOU et al., 2002).

Several works in the literature report that edible composite coatings based on hydroxypropyl methylcellulose (HPMC) and lipids such as beeswax (BW), carnauba wax, or resin (shellac) preserved the postharvest quality of citrus fruit by reducing weight loss and keeping firmness and sensory quality of coated fruit (PÉREZ-GAGO et al., 2002; NAVARRO-TARAZAGA et al., 2007; NAVARRO-TARAZAGA et al., 2008). The inhibitory activity of hydroxypropyl methylcellulose (HPMC) associated with nisin was confirmed in *Listeria inoccua* and *Staphylococcus aureus* by Sebti and Voma (2002). In recent work, *in vivo* selected edible composite coatings based on hydroxypropyl methylcellulose (HPMC) associated with generally recognized as safe (GRAS) compounds, reduced the incidence and severity of GM and BM on 'Clemenules' clementine mandarins, 'Ortanique' hybrid mandarins, and 'Valencia' oranges stored at 20 °C (VALENCIA-CHAMORRO et al., 2009). HPMC-based coatings containing sorbic acid (0.4%) enhanced the inactivation of *Salmonella montevideo* on the surface of tomatoes (ZHUANG et al., 1996). Functional properties and antimicrobial effects of chitosan are related to its desacetylation degree and molecular weight. Chitosan inhibits the growth of a wide variety of fungi, yeasts and bacteria. Due to its film forming property, chitosan is used to prepare films and coatings (NO et al., 2007). Ali et al. (2010), showed that using 10% gum arabic as an edible coating, delay the ripening process of tomatoes stored at 20 °C, and at shelf life can be extended up to 20 days without any spoilage and off-flavour.

Nevertheless, there is little information focused on the effect of coatings containing food preservatives on the control of postharvest *B. cinerea* on cherry tomatoes and the impact of these coatings on the postharvest quality of fresh vegetable during cold storage. Therefore, the objective of this work was to determine the effect of selected HPMC-lipid edible composite coatings containing food additives with

antifungal properties on the development of *B. cinerea* and the physico-chemical and sensory quality of cherry tomatoes during cold storage.

8.2. Materials and methods

8.2.1. Materials

HPMC (Methocel E15) was purchased from Dow Chemical Co. (Midland, MI, USA). Beeswax (BW) (grade 1) were supplied by Fomesa Fruitech, S.L. (Beniparrell, València, Spain). Oleic acid and glycerol were from Panreac Química, S.A (Barcelona, Spain). Food preservatives used in this work, solid concentration, viscosity and pH are shown in Table 8.1. All are likewise classified as food additives or GRAS compounds by the United States Food and Drug Administration (US FDA). Laboratory reagent grade preservatives (99% minimum purity) were purchased from Panreac Química S.L.U.

Table 8.1. Characteristics of hydroxypropyl methylcellulose (HPMC)-lipid edible composite coatings containing antifungal food preservatives

HPMC-lipid edible coatings with food preservative	Solid concentration (%)	Viscosity (cp)	pH
Sodium propionate	8.0	103.3	6.68
Potassium carbonate	10.0	123.8	10.98
Ammonium phosphate	6.5	123.1	7.87
Ammonium carbonate	10.0	147.5	9.40

8.2.2. Emulsions preparation

HPMC-lipid edible composite emulsions were prepared combining the hydrophilic phase (HPMC) and the hydrophobic phase (BW) suspended in water. Glycerol and oleic acid were used as plasticizer and emulsifier, respectively. Ratios of HPMC-glycerol (3:1) (dry basis, db) and BW-oleic acid (5:1) (db) were kept constant throughout the study. Tween 80 was also added to the formulations at a concentration of 1.5% (w/w) to improve wetting of the coating and adherence to the tomato fruit. All formulations contained 2% (w/w) of food preservative. Emulsions were prepared as described by Valencia-Chamorro et al. (2008). Briefly, an aqueous solution of HPMC (5% w/w) was prepared by dispersing the HPMC in hot water at 90 ºC and

later hydration at 20 ºC. The corresponding food preservative, BW, glycerol, oleic acid, and water were added to the HPMC solution and heated at 98 °C to melt the lipids. Samples were homogenized with a high-shear probe mixer (Ultra-Turrax model T25, IKA-Werke, Steufen, Germany) for 1 min at 12,000 and 3 min at 22,000 rpm. Emulsions were cooled under agitation to a temperature lower than 25 ºC by placing them in a water bath and agitation was continued during 25 min to ensure complete hydration of the HPMC. The final solid concentration of the emulsions were optimized to obtain formulations with a viscosity range of 100-150 cp. Emulsions were kept 1 day at 5 °C before use. The formulations were tested for stability and phase separation.

8.2.3. Effect of coatings on disease development

8.2.3.1. Fungal inoculum

The strain TAA-1 of *B. cinerea*, obtained from decayed tomatoes in Valencia packinghouses, was isolated, identified, and maintained in the IVIA culture collection of postharvest pathogens. Prior to each experiment, the isolate was grown on potato dextrose agar (PDA; Sigma-Aldrich Chemie, Steinheim, Germany) in Petri dishes at 25 °C for 7-14 days. Depending on the experiment, mycelial plugs from this culture was used or high-density conidial suspension was prepared in Tween 80 (0.05%, w/v; Panreac-Química S.A., Barcelona, Spain) in sterile water, passed through two layers of cheesecloth, measured with a haemacytometer, and diluted with sterile water to achieve an inoculum density of 1 x 10^6 spores/mL of *B. cinerea*.

8.2.3.2. Fruit inoculation and coating application

Cherry tomatoes (*Lycopersicon esculentum* L.) used in the experiments were commercially grown and collected in the Valencia area (Spain) and stored up to 24 h at 5 °C until use. Fruit were free from previous postharvest treatments or coatings. Before each experiment, fruit were selected, randomized, washed with fruit biodegradable detergent (Essasol V., Didsa, Potries, Valencia), rinsed with tap water, and allowed to air-dry at room temperature. Cherry tomatoes were superficially wounded once in the equator with a stainless steel rod with a probe tip 1 mm wide and 2 mm in length. This wound was inoculated with the pathogen by placing 10 µL of a spore suspension containing 1 x 10^6 spores/mL of *B. cinerea*. After incubation at 20 °C for 24 h,

inoculated fruit were coated by immersion for 30 s in the selected HPMC-lipid edible composite emulsions, drained, and allowed to air-dry at 20 °C. Inoculated but uncoated fruit were used as controls. Coated fruit were placed on plastic trays on corrugated cartons and then incubated up to 14 days at 20 °C, followed by 7 d at 20 °C and 85-90% RH. In every experiment, each treatment was applied to 3 replicates of 10 fruit each. The experiments were repeated twice.

8.2.3.3. Determination of disease incidence and severity

Disease incidence of BC was calculated as the percentage of decayed fruit. Disease severity was determined as the diameter of the lesion (mm). Incidence and severity were assessed in 7 and 14 d during the storage period at 5 ºC, and also after a shelf-life period of 7 d at 20 ºC following cold storage.

8.2.4. Effect of coating on fruit quality

8.2.4.1. Fruit coating and storage

For the quality study, before each experiment, fruit were selected, randomized, washed with fruit biodegradable detergent (Essasol V., Didsa, Potries, Valencia), rinsed with tap water, and allowed to air-dry at room temperature. Fruit were divided into five groups of 120 fruit each, which corresponded to the four coating treatments described in Table 8.1 and one control (uncoated fruit). The cherry tomatoes were coated as described above, drained of excess coating, dried and stored for up to 15 d at 5 °C and 90-95 % RH. Physico-chemical and sensory fruit quality was assessed in 10 and 15 d at 5 °C plus a shelf life period of 5 d at 20 °C.

8.2.5. Assessment of fruit quality

8.2.5.1 Internal quality

Internal quality was expressed through of the parameters as soluble solids content (SSC) determinate using the juice fruits pulp and content read by digital refractometer (model PR1; AtagoCo. Ltd, Japan), values were expressed as °Brix. The titratable acidity (TA) of the fruit juice was determined by titrating 5 mL of juice sample with 0.1 mol L^{-1} sodium hydroxide end point of pH 8.1 and expressed as percentage of citric acid. The pH of the cherry tomatoes was determined

in the fruits juice by using a pH-meter (Consort C830). For each treatment was prepared three juices, and each performed three readings.

8.2.5.2. Color

The color of the skin cherry tomatoes was measured with a Minolta (Model CR-400, Minolta, Tokyo, Japan) on 20 fruits per treatment, using the CIELAB color parameters, L*, a*, b*, chroma (C) and hue angle (h). Each measurement was taken at three locations for each cherry tomato. A standard white calibration plate was employed to calibrate the spectrophotometer.

8.2.5.3. Weight loss

Lots of 30 fruit per treatment were used to measure weight loss. The same marked cherry tomato were weighted at the beginning and at the end of each storage period. The results were expressed as the percentage of initial weight lost.

8.2.5.4. Fruit firmness

Firmness of 20 fruit per treatment was determined at the end of each storage period using an Instron Universal testing machine (Model 4301, Instron Corp., Canton, MA, USA). Each fruit was compressed between two flat surfaces closing together at therate of 5 mm min-1. The machine gave the deformation (mm) after application of a load of 9.8 N to the equatorial region of the fruit. Results were expressed as percentage of deformation, related to initial diameter.

8.2.5.5. Respiration rate

Samples of cherry tomatoes, coated and uncoated, were placed in sealed containers for measures the gas concentration. Aliquot the amount of O_2 and CO_2 produced by the fruit storage for 3 h at 20 °C was taken from the headspace. The gas sample was injected into a gas chromatograph (GC) (Thermo Trace, Thermo Fisher Scientific, Inc. Waltham, MA, USA) equipped with a thermal conductivity detector (TCD) and fitted with a Poropack QS 80/100 column (1.2 m x 0.32 cm i.d.). Temperatures were 35, 115, and 150 ºC, respectively for the oven, injector, and thermal conductivity detector. Helium was used, as carrier gas at a flow rate of 22 mL min-1. The respiration rate concentration was calculated using peak area obtained from standard gas mixtures of

15.0:2.5 % O_2:CO_2. Results were expressed as (mg O_2 / kg h) and (mg CO_2 / kg h). Three closed containers per treatment were analyzed.

8.2.5.6. Ethanol and acetaldehyde contents

Ethanol and acetaldehyde were analysed from the head-space of juice from samples using a GC (Thermo Trace, Thermo Fisher Scientific) equipped with an auto-sampler (Model HS 2000), flame ionization detector (FID), and 1.2 m x 0.32 cm (i.d.) Poropack QS 80/100 column. The injector was set at 175 ºC, the column at 150 ºC, the detector at 200 ºC, and the carrier gas at 28 mL min-1. A composite juice of three replicates of ten fruit per treatment was analyzed. Five mL of juice were transferred to 10-mL vials with crimptop caps and TFE/silicone septum seals. Samples were frozen and stored at –18 ºC until analyses. A 1-mL sample of the headspace was withdrawn from vials previously equilibrated in a water bath at 20 ºC for 1 h, followed by 15 min at 40 ºC, to reach equilibrium in the headspace, and then injected into the GC. Ethanol and acetaldehyde was identified by comparison of retention times with standards. Results were expressed as mg of gas per 1 L of juice.

8.2.5.7. Sensory evaluation

Sensory quality of treated samples was evaluated by 10 trained judges at the end of each storage period. Judges rated flavor on a 9-point scale where 1 = very poor and 9 = optimum. Each judge was given samples from each batch and requested to evaluate off-flavor on a 5-point scale where 0 = absence of off-flavor and 5 = high presence of off-flavor. Five fruit per treatment were halved cut and separated into individual segments. Two segments from two different fruit were presented to judges in trays labeled with 3-digit random codes and served to them at room temperature. The judges had to taste several segments of each sample in order to compensate, as far as possible, for biological variation of the material. Spring water was provided for palate rinsing between samples. External aspect of treated fruit (coating cracks, spots, etc.) was also evaluated by the panelists. A 3- point scale was used in which the aspect was classified as 1 = bad, 2 = acceptable, and 3 = good. Panelists were also asked to rank visually the treatments from highest to lowest gloss.

8.2.6. Statistical analysis

Statistical analysis was performed using Statgraphics 5.1. (Manugistics Inc., Rockville, MD, USA). Specific differences between means were determined by Fisher's protected least significant difference test (LSD, $P < 0.05$) applied after an analysis of variance (ANOVA). For sensory gloss, specific differences were determined by Friedman test, which is recommended for ranking by the UNE 87023 (AENOR, 1997). For disease incidence data, the ANOVA was applied to the arcsine of the square root of the percentage of infected fruit in order to assure the homogeneity of variances. Non-transformed means are shown.

8.3. Results and Discussion

8.3.1. Effect of coatings on disease development

The effect of different edible coatings containing food preservatives on gray mold development on cherry tomato artificially inoculated with *B. cinerea*, and stored for 14 d at 5 °C followed by 7d at 20 °C is shown in Figure 8.1. After 2 wks of cold storage at 5 °C, all the coatings reduced gray mold incidence and severity compared with uncoated samples. Although, severity increase was observed after 14 d at 5 °C followed by 7d at 20 °C, all the coatings reduced the disease compared with control. In the first week at 5 °C, SP, AC and Aph coating were the most effective to reduce the disease severity of gray mold. In general, the reduction of disease severity was considerably higher than the reduction of disease incidence. The disease incidence on coated samples increased during the entire cold storage period, but it was lower than on control samples after 2 wks ($P < 0.05$). The coating containing SP was most effective to reduce the disease incidence of gray mold after 2 wks of cold storage (reduction of 60% after 7 d and 30% after 14 d). After 2 wks of cold storage at 5 °C followed by storage at 20 °C for 7 d, the data obtained showed that the antifungal emulsions did not prevent the onset of fungal diseases, since the values of disease incidence were of 100% in every case. This result might have been influenced by the high concentration of fungal inoculum that was used in these trials (10^6 spores/mL).

From results of disease incidence and severity, found that the food preservatives were fungistatics but no fungicidal, because growth slowed but did not eliminated the fungus. In general, comparable differences on performance depending on the fruit species or cultivars

have been observed with most of the alternative antifungal treatments which mode of action is rather fungistatic than fungicidal (PALOU et al., 2008)

In this work, an HPMC-lipid edible coating with SP additive was the best antifungal against the pathogen BC. The propionate are classical preservation agents, Droby et al. (2003) showed that calcium propionate completely inhibited mycelial growth of *B. cinerea* at a level of 5% (w/v). The food preservatives AC, APh and PC presented reduction of BC grown during cold storage. According to Sivakumar et al. (2002), ammonium carbonate (3%) incorporated into the wax formulation effectively reduced anthracnose incidence by 70% in naturally infected papaya and extended the storage life. The activity of carbonates in inhibiting spore germination, germ tube elongation, and production of pectinolytic enzymes in several pathogens is well recognized (PALOU et al., 2002; SMILANICK et al., 2005). These salts strongly inhibited mycelial growth and spore germination of *B. cinerea* as well as polygalacturonase activity. Considering that the proportion of CO_3^{2-} ion is elevated at high pH (>11), the CO_3^{2-} form has been suggested to be responsible in aqueous solutions of the inhibitory activity that leads to reductions of mycelial growth and spore germination (Palmer et al., 1997). Liu et al. (2007) evaluated effect antifungal of chitosan in tomatoes, and the results indicated that chitosan at 0.5 and 1% could significantly decrease gray mould and blue mould caused by *B. cinerea* and *P. expansum* in tomato fruit stored at 25 and 2 °C, respectively.

The application of HPMC-lipid edible composite coatings containing food preservatives is a simple and environmentally-friendly method to reduce the losses caused by postharvest diseases. Thus, these coatings could be used as a commercial alternative to synthetic chemical fungicides for decay control, especially in combination with other postharvest treatments that provide complementary activity.

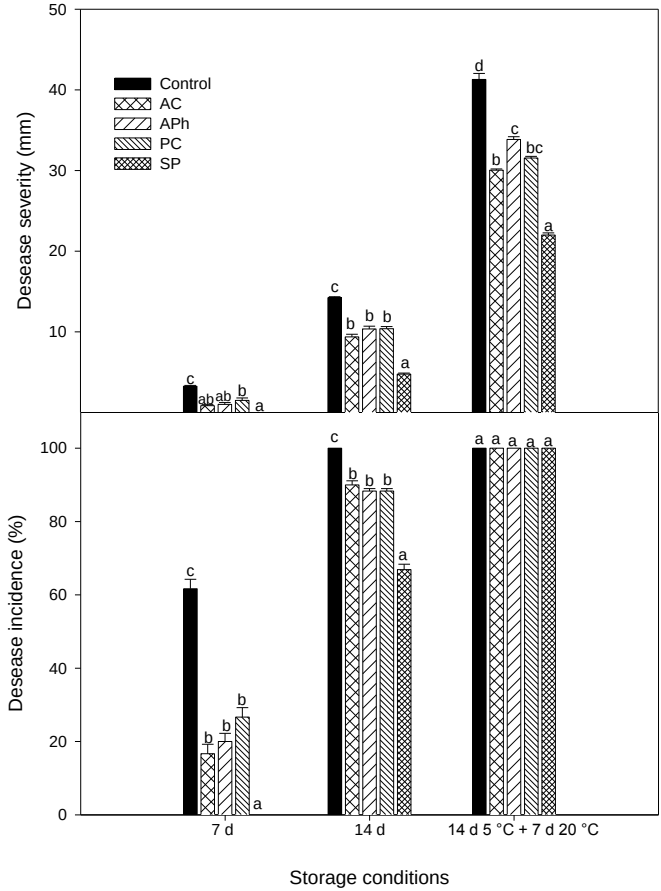

Figure 8.1. Disease incidence and severity of gray mold, on cherry tomatoes artificially inoculated with *Botrytis cinerea*, uncoated (control), or coated 24 h later with hydroxypropyl methylcellulose (HPMC)-lipid edible composite coatings containing ammonium carbonate (AC), ammonium phosfate (APh), sodium propionate (SP) and potassium carbonate, stored at 5 ºC for 14 d followed by 7d at 20 °C. For each storage period, columns with different letters are significantly different by Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA. For disease incidence, the ANOVA was applied to arcsine-transformed values. Non-transformed means are shown.

8.3.2 *Effect of coating on fruit quality*

8.3.2.1 Weight loss

Figure 8.2 shows the weight loss on coated and uncoated samples stored for 10 and 15 d at 5 ºC, followed by 5 d at 20 ºC. At the end the first and second storage period, weight loss for the all samples were around 1.54-2.98% and 1.95-3.25% respectively. In general, weight loss slightly increased with storage time. The coatings containing AC significantly reduced weight loss of coated cherry tomatoes after the first and second storage period, which indicates the effectiveness of these coatings as a moisture barrier. During entire storage period weight loss of cherry tomatoes treated with coatings containing APh was not significantly different of the control samples. In contrast, tomatoes coated with SP and PC coatings presented higher weight loss than uncoated samples.

Many works had reported the edible coating application with and without significant effects on weight loss of fruits. Das et al. (2013) studying coatings containing starch, glycerol and lipid verified that weight loss was lowest in coated tomatoes indicating that lipid in the coating film was effective in reducing water loss. Sánches- González et al. (2011) reported the effect of hydroxypropylmethylcellulose coatings with and without bergamot essential oil cold-stored grapes and verified that both provided a significant water vapour barrier, showing lower weight losses than the uncoated samples.

Navarro-Tarazaga et al. (2008) observed that HPMC-BW coatings containing different types of plasticizers did not reduce weight loss of ‘Angeleno’ plums as compared with uncoated samples. Quality of table grapes coated with hydroxypropylmethylcellulose edible coatings containing propolis extract assessed by Pastor et al. (2011) showed that weight loss of the grapes was significantly higher in uncoated samples. In another study, Valencia-Chamorro et al. (2009) found that weight loss of oranges treated with most of the coatings, HPMC coatings containing food preservatives, was not significantly different from that of control samples. These same authors, based on data obtained in Valencia-Chamorro et al. (2008) state that the mechanical properties of the film could also explain the performance of the coating after a long storage period. From this it is likely that coatings containing some food preservatives could more easily form pits or

cracks on the fruit surface that might enhance water loss leading to high weight loss.

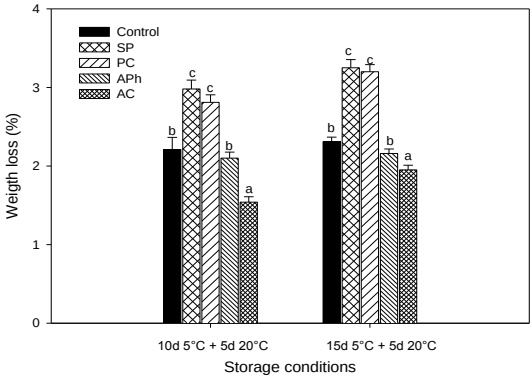

Figure 8.2. Weight loss of cherry tomatoes uncoated (control) or coated with hydroxypropyl methylcellulose (HPMC)-lipid edible composite coatings containing ammonium carbonate (AC), ammonium phosfate (APh), sodium propionate (SP) and potassium carbonate, stored at 5 ºC followed by 5d at 20 °C. For each storage period, columns with different letters are different by Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

8.3.2.2. Fruit firmness

Firmness values of the cherry tomatoes coated not significantly different with uncoated samples, although samples treated with coatings containing AC and APh showed lower deformation values compared to control. Fruit deformation after both storage periods was around 17%. According to Valencia-Chamorro et al. (2009) the firmness of 'Valencia' oranges were not modified on coated fruit after both storage periods as compared to uncoated samples. An another study, the coatings hydroxypropyl methylcellulose-based content different beeswax concentration, maintained firmness of coated samples compared to uncoated samples when they were stored 4 weeks at 1 °C followed by 2 and 3 weeks at 20 °C (NAVARRO-TARAGAZA et al., 2011). Ahmed et al. (2013) evaluated the application of delactosed whey permeate in tomatoes during 21d of storage at 15°C and verified that the treatment significantly ($p < 0.05$) inhibited fruit softening and

maintained higher levels of firmness throughout the storage compared to control.

The effect of coatings on the maintenance of fruit firmness is usually related to their control of weight loss. The samples with highest weight loss (coatings containing SP and PC) showed a greater reduction of texture. Softening of fruit is due to deterioration in the cell structure, cell wall composition and intracellular materials (SEYMOUR et al., 1993) and a biochemical process involving the hydrolysis of pectin and starch by enzymes (YAMAN and BAYOINDIRLI, 2002). Low respiration rate can limit the activities of these enzymes and allow retention of the firmness during storage (SALUNKHE et al., 1991). Park et al. (1994) reported consumption O_2 of corn-zein coated tomatoes were lower than for non-coated tomatoes. In this work, samples coated containing AC and APh presented lower O_2 consumption and deformation, indicating the possible influence of respiration rate in the texture of the product.

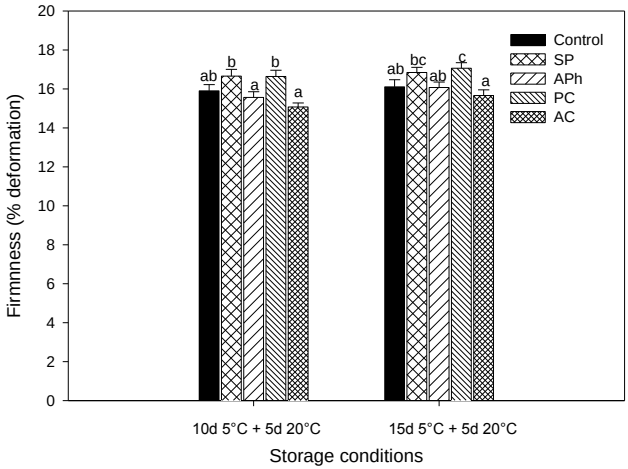

Figure 8.3. Firmness of cherry tomatoes uncoated (control) or coated with hydroxypropyl methylcellulose (HPMC)-lipid edible composite coatings containing ammonium carbonate (AC), ammonium phosfate (APh), sodium propionate (SP) and potassium carbonate (PC), stored at 5 ºC followed by 5 d at 20 °C. For each storage period, columns with different letters are different by Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

8.3.2.3. Respiration rate

The effect of coating on fruit respiration rate was evaluated through O_2 consumption and CO_2 generation. Figure 8.4 shows the development of respiration rates as a function of the cold storage time. For oxygen consumption, significant differences among samples were observed, in all storage conditions. In the first and second storage period, cherry tomatoes treated with edible coating content AC and APh showed lower O_2 consumption compared with control. This indicates that coatings represent an oxygen barrier that limits the acceleration of aerobic respiration rates. Sample that presented lower consumption of O_2 showed lower weight loss. In general, respiration rates are linked to sample weight loss, as has been reported in previous studies (FALLIK et al., 2005; VALVERDE et al., 2005).

All the samples showed an increase in the CO_2 generation with the increase of cold storage which agrees with the increase in the metabolic activity of samples at long storage times related with tissue senescence and cell breakdown (PASTOR et al., 2011). The results showed lower CO_2 production for samples covered by coating contained AC, in the first storage period. The samples with lower CO_2 generation in second storage period were tomatoes covered with coating contained AC and APh. Ali et al. (2010) reduced the respiratory rate of tomatoes using a covering of gum arabic (10%) and suggests that edible coating exerted a barrier to the gaseous exchange.

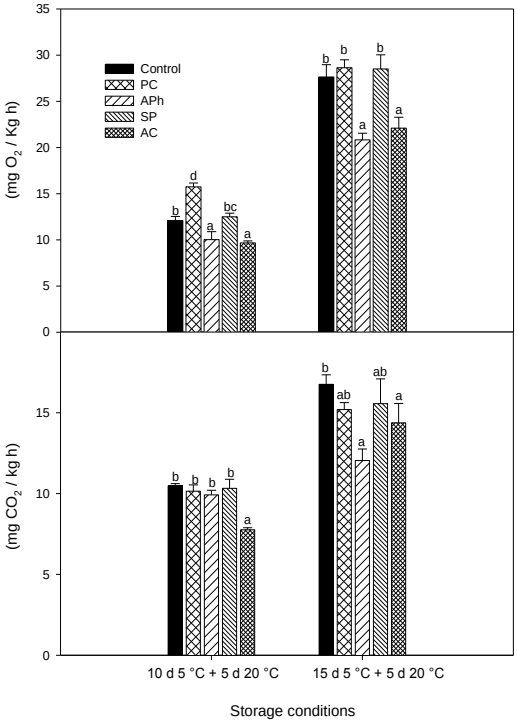

Figure 8.4. Respiration rate of cherry tomatoes uncoated (control) or coated with hydroxypropyl methylcellulose (HPMC)-lipid edible composite coatings containing ammonium carbonate (AC), ammonium phosfate (APh), sodium propionate (SP) and potassium carbonate, stored at 5 ºC followed by 5 d at 20 ºC. For each storage period, columns with different letters are different by Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

8.3.2.4. Ethanol and acetaldehyde content

The application of HPMC-lipid coatings increased ethanol content in the juice of coated cherry tomatoes ($P < 0.05$; Figure 8.5). Thus, the creation of a modified atmosphere with the fruit was confirmed. After the first and second storage period, ethanol and acetaldehyde content in the juice of coated tomato was very varied, and the highest level was found in cherry tomatoes treated with the PC based

coating ($P < 0.05$). In general, the concentration of ethanol in the juice of coated cherry tomatoes after both storage periods was in the range of 1.24-2.95 mg L-1, while it was in the range of 0.24-0.81 mg L-1 in uncoated samples. The concentration of acetaldehyde in the juice of coated cherry tomatoes after both storage periods was in the range of 0.33-0.82 mg L-1, while it was in the range of 0.20-0.36 mg L-1 in uncoated samples.

Different workers have reported higher amount of ethanol content on coated fruit after cold storage fruit. According of Ayala-Zavala et al. (2011) edible coating mineral oil wax-based promoted a accumulation of acetaldehyde and ethanol on treated tomatoes stored at 10 °C for 28 days, which was statistically different from the acetaldehyde contents of carnauba-wax-treated and control fruits, it appears that those levels (acetaldehyde and ethanol) were not sufficient to produce off-flavors that could affect the acceptability of the product.

Baldwin et al. (1999) evaluated the effect of two different edible coatings, one based on polysaccharides and the other based on carnauba wax, on the volatile content of mango fruit. According with this author both edible coatings decreased fruit deterioration, although the polysaccharide coating increased the ethanol and acetaldehyde contents compared to the carnauba wax coating and control fruits. This increase in off-flavor compounds was attributed to the low permeability of the edible coating used.

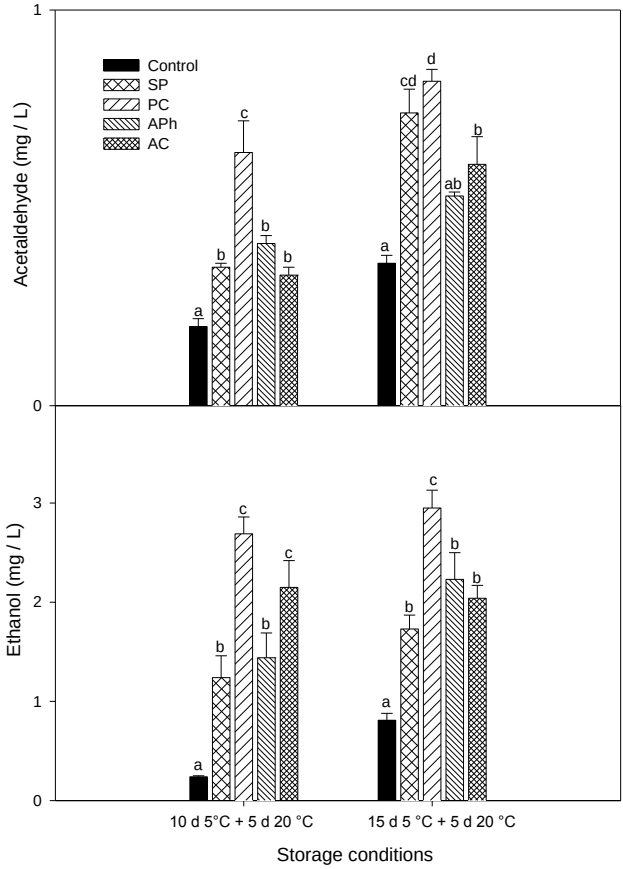

Figure 8.5. Ethanol and acetaldehyde content in the juice of cherry tomatoes uncoated (control) or coated with hydroxypropyl methylcellulose (HPMC)-lipid edible composite coatings containing ammonium carbonate (AC), ammonium phosfate (Aph), sodium propionate (SP) and potassium carbonate, stored at 5 ºC followed by 5 d at 20 °C. For each storage period, columns with different letters are different by Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

8.3.2.5. Fruit internal quality

Coating application did not affect TA, TSS, and pH of cherry tomatoes. The effect of coating application on internal quality parameters has been shown to depend on coating type, fruit cultivar and storage conditions. Some authors have found no differences in these parameters after coating application on different citrus cultivars (BALDWIN et al., 1995; OBENLAND et al., 2008); whereas others have found a decrease in SSC and TA losses compared to uncoated fruits, which was always related to a decrease in weight loss and respiration rate (TOGRUL and ARSLAN, 2004).

Das et al. (2013) found greater values for TA of the uncoated compared with coated tomatoes, according the authors this can be attributed to the increase in ethylene production and respiration rate during the advent of ripening. The same authors observed higher values of pH and TSS of uncoated tomatoes. The pH increase has been attributed to the loss of citric acid in tomatoes by Anthon et al. (2011). Ali et al. (2010) observed that the lowest TSS at the end of the storage period was recorded in tomatoes coated with 20% gum arabic, and showed that the coatings provided an excellent semi-permeable film around the fruit, modifying the internal atmosphere by reducing O_2 and/or elevating CO_2 and suppressing ethylene production. Decreased respiration rates also slow down the synthesis and use of metabolites resulting in lower SSC (YAMAN and BAYOINDIRLI, 2002).

8.3.2.6. Color

Table 8.2 shows the results obtained for the color of cherry tomatoes. The color result is an important indication of the shelf life of fruits and vegetables. The maturation stage can be characterized subjectively by the level of skin color, which is an important parameter to predict the shelf life of fruits. Lightness (L) and hue (h) no presented significant difference among samples coated and uncoated.

In other hand, a*, b* and C* parameters presented difference among samples with and without coating. Only samples treated with coatings containing PC showed significant differences when compared uncoated samples, what may indicate that these samples had an increase in red coloration. The difference between the crhoma (C) values of samples with and without coverage can indicate slight saturation of red color of samples coated, although the chroma not a good indicator of tomato ripening because it essentially is an expression of the purity or

saturation of a single color (different colors may have the same chroma values).

In the case of tomato ripening, different colors are present simultaneously since chlorophyll is degraded from green to colorless compounds at the same time that carotenoids are synthesized from colorless precursor (phytoene) to carotene (paleyellow), lycopene (red), β-carotene (orange) xanthophylls and hydroxylated carotenoids (yellow) (GIULIANO et al., 1993). Ali et al. (2010) observed significant differences in color parameters of tomatoes uncoated and coated with gum arabic edible coating.

Table 8.2. Total soluble solid (TSS), titratable acidity, pH and color index (L*, a*, b*, chroma and hue) of cherry tomatoes coated with hydroxypropyl methylcellulose (HPMC)-lipid edible composite coatings containing antifungal food preservatives and stored at 5 ºC followed by 5 d of shelf life at 20 ºC.

Food preservative	15 d 5 °C +5 d 20 °C							
	TA (g citric acid/L)	TSS(°Brix)	pH	L	a*	b*	C*	h
Control[v]	5.65 ab[z]	8.77 a	4.54 ab	34.56 a	14.29 a	18.16 a	23.14 a	51.89 a
Sodium propionate	5.95 b	8.80 a	4.51 a	34.45 a	14.69 ab	19.45 b	24.42 b	52.99 a
Potassium carbonate	5.94 b	9.00 a	4.59 bc	34.41 a	15.55 b	19.81 b	25.21 b	51.95 a
Ammonium carbonate	5.83 b	8.70 a	4.51 a	34.37 a	15.11 ab	19.79 b	24.94 b	52.70 a
Ammonium phosphate	5.42 a	8.77 a	4.61 c	33.51 a	15.18 ab	19.24 b	24.53 b	51.77 a

[v] Control = uncoated. L*= lightness; a*= red/green; b*= yellow/blue; C*=chroma; h=hue. [z] Means in columns with different letters are significantly different according to Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA

8.3.2.7. Sensory evaluation

HPMC-lipid based coatings containing food preservatives no modified the flavor of cherry tomatoes compared to uncoated samples, as determined by the semi-trained judges of the sensory panel (data not shown). Increases in the ethanol content of the juice beyond a minimal value of 2,000 mg L-1 have been associated with off-flavors in citrus fruit (KE and KADER, 1990). In this study, ethanol values on coated samples were higher than this limit value (Figure 8.5), but the panelists no detected off-flavor after 15 d of storage at 5 ºC plus 5 d at 20 ºC, and observing no differences between coated and uncoated samples, which indicates that the coatings did not induce off-flavor.

The addition of food preservatives to HPMC-lipid emulsion resulted in stable emulsions, but some coated fruit presented small white spots on their surface that reduced the general good appearance of the samples. Among all coated samples, fruit coated with APh-based coatings was evaluated with the highest external appearance value after 15 d at 5 ºC plus 7 d of shelf life at 20 ºC (Table 8.4).

In general, after 15 d at 5 ºC plus 5 d at 20 ºC, coated samples were evaluated as acceptable, no sample coverage was evaluated as bad. After both storage periods, none of the tested coatings provided higher gloss than the uncoated control, and the cherry tomatoes coated with PC and SP were significantly less glossy than the control after 15 d at 5 ºC plus 5 d at 20 ºC (Table 8.3). This behavior could be related to the macroemulsion character of the coating formulations (HAGENMAIER and BAKER, 1994). Ali et al. (2010) studying flavour and overall acceptability of tomatoes with 10% gum arabic coated fruit had the highest scores in all parameters after 20 d of storage, while tomatoes coated with 15 and 20% gum had off-flavour and were not acceptable to the panel of experts. Ahmed et al. (2013) evaluated the application of delactosed whey permeate in tomatoes during 21d of storage at 15°C and at the end of storage, treated tomatoes kept a good appearance and overall quality while in control fruit these parameters fell below the limit of marketability.

Table 8.3. Ranked fruit gloss of cherry tomatoes coated with hydroxypropyl methylcellulose (HPMC)-lipid edible composite coatings containing antifungal food preservatives and stored at 5 ºC followed by 5 d of shelf life at 20 ºC.

Gloss rank	10 d 5 °C + 5 d 20 °C		15 d 5 °C + 5 d 20 °C	
More Glossy	Control[x]	a[y]	Control	a
	APh	ab	APh	ab
	AC	bc	AC	abc
	SP	bc	PC	bc
Less Glossy	PC	c	SP	c

[x] Control = uncoated; APh= ammonium phosphate; AC = ammonium carbonate; PC = potassium carbonate; SP = sodium propionate. [y] Treatments in columns with different letters are significantly different according to Friedman test.

Table 8.4. Flavor, off-flavor and coating appearance of cherry tomatoes coated with hydroxypropyl methylcellulose (HPMC)-lipid edible composite coatings containing antifungal food preservatives and stored at 5 ºC followed by 5 d of shelf life at 20 ºC.

Food preservative	10 d 5 °C + 5 d 20 °C			15 d 5 °C + 5 d 20 °C		
	Flavor	Off-flavor	Coating appearance	Flavor	Off-flavor	Coating appearance
Control[x]	6.9 a[y]	0.1a	3.9a	6.1 a	0.3a	3.6a
Sodium propionate	7.0 a	0.2a	3.8a	6.5 a	0.6a	2.0b
Potassium carbonate	6.5 a	0.4b	3.4b	6.9 a	0.4a	3.0a
Ammonium phosfate	6.6 a	0.2a	3.9a	6.8 a	0.3a	2.4b
Ammonium carbonate	6.9 a	0.1a	4.0a	6.1 a	0.3a	2.1b

[x] Control = uncoated; APh= ammonium phosphate; AC = ammonium carbonate; PC = potassium carbonate; SP = sodium propionate. [y] Treatments in columns with different letters are significantly different according to Friedman test.

8.4 Conclusion

All coatings effectively reduced BC on artificially inoculated and coated cherry tomatoes during cold storage, and the SP-based coating was the most effective at inhibiting molds during storage at 5 ºC. Therefore, HPMC-lipid edible composite coatings containing antifungal could be a promising treatment for tomatoes that should be kept in cold storage. The cover containing AC was the only one that reduced the weight loss. Although the coatings did not reduce weight loss or improve fruit gloss, they did not adversely affect the physico-chemical and sensory quality of cherry tomatoes. Further research should be conducted to improve the physical characteristics of these HPMC-lipid edible composite coatings in order to obtain better water loss control and enhance gloss and visual quality of coated fruit and also on their combination with other control methods alternative to chemical synthetic fungicides in order to find synergistic and /or complementary activities.

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CAPÍTULO 9:
PERFORMANCE OF HYDROXYPROPYL METHYLCELLULOSE (HPMC)-LIPID EDIBLE COMPOSITE COATINGS CONTAINING FOOD ADDITIVES WITH ANTIFUNGAL PROPERTIES DURING COLD STORAGE OF CHERRY TOMATOES

Performance of hydroxypropyl methylcellulose (HPMC)-lipid edible composite coatings containing food additives with antifungal properties during cold storage of cherry tomatoes

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Abstract

Edible composite coatings based on hydroxypropyl methylcellulose (HPMC), hydrophobic components (beeswax), and food preservatives with antifungal properties were evaluated on cherry tomatoes during cold storage. Selected food preservatives included: sodium propyl paraben (SPP), sodium methyl paraben (SMP), sodium ethyl paraben (SEP) and sodium benzoate (SB). Cherry tomatoes artificially inoculated with *Alternaria alternata* (AA) were coated and stored up to 21 d at 5 °C followed by 4 d of shelf life at 20 °C. All antifungal HPMC-lipid coatings reduced the incidence and severity of black rot molds on inoculated and cold-stored cherry tomatoes and SB-based coating was the most effective to control of the growing of fungal. Analytical and sensory fruit quality was evaluated on intact tomatoes. After 10 and 15 d at 5 °C plus 5 d at 20 °C, weight loss, firmness, color, respiration rate, ethanol and acetaldehyde content of the juice, and fruit appearance were not improved by the application of the antifungal coatings. However, further studies should follow to change some coating physical characteristics in order to provide better water loss control and higher gloss on coated cherry tomatoes.

Keywords: cherry tomatoes, coating, food preservatives, conservation, sensory quality.

9.1. Introduction

Tomato fruit have a relatively short postharvest life and, during fruit ripening many processes affecting quality take place. There is a large annual loss due to spoilage and this means that a method to control ripening would be of great economic importance (HOEBERICHTS et al., 2002). Postharvest rots of cherry tomatoes are mainly caused by fungal pathogens such as *Alternaria alternate* (EL GHAOUTH, et al., 1992). The use of synthetic preservatives as antimicrobial agents to control fungal spoilage of food has been practiced for many years. However, this has led to a number of environmental and health problems because of the carcinogenicity, teratogenicity, high and acute toxicity, and long degradation periods of the synthetic preservatives (LINGK, 1991).

New methods are needed because of concerns about environmental contamination and human health risks associated with fungicide residues and because the widespread use of these chemicals in commercial packinghouses has led to the proliferation of resistant strains of the pathogens (PALOU et al., 2002). Alternative methods that have been proposed for the control of postharvest diseases include biological control, physical methods such as heat or radiations, and the use of safe low-toxicity chemicals such as food additives (PALOU et al., 2002, 2008; MONTESINOS-HERRERO et al., 2009; VALENCIA-CHAMORRO et al., 2009).

Alternative chemical control methods comprise the use of natural or synthetic compounds with known and low toxicity, usually classified as food additives or 'generally recognized as safe' (GRAS) substances by most of food and drug Administrations worldwide (LARRIGAUDIÈRE et al., 2002; PALOU et al., 2002). Parabens and some of their salts are classified as "generally regarded as safe" (GRAS) compounds and approved for use in foods by the US Food and Drug Administration (FDA) and European Union (EU) regulations (MILS et al., 2004).

Consumer interest towards natural healthy products has led researchers to develop new edible films and coatings as an environmentally-friendly technology that may enhance food quality, safety, stability, and the mechanical handling properties by providing a semi-permeable barrier to water vapor, oxygen, and carbon dioxide between the food and the surrounding atmosphere (GREENER-DONHOWE and FENNEMA, 1994).

Edible films are biodegradable as the films are produced exclusively from renewable, edible biological components, as polysaccharides, proteins and lipids or a mixture of these (DAS et al., 2013). Starch is used in edible films and coatings (XU et al., 2005) because of its good mechanical properties. Lipids like beeswax, mineral oil, vegetable oil, surfactants, acetylated monoglycerides, carnauba wax and paraffin wax strongly affect the permeability of films and coatings (KESTER and FENNEMA, 1986). Glycerol imparts pliability and flexibility for improved handling and is a widely used plasticizer for making starch-based films and coatings (CHANG et al., 2010). The functionality of edible coatings can be expanded by incorporating antimicrobials to protect food products from microbial spoilage, extend their shelf-life and enhance their safety (FRANSSEN and KROCHTA, 2003). (VALENCIA-CHAMORRO et al., 2008), reported that a wide variety of food additives such as mineral salts, organic acid salts and their mixtures, and sodium salts of parabens and their mixtures, added to stand-alone hydroxypropyl methylcellulose (HPMC)-lipid edible composite films, exhibited antifungal properties against *Penicillium digitatum* and *Penicillium italicum* (PI). HPMC-based coatings containing sorbic acid (0.4%) enhanced the inactivation of *Salmonella montevideo* on the surface of tomatoes (ZHUANG et al., 1996). Feng et al. (2011) assess antifungal effects of thyme oil against *A. alternate*, and verified that thyme oil at 500 mL/L showed a significant contact inhibition effect on *A. alternata* of cherry tomatoes stored at 25 °C for 3 days.

Chitosan and grapefruit seed extract, alone and in combination, produced changes in weight loss, color change, ripening, and improved sensory quality of grapes (XU et al., 2007). Tomatoes coated with rice starch-based edible coating formulation containing coconut oil and tea leaf extract were studied for the effect of coating on biochemical changes during storage for 20 days. Coconut oil and tea leaf extract in the edible coating clearly delayed ripening effects on tomatoes (DAS et al., 2013). Several works in the literature report that edible composite coatings based on hydroxypropyl methylcellulose (HPMC) and lipids such as beeswax (BW), carnauba wax, or resin (shellac) preserved the postharvest quality of citrus fruit by reducing weight loss and keeping firmness and sensory quality of coated fruit (PÉREZ-GAGO et al., 2002; NAVARRO-TARAZAGA et al., 2007; NAVARRO-

TARAZAGA et al., 2008). However, no information is available on the performance of this type of edible coatings on cold-stored tomatoes.

Therefore, the objective of this work was to study the effect of new edible composite coatings prepared with HPMC-lipid containing food additives with antifungal properties on the development of and the physico-chemical, and sensory quality of cherry tomatoes during cold storage.

9.2. Materials and methods

9.2.1. Materials

HPMC (Methocel E15) was purchased from Dow Chemical Co. (Midland, MI, USA). Beeswax (BW) (grade 1) were supplied by Fomesa Fruitech, S.L. (Beniparrell, València, Spain). Oleic acid and glycerol were from Panreac Química, S.A (Barcelona, Spain). Food preservatives used in this work, solid concentration, viscosity and pH are shown in Table 9.1. All of them are likewise classified as food additives or GRAS compounds by the United States Food and Drug Administration (US FDA). Laboratory reagent grade preservatives (99% minimum purity) were purchased from Fluka Chemie AG (Buchs, Switzerland), and Merck KGaA (Darmstadt, Germany).

Table 9.1. Characteristics of hydroxypropyl methylcellulose (HPMC)-lipid edible composite coatings containing antifungal food preservatives

HPMC-lipid edible coatings with food preservative	Solid concentration (%)	Viscosity (cp)	pH
Sodium propyl paraben	9.0	116.5	10.12
Sodium methyl paraben	10.0	140.4	9.60
Sodium ethyl paraben	10.0	147.0	9.70
Sodium benzoate	10.0	142.3	6.39

9.2.2. Emulsions preparation

HPMC-lipid edible composite emulsions were prepared combining the hydrophilic phase (HPMC) and the hydrophobic phase (BW) suspended in water. Glycerol and oleic acid were used as

plasticizer and emulsifier, respectively. Ratios of HPMC-glycerol (3:1) (dry basis, db) and BW-oleic acid (5:1) (db) were kept constant throughout the study. Tween 80 was also added to the formulations at a concentration of 1.5% (w/w) to improve wetting of the coating and adherence to the tomato fruit. All formulations contained 2% (w/w) of food preservative. Emulsions were prepared as described by Valencia-Chamorro et al. (2008). Briefly, an aqueous solution of HPMC (5% w/w) was prepared by dispersing the HPMC in hot water at 90 °C and later hydration at 20 °C. The corresponding food preservative, BW, glycerol, oleic acid, and water were added to the HPMC solution and heated at 98°C to melt the lipids. Samples were homogenized with a high-shear probe mixer (Ultra-Turrax model T25, IKA-Werke, Steufen, Germany) for 1 min at 12.000 and 3 min at 22.000 rpm. Emulsions were cooled under agitation to a temperature lower than 25 °C by placing them in a water bath and agitation was continued during 25 min to ensure complete hydration of the HPMC. The final solid concentration of the emulsions were optimized to obtain formulations with a viscosity range of 100-150 cp. Emulsions were kept 1 day at 5 °C before use. The formulations were tested for stability and phase separation.

9.2.3. Effect of coatings on disease development

9.2.3.1. Fungal inoculum

The strain TAV-6 of *A. alternata*, obtained from decayed tomatoes in Valencia packinghouses, was isolated, identified, and maintained in the IVIA culture collection of postharvest pathogens. Prior to each experiment, the isolate was grown on potato dextrose agar (PDA; Sigma-Aldrich Chemie, Steinheim, Germany) in petri dishes at 25 °C for 7-14 days. Mycelial plugs from this culture was used and high-density conidial suspension was prepared in Tween 80 (0.05%, w/v; Panreac-Química S.A., Barcelona, Spain) and sterile water. This suspension was passed through two layers of cheesecloth, measured with a haemacytometer, and diluted with sterile water to achieve an inoculum density of 1×10^6 spores/ml of *A. alternate*.

9.2.3.2. Fruit inoculation and coating application

Cherry tomatoes (*Lycopersicon esculentum* L.) used in the experiments were commercially grown and collected in the Valencia area (Spain) and stored up to 24 h at 5 °C until use. Fruit were free from

previous postharvest treatments or coatings. Before each experiment, fruit were selected, randomized, washed with fruit biodegradable detergent (Essasol V., Didsa, Potries, Valencia), rinsed with tap water, and allowed to air-dry at room temperature. Cherry tomatoes were superficially wounded once in the equator with a stainless steel rod with a probe tip 1 mm wide and 2 mm in length. This wound was inoculated with the pathogen by placing 10 µl of a spore suspension containing 1 x 10^6 spores/ml of *B. cinerea*. After incubation at 20 ºC for 24 h, inoculated fruit were coated by immersion for 30 s in the selected HPMC-lipid edible composite emulsions, drained, and allowed to air-dry at 20 ºC. Inoculated but uncoated fruit were used as controls. Coated fruit were placed on plastic trays on corrugated cartons and then incubated up to 21 d at 5 ºC, followed by 4 d at 20 ºC and 85-90% RH. In every experiment, each treatment was applied to 3 replicates of 10 fruit each. The experiments were repeated twice.

9.2.3.3. Determination of disease incidence and severity

Disease incidence of BC was calculated as the percentage of decayed fruit. Disease severity was determined as the diameter of the lesion (mm). Incidence and severity were assessed in 7, 14 and 21 d during the storage period at 5 ºC, and also after a shelf-life period of 4 d at 20 ºC.

9.2.4. Effect of coating on fruit quality

9.2.4.1. Fruit coating and storage

For the quality study, before each experiment, fruit were selected, randomized, washed with fruit biodegradable detergent (Essasol V., Didsa, Potries, Valencia), rinsed with tap water, and allowed to air-dry at room temperature. Fruit were divided into five groups of 120 fruit each, which corresponded to the four coating treatments described in Table 1 and one control (uncoated fruit). The cherry tomatoes were coated as described above, drained of excess coating, dried and stored for up to 15 d at 5 ºC and 90-95 % RH. Physico-chemical and sensory fruit quality was assessed in 10 and 15 d at 5 ºC plus a shelf life period of 5 d at 20 ºC.

9.2.4.2. Assessment of fruit quality

9.2.4.2.1 Internal quality

For the determination of the internal quality was obtained 3 juices fruit pulp with a crusher. In the juice was determined the soluble solids content with a digital refractometer (Brix), acidity as percentage of citric acid by titration with 0.1 N NaOH. The pH of the cherry tomatoes was determined in the juice by using a pH-meter (Consort C830). For each treatment was prepared three juices, and each performed three readings.

9.2.4.2.2. Color

The color of the skin cherry tomatoes was measured with a Minolta (Model CR-400, Minolta, Tokyo, Japan) on 20 fruits per treatment, using the CIELAB color parameters, L*, a*, b*, chroma (C) and hue angle (h). Each measurement was taken at three locations for each cherry tomato. A standard white calibration plate was employed to calibrate the spectrophotometer.

9.2.4.2.3. Weight loss

Lots of 30 fruit per treatment were used to measure weight loss. The same marked cherry tomato were weighted at the beginning and at the end of each storage period. The results were expressed as the percentage of initial weight lost.

9.2.4.2.4. Fruit firmness

Firmness of 20 fruit per treatment was determined at the end of each storage period using an Instron Universal testing machine (Model 4301, Instron Corp., Canton, MA, USA). Each fruit was compressed between two flat surfaces closing together at the rate of 5 mm min^{-1}. The machine gave the deformation (mm) after application of a load of 9.8 N to the equatorial region of the fruit. Results were expressed as percentage of deformation, related to initial diameter.

9.2.4.2.5. Respiration rate

Samples of cherry tomatoes, coated and uncoated, were placed in sealed containers for measures the gas concentration. Aliquot the amount of O_2 and CO_2 produced by the fruit storage for 3 h at 20 °C was taken from the headspace. The gas sample was injected into a gas chromatograph (GC) (Thermo Trace, Thermo Fisher Scientific, Inc. Waltham, MA, USA) equipped with a thermal conductivity detector

(TCD) and fitted with a Poropack QS 80/100 column (1.2 m x 0.32 cm i.d.). Temperatures were 35, 115, and 150 °C, respectively for the oven, injector, and thermal conductivity detector. Helium was used, as carrier gas at a flow rate of 22 mL min-1. The respiration rate concentration was calculated using peak area obtained from standard gas mixtures of 15.0:2.5 % O_2:CO_2. Results were expressed as (mg O_2 / kg h) and (mg CO_2 / kg h). Three closed containers per treatment were analyzed.

9.2.4.2.6. Ethanol and acetaldehyde contents

Ethanol and acetaldehyde were analysed from the head-space of juice from samples using a GC (Thermo Trace, Thermo Fisher Scientific) equipped with an auto-sampler (Model HS 2000), flame ionization detector (FID), and 1.2 m x 0.32 cm (i.d.) Poropack QS 80/100 column. The injector was set at 175 °C, the column at 150 °C, the detector at 200 °C, and the carrier gas at 28 mL min-1. A composite juice of three replicates of ten fruit per treatment was analyzed. Five mL of juice were transferred to 10-mL vials with crimptop caps and TFE/silicone septum seals. Samples were frozen and stored at –18 °C until analyses. A 1-mL sample of the headspace was withdrawn from vials previously equilibrated in a water bath at 20 °C for 1 h, followed by 15 min at 40 °C, to reach equilibrium in the headspace, and then injected into the GC. Ethanol and acetaldehyde was identified by comparison of retention times with standards. Results were expressed as mg of gas per 1 L of juice.

9.2.4.2.7. Sensory evaluation

Sensory quality of treated samples was evaluated by 10 judges at the end of each storage period. Judges rated flavor on a 9-point scale where 1 = very poor and 9 = optimum. Each judge was given samples from each batch and requested to evaluate off-flavor on a 5-point scale where 0 = absence of off-flavor and 5 = high presence of off-flavor. Five fruit per treatment were halved and separated into individual segments. Two segments from two different fruit were presented to judges in trays labeled with 3-digit random codes and served to them at room temperature. The judges had to taste several segments of each sample in order to compensate, as far as possible, for biological variation of the material. Spring water was provided for palate rinsing between samples. External aspect of treated fruit (coating cracks, spots, etc.) was also evaluated by the panelists. A 3-point scale was used in

which the aspect was classified as 1 = bad, 2 = acceptable, and 3 = good. Panelists were also asked to rank visually the treatments from highest to lowest gloss.

9.2.5. Statistical analysis

Statistical analysis was performed using Statgraphics 5.1. (Manugistics Inc., Rockville, MD, USA). Specific differences between means were determined by Fisher's protected least significant difference test (LSD, $P < 0.05$) applied after an analysis of variance (ANOVA). For sensory gloss, specific differences were determined by Friedman test, which is recommended for ranking by the UNE 87023 (AENOR, 1997). For disease incidence data, the ANOVA was applied to the arcsine of the square root of the percentage of infected fruit in order to assure the homogeneity of variances. Non-transformed means are shown.

9.3. Results and Discussion

9.3.1. Effect of coatings on disease development

During of cold storage at 5 °C, all the coatings reduced black rot incidence and severity compared with uncoated samples (Figure 9.1). Although, a severity increase during the storage period, all the coatings reduced the disease compared with control. Until the third week at 5 °C, SPP, SEP and SB coating were the most effective to reduce the disease severity of black rot. In general, the reduction of disease severity was considerably higher than the reduction of disease incidence. Although disease incidence on coated samples increased during the entire cold storage period, it was lower than on control samples after 3 wks ($P < 0.05$), except for SMP. The coating containing SB was most effective to reduce the disease incidence of black rot during of cold storage (reduction around of 100% after 7 d, around of 70% after 14 d and 30% after 20 d of storage). After 3 wks of cold storage at 5 °C followed by 5 d at 20 °C, the data obtained showed that the antifungal emulsions did not prevent the onset of fungal diseases, since the values of disease incidence were of 100% in the most case. This result might have been influenced by the high concentration of fungal inoculum that was used in these trials (10^6 spores/mL). From results of disease incidence and severity, found that the food preservatives were fungistatics but no fungicidal, because growth slowed but did not eliminated the fungus. In general, comparable differences on performance depending on the fruit

species or cultivars have been observed with most of the alternative antifungal treatments which mode of action is rather fungistatic than fungicidal (PALOU et al., 2008).

In this work, an HPMC-lipid edible coating with SB additive was the best antifungal against the pathogen AA, after the SB, the parabens showed the best results for reducing the black rot. Propyl paraben, methyl paraben, and ethyl paraben and their sodium salts are GRAS compounds of increasing interest as means to control postharvest decay in fresh horticultural products (MOSCOSO-RAMÍREZ, et al., 2013; VALENCIA-CHAMORRO et al., 2011). Parabens are in the undissociated form at pH values of most foods (p*K*a = 8.5) and are effective over a wide pH range of 4–8 (THOMPSON, 1994). Paraben salts like SMP, SEP, or SPP are more soluble in water than their correspondent parabens and they might interfere on both the germinative and vegetative phases of microbial development, but it has been reported that in fungi spore germination is much more susceptible than vegetative growth (WATANABE and TAKESUE, 1976). It has been suggested that the general mode of action of these salts is through an uncoupling of oxidative phosphorylation, inhibition of NAD+ and FAD-linked mitochondrial respiration, or the reduction of mitochondrial membrane potential (SONI et al., 2001).

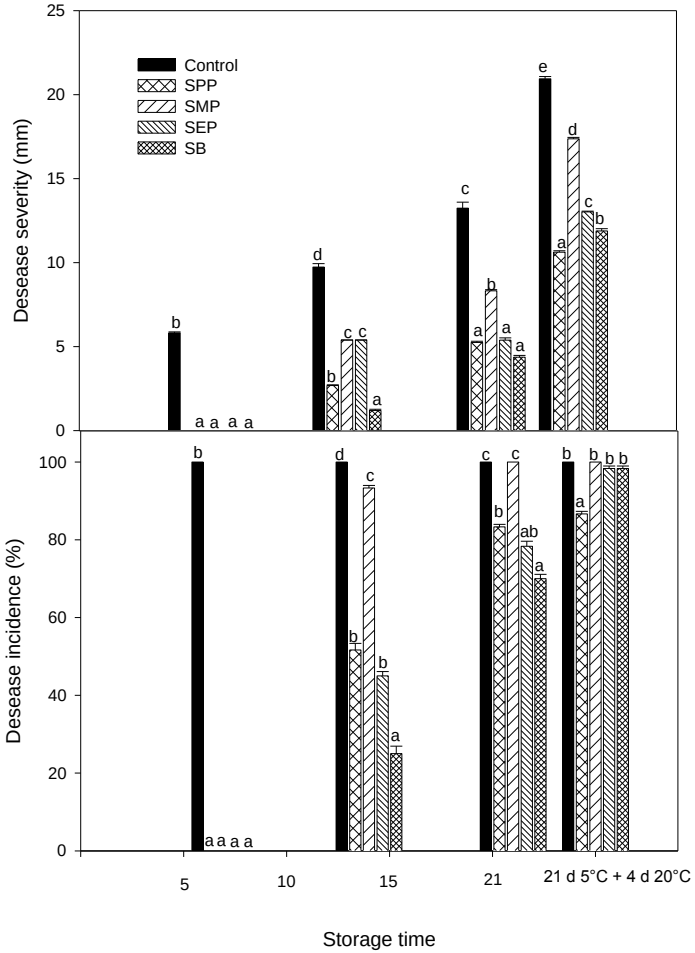

Figure 9.1. Disease incidence and severity of black rot, on cherry tomatoes artificially inoculated with *Alternaria alternata*, uncoated (control), or coated 24 h later with hydroxypropyl methylcellulose (HPMC)-lipid edible composite coatings containing Sodium propyl paraben (SPP), Sodium methyl paraben (SMP), Sodium ethyl paraben (SEP) and sodium benzoate (SB), stored at 5 °C for 21 d followed by 4 d at 20 °C. For each storage period, columns with different letters are significantly different by Fisher's protected LSD test ($P < 0.05$) applied

after an ANOVA. For disease incidence, the ANOVA was applied to arcsine-transformed values. Non-transformed means are shown.

9.3.2 Effect of coating on fruit quality

9.3.2.1 Weight loss

Figure 9.2 shows the weight loss on coated and uncoated samples stored for 10 and 15 d at 5 °C, followed by 5 d at 20 °C. There was increased of the weight loss during storage period. At the end of the first and second storage period, weight losses ranging from 2.91-4.28% and 3.30-4.90%, respectively. Coating containing SB showed the best results between the samples coated, presented average weight loss of the 3.17 and 3.57 % at the end of the first and second storage period. In contrast, tomatoes coated with SPP, SMP and SEP- based coating showed the greatest weight losses at the both storage period. This result probably is due the low barrier at water vapor provided by the coating thickness used. Ali et al. (2010) showed a significantly higher weight loss in 5% gum arabic coatings which could be explained by the thickness of coatings. The 5% gum arabic coating was not so thick that it provided a sufficient barrier against moisture loss.

The basic mechanism of weight loss from fresh fruit and vegetables is by vapor pressure at different locations (YAMAN and BAYOINDIRLI, 2002), although respiration also causes a weight reduction (PAN and BHOWMILK, 1992). This reduction in weight loss was probably due to the effects of the coating as a semi-permeable barrier against O_2 and CO_2, moisture and solute movement, thereby reducing respiration, water loss and oxidation reaction rates (BALDWIN et al., 1999; PARK, 1999).

Many works had reported the edible coating application with and without significant effects on weight loss of fruits. Navarro-Tarazaga et al. (2008) observed that HPMC-BW coatings containing different types of plasticizers did not reduce weight loss of ‘Angeleno’ plums as compared with uncoated samples. Quality of table grapes coated with HPMC edible coatings containing propolis extract assessed by Pastor et al. (2011) showed that weight loss of the grapes was significantly higher in uncoated samples. In another study, Valencia-Chamorro et al. (2009) found that weight loss of oranges treated with most of the coatings, HPMC coatings containing food preservatives, was not significantly different from that of control samples. These same

authors, based on data obtained in Valencia-Chamorro et al. (2008) state that the mechanical properties of the film could also explain the performance of the coating after a long storage period. From this it is likely that coatings containing some food preservatives could more easily form pits or cracks on the fruit surface that might enhance water loss leading to high weight loss.

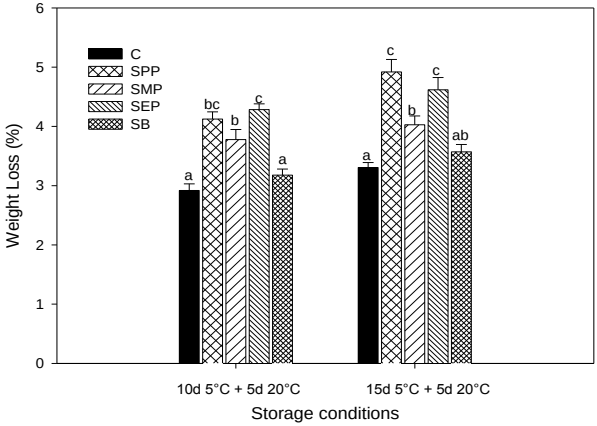

Figure 9.2. Weight loss of cherry tomatoes uncoated (control) or coated with hydroxypropyl methylcellulose (HPMC)-lipid edible composite coatings containing Sodium propyl paraben (SPP), Sodium methyl paraben (SMP), Sodium ethyl paraben (SEP) and sodium benzoate (SB), stored at 5 °C for 15 d followed by 5d at 20 °C. For each storage period, columns with different letters are different by Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

9.3.2.2. Fruit firmness

The results of firmness, expressed by % deformation of coated and uncoated samples are shown in Figure 9.3. Tomatoes coated with SB-based coating (first period:16.63%, second period: 17.97%) and control (first period: 16.12%, second period:16.35%) presented similar firmness value and the coating content SPP, SMP and SEP presented high deformation values and consequently lower firmness. The effect of coatings on the maintenance of fruit firmness is usually related to their control of weight loss. The samples with highest weight loss

(coatings containing SPP, SMP and SEP) showed a greater reduction of texture.

Softening of fruit is due to deterioration in the cell structure, cell wall composition and intracellular materials (SEYMOUR et al., 1993) and a biochemical process involving the hydrolysis of pectin and starch by enzymes (YAMAN and BAYOINDIRLI, 2002). Low respiration rate can limit the activities of these enzymes and allow retention of the firmness during storage (SALUNKHE et al., 1991). Park et al. (1994) reported consumption O_2 of corn-zein coated tomatoes were lower than for non-coated tomatoes. Many works had reported the edible coating application with and without significant effects on firmness of fruits. Ahmed et al. (2013) evaluated the application of delactosed whey permeate in tomatoes during 21d of storage at 15°C and verified that the treatment inhibited significantly ($p<0.05$) fruit softening and maintained higher levels of firmness during the storage period as compared to control. According to Valencia-Chamorro et al. (2009) the firmness of 'Valencia' oranges were not modified on coated fruit after both storage periods as compared to uncoated samples. An another study, coatings hydroxypropyl methylcellulose-based content different beeswax concentration, maintained firmness of coated samples compared to uncoated samples when they were stored 4 weeks at 1 °C followed by 2 and 3 weeks at 20 °C (NAVARRO-TARAGAZA et al., 2011).

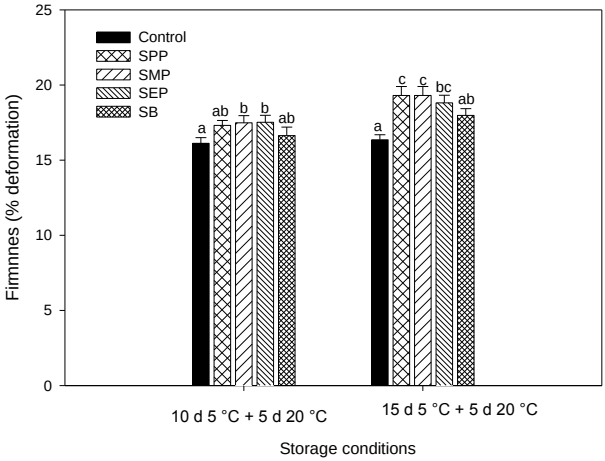

Figure 9.3. Firmness of cherry tomatoes uncoated (control) or coated with hydroxypropyl methylcellulose (HPMC)-lipid edible composite coatings containing Sodium propyl paraben (SPP), Sodium methyl paraben (SMP), Sodium ethyl paraben (SEP) and sodium benzoate (SB), stored at 5 °C for 15 d followed by 5d at 20 °C. For each storage period, columns with different letters are different by Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

9.3.2.3. Respiration rate

The effect of coating on fruit respiration rate was evaluated through O_2 consumption and CO_2 generation. Figure 9.4 shows the development of respiration rates as a function of storage time cold. For oxygen consumption, significant differences among samples were observed, in all storage conditions. In the first and second storage period, cherry tomatoes treated with edible coating content SB and SMP showed O_2 consumption similar to the control. Tomatoes treated with coatings containing SPP and SEP showed higher O_2 consumption. This indicates that some coatings no represent an oxygen barrier and doesn't limits the acceleration of aerobic respiration rates. Sample that presented lower O_2 consumption showed lower weight loss.

In general, respiration rates are linked to sample weight loss, as has been reported in previous studies (FALLIK et al., 2005;

VALVERDE et al., 2005). All the samples showed an increase in the CO_2 generation with the increase of cold storage which agrees with the increase in the metabolic activity of samples at long storage times related with tissue senescence and cell breakdown (PASTOR et al., 2011). A reduction of CO_2 production in coated fruit has been described in other studies; on the muscatel cultivar (SÁNCHEZ-GONZÁLEZ et al., 2011), other grape cultivars (VALVERDE et al., 2005) and other fruit such as avocado (MAFTOONAZAD and RAMASWAMY, 2005) and sweet cherry (ALONSO and ALIQUE, 2004). Ali et al. (2010) reduced the respiratory rate of tomatoes using a covering of gum arabic (10%) and suggests that edible coating exerted a barrier to the gaseous exchange.

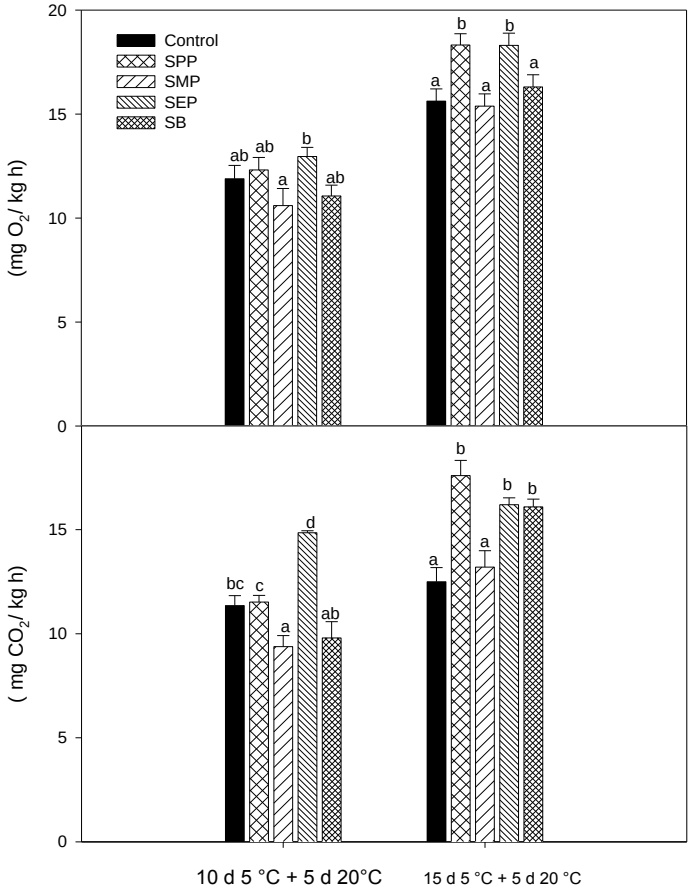

Figure 9.4. Respiration rate of cherry tomatoes uncoated (control) or coated with hydroxypropyl methylcellulose (HPMC)-lipid edible composite coatings containing Sodium propyl paraben (SPP), Sodium methyl paraben (SMP), Sodium ethyl paraben (SEP) and sodium benzoate (SB), stored at 5 °C for 15 d followed by 5 d at 20 °C. For each storage period, columns with different letters are different by Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

9.3.2.5. Ethanol and acetaldehyde content

Ethanol and acetaldehyde content of the cherry tomatoes are shown in Fig 9.5. The application of HPMC-lipid coatings increased ethanol content in the juice of coated cherry tomatoes ($P < 0.05$; Fig. 9.5). Thus, the creation of a modified atmosphere with the fruit was confirmed. After the first and second storage period, ethanol and acetaldehyde content in the juice of coated tomato was very varied. The highest level was found in cherry tomatoes treated with the SMP based coating, in second period.

In general, the concentration of acetaldehyde in the juice of coated cherry tomatoes after both storage periods was in the range of 0.76-1.30 mg L-1, while it was in the range of 0.43- 0.55 mg L-1 in uncoated samples. The concentration of ethanol in the juice of coated cherry tomatoes after both storage periods was in the range of 4.2-21 mg L-1, while it was in the range of 0.32-0.66 mg L-1 in uncoated samples.

Acetaldehyde, a natural aroma component in almost every fruits, accumulates during ripening, even under aerobic conditions. Both acetaldehyde and ethanol are precursors of natural aroma compounds (KNEE and HATFIELD, 1981). However, ethanol accumulation in the fruits and vegetables can be negative effect due off-flavor. In this study, the sensory evaluation detected a slight off-flavor after storage but no differences between coated and uncoated samples were observed, which indicates that the coatings did not induce off-flavor. Different workers have reported higher amount of ethanol content on coated fruit after cold storage fruit. Mandarins coated with HPMC-lipid (20 % lipid content) reached ethanol values between 3.000 and 4.000 mg L-1 after 30 d at 9 °C plus 7 d at 20 °C (PÉREZ-GAGO et al., 2002).

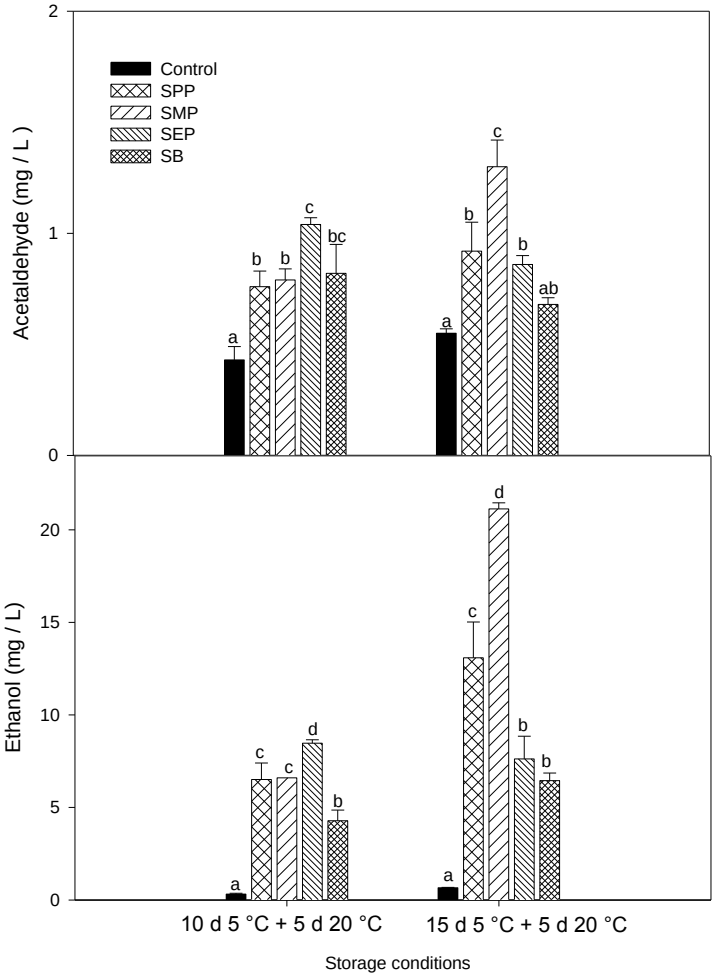

Figure 9.5. Ethanol and acetaldehyde content in the juice of cherry tomatoes uncoated (control) or coated with hydroxypropyl methylcellulose (HPMC)-lipid edible composite coatings containing Sodium propyl paraben (SPP), Sodium methyl paraben (SMP), Sodium ethyl paraben (SEP) and sodium benzoate (SB), stored at 5 °C for 15 d followed by 5d at 20 °C. For each storage period, columns with different

letters are different by Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA

9.3.2.6. Fruit internal quality

The results to titratable acidity (TA), total soluble solids (TSS) and pH of cherry tomatoes, are showed in Table 9.2. There was no significant difference between the control and the samples with coatings for TA, SSC and pH. This result may indicate that coating application did not affect TA, TSS, and pH of cherry tomatoes. Generally the use of technologies retard the transformations that occur during ripening, as in the sugar and organic acids. These changes reduce the TA and increase TSS. According to Sadler and Murphy (1998), in both post-harvest and storage periods, the concentration of organic acids decreases due to their use as a substrate in the respiration or their transformation into sugars. TSS values ranged from 6.77 to 27.7 Brix for the samples with and without coatings. Guillén et al. (2006) assessed cherry tomatoes in two different stages of maturation and found values of soluble solids ranging from 7.11 to 7.23 °Brix, this result is similar to the values found in this work. Decreased respiration rates also slow down the synthesis and use of metabolites resulting in lower TSS (YAMAN and BAYOINDIRLI, 2002). Ali et al. (2010) observed that the lowest TSS at the end of the storage period was recorded in tomatoes coated with 20% gum arabic, and showed that the coatings provided an excellent semi-permeable film around the fruit, modifying the internal atmosphere by reducing O_2 and/or elevating CO_2 and suppressing ethylene production.

Das et al. (2013) found greater values for TA of the uncoated compared with coated tomatoes, according the authors this can be attributed to the increase in ethylene production and respiration rate during the advent of ripening. The effect of coating application on internal quality parameters has been shown to depend on coating type, fruit cultivar and storage conditions. Some authors have found no differences in these parameters after coating application on different citrus cultivars (BALDWIN et al., 1995; OBENLAND et al., 2008); whereas others have found a decrease in TSS and TA losses compared to uncoated fruits, which was always related to a decrease in weight loss and respiration rate (TOGRUL and ARSLAN, 2004). The same authors observed higher values of pH and TSS of uncoated tomatoes. The pH

increase has been attributed to the loss of citric acid in tomatoes by Anthon et al. (2011).

9.3.2.7. Color

Table 9.2 shows the results obtained for the color of cherry tomatoes. The color change occurs during ripening of many fruits, and composes one of the most important criteria used by consumers to judge their maturity. The most common change is the disappearance of green color, followed by the appearance of various colors ranging from yellow to red (AWAD, 1993). The "a" parameter no presented significant difference among samples coated and uncoated. This result indicates that there was no difference in the increase of red coloring for the samples with and without coating. In other hand, L, b*, C* and h parameters presented difference among samples with and without coating. The difference between the crhoma (C) values of samples with and without coverage can indicate slight saturation of red color of samples coated, although the chroma not a good indicator of tomato ripening because it essentially is an expression of the purity or saturation of a single color (different colors may have the same chroma values).

The red color is the quality attribute most visible and important some ripe fruits for fresh consumption and processing. As the market for fresh tomatoes, the color of the fruit has a significant effect on their marketing. This color is the result of a combination of carotenoid pigments, lycopene is the most abundant, followed by carotenes and xanthophylls (LÓPEZ et al., 2001). Ali et al. (2010) observed significant differences in color parameters of tomatoes uncoated and coated with gum arabic edible coating.

Table 9.2. Soluble solid content, titratable acidity, pH and color index (L*, a*, b*, chroma and hue) of cherry tomatoes coated with hydroxypropyl methylcellulose (HPMC)-lipid edible composite coatings containing food preservatives and stored at 5 ℃ for 15 d followed by 5 d of shelf life at 20 ℃.

Food preservative	15 d 5 ℃ + 5 d 20 ℃							
	pH	TSS(°Brix)	TA (g citric acid/ L)	L	a*	b*	C*	h
Control[v]	4.47 a	7.27 a	4.05 ab	35.60 a	15.23 a	18.04 a	23.63 a	49.86 a
Sodium propyl paraben	4.50 ab	6.92 a	3.97 ab	33.96 b	15.25 a	19.52 b	24.80 b	52.04 b
Sodium methyl paraben	4.47 a	7.15 a	4.04 ab	33.96 b	14.89 a	19.59 b	24.64 ab	52.83 b
Sodium ethyl paraben	4.45 a	6.77 a	4.15 b	34.48 b	14.63 a	19.86 b	24.73 b	53.75 b
Sodium benzoate	4.54 b	7.07 a	3.84 a	34.38 b	15.00 a	19.47 b	24.63 ab	52.54 b

[v] Control = uncoated. L*= lightness; a*= red/green; b*= yellow/blue; C*=chroma; h=hue. [z] Means in columns with different letters are significantly different according to Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

9.3.2.8. Sensory evaluation

HPMC-lipid based coatings containing food preservatives no modified the flavor of cherry tomatoes compared to uncoated samples. The panellists considered the flavor as acceptable irrespective of the treatments and the storage time (data not shown). At the end of the storage period, after 15 d of storage at 5 °C plus 5 d at 20 ºC of shelf life, flavor scores were around 5.6-6.4 (considered as acceptable) and no differences were detected among coated samples.

In this study, the panellists detected a slight off-flavor after storage but no differences between coated and uncoated samples were observed, which indicates that the coatings did not induce off-flavor. Ali et al. (2010) studying flavour and overall acceptability of tomatoes with 10% gum arabic coated fruit had the highest scores in all parameters after 20 d of storage, while tomatoes coated with 15 and 20% gum had off-flavour and were not acceptable to the panel of experts.

The addition of food preservatives to HPMC-lipid emulsion resulted in stable emulsions. Among all coated samples, fruit coated with SEP-based coatings was evaluated with the highest external appearance value after 15 d at 5 ºC plus 5 d of shelf life at 20 °C (data not shown). Valencia-chamorro et al. (2009) verified that some coated fruit presented small white spots on their surface, reducing the general good appearance of the oranges coated with HPMC-lipid emulsion containing food preservatives. In this work, after storage period, coated samples were evaluated as acceptable, no sample coverage was evaluated as bad appearance.

After both storage periods, none of the tested coatings provided higher gloss than the uncoated control, and every cherry tomatoes coated showed significantly less glossy than the control after 15 d at 5 °C plus 5 d at 20 °C (Table 9.3). Ahmed et al. (2013) evaluated the application of delactosed whey permeate in tomatoes during 21d of storage at 15°C and at the end of storage, treated tomatoes kept a good appearance and overall quality while in control fruit these parameters fell below the limit of marketability.

Table 9.3. Ranked fruit gloss of cherry tomatoes coated with hydroxypropyl methylcellulose (HPMC)-lipid edible composite coatings containing antifungal food preservatives and stored at 5 ºC followed by 5 d of shelf life at 20 ºC.

Gloss rank	10 d 5 °C + 5 d 20 °C		15 d 5 °C + 5 d 20 °C	
More Glossy	Control[x]	a[y]	Control	a
	SPP	b	SPP	b
	SMP	b	SMP	b
	SEP	b	SEP	b
Less Glossy	SB	b	SB	b

[x] Control = uncoated; Sodium propyl paraben (SPP), Sodium methyl paraben (SMP), Sodium ethyl paraben (SEP) and sodium benzoate (SB). [y] Treatments in columns with different letters are significantly different according to Friedman test.

9.4. Conclusion

During of cold storage at 5 °C, all the coatings reduced black rot incidence and severity compared with uncoated samples, and the SB-based coating was the most effective at inhibiting molds during storage. Therefore, HPMC-lipid edible composite coatings containing antifungal could be a promising treatment for tomatoes kept in cold storage. HPMC-lipid edible composite coatings no improve the weight loss and firmness, although the coatings did not adversely affect the sensory quality of cherry tomatoes.

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CONCLUSÕES FINAIS

A partir dos resultados discutidos e das considerações apresentadas ao final de cada capítulo podem ser destacadas as seguintes conclusões:

- O hipoclorito de sódio e o ozônio, em todas as concentrações testadas, apresentaram reduziram a contagem microbiana em tomates. Portanto é possível realizar a higienização do fruto optando por tratamentos com menor concentração de ozônio (0,5 µg.mL-1) e menor tempo de contato do produto com a água ozonizada (1 min).

- O analisador de gases é um equipamento que reproduz resultados confiáveis e pode substituir o cromatografo gasoso na quantificação de CO_2 e O_2 em embalagens contendo alimentos.

- O presente estudo mostrou que a embalagem de PPBO / PEBD foi a mais apropriada para acondicionar tomates cereja sob atmosfera modificada. A atmosfera modificada contendo 5% de O_2 + 5% de CO_2 com balanço de N_2 apresenta a melhor composição gasosa para o armazenamento de tomate cereja, na temperatura de 5 °C durante 25 dias, e a 10 °C por 20 dias, resposta obtida através dos parâmetros físico-químicos e de taxa de respiração do fruto.

- A atmosfera modificada contendo 5% de O_2 + 5% de CO_2 com balanço de N_2 reduziu a taxa de respiração e a produção de etileno das amostras de tomate cereja e, consequentemente, manteve a firmeza dos tecidos, inibiu as alterações que ocorrem em compostos como açúcares e ácidos orgânicos, além de manter o teor de licopeno e a cor do produto.

- A aplicação de coberturas a base de HPMC com adição de antifúngicos mostrou-se promissor, como um método não poluente, para reduzir as perdas causadas por doenças pós-colheita em tomate. Entre antifúngicos testados, os compostos que apresentaram as melhores respostas para controlar doenças em tomates cereja causadas por *B. cinerea* foram carbonato de potássio, fosfato de amônio, bicarbonato de potássio e o carbonato de amônio, enquanto que os parabenos de sódio foram os mais apropriados contra deterioração causada por *A. alternata*.

- Os testes realizados para avaliar a manutenção das características físico-químicas do tomate cereja, após aplicação da cobertura a base de HPMC contendo antifúngico, mostrou que as coberturas não reduziram a perda de peso nem aumentaram o brilho, porém não afetaram negativamente a qualidade sensorial dos frutos.

- As duas tecnologias testadas são promissoras para o uso no armazenamento pós-colheita do tomate cereja. O armazenamento em atmosfera mostrou melhor resultado para manutenção da qualidade do fruto.

- Outras pesquisas podem ser realizadas com objetivo de melhorar as características físicas da cobertura a base de HPMC, obter maior controle da perda de água e melhorar a qualidade visual da fruta revestida. A combinação com outros métodos de controle ou até mesmo a adição de outros componentes na formulação representam uma alternativa para promover a aplicabilidade comercial deste recobrimento.

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ANEXOS

Foto 1: Avaliação da composição gasosa em analisador de gases.

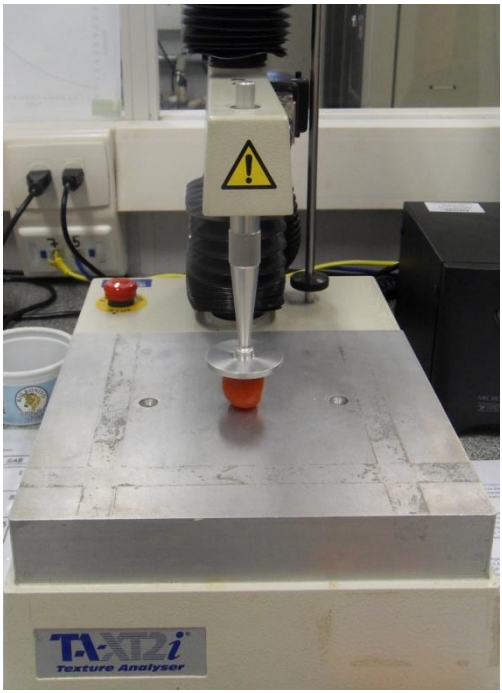

Foto 2: Avaliação da textura de tomate cereja em texturômetro

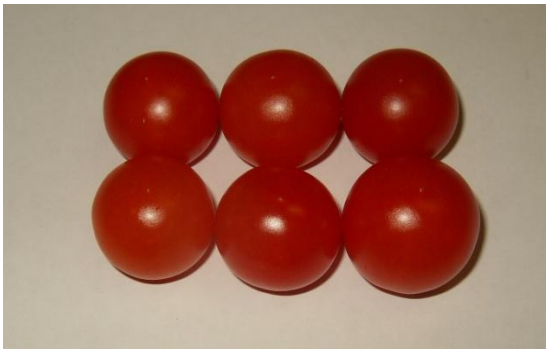

Foto 3: Tomates armazenados em atmosfera gasosa contendo ar sintético em 25 dias a 5°C

Foto 4: Tomates armazenados em atmosfere contendo 5% O_2 + 95% N_2 em 20 dias de armazenamento a 5°C

Foto 5: Tomate armazenados em atmosfera contendo 5% O_2 + 5% CO_2 + 90% N_2 em 25 dias de armazenamento a 5°C

Foto 5: Tomate armazenados em atmosfera contendo 5% O_2 + 10% CO_2 + 85% N_2 em 25 dias de armazenamento a 5°C