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Bruna Klein

COMPOSIÇÃO DAS CERAS CUTICULARES DE MAÇÃS 'CRIPPS PINK', 'MAXI GALA' E 'ELSTAR': CONSEQUÊNCIAS NO METABOLISMO E QUALIDADE DO FRUTO APÓS O ARMAZENAMENTO

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Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Ciência e Tecnologia dos Alimentos, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do título de **Doutora em Ciência e Tecnologia dos Alimentos.**

Orientador: Prof. Dr. Roger Wagner
Co-orientador: Prof. Dr. Auri Brackmann

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Aprovada em 26 de fevereiro de 2021:

ROGER WAGNER

Roger Wagner, Prof. Dr. (UFSM)
(Presidente/Orientador)

Anderson Weber, Prof. Dr. (UNIPAMPA)

Daniel Alexandre Neuwald, Dr. (KOB - Germany)

Fabio R. Thewes

Fabio Rodrigo Thewes, Dr. (UFSM)

Rogerio de Oliveira Anese, Prof. Dr. (IFSC)

Santa Maria, 26 de fevereiro de 2021

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Dedico esta tese aos meus pais Elton e Dileta, pelo amor, carinho e dedicação incondicional e ao meu irmão Rodrigo, que foram essenciais no percorrer dos caminhos que me trouxeram até aqui. À minha avó, Emma Pereira (*in memoriam*), pelo exemplo de força, coragem e aprovação entusiástica da vida.

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“A utopia está lá no horizonte. Me aproximo dois passos, ela se afasta dois passos. Caminho dez passos e o horizonte corre dez passos. Por mais que eu caminhe, jamais alcançarei. Para que serve a utopia? Serve para isso: para que eu não deixe de caminhar. ”

Eduardo Galeano

RESUMO

COMPOSIÇÃO DAS CERAS CUTICULARES DE MAÇÃS 'CRIPPS PINK', 'MAXI GALA' E 'ELSTAR': CONSEQUÊNCIAS NO METABOLISMO E QUALIDADE DO FRUTO APÓS O ARMAZENAMENTO

AUTORA: Bruna Klein
ORIENTADOR: Prof. Roger Wagner

O cultivo da maçã tem a colheita restrita a poucos meses do ano, resultando em períodos de alta e baixa oferta da fruta. Por sua sazonalidade, o armazenamento é importante para ofertar frutas de qualidade no período entressafra. Como alternativa ao armazenamento em atmosfera controlada (AC), novas tecnologias de armazenamento vêm sendo desenvolvidas, estas utilizam técnicas capazes de reduzir a pressão parcial de O_2 de maneira segura pelo monitoramento do limite mínimo de O_2 (LMO), o qual está associado ao metabolismo do fruto. Esses sistemas de armazenamento são conhecidos como atmosfera controlada dinâmica (ACD). Sistemas de ACD já mostram vantagens quanto à manutenção da qualidade pós-colheita de frutas por um período de tempo maior. Estudos recentes têm mostrado aspectos importantes em relação à cutícula da epiderme e ao desempenho pós-colheita dos frutos. Entretanto, a avaliação de composição cuticular associada às técnicas de ACD não foram reportados até o presente momento. Em vista disso, no presente trabalho foram desenvolvidos 3 artigos científicos com os objetivos de: [1] avaliar as mudanças na concentração e composição química de ceras de casca de maçã 'Cripps Pink' após armazenamento sob condições AC, ACD monitorada pela fluorescência de clorofilas (ACD-FC) e ACD monitorado pelo quociente respiratório (ACD-QR) e a relação destas alterações com o metabolismo, incidência de podridões e desenvolvimento da oleosidade na epiderme da fruta; [2] avaliar os efeitos da interação entre os métodos ACD (FC e QR) e 1-MCP na concentração, composição química da cera e na qualidade geral de maçãs 'Maxi Gala' após o armazenamento; [3] avaliar a concentração e a composição química de ceras da casca de maçãs 'Elstar' armazenadas sob AC, ACD-CF, ACD-QR e comparar com ACD-DC (um método recentemente desenvolvido de ACD, baseado na produção de CO_2 das frutas) e relacionar as alterações com o metabolismo, incidência de podridões e qualidade geral dos frutos. O armazenamento em ACD de maçãs 'Cripps Pink' aumentou o teor de cera total ao longo da vida de prateleira, embora as maçãs armazenadas em ACD-QR não desenvolveram oleosidade na epiderme, que é considerada um distúrbio fisiológico de armazenamento. Ésteres graxos, ácido ursólico, ácido oleanólico e ácido palmítico foram correlacionados com maior oleosidade na epiderme. ACD-QR1,3 apresentou a menor taxa respiratória, evidenciando um menor metabolismo e corroborando com o maior número de frutos sadios e, consequentemente, uma melhor condição de armazenamento. O armazenamento com menor pressão parcial de oxigênio (pO_2), como em ACD-QR, pode ter favorecido mecanismos de adaptação contra o baixo pO_2, induzindo a formação de compostos como 10-nonacosanol e octacosanol, que confere uma estrutura cristalina à cutícula. Para maçãs 'Maxi Gala', a aplicação de 1-MCP foi eficaz na redução da produção de etileno em AC, embora ACD, especialmente ACD-QR, também tenha se mostrado eficiente na redução do metabolismo geral da fruta. Quanto à cera cuticular, as atmosferas de armazenamento e o tratamento com 1-MCP não influenciaram o teor total de cera. As composições de cera das maçãs armazenadas em ACD foram semelhantes quando o 1-MCP foi aplicado. Entretanto, o 1-MCP pode estar associado a uma supressão na biossíntese de alcanos e, consequentemente, maior perda de massa nos frutos armazenados em ACD. Para maçãs 'Elstar', o armazenamento sob ACD-QR e ACD-DC ou AC+1-MCP reduziu a produção de etileno e retardou o amadurecimento dos frutos. ACD resultou menor incidência à podridão em relação à AC. ACD-QR e -DC resultaram em frutos mais verdes (ºHue) e mais opacos (C*) quando comparados, principalmente ao AC, foram os tratamentos com os teores de cera mais baixos e apresentaram similaridade na composição química da cera. De maneira geral, as alterações na composição de cera mostraram variações significativas relacionadas ao armazenamento e especificidades das cultivares. Entretanto, o entendimento das relações entre características pós-colheita e componentes químicos específicos ainda é preliminar. Novos estudos podem levar a uma melhor compreensão dos mecanismos subjacentes à grande variação no potencial pós-colheita de maçãs relacionados à camada cuticular.

Palavras chave: composição de cera cuticular; cromatografia em fase gasosa; *Malus domestica* Borkh.; qualidade pós-colheita; quimiometria.

ABSTRACT

COMPOSITION OF CUTICULAR WAXES OF APPLES 'CRIPPS PINK', 'MAXI GALA' AND 'ELSTAR': CONSEQUENCES ON METABOLISM AND FRUIT QUALITY AFTER STORAGE

AUTHOR: Bruna Klein
ADVISER: Prof. Roger Wagner

Apple cultivation has a restricted harvest to a few months of the year, resulting in periods of high and low supply of fruit. Due to its seasonality, storage is an important factor in offering quality fruit in the off-season. As an alternative to controlled atmosphere (CA) storage, new storage technologies have been developed, that use techniques capable of safely reducing partial O_2 pressure by monitoring the lowest O_2 limit (LOL), associated with fruit metabolism. These storage systems are known as dynamic controlled atmosphere (DCA). DCA systems already show advantages in maintaining post-harvest quality of fruit for a longer period of time. Recent studies have shown important aspects in relation to the cuticle of the epidermis and the post-harvest performance of the fruit. However, the assessment of cuticular composition associated with DCA techniques has not been reported to date. In view of this, in the present work, three scientific articles were developed with the objectives of: [1] Evaluate the changes in the concentration and chemical composition of 'Cripps Pink' apple peel wax after storage under CA, DCA-CF and DCA-RQ conditions and the relationship of these changes with metabolism, decay incidence, and greasiness development in the skin of the fruit; [2] Evaluate the effects of the interaction between the DCA (CF and RQ) and 1-MCP methods on the concentration, chemical composition of the wax and on the general quality of 'Maxi Gala' apples after storage; [3] evaluate the concentration and chemical composition of waxes from 'Elstar' apple peels stored under CA, DCA-CF, DCA-RQ and compare with DCA-CD (a new DCA method, based on the CO_2 production of fruit) and relate the changes with the metabolism, decay incidence, and general quality of the fruit. DCA storage of 'Cripps Pink' apples increased the total wax content over the shelf life, although apples stored in DCA-RQ did not develop greasiness in the epidermis, which is considered a physiological storage disorder. Fatty esters, ursolic acid, oleanolic acid and, palmitic acid were correlated with greater greasiness in the epidermis. DCA-RQ1.3 showed the lowest respiratory rate, showing a lower metabolism and corroborating with the greater number of healthy fruit and consequently a better storage condition. Storage with less pO_2, as in DCA-RQ, may have favored adaptation mechanisms against low pO2, inducing the formation of compounds such as 10-nonacosanol and octacosanol, which gives the cuticle a crystalline structure. For 'Maxi Gala' apples, the application of 1-MCP was effective in reducing ethylene production in CA, although DCA, especially DCA-RQ, has also been shown to reduce the overall metabolism of the fruit. As for cuticular wax, the storage atmospheres and treatment with 1-MCP did not influence the total wax content. The wax compositions of the apples stored in DCA were similar when 1-MCP was applied. However, 1-MCP may be associated with a suppression in alkane biosynthesis and, consequently, greater loss of mass in the fruit stored in DCA. For 'Elstar' apples, storage under DCA-RQ and DCA-CD or CA+1-MCP reduced ethylene production and delayed fruit ripening. DCA-RQ1.5 resulted in a lower decay incidence and was associated with a greater accumulation of compounds of anaerobic metabolism. ACD-QR and -DC resulted in greener (^{o}Hue) and more opaque (C *) fruits when compared, mainly to AC, were the treatments with the lowest wax levels and showed similarity in the chemical composition of the wax. In general, changes in wax composition showed significant variations related to storage and cultivar specificities. However, the understanding of the relationship between post-harvest characteristics and specific chemical components is still preliminary. New studies may lead to a better understanding of the mechanisms underlying the wide variation in the post-harvest potential of apples related to the cuticular layer.

Keywords: cuticular wax composition; gas chromatography; *Malus domestica* Borkh.; post-harvest quality; chemometry.

LISTA DE FIGURAS

ARTIGO 1

ARTIGO 2

ARTIGO 3

LISTA DE TABELAS

ARTIGO 1

ARTIGO 2

ARTIGO 3

LISTA DE ABREVIATURAS E SIGLAS

°C - Temperatura em graus Celsius
1-MCP - 1-metilciclopropeno
AC - Atmosfera controlada
ACC - Ácido 1-aminociclopropano-1-carboxílico
ACC oxidase - Ácido 1-aminociclopropano-1-carboxílico oxidase
ACC sintase - Ácido 1-aminociclopropano-1-carboxílico sintase
ACD - Atmosfera controlada dinâmica
ACD-FC - Atmosfera controlada dinâmica monitorada pela fluorescência de clorofilas
ACD-QR - Atmosfera controlada dinâmica monitorada pelo quociente respiratório
ATP - Adenosina trifosfato
CA - Controlled atmosphere
CO_2 - Dióxido de carbono (gás carbônico)
DCA - Dynamic controlled atmosphere
DCA-CD - Dynamic controlled atmosphere monitored by CO2 release
DCA-CF - Dynamic controlled atmosphere – chlorophyll fluorescence
DCA-RQ - Dynamic controlled atmosphere – respiratory quotient
DPA – Difenilamina, do inglês diphenylamine
FID - Detector por ionização em chama, do inglês flame ionization detector
Fo - Fluorescência mínima
g – grama
GC/MS- Cromatógrafo a gás acoplado a um espectrômetro de massas, do inglês gas chromatography coupled to mass spectrometer
GC-FID - Cromatógrafo a gás com detector por ionização em chama, do inglês gas chromatography equiped with flame ionization detector
ILOS - Initial Low Oxygen Stress
Kg - Quilograma
kPa – Kilopascal
LMO/LOL - Limite mínimo de oxigênio/Lowest oxygen limit
LOX – Lipoxigenase
MdACO1 - Gene para ACC oxidase em maçã
MdACS1 - Gene para ACC sintase em maçã
Meq - Miliequivalente
mL – Mililitro
N - Newton ou Normal
NADH - Nicotinamida adenina dinucleotídeo oxidada/ reduzida
NADPH - Nicotinamida adenina dinucleotídeo fosfato reduzida
NaOH - Hidróxido de sódio
NPP - Núcleo de Pesquisa em Pós-Colheita da Universidade Federal de Santa Maria
OA - Oleanolic acid
PCA – Análise de componente principal, do inglês Principal Component Analysis
PcA - Ponto de compensação anaeróbico
pH - Potencial hidrogeniônico
pO_2 – Pressão parcial de oxigênio
RH - Relative humidity
ROS - reactive oxygen species
SPI - Starch pattern index
TA - Titratable acidity
UA - Ursolic acid

ULO - Ultra Low Oxygen (Ultrabaixo oxigênio)
UR - Umidade relative
VLCFA - very long chain fatty acids
µg - Micrograma
µL L^{-1} - Microlitro por litro

SUMÁRIO

1 INTRODUÇÃO E JUSTIFICATIVA

A produção brasileira de maçãs na safra 2017/18 foi de 1.203.007 toneladas. Na safra 2018/19 este valor alcançou 1.222.979 toneladas (FAOSTAT, 2020). Nas unidades federativas, aproximadamente 603 mil toneladas foram produzidas no Rio Grande do Sul (RS), 586 mil toneladas em Santa Catarina (SC), 26 mil toneladas no Paraná (PR) e 7 mil toneladas nos demais Estados (IBGE, 2019). Esses montantes evidenciam a importância econômica dessa cultura para o país. Levantamentos feitos pela Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (EPAGRI, 2019) mostram que a média de participação de cultivares na produção brasileira de maçã, nos últimos cinco anos, foi de 56% do cultivar Gala, 39% de Fuji; e 5% de outros cultivares.

A colheita da maçã é concentrada nos meses de fevereiro a abril, sendo necessário o armazenamento para a comercialização da fruta na entressafra. De toda a produção, apenas uma pequena porcentagem é comercializada imediatamente, sendo a maior parte armazenada em câmaras frigoríficas sob atmosfera controlada (AC, do inglês, *controlled atmosphere*, CA). A técnica de AC, além do controle de temperatura e umidade, utiliza baixas pressões parciais de oxigênio (pO_2) e altas de CO_2. As baixas pO_2 acarretam a redução na produção de etileno e na taxa respiratória e, por consequência, retardam a maturação e senescência, e reduzem a ocorrência de distúrbios fisiológicos (WRIGHT; ARUL; PRANGE, 2015). Entretanto, os níveis de oxigênio e gás carbônico são definidos previamente e mantidos constantes durante o armazenamento, com isso, não levam em consideração a variação do metabolismo do fruto que ocorrerá ao longo do armazenamento (ARGENTA; FAN; MATTHEIS, 2000; BRACKMANN et al., 2008).

Uma nova tendência é a utilização do armazenamento sob atmosfera controlada dinâmica (ACD, do inglês, *dynamic controlled atmosphere*, DCA), onde a pO_2 é variável a partir do monitoramento do limite mínimo de O_2 (LMO do inglês, *lower oxygen limit*, LOL), que é a pressão parcial de O_2 mínima tolerado pelo fruto durante o armazenamento, reduzindo assim ao máximo a respiração sem prejuízo à sua qualidade (BESSEMANS et al., 2016; BRACKMANN; WEBER; BOTH, 2015; PRANGE et al., 2015; WRIGHT et al., 2012). Para esse monitoramento, pode-se utilizar a produção de etanol pelas frutas (DEUCHANDE et al., 2016; SCHOUTEN et al., 1998; VELTMAN; VERSCHOOR; VAN DUGTEREN, 2003), a fluorescência de clorofila (ACD-FC) (PRANGE; DELONG; HARRISON, 2005; PRANGE et al., 2005b; 2007; 2015) ou o quociente respiratório (ACD-QR), baseado na razão entre a produção de CO_2 e o consumo de O_2 pelo metabolismo respiratório dos frutos (BESSEMANS

et al., 2016; BRACKMANN; WEBER; BOTH, 2015; WEBER et al., 2015). Porém, a falta de estanqueidade das câmaras de armazenamento pode interferir na determinação do LMO, que é um dos problemas enfrentados na ACD. Nesse caso, recentemente, Thewes et al. (2020) desenvolveram uma técnica para monitorar o metabolismo das frutas apenas pela respiração (produção de CO_2) (ACD-DC), uma vez que a produção de CO_2 diminui com a redução de pO_2 na câmara até o ponto de compensação anaeróbico, a partir do qual haverá aumento na liberação de CO_2 pela fruta (NEUWALD et al., [s.d.]). Essa alternativa facilita a medição do LMO em nível comercial sem a necessidade de equipamentos adicionais nas câmaras de AC, além da pouca influência do ambiente externo em sua determinação.

Os benefícios do armazenamento de maçãs em níveis de O_2 seguros e extremamente baixos (< 0,5 kPa) favorecidos pela ACD quando comparados à AC, já têm sido extensivamente apresentados: ACD-FC reduz a ocorrência de distúrbios fisiológicos, especialmente da escaldadura superficial (MDITSHWA et al., 2017a, 2017b; MDITSHWA; FAWOLE; OPARA, 2018), degenerescência de polpa (BOTH et al., 2017; THEWES et al., 2015) e maior firmeza de polpa (TRAN et al., 2015; WEBER et al., 2019); ACD-QR é capaz de induzir o metabolismo anaeróbico de maneira controlada o que resulta em um leve incremento na produção de etanol, capaz de reduzir a produção de etileno, retardando o amadurecimento (ASODA et al., 2009; WEBER et al., 2016, 2020). Associado a isso, as frutas armazenadas em ACD-QR apresentam menor incidência de escaldadura superficial (BESSEMANS et al., 2016) e degenerescência de polpa, maior firmeza de polpa e frutos sadios (BOTH et al., 2017; THEWES et al., 2017b; WEBER et al., 2015, 2019) e maior emissão de compostos voláteis, especialmente ésteres (BOTH et al., 2017; THEWES et al., 2017a, 2017b).

Apesar de todo o benefício de prolongar a vida de prateleira da fruta, também estão associadas às tecnologias de armazenamento algumas alterações fisiológicas que podem comprometer a qualidade da fruta (RAFFO et al., 2009). De um modo geral, a qualidade ou o potencial de armazenamento desse produto é comumente avaliado com base na aparência visual e nas suas características físico-químicas, como mencionado acima. No entanto, produtos frescos são organismos vivos e, portanto, as vias de biossíntese e catabolismo continuam a operar mesmo durante o armazenamento (MALTAS et al., 2009). Esses eventos metabólicos que ocorrem durante a maturação, amadurecimento e desenvolvimento pós-colheita no fruto, causam mudanças significativas que reduzem o volume comercial do produto colhido, o apelo visual e organoléptico para o consumidor, o nível de vitaminas e antioxidantes disponíveis e a capacidade de armazenamento (LARA; BELGE; GOULAO, 2014). Além dos parâmetros de qualidade comumente avaliados, outras frações merecem atenção devido à sua importância para

a qualidade da maçã, como a cutícula, que recobre a camada mais externa da epiderme. Essa fração pode ser considerada uma importante moduladora da qualidade pós-colheita de frutos.

A cutícula, uma camada hidrofóbica, cobre todos os órgãos aéreos primários da planta e, assim, medeia a interação da planta com o ambiente circundante (BHANOT; FADANAVIS; PANWAR, 2021), é depositada sob a parede celular epidérmica externa e desempenha papéis críticos na fisiologia e no desenvolvimento das plantas, mantendo a resistência mecânica, protegendo da radiação ultravioleta e, principalmente, limitando a perda de água não estomática, permitindo a manutenção de uma quantidade de água adequado e atividades celulares (MARTIN; ROSE, 2014; SCHUSTER et al., 2016). Essa membrana lipofílica, foi amplamente desconsiderada no que diz respeito à sua influência na modulação do desenvolvimento, amadurecimento e desempenho pós-colheita dos frutos (LARA, 2018; LARA; BELGE; GOULAO, 2014). No entanto, esse cenário está sendo alterado pelas evidências experimentais crescentes sobre a importância desta camada para a manutenção da qualidade pós-colheita de frutos (LARA; BELGE; GOULAO, 2014; MARTIN; ROSE, 2014). Estudos demonstram que a composição cuticular é afetada por fatores ambientais, como vento, temperatura, luz, ensacamento pré-colheita, atmosfera controlada (LI et al., 2012, DONG et al., 2012). Estudos sobre maçã demonstraram que a composição da cera cuticular muda continuamente durante o desenvolvimento e após a colheita (BELDING et al., 1998; DONG et al., 2012; JU; BRAMLAGE, 2001). Já foram avaliadas as alterações da cutícula bem como suas implicações na qualidade pós-colheita de maçãs de diferentes cultivares (CHAI et al., 2020; LEIDE et al., 2018), durante a vida de prateleira (YANG et al. 2017b), armazenadas sob refrigeração (MORICE; SHORLAND, 1973; LI et al., 2017), atmosfera controlada (VERAVERBEKE et al. 2001) e combinado com o uso do 1-metilciclopropeno (1-MCP) (CURRY, 2008; DONG et al. 2012; YANG et al. 2017a). Entretanto, o efeito da ACD ainda é pouco explorado.

Desta forma, fica claro que a camada cuticular exerce um papel modulador em várias características essenciais de qualidade de frutos, o que confere ao estudo da cutícula um interesse especial no campo da pesquisa pós-colheita. Entretanto, estudos que relacionam essa camada cuticular, seus aspectos químicos, biofísicos à qualidade pós-colheita de frutas, especialmente maçãs, com as novas tecnologias de armazenamento, como ACD, ainda são incipientes. Assim, pretende-se estudar os efeitos do uso de diferentes métodos de ACD, com estresses por baixo O_2, na composição química da cera cuticular de maçãs após longos períodos de armazenamento, afim de compreender as alterações composicionais e os impactos destas alterações sob a qualidade pós-colheita de maçãs.

1.1. OBJETIVOS

Avaliar a influência das ceras cuticulares sob o metabolismo e parâmetros de qualidade de maçãs ‘Cripps Pink’ após oito meses de armazenamento em AC e ACD (FC e RQ) mais 7 e 14 dias de vida de prateleira a 20 °C.

Identificar a relação entre ceras cuticulares e a oleosidade da casca de maçãs, avaliando as alterações nos compostos da cera de maçãs ‘Cripps Pink’ após oito meses de armazenamento em AC e ACD (FC e RQ) mais 7 e 14 dias de vida de prateleira a 20 °C.

Verificar a interação entre o armazenamento em AC, ACD – FC e ACD – QR com a aplicação de 1-MCP sobre a manutenção da qualidade, concentração e composição da cera de maçãs ‘Maxi Gala’ após nove meses de armazenamento mais 7 dias de vida de prateleira a 20 °C.

Avaliar o efeito do armazenamento em ACD – DC de maçãs ‘Elstar’ sobre a qualidade, metabolismo anaeróbico, concentração e composição de cera cuticular em comparação a AC e outros métodos de ACD.

2 REVISÃO DA LITERATURA

2.1. A PRODUÇÃO BRASILEIRA DE MAÇÃS

A cultura da macieira apresenta importância econômica no cenário mundial devido ao volume de produção, estimado em 87,2 milhões de toneladas na safra de 2019 (FAOSTAT, 2020). Considerada uma das mais importantes frutas comercializadas *in natura*, tanto no contexto internacional como nacional (GIRARDI, 2004), a maçã (*Malus domestica* Borkh.) pertence à família Rosaceae, sendo uma frutífera de clima temperado.

Na safra de 2018/19 o Brasil colheu em média mais 1,2 milhões de toneladas (FAOSTAT, 2020). Desta produção total, nos últimos anos a maioria foi destinada para o consumo *in natura* interno (67,6%), consumo *in natura* externo (7%) e industrialização para produção de suco e outros alimentos (25,4%) (ARGENTA et al., 2015). Segundo dados da Associação Brasileira dos Produtores de Maçã (ABPM, 2019), a produção nacional de maçãs está localizada nos três estados do Sul do Brasil em função do clima favorável. A produção somada destes estados correspondeu a mais de 90% da produção nacional de maçãs na safra de 2017/18.

Neste mesmo ano, das cultivares colhidas, em média, 674,1 mil toneladas foram da variedade ‘Gala’, em fase de colheita no mês de fevereiro e 360,5 mil toneladas de ‘Fuji’, que são de ciclo tardio. Ambas representam 95% da produção nacional de maçãs, na qual dentre esses dois grupos de variedades, a ‘Gala’ e suas mutantes perfazem aproximadamente 60% da produção nacional (ABPM, 2019). As mutantes de ‘Gala’, como a ‘Royal Gala’, ‘Galaxy’, ‘Maxi Gala’, ‘Imperial Gala’, ‘Mondial Gala’, além de outras, apresentam a vantagem de possuírem maior recobrimento da epiderme com coloração vermelha, o que as tornam mais atrativas ao consumidor (CAMILO; DENARDI, 2002).

Apesar de menor relevância em relação às cultivares, ‘Gala’ e ‘Fuji’, a cultivar ‘Cripps Pink’, comercializada como ‘Pink Lady®’, tem aumentado sua produção. Na safra 2017/2018 a produção de ‘Cripps Pink’ foi de 16.563 toneladas, cultivada principalmente em pomares localizados no Rio Grande do Sul (AGAPOMI, 2018).

2.2. ARMAZENAMENTO PÓS-COLHEITA

De todo o volume de maçãs produzido, grande parte é armazenado para abastecer o mercado na entressafra. Algumas técnicas são utilizadas para prolongar o período pós-colheita

de maçãs. Atualmente, a baixa temperatura durante o armazenamento refrigerado e os sistemas de atmosfera controlada (AC), com redução dos níveis de O_2 e aumento de CO_2, são as técnicas mais utilizadas.

Apesar da redução da temperatura e dos níveis de O_2 atuarem na redução do metabolismo como consequência direta da redução da respiração dos frutos armazenados (WRIGHT; ARUL; PRANGE, 2015), a pressão parcial de O_2 é mantida acima da considerada ideal para alcançar um metabolismo mínimo dos frutos, como precaução para não induzir o metabolismo anaeróbico que depreciaria a qualidade dos frutos pelo acúmulo excessivo de compostos *off-flavour* como etanol, acetaldeído e acetato de etila (TRAN et al., 2015; WRIGHT; ARUL; PRANGE, 2015). Entretanto, desenvolveu-se uma melhoria da tecnologia AC através do desenvolvimento da atmosfera controlada dinâmica (ACD) em que a concentração de O_2 varia durante o armazenamento em função do LMO, o que pode ativar de forma controlada o metabolismo anaeróbico, e como consequência, redução da ocorrência de distúrbios fisiológicos e mantendo a qualidade dos frutos mais eficientemente que a AC convencional (PRANGE et al., 2003; ZANELLA et al., 2005; WRIGHT et al., 2012; WEBER et al., 2015; THEWES et al., 2017c). O monitoramento do LMO identifica o momento em que as frutas estão em um nível crítico de estresse, por exemplo pela alta produção de etanol, o que pode danificar seus tecidos em concentrações mais elevadas (GASSER et al., 2010). Portanto, essa tecnologia monitora instantaneamente o nível de estresse ao qual a fruta é exposta e fornece uma maneira segura de atingir os níveis mais baixos de oxigênio sem danificar a fruta.

Apesar disso, tem-se que a aplicação de etanol diminui a expressão gênica da ácido 1-aminociclopropano-1-carboxílico oxidase (ACC oxidase) em brócolis, levando à redução da produção de etileno (ASODA et al., 2009). A aplicação de etanol na dose de 0,3 mL kg^{-1} $mês^{-1}$ de maçãs também melhorou a manutenção da qualidade das maçãs "Royal Gala" durante a vida de prateleira a 20 °C, reduzindo expressivamente a produção de etileno (WEBER et al., 2016). A aplicação de vapor de etanol ou produção endógena reduziu a biossíntese de etileno em maçãs "Granny Smith" (PESIS et al., 2010; SCOTT; YUEN; GHAHRAMANI, 1995). Portanto, tecnologias que permitam ativação controlada do metabolismo anaeróbico de frutas parecem ser favoráveis na manutenção da qualidade durante o armazenamento.

2.2.1. Atmosfera controlada (AC)

Níveis baixos de O_2, juntamente com a refrigeração e o aumento da concentração de CO_2, são comumente aplicados para prolongar a vida de prateleira de frutas e vegetais nos

sistemas de atmosfera controlada (AC). De acordo com a Associação Gaúcha dos Produtores de Maçã (AGAPOMI, 2019), em média 66% do total de maçãs produzidas no Brasil, são armazenadas em AC. Desde as primeiras aplicações comerciais, os benefícios da tecnologia AC no armazenamento de maçãs, em comparação com o armazenamento em atmosfera regular estão relacionadas ao atraso do amadurecimento e da senescência (ARGENTA; FAN; MATTHEIS, 2000; BRACKMANN et al., 2008). Além disso, há uma melhor manutenção da qualidade dos frutos devido aos efeitos sinérgicos da baixa temperatura, aumento da concentração de CO_2 e níveis reduzidos de O_2 (LUMPKIN et al., 2015; STEFFENS et al., 2013; WEBER et al., 2011; YAHIA, 2009).

A AC tem como princípio a alteração e controle da composição gasosa da atmosfera de armazenamento, associada à redução da temperatura e controle da umidade relativa (BRACKMANN et al., 2005a, 2008; CORRÊA et al., 2010; LUMPKIN et al., 2014, 2015). Os níveis reduzidos de O_2 limitam o oxigênio molecular para a conversão do ácido 1-aminociclopropano-1-carboxílico (ACC) em etileno pela enzima ACC oxidase (ASODA et al., 2009; YANG; HOFFMAN, 1984) e, portanto, exercem efeito na biossíntese de etileno. Além disso, a respiração dos frutos reduz, pois durante a respiração aeróbica, ocorre a inibição da enzima citocromo *c* oxidase que utiliza o O_2 como o aceptor final de elétrons (TAIZ; ZEIGER, 2013; WRIGHT; ARUL; PRANGE, 2015). Já foi estabelecido experimentalmente que a pressão parcial de O_2 em AC para maçãs das cultivares Gala e Fuji e seus mutantes, varia entre 1,0 a 1,2 kPa (ARGENTA; FAN; MATTHEIS, 2000; BRACKMANN et al., 2005a; 2008; 2009a; CORRÊA et al., 2010; WEBER et al., 2011). Outras cultivares como Cripps Pink, Elstar, Nicoter, Braeburn e Jonagold, as pressões parciais de O_2 são geralmente mais altas, variando de 1,0 a 2,0 kPa de O_2 (HO et al., 2013; BRACKMANN et al., 2005b; KÖPCKE, 2015; VELTMAN; VERSCHOOR; VAN DUGTEREN, 2003).

A descoberta de que concentrações de O_2 de 1 kPa ou abaixo disso melhoraram a capacidade de armazenamento levou à aplicação de protocolos de ultrabaixo oxigênio (do inglês, *Ultra Low Oxygen*, ULO, 0,8-1,2 kPa) (DILLEY, 2006). Em geral, essas condições hipóxicas, assim como na AC, são mantidas desde o início até o final do armazenamento. Essa abordagem estática nem sempre fornece resultados satisfatórios de qualidade da maçã pós-armazenamento (PRANGE et al., 2013). O armazenamento de maçãs em ultrabaixo oxigênio é baseado no princípio da redução do O_2 até níveis próximos (um pouco acima) do ponto de compensação anaeróbica, que é o nível de O_2 em que a produção de CO_2 é mínima (GASSER et al., 2008; HOEHN et al., 2009) a fim de reduzir ao máximo a respiração, objetivando, portanto, manter a qualidade dos frutos (THEWES et al., 2015; WEBER et al., 2011).

Experimentalmente estabelecidos, os níveis fixos de O_2 e CO_2 usados sob o armazenamento em AC ou AC-ultrabaixo oxigênio são um compromisso empírico. Em condições muito rigorosas (baixas concentrações de O_2/altas concentrações de CO_2) mantidas estaticamente, distúrbios fisiológicos podem se desenvolver, enquanto em condições de armazenamento moderadas a qualidade da maçã diminui rapidamente, limitando a capacidade de armazenamento (THEWES et al., 2021).

O limite mínimo de oxigênio (LMO) representa a concentração abaixo da qual o metabolismo anaeróbico torna-se excessivamente elevado, causando danos nos tecidos e perda de qualidade dos frutos. Enquanto as concentrações de O_2 que correspondem ao LMO, ou ligeiramente acima do LMO, resultam em respiração aeróbica reduzida e armazenamento máximo de maçãs (PRANGE et al., 2003). Entretanto, o risco de perdas de qualidade graves devido ao metabolismo anaeróbico excessivo (por exemplo, a fermentação) e condições de estresse, são aumentadas. Neste contexto, a avaliação da atividade metabólica da fruta por meio do monitoramento de sinais fisiológicos, torna-se crucial.

Os parâmetros comumente utilizados para monitorar as respostas metabólicas da fruta e definir o LMO são a produção de etanol, fluorescência de clorofila (ACD-FC), o quociente respiratório (ACD-QR) (MAXWELL, 2000; PRANGE et al., 2002; VELTMAN et al., 2003; GASSER et al., 2008; BESSEMANS et al., 2016) e a produção de dióxido de carbono (ACD-DC) (THEWES et al., 2020). Com base nessas respostas fisiológicas, a concentração de O_2 é definida para atingir níveis considerados seguros.

2.2.2. Atmosfera controlada dinâmica monitorada pela detecção da produção de etanol

Quando as pressões parciais de O_2 são reduzidas nas câmaras de armazenamento, nesse sistema de ACD, o etanol produzido pelos frutos é monitorado. Com o objetivo de monitorar o LMO, o controle das condições de armazenamento com base na resposta desse marcador químico, fornece informações sobre o metabolismo da fruta em um momento específico (SCHOUTEN, et al., 1998; DEUCHANDE, et al. 2016). O etanol é um indicador da ativação do metabolismo anaeróbico, quando em altas concentrações causa *off*-flavour e degenerescência, depreciando a qualidade dos frutos (KE et al., 1991; KE et al. 1993; PEPPELENBOS; OOSTERHAVEN, 1998; TRAN et al. 2015; WRIGHT et al., 2015). Quando o etanol supera o nível estabelecido, o nível de O_2 na câmara é levemente aumentado para que reduza o metabolismo anaeróbico e a concentração de etanol volte ao normal (VELTMAN; VERSCHOOR; VAN DUGTEREN, 2003).

Esse nível de etanol pode ser determinado tanto no *headspace* da câmara de armazenamento (*Dynamic Control System* - DCS®), um sistema que permite um monitoramento contínuo desenvolvido na Holanda (SCHOUTEN et al., 1998; VELTMAN; VERSCHOOR; VAN DUGTEREN, 2003), quanto na polpa dos frutos, através do suco (ILOS-Plus®) por um biossensor enzimático de etanol (MARVIL, 2015), desenvolvido na Itália.

No entanto, ambas as determinações possuem um inconveniente em comum, visto que o etanol produzido pelos frutos é um metabólito intermediário que pode ser convertido rapidamente em ésteres etílicos, como o acetato de etila (BRACKMANN; STREIF; BANGERTH, 1993; JIN et al., 2013; LIU et al., 2012). Portanto, torna-se um método pouco prático em nível comercial, devido aos resultados contraditórios na determinação do LMO que podem ocorrer durante o armazenamento, utilizando o etanol como marcador.

2.2.3. Atmosfera controlada dinâmica monitorada pela fluorescência de clorofila (ACD-FC)

A tecnologia ACD-FC é fundamentada na relação entre a emissão da fluorescência mínima (Fo) pelas clorofilas presentes na epiderme dos frutos e uma mudança metabólica de metabolismo predominantemente aeróbico para anaeróbico, ou seja, um indicativo do LMO do produto fresco armazenado (MAHAJAN et al., 2021).

A fluorescência das clorofilas ocorre com maior intensidade em situações de estresse, que impossibilitam a transferência da energia luminosa captada pela clorofila para os centros de reação (TAIZ; ZEIGER, 2013). Quando o fruto está sob estresse por baixo O_2, ou seja, quando o nível de O_2 atinge o limite mínimo, ocorre o aumento da emissão da fluorescência mínima (Fo) das plantas pelas clorofilas quando estas recebem energia luminosa (HARRIS; HEBER, 1993; PRANGE; SCHOUTEN; VAN KOOTEN, 1997; PRANGE et al., 2002). Desta forma, é possível promover um sistema com ajuste dinâmico, de forma rápida e não destrutiva, das condições de AC para o estado fisiológico dos frutos e preservar a qualidade de maçãs armazenadas (PRANGE et al. 2005b; ZANELLA; CAZZANELLI; ROGGI, 2008; KITTEMANN; MCCORMICK; NEUWALD, 2015).

A explicação para esse fenômeno fisiológico de emissão de fluorescência pelas clorofilas ainda é baseada em hipóteses como a acidificação do citoplasma, pois a baixa pO_2 é capaz de reduzir o pH citoplasmático, que ocorre simultaneamente com a hidrólise do ATP e formação de ácido fosfórico (PRANGE et al., 2005a). Além disso, há formação de ácido lático em decorrência do metabolismo anaeróbico, portanto a presença desse ácido torna o pH do

citosol reduzido. PRANGE et al. (2005a) também consideraram que com a menor respiração aeróbica ocorre menor disponibilidade de ATP para a H+-ATPase bombear H+ para o vacúolo, causando a acúmulo de H+ no citoplasma. Outros autores reportam que há menor produção de energia quando a supressão de oxigênio ocorre e compostos reduzidos, como NADH, são acumulados no citoplasma devido à inibição da fosforilação oxidativa, sendo utilizados para redução do *pool* de plastoquinona (PQ), o que pode frear o transporte da energia luminosa (elétrons) e, consequentemente, aumentar a fluorescência de clorofilas (WRIGHT et al., 2010; 2011; 2015). Outra hipótese refere-se ao ciclo das xantofilas. Estudos reportaram que a a conversão da xantofila em zeaxantina é fundamental para que o excesso de energia seja dissipado na forma de calor, quando compostos reduzidos estão presentes em excesso. Assim, a acidificação do estroma inibe a zeaxantina epoxidase, enzima que age na interconversão da xantofila em anteroxantina e desta para zeaxantina (TAIZ; ZEIGER, 2013). Quando esta interconversão é reduzida, ocorre a emissão de fluorescência de clorofila para dissipar o excesso de energia (WRIGHT et al., 2011). Além disso, a redução das pressões parciais de O_2 a níveis extremamente baixos resulta na produção e acúmulo de acetaldeído, etanol e acetato de etila, que podem causar danos nas membranas celulares e suas organelas, como os cloroplastos, danificando os fotossistemas (FS), dificultando a transferência da energia luminosa e resultando no aumento da fluorescência (MAXWELL; JOHNSON, 2000).

Na câmara de armazenamento são colocados sensores FIRM (*Fluorescence Interactive Response Monitor)* com o intuito de monitorar a emissão de fluorescência pela clorofila na epiderme de amostras representativas de toda câmara comercial, em função do estresse causado pelo baixo O_2. Quando o nível de O_2 atinge o limite mínimo (estresse no fruto), a emissão de fluorescência ocorre, a qual é detectada pelos sensores (DeELL et al., 1999), momento no qual se eleva a pressão parcial de O_2 na câmara em 0,2 a 0,3 kPa (PRANGE et al., 2005; WRIGHT et al., 2012), permanecendo assim até o final do armazenamento ou até que um novo pico de fluorescência seja detectado, quando o nível de O_2 deve ser novamente incrementado. Considera-se que o nível de O_2 deve permanecer sempre acima de 0,4 kPa após o pico de emissão de fluorescência pelas clorofilas (PRANGE et al., 2005; WRIGHT et al., 2012).

Patenteada com nome de HarwestWatch™ (PRANGE et al., 2007), essa tecnologia foi incialmente comercializada pela empresa italiana Isolcell. Além desta, a Besseling, uma empresa Holandesa, comercializa a tecnologia de ACD-FC com o nome de FruitObserver® (PRANGE, 2018). É uma das técnicas de ACD mais utilizadas comercialmente no mundo e estudos mostram sua efetividade na manutenção da qualidade de maçãs e outras frutas em

relação à AC (EREN et al., 2015; BOTH et al., 2017; MDITSHWA et al., 2017a, 2017b; MDITSHWA; FAWOLE; OPARA, 2018; RIZZOLO et al., 2015; THEWES et al., 2015).

Para maçãs ‘Golden Delicious’, a ACD-FC resultou em melhor manutenção da qualidade em comparação com AC isolada (GABIOUND REBEAUD; GASSER, 2015). ACD-FC resultou em maior emissão/concentração de ésteres em comparação com AC+1-MCP (RAFFO et al., 2009). Ainda em comparação com a AC, são reportados redução na ocorrência de distúrbios fisiológicos, especialmente da escaldadura superficial (EREN et al., 2015; MDITSHWA et al., 2017a, 2017b; MDITSHWA; FAWOLE; OPARA, 2018), degenerescência de polpa (BOTH et al., 2017; THEWES et al., 2015a), manchas na epiderme (KÖPCKE, 2015) e maior manutenção da firmeza de polpa e da porcentagem de frutos sadios (BOTH et al., 2017; GABIOUD REBEAUD; GASSER, 2015; KÖPCKE, 2015; WEBER et al., 2019).

Entretanto, o emprego de ACD – FC tem pouca representatividade do número de frutas utilizados por sensor (de 6 a 8 frutas por sensor). Além disso, a detecção do estresse é realizada nas primeiras camadas de células da epiderme (WRIGHT et al., 2015), local onde tem maior disponibilidade de O_2 em comparação ao interior do fruto (HO et al., 2013), o que indica que internamente o fruto já pode ter ativado o metabolismo anaeróbico. Assim, as determinações do LMO por técnicas mais precisas podem trazer vantagens em relação à qualidade dos frutos.

2.2.4. Atmosfera controlada dinâmica monitorada pelo quociente respiratório (ACD-QR)

Uma alternativa mais recente é determinação do LMO dos frutos pelo monitoramento do quociente respiratório (QR), baseado na razão entre a produção de CO_2 e o consumo de O_2 pelo metabolismo respiratório dos frutos (BESSEMANS et al., 2016; WEBER et al., 2015).

Nas câmaras de armazenamento, conforme a pressão parcial de O_2 é reduzida, a respiração mitocondrial também diminui, fazendo com que ocorra a redução da quantidade de CO_2 produzido. Abaixo de uma determinada concentração de O_2 (ponto de compensação anaeróbico, PCA) ocorre um aumento da produção de CO_2 pelo metabolismo anaeróbico, em virtude da glicólise ser mais pronunciada como forma de aumentar a produção de energia (BOERSING; KADER; ROMANI, 1988).

Neste sentido, é possível avaliar o quociente respiratório (QR) de uma amostra de frutos armazenados em condições de níveis extremamente baixos de O_2. Quando os valores da razão são próximos de 1,0, há indicação de que ocorre respiração aeróbica e, quanto maior este valor, maior o processo fermentativo (respiração anaeróbica) (BOERSING; KADER; ROMANI,

1988; GOYETTE et al., 2012). Assim, a medida que o O_2 é insuficiente para o metabolismo aeróbico, o fruto inicia o metabolismo anaeróbico e aumenta o QR. Quando estabelecido o QR ideal, esta pode ser considerada uma técnica que mais se aproxima do conceito de ACD, visto que é possível determinar a variação diária de O_2, de forma que se adeque ao mínimo o metabolismo dos frutos.

WEBER et al. (2015) propuseram uma metodologia para estabelecer um ponto de ajuste do O_2 em função do QR para maçãs armazenadas em ACD. O QR é determinado a partir da pressão parcial de O_2 e CO_2 que é medida imediatamente após o fechamento das as câmaras. Após 24 h, a pressão parcial dos gases é medida novamente e o QR é calculado como a relação entre o CO_2 liberado e o O_2 consumido dentro de um período de 24 h. A pressão parcial de O_2 é controlada por variações do QR relacionado a um QR pré-estabelecido. Se o QR calculado for superior ao valor pré-estabelecido, aumenta-se a pressão parcial de O_2 para reduzir a fermentação e reduzir QR. Já se o QR calculado for inferior ao QR pré-estabelecido, a pressão parcial de O_2 é reduzida para permitir uma maior fermentação. Depois de estabelecidas as condições de QR, as pressões parciais de oxigênio e dióxido de carbono são monitoradas e corrigidas por um sistema de controle automático. O equipamento compara a pressão parcial de oxigênio e dióxido de carbono em um ponto de ajuste. Se a pressão parcial de oxigênio estiver abaixo do ponto de ajuste, o O_2 é injetado até o ponto de ajuste.

Muitos estudos já evidenciam resultados positivos comparando a tecnologia de ACD – QR com AC e ACD – FC (BOTH et al., 2017; BRACKMANN; WEBER; BOTH, 2015; THEWES et al., 2017c; WEBER et al., 2015). ACD – QR proporciona maçãs com menor incidência de escaldadura superficial (BESSEMANS et al., 2016), degenerescência de polpa, maior firmeza de polpa e frutos sadios quando comparado à AC convencional (BOTH et al., 2017; THEWES et al., 2017c; WEBER et al., 2015, 2019) e maior emissão de compostos voláteis (BOTH et al., 2017; THEWES et al., 2017b, 2017c), independentemente do estádio de maturação na colheita (THEWES et al., 2017a, 2017b).

2.2.5. Atmosfera controlada dinâmica monitorada pelo dióxido de carbono (ACD-DC)

As técnicas de ACD descritas acima são aplicadas comercialmente e as tecnologias são protegidas quanto ao direito de uso, mas com seus princípios já bem estudados (WRIGHT; ARUL; PRANGE, 2015). Por outro lado, a ACD-DC é uma técnica ainda com divulgação protegida, uma vez que seu desenvolvimento é recente e está depositado em um pedido para obtenção do registro como patente.

Diferente das tecnologias de armazenamento supracitadas, que tem seu princípio baseado no monitoramento do LMO dos frutos armazenados a partir dos níveis O_2 consumido pelos frutos, a ACD-DC monitora apenas a emissão de CO_2 ao longo do armazenamento, objetivando, da mesma forma, manter a qualidade dos frutos durante o período pós-colheita evitando distúrbios atribuídos a intensa respiração anaeróbica. Determinar o LMO a partir da emissão de CO_2 é possível pois, com a redução da pressão parcial de O_2 na câmara de armazenamento abaixo do PcA, a produção de CO_2 tende a aumentar pelo metabolismo anaeróbico (BOERSIG; KADER; ROMANI, 1988; GASSER et al., 2003).

O desenvolvimento de um software por pesquisadores do Núcleo de Pesquisa em Pós-colheita (NPP) da Universidade Federal de Santa Maria visa monitorar a emissão de CO_2, para que através um algoritmo permita predizer o LMO tolerado pelos frutos armazenados e ajustar a concentração o O_2 nas câmaras durante o armazenamento (THEWES et al., 2020).

Essa técnica foi desenvolvida com o propósito de superar algumas limitações ainda inerentes as técnicas de ACD supracitadas. Por exemplo, na determinação do LMO, podem ocorrer interferências pela falta de estanqueidade das câmaras de armazenamento e entrada de O_2, além disso, essa alternativa facilita a medição do LMO em nível comercial sem a necessidade de equipamentos adicionais nas câmaras de AC.

O primeiro estudo que utilizou essa técnica para o armazenamento de maçãs ‘Imperial Gala’, ‘Fuji Suprema’, ‘Golden Delicious’ e ‘Cripps Pink’ foi publicado recentemente (THEWES et al., 2020). ACD-DC foi comparada com AC-ultrabaixo oxigênio, ACD - FC e ACD - QR após 9 meses de armazenamento. Além de estimar corretamente o LMO para as cultivares testadas, permitiu a indução de compostos anaeróbios em níveis seguros sem causar danos às membranas celulares, diminuiu a incidência de degenerescência de polpa e manteve a firmeza dos frutos. Em maçãs ‘Imperial Gala’ e ‘Golden Delicious’, ACD-DC reduziu a incidência de podridão.

2.2.6. Efeito do 1-metilciclopropeno (1-MCP) no armazenamento de maçãs

O 1-metilciclopropeno (1-MCP) tem sido aplicado para a conservação pós-colheita de frutos climatéricos e tem sido utilizado mundialmente por empresas armazenadoras de maçãs (BRACKMANN et al., 2009b). É considerado um retardador químico de amadurecimento que se liga irreversivelmente aos receptores de etileno nas membranas celulares (WATKINS, 2006) e é, portanto, capaz de reduzir os efeitos do etileno e/ou interferir a sua ação em plantas, bloqueando a cascata de sinais de transdução que leva à expressão de genes relacionados a

enzimas produtoras e receptores de etileno (IN et al., 2013). 1-MCP tem uma afinidade para os receptores de etileno que foi relatado como sendo 10 vezes maior do que a do próprio etileno e atua como inibidor em concentrações tão baixas quanto 5 nL L^{-1} -100 μL L^{-1} (BLANKENSHIP e DOLE, 2003).

A aplicação de 1-MCP, resulta no retardamento da senescência das frutas e, consequentemente, em frutas com melhor manutenção da firmeza de polpa (BRACKMANN et al., 2013; NOCK; WATKINS, 2013; WATKINS, 2006), menor taxa respiratória (NOCK; WATKINS, 2013; PRE-AYMARD; WEKSLER; LURIE, 2003; THEWES et al., 2015b, 2018; WATKINS, 2006) e resultados melhores para sólidos solúveis totais e acidez para maçãs tratadas em relação às não tratadas (BRACKMANN et al., 2013; WATKINS, 2006).

A ACD é capaz de inibir a síntese de etileno de maneira similar a aplicação de 1-MCP (BOTH et al., 2018; THEWES et al., 2017b), entretanto, resulta no acúmulo de compostos do metabolismo anaeróbico, como o etanol, que possui diversos efeitos no metabolismo das frutas (ASODA et al., 2009; JIN et al., 2013; LIU et al., 2012; WEBER et al., 2020). Apesar disso, ainda não está bem clara a interação do armazenamento em ACD, com a aplicação de 1-MCP sobre a qualidade geral das frutas.

2.3. REVESTIMENTOS HIDROFÓBICOS DAS FRUTAS

A transição de algas do ecossistema aquático para a ocorrência de plantas no ecossistema terrestre exigiu o desenvolvimento de avanços fisiológicos e morfológicos para possibilitar a sobrevivência e a colonização (BHANOT; FADANAVIS; PANWAR, 2021). O desenvolvimento da cutícula, foi uma das adaptações mais importantes.

Estrategicamente localizada na interface planta/ar, a cutícula da planta é uma membrana extracelular contínua à prova de água, translúcida e fina (JEFFREE, 1996) a qual abrange os principais órgãos acima do solo (por exemplo, flores, folhas, caules, frutos) de todas as plantas inferiores e superiores da terra (KOLATTUKUDY, 1980). Desta forma, desempenha funções essenciais nas interações da planta com o meio ambiente. Suas capacidades de proteção são baseadas nas propriedades físicas e bioquímicas dos seus componentes altamente hidrofóbicos.

O principal componente que forma a cutícula é a cutina, enquanto a superfície da planta é coberta por ceras. Esta cera incorporada na cutina é chamada de “cera intracuticular” enquanto que a cera sobreposta sobre a cutina é chamada de “cera epicuticular” (HOLLOWAY, 1994; HOLLOWAY, 1982; LARA; BELGE; GOULAO, 2015). O polímero de cutina e as ceras intracuticulares constituem a cutícula adequada que está ligada à parede da célula através de

uma camada cuticular feito de cutina, cera e polissacarídeos. Cobrindo a cutina, a camada mais externa da cutícula é formada por cera epicuticular que pode ser micro estruturada em cristais de cera (BERNARD; JOUBÈS, 2013).

A cutina, definida como um polímero poliéster composto principalmente de ácidos graxos esterificados hidroxilados e epóxi-hidroxilados de cadeia longa (C16 e C18) (LARA; BELGE; GOULAO, 2015) originam-se geralmente a partir de precursores C16:0 e C18:1 sintetizados nos plastídios (KOLATTUKUDY, 1980). Os monômeros C16 mais comuns da cutina são os ácidos 9,16- e 10,16-dihidroxihexadecanóico, enquanto que os ácidos 9,10,18-trihidroxioctadecanóico e 9,10-epóxi, 18-hidroxioctadecanóico são os monômeros mais característicos da classe C18. Além de derivados de ácidos graxos, a cutina também contém quantidades variáveis de compostos fenólicos, ácidos dicarboxílicos, e glicerol (STARK, TIAN, 2006).

As ceras consistem em uma mistura de hidrocarbonetos alifáticos e seus derivados, com comprimentos de cadeia entre 20 e 40 carbonos. As principais classes de componentes são geralmente álcoois primários e secundários, cetonas, ácidos graxos, e aldeídos. Os alcanos são amplamente distribuídos. No entanto, muitas ceras vegetais não coincidem com a definição química verdadeira de ceras. Triterpenóides, por exemplo, ocorrem em concentrações elevadas nos revestimentos cuticulares de maçãs (*Malus* x *domestica* Borkh.) (BELDING et al., 1998).

A biossíntese da cutícula durante o desenvolvimento dos frutos é relatada morfologicamente como um processo que cessa cedo, antes do início do processo de amadurecimento e, muitas vezes, antes que a fruta tenha atingido o tamanho máximo. Como consequência disso, ocorre uma diminuição da quantidade de cutícula por área superficial, bem como, espessura mais fina da cutícula no fruto maduro (ROSENQUIST, 1988, apud LARA; BELGE; GOULAO, 2014). Entretanto, o estudo de Kosma e colaboradores (2010) comprovaram um aumento contínuo de cera cuticular e monômeros de cutina durante o desenvolvimento de tomates.

Os componentes cuticulares e suas alterações durante a maturação dos frutos já foram relatados em algumas espécies de frutas, incluindo maçãs (BELDING et al., 1998; DONG et al., 2012). Essas mudanças na composição durante o armazenamento ou, em resposta a determinadas condições de pós-colheita revelam uma considerável variabilidade associada às cultivares distintas da mesma espécie (VERAVERBEKE et al., 2001a; MORICE; SHORTLAND, 1973).

As cutículas são barreiras de transporte muito eficientes, onde suas principais funções fisiológicas são a proteção contra a perda de água não controlada pela transpiração

(BURGHARDT; RIEDERER, 2006, RIEDERER; SCHREIBER, 2001; KERSTIENS, 1996), redução da lixiviação de solutos essenciais do interior das células, por exemplo, íons e solutos orgânicos polares e proteção a planta contra a irradiação ultravioleta (UV) (SCHÖNHERR, 2000 e SCHÖNHERR, 2001). Além de, proporcionar uma barreira eficaz a qual seria capaz de resistir a ataques de patógenos (SERRANO et al., 2014; REINA-PINTO; YEPHREMOV, 2009). A preservação de todas estas funções requer integridade estrutural da cutícula durante todo o desenvolvimento do fruto.

As propriedades funcionais associadas às cutículas são fisiologicamente muito relevantes e, além disso, revelam uma importância econômica considerável, uma vez que influenciam na vida de prateleira pós-colheita do fruto (LARA; BELGE; GOULAO, 2014). Devido à natureza hidrofóbica dos componentes da cutícula, essa impermeabilização dos frutos regula a perda de peso durante o armazenamento em função da respiração e difusão de vapor de água através da cutícula dos frutos. Estudos já mostraram a capacidade da camada de cera cuticular de espalhar-se sobre a superfície cobrindo microfissuras, provavelmente como uma proteção contra a perda de umidade (VERAVERBEKE, 2001b).

Em relação à espessura da cutícula como contribuinte contra a perda de peso durante o armazenamento, existem divergências nos resultados relacionados a diferentes espécies, uma vez que, estudos demonstraram que diferenças significativas na espessura da cutícula de ameixas e pimentas contribuíram para a perda de peso em taxas significativamente mais baixas (CRISOSTO et al. 1993; LOWNDS et al., 1993). Porém em outros casos, os efeitos positivos sobre a perda de peso, mostraram ser independentes da espessura das cutículas (RIEDERER e SCHREIBER, 2001). As ceras geralmente são relatadas como os principais componentes cuticulares representando a sua função como uma barreira de impermeabilidade (LARA; BELGE; GOULAO, 2014). Variedades de maçã com cera cuticular mais espessa sofrem menos perda de água e podem ser armazenadas por um período mais longo em comparação com aquelas com camadas de cera mais finas (KNOCHE; GRIMM, 2008; CURRY, 2012).

As frutas tornam-se progressivamente mais suscetíveis a ferimentos e danos mecânicos, em geral, após longos períodos de armazenamento, favorecendo vias para o desenvolvimento de infecções. Algumas destas lesões podem ser microscópicas, tais como as decorrentes de microfissuras à superfície que algumas espécies de frutas particularmente susceptíveis, e que também está diretamente dependente da quantidade, composição e propriedades mecânicas da cutícula (LARA; BELGE; GOULAO, 2014). Em maçãs, existe uma relação entre a susceptibilidade à infecção e a estrutura e espessura da cutícula (KONARSKA, 2012).

Entretanto, a literatura ainda é escassa em relatar especificamente quais são os componentes da cutina ou das ceras capazes de resistir às infecções.

A firmeza, que representa um dos principais atributos de qualidade de frutos, é investigada há longos anos e suas alterações são principalmente relacionadas ao metabolismo da parede celular (GOULAO; OLIVEIRA, 2008). Recentemente, estudos que suprimiram geneticamente a expressão de várias proteínas relacionadas à modificação da parede celular relacionada ao amadurecimento, em geral, não comprovaram se houve uma conservação da firmeza da polpa na matriz avaliada. Com isso, concluíram que a modificação da parede celular em função do amadurecimento ocorre de maneira cooperativa pelas ações de várias atividades diferentes. Em função disso, sugere-se que a composição da cutícula bem como sua estrutura pode representar grande importância para respostas às alterações de firmeza das frutas, a qual acrescentaria um suporte mecânico para a integridade do fruto (LARA, BELGE, GOULAO, 2014). Entretanto, mais conhecimento sobre um possível papel da cutícula da fruta relacionada a mudanças de firmeza se faz necessário.

Em face disso, é possível observar que o conhecimento sobre a estrutura e a composição de ceras cuticulares está elucidado, de uma maneira geral, a todas as plantas. Porém, os conhecimentos sobre as especificidades dessa camada cuticular, bem como suas verdadeiras funções e interações com o meio, ainda incitam investigações e ilustram a necessidade de realizar estudos direcionados exclusivamente a uma espécie vegetal, devido a essa grande variabilidade evidenciada.

2.4. ALTERAÇÕES NAS CERAS CUTICULARES DE FRUTAS SOB INFLUÊNCIA DO ARMAZENAMENTO

As investigações sobre as alterações da composição de ceras cuticulares de frutas, especialmente maçãs, submetidas à diferentes condições de armazenamento, e a sua relação com parâmetros de qualidade, já são consistentes na literatura (MORICE; SHORLAND. 1973; VERARDO et al., 2003; WANG et al., 2021; DONG et al., 2012; VERAVERBEKE et al., 2001a; 2001b; CURRY, 2008; FAN; MATTHEIS; BLANKENSHIP, 1999; YANG et al., 2017a; 2017b).

A cera cuticular de peras ‘Korla’ armazenada sob refrigeração e diferentes umidades relativa (0±1 ℃, 50–55%, 70–75% e 90–95%) por 90 dias foi avaliada em termos de conteúdo total de cera, composição química e morfologia do cristal (WANG et al., 2021). Os autores observaram que a alta umidade manteve o conteúdo de cera total, especialmente alcanos e

aldeídos em níveis mais elevados, e estes poderiam contribuir com a capacidade de preservação da água, mantendo a integridade da parede celular e retardando a senescência. Além disso, a alta umidade atrasou a transformação da estrutura da cera e retardou o amadurecimento de peras 'Korla' (WANG et al., 2021). A quantidade total de cera diminuiu drasticamente em maçãs 'Red Fuji' durante o armazenamento a 0 °C por sete meses, e a composição da cera também foi modificada, principalmente pela diminuição da fração de alcanos (DONG et al., 2012).

Maçãs 'Elstar', 'Jonagold' e 'Jonagored' foram armazenadas sob condições de AC, e a composição de cera foi avaliada após 4 e 8 meses de armazenamento mais 7 e 14 dias de vida de prateleira a 20 °C (VERAVERBEKE et al., 2001a). O armazenamento e o subsequente vida de prateleira afetaram as propriedades da cera e causaram alterações na composição química. As alterações na composição da cera de 'Elstar', foram menores do que aquelas em 'Jonagold' e 'Jonagored', possivelmente associadas a menor resistência à difusão e mudanças estruturais mais limitadas da cera 'Elstar'. Períodos mais longos de armazenamento em AC aceleraram as mudanças na composição da cera e os componentes responsáveis foram, principalmente, as frações alcanos e ésteres (VERAVERBEKE et al., 2001a).

Além de suas características de barreira protetora, a cera cuticular da maçã determina diretamente a qualidade do brilho das maçãs e algumas cultivares, como 'Jonagold', 'Royal Gala', 'Granny Smith' e 'Cripps Pink' podem desenvolver uma superfície desagradável, com aspecto oleoso durante o armazenamento (CURRY, 2008; FAN; MATTHEIS; BLANKENSHIP, 1999; YANG et al., 2017a; 2017b). Mudanças na composição da cera de maçãs em resposta ao etileno e ao tratamento com 1-MCP também já foram relatadas durante o armazenamento refrigerado (CURRY, 2008, DONG et al., 2012; LI et al., 2017). Em geral, o 1-MCP inibiu a produção de etileno e atrasou o desenvolvimento de certos constituintes da cera, principalmente em ésteres de linoleato e oleato e quantidades menores de ácidos linoleico e oleico livres, considerados responsáveis pela oleosidade da fruta durante o armazenamento refrigerado (YANG et al., 2017a). Genes relacionados à biossíntese e exportação de constituintes de cera fluída, mostraram aumentos significativos na expressão durante o amadurecimento pós-colheita dos frutos. Em contrapartida, seus níveis de transcrição foram consideravelmente suprimidos em frutas tratadas com 1-MCP (YANG et al., 2017a). Outros autores, associaram a biossíntese de voláteis ao desenvolvimento da oleosidade da epiderme, possivelmente, por disponibilizar substratos de álcoois voláteis para a produção de ésteres gordurosos (YANG et al., 2017b).

Até agora, os componentes principais, a estrutura cristalina e as vias metabólicas da cera cuticular da maçã estão esclarecidas, vários genes estruturais e fatores de transcrição também

foram identificados e relacionados às vias regulatórias da cera cuticular da maçã. No entanto, apesar da avaliação da produção e composição da cera da casca de maçã fornecer dados importantes sobre o metabolismo dos frutos e sua relação com atributos de qualidade, ainda não existiam investigações sobre essa variabilidade composicional e sua relação da qualidade pós-colheita de frutos quando armazenados por longos períodos sob diferentes métodos de DCA.

Os resultados apresentados neste trabalho são os primeiros a nortear a compreensão da relação dos constituintes de cera cuticular de maçãs e os efeitos benéficos relacionados ao armazenamento em condições de pO_2 cada vez mais baixas. Ademais, poder relacionar esses constituintes de cera com parâmetros de qualidade, associados às tendências do armazenamento dinâmico no período pós-colheita, pode auxiliar no desenvolvimento de novas tecnologias ou aprimorar àquelas já existentes para cultivares específicas.

3. ARTIGO 1

3.1. DYNAMIC CONTROLLED ATMOSPHERE: EFFECTS ON THE CHEMICAL COMPOSITION OF CUTICULAR WAX OF 'CRIPPS PINK' APPLES AFTER LONG-TERM STORAGE[1]

Abstract

The effects of controlled atmosphere (CA) and dynamic controlled atmosphere based on chlorophyll fluorescence (DCA-CF) and respiratory quotient (DCA-RQ; RQ = 1.3 and 1.5) on the metabolism, decay incidence, concentration and chemical composition of 'Cripps Pink' apple peel wax after 8 months of storage plus shelf life at 20 °C were studied. DCA-RQ1.3 stored fruit had the lowest respiration rate, evidencing low metabolism, corroborating with the highest number of healthy fruit and consequently being the best storage condition. The mean wax concentration found 21.23 g m^{-2}, although it was lower for the DCA-RQ1.5 condition after 7 d. There was increase in wax concentration for DCA treatments from 7 to 14 d of shelf life. Chromatographic analysis allowed the identification of palmitic, stearic, oleic and linoleic fatty acids, with an increase in cis-11,14-eicosadienoic acid in all treatments and in palmitic acid in CA at 14 d. Triterpenoids, such as ursolic acid and oleanolic acids, were higher in CA, while alcohols such as 10-nonacosanol was higher in both DCA-RQ treatments. All treatments had high concentrations of nonacosane and tetracosanal. DCA-RQ decrease the incidence of greasiness. The supposed induction of anaerobic metabolism by extremely low oxygen levels and consequently higher concentration of ethanol, which were monitored by the two levels of RQ, induced the formation of compounds that may favor some mechanisms of adaptation against the low oxygen partial pressure (pO_2).

Keywords: Apple peel wax; Chlorophyll fluorescence; Fruit; 'Pink Lady'; Postharvest; Respiratory quotient.

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3.1.1. Introduction

‘Cripps Pink’ (*Malus* × *domestica* Borkh) was developed by crossing ‘Golden Delicious’ and ‘Lady Williams’ apples. This is a promising cultivar due to its sensorial attributes, which are mainly related to flavor and crunchy texture (Cripps et al., 1993). Apples are seasonal and perishable fruit, although they can be stored for long periods, significantly increasing commercial interest by promoting the development of novel storage technologies that increase the availability of fresh apples and preserve the quality of the final product (Mditshwa et al., 2018).

Controlled atmosphere (CA) is one of the most used methods to store apples and uses low (1 kPa) (Brackmann et al., 2005b) or ultra-low (0.8, 0.7 and 0.5 kPa) (Both et al., 2014) O_2 partial pressure (pO_2) combined with similar CO_2 partial pressure (pCO_2). However, these gases are maintained at static concentration, and do not reflect physiological changes of the fruit throughout the storage period, and are above the lower oxygen limit (LOL). The quality of the apple is maintained when it is closer to LOL, due to lower mitochondrial respiration (Wright et al. 2015). Several dynamic controlled atmosphere (DCA) systems have been proposed to determine LOL and maintain fruit at safe concentrations during storage. Thus, LOL can be monitored based on ethanol production by fruit (DCA-ethanol) (Veltman et al., 2003), fruit chlorophyll fluorescence emission (DCA-CF) (Prange et al., 2007), or respiratory quotient (DCA-RQ) (Brackmann, 2015; Van Schaik et al., 2015; Weber et al., 2015; Bessemans et al., 2016).

Recent studies have shown important aspects regarding the cuticle of the epidermis and the postharvest performance of fruit: permeability and water loss, susceptibility to infections and physiological disorders, and mechanical properties that define texture (Lara, 2018; Lara et al., 2014). Cuticular wax is composed of very long chain fatty acids (VLCFAs) and their derivatives, including primary and secondary alcohols, aldehydes, alkanes, ketones, wax esters, as well as those derived from other precursors such as triterpenoids (Lara et al., 2015). Therefore, it is necessary to relate the properties and/or composition of this cuticular layer with the quality attributes during the postharvest period.

‘Cripps Pink’ apple is characterized by a very dense pulp as well as a greasy and waxy cuticle that develops mainly during the ripening and storage. Besides ‘Pink Lady’, other apple cultivars have been reported to become greasy during storage, including ‘Jonagold’, ‘Royal Gala’, and ‘Granny Smith’ (Fan et al., 1999; Veraverbeke et al., 2001; Curry, 2008; Yang et al., 2017b, 2017a). Excessive greasiness is considered a disagreeably sensorial quality attribute

for fresh fruit consumers (Richardson-Harman et al., 1998). Greasiness is a physical and chemical fruit-surface phenomenon that may occur both in development and during storage and is associated with changes in wax constituents (Curry, 2008; Dong et al., 2012). Many of these changes have been shown to be regulated by ethylene (Li et al., 2017; Yang et al., 2017a). DCA use, mainly DCA-RQ, reduces ethylene production (Both et al., 2018; Donadel et al., 2019), and may affect the characteristics and chemical composition of this cuticular layer of stored apples.

The production and composition evaluation of apple peel wax will provide important data on fruit metabolism and its relation to quality attributes, especially when stored for long-term periods under different DCA methods. Therefore, the objective of this study was to evaluate the changes in the concentration and chemical composition of 'Cripps Pink' apple peel waxes after storage under CA, DCA-CF and DCA-RQ conditions for eight months of storage plus 7 and 14 d shelf life at 20 °C. In addition, the results of the concentration and chemical composition of apple peel wax were related to the metabolism, decay incidence, and greasiness of fruit.

3.1.2. Material and methods

3.1.2.1. Plant material and experimental design

The experimental material was composed of 'Cripps Pink' apple harvested at physiologically maturity (commercial harvest peak: starch pattern index (SPI) 6.9 and titratable acidity (TA) 4.2 g L^{-1} of malic acid) in a commercial orchard located at Vacaria, RS, Brazil. The apples were then transported to the Postharvest Research Center of the Federal University of Santa Maria, RS, Brazil. Fruit with any defects were discarded and homogenize on size between treatments. The apples were randomly sampled, 24 samples of 25 fruit, with each treatment composed by three replications of 25 fruit. The experiment was carried out with four treatments: [1] CA – control treatment, with 1 kPa of O_2 and 1 kPa of CO_2; [2] DCA-CF, with variable O_2 according to CF and 1 kPa of CO_2; [3] DCA-RQ1.3: variable O_2 according to the RQ defined in 1.3 and 1 kPa of CO_2; and [4] DCA-RQ1.5: variable O_2 according to the RQ defined in 1.5 and 1 kPa of CO_2.

3.1.2.2. Installation and maintenance of treatments

Treatments were applied in chambers with a volume of 233 L and hermetically closed. Temperature was set at 5.0 ± 0.1 °C in the first storage day and, thereafter, was reduced gradually (0.6 °C d^{-1}) to the desired storage temperature of 2.0 ± 0.1 °C in 5 d. The temperature was measured in the flesh of three fruit.

CA and DCA storage conditions were applied by flushing the chambers with N_2 until the pO_2 pre-established for CA (1.0 kPa) and reduced to 0.5 kPa for the DCA conditions, at the time which the RQ determination started. The target level of CO_2 was obtained by fruit respiration. PO_2 and pCO_2 were daily monitored with a gas analyzer (Siemens®, model Ultramat, Germany) and corrected by an automatic CA and DCA-RQ control system (Valis®, Lajeado, RS, Brazil). The system measures the pO_2 and pCO_2 in relation to an established set point for each treatment. If the O_2 measured was lower than the set point, it was corrected with the injection of atmospheric air up to the desired pO_2. The CO_2 was absorbed with a lime scrubber.

In DCA-RQ conditions the pO_2 was set according to fruit metabolism, as proposed by Brackmann et al. (2015) and Weber et al. (2015). The RQ was adjusted at 1.3 and 1.5 (DCA-RQ1.3 and DCA-RQ1.5, respectively) and the pO_2 changed accordingly, to obtain this RQ during the entire storage time (Fig. 1). The RQ was calculated as the ratio of CO_2 production and O_2 uptake, between the first and second determination of the chamber gas concentration in a period of 13 hours closing without correction of the gases composition.

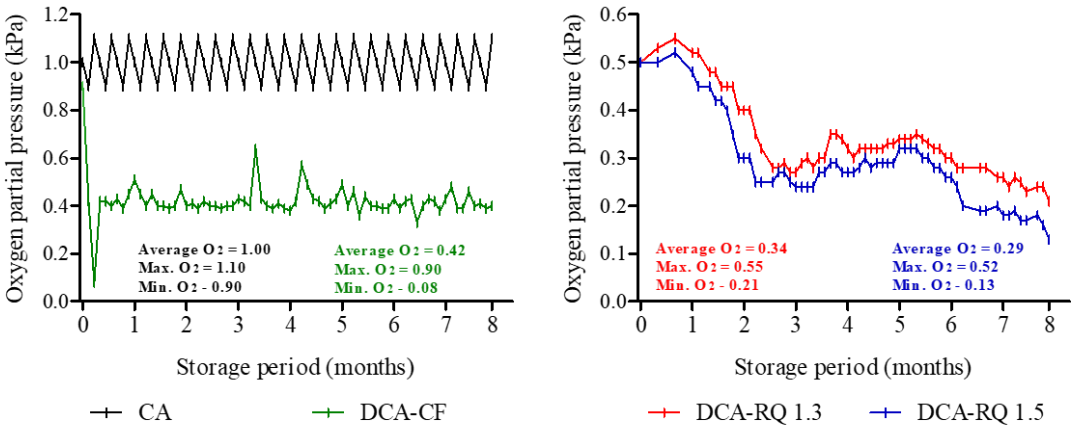

Figure 1. Oxygen set point variation for CA, DCA-CF, DCA-RQ1.3 and DCA-RQ1.5 during 8 months of storage of 'Cripps Pink' apple.

The monitoring and correction of pO_2 in DCA-CF was according to Prange et al. (2007), where a sensor was utilized to monitor CF and indicated the LOL during storage. In all

treatments, the relative humidity was maintained at 94 ± 2 % during storage period by calcium chloride addition.

3.1.2.3. Decay incidence, ethylene production and respiration rate

The apples were evaluated after eight months of storage according to each treatment plus 7 and 14 d of shelf life at 20 ± 2 °C.

Decay incidence was evaluated immediately after opening of the chambers and after shelf life by counting fruit with decay lesions higher than 5 mm in relation to total number of fruit per replicate (n = 25). Results were obtained from three replicates for each treatment and expressed in percentage of total fruit.

For ethylene production and respiration rate determination, approximately 1.5 kg fruit was placed into a 5 L flasks and hermetically closed for about 2 h. Two samples of 1 mL of headspace were taken from the container and injected into a Varian Star CX 3400 (Varian Inc, Palo Alto, CA, USA) gas chromatograph equipped with a flame ionization detector (GC-FID) and a Porapak N80/100 packaged column. Column, injector and detector temperatures were maintained at 90, 140 and 200 °C, respectively. Results were obtained from three replicates for each treatment and expressed in ng kg^{-1} s^{-1}.

The same sample headspace of container used for ethylene quantification was circulated throughout an electronic gas analyzer (Schele®, model KB7) to determine fruit respiration as the CO_2 production. Respiration rate were obtained from three replicates for each treatment and expressed in µg kg^{-1} s^{-1}.

3.1.2.4. Apple cuticular wax extraction

Sample preparation and extraction of the wax compounds followed the method of Klein et al. (2018). Peel was sampled by removing the epidermis and 2-3 mm of cortex from the equatorial part of 10 fruit. A standardized sample in the 1 cm^2 quadrangular shape of the peel (each fruit) was transferred into a tube of 50 mL and frozen at -30 °C until analysis. In the samples were added 5 mL of distilled water. After, extraction was carried out with 4 mL of chloroform used as extraction solvent (2 mL on the first extraction, followed by another two partitions with 1 mL each) and 4 mL of acetone as the disperser solvent added at first partition. The mixture containing sample and organic solvents was shaken (SL183, Solab, Brazil) for 30 min in each partition. Then, it was centrifuged for phase separation and the organic fraction

retained. The volume of organic phase recovered from the extractions was transferred to a 10 mL volumetric flask, to which was added 250 μL of a solution of methyl tricosanoate (C23:0, 4.006 g L^{-1}, Sigma-Aldrich, St. Louis, USA), used as internal standard (IS) and the volume adjusted with acetone. From this volume, 5 mL was used to analyze total wax extraction, measured gravimetrically on analytical balance (AUW-220, precision of 10 μg; Shimadzu Corporation, Kyoto, Japan), after evaporation of organic phase and drying at 105 °C in a conventional oven (De Leo e Cia Ltda, Brazil) to constant mass. Results were obtained from three replicates for each treatment and expressed in g m^{-2} of wax. The remainder was used to analyze wax composition by determination of fatty acid profile, direct analysis of the concentrated organic fraction and derivatization for high boiling compounds determination.

3.1.2.4.1. Apple cuticular wax characterization

An aliquot of 4 mL was transferred into a tube and solvent was evaporated under N_2 continuous flow at 40 °C to obtain the lipid fraction 1 (F1). Fatty acid methylation (Hartman and Lago, 1973) was performed. Fatty acid methyl esters (FAME) were determined using a gas chromatograph equipped with a flame ionization detector (GC-FID, Varian Star 3400 CX, Walnut Creek, USA). A volume of 1 μL was injected in splitless mode (split valve closed for 1 min and then split 20:1) at 250 °C. The FAMEs were separated using capillary column CP-Wax 52CB (Chrompack, Middelburg, 50 m × 0.32 mm × 0.20 μm). The initial column temperature was 50 °C, remaining for 1 min, increasing to 180 °C at 10 °C min^{-1}. The temperature increase rate was 2 °C min^{-1} until 200 °C was reached and then 10 °C min^{-1} to 230 °C, and maintained in isotherm for 5 min. The detector was maintained at 240 °C. The FAMEs were identified by comparison of the retention times of the analytes with those found in the FAME Mix-37 standard (P / N 47885-U, Sigma-Aldrich, St. Louis, USA). The FAMEs were quantified using IS as a reference and both FID response and ester to acid conversion factor was applied as described by Simionato et al. (2010).

A volume of 500 μL of the solution containing the wax was transferred into 2 mL glass vials and the solvent was evaporated under N_2 continuous flow at 40 °C. The residue was reconstituted with 50 μL of chloroform and vigorously vortexed, obtaining the fraction 2 (F2). The compounds determination with high boiling point (fraction 3 – F3) occurred with derivatization according to Oms-Oliu et al. (2011). Thus, 100 μL of the extract was transferred into 2 mL vials and the solvent was evaporated under N_2 continuous flow at 40 °C. The residue was derivatized with 40 μL of methoxyamine hydrochloride solution (20 g L^{-1} in pyridine) and

60 µL N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA; Sigma-Aldrich) at 40 °C.

The F2 and F3 extracts were analyzed using GC-FID (Varian, Star model 3400CX, CA, USA). Compounds were separated using capillary column BPX-5 of nonpolar phase (SGE, Australia, 25 m × 0.22 mm; 0.25 µm of thickness film). H_2 was used as carrier gas at a constant pressure of 103 kPa. The injector remained in splitless mode (1 min) and temperature of 300 °C. The column initial temperature was 50 °C, where it remained 1 min, increasing to 200 °C at a rate of 10 °C min^{-1} and again increasing to 320 °C at a rate of 3 °C min^{-1}, remaining isothermic for 10 min. The detector was maintained at 280 °C. For fractions 2 and 3, the concentration of the compounds was calculated by internal standardization (C23:0) considering a response factor of 1 and the results were obtained from three replicates for each treatment and expressed in g kg^{-1} wax.

Wax compound identification was performed by GC coupled to mass spectrometer (GC/MS, Shimadzu QP2010 Plus, Kyoto, Japan). For these analyses, the same chromatographic conditions described above were used and He was used as the carrier gas. The MS operated in the electron impact ionization mode with ionization energy of 70 eV and single quadrupole type analyzer operating in scan mode in a mass range of 35-450 m/z. The analytes were identified based on the comparison of mass spectra obtained with those available from the National Institute of Standards and Technology (NIST) and the literature.

3.1.2.5. Determination of ethanol in apple juice

After storage plus 7 and 14 d of shelf life at 20 °C, apples were cooled to 0.5 °C and the juice prepared and extracted according to Both et al. (2014). The juice was immediately stored at -30 °C until GC analyses. The ethanol extraction was carried out by HS-SPME. A 10 mL aliquot of this juice was placed inside a 20 mL vial following the addition of 3 g of NaCl and 10.0 µL of a 3-octanol standard solution (0.0822 g L^{-1}). The vial was sealed with a screw cap and septum coated with PTFE/silicone, and submerged in a water bath at 40 °C for 15 min. The fiber was then exposed to the headspace of the sample for 60 min under constant stirring at the same temperature for sorption and submitted to GC analysis. Divinylbenzene/carboxen/polydimethylsiloxane (DVB/Car/PDMS) fiber (Supelco, 50/30µm×20mm) has used for extraction. The ethanol was identified and quantified on a GC coupled to a mass spectrometer (Shimadzu QP2010 Plus - GC/MS, Shimadzu Corporation, Kyoto, Japan). The chromatographic conditions employed were according to Thewes et al.

(2017b) with slight modifications. The fiber was desorbed at 250 °C for 10 min and the injection split ratio was 1:5. The compounds were separated on a ZB-WAX polar phase capillary column (Zebron, Phenomenex, USA; 60 m × 0.25 mm × 0.25 μm). He was used as carrier gas at a constant pressure. The column temperature began at 35 °C, kept for 3 min, and increased at 2 °C min^{-1} to 80 °C and then increased at 5 °C min^{-1} to 230 °C. The column temperature remained isothermal for 5 min. The GC/MS interface and MS ion source temperature were maintained at 230 °C. The detector operated in the electron impact ionization mode with ionization energy of +70 eV and scan mass range from 35 to 350 m/z. A series of homologous n-alkanes was analyzed under the same conditions to calculate the linear retention index (LRI). Ethanol was identified by comparing mass spectra with the standard. Quantification was performed with the use of 3-octanol as internal standardization (Both et al., 2014).

3.1.2.6. Assessment of skin greasiness

Skin greasiness was evaluated immediately after opening of the chambers, 7 and 14 d of shelf life at 20 ± 2 °C by counting fruit with perceived greasiness after rubbing the fruit against the hand (n = 25). Results were obtained from three replicates for each treatment and expressed in percentage of total fruit.

3.1.2.7. Statistical analysis

All data were submitted to analysis of variance (ANOVA) and means of the treatments were compared applying the Tukey test ($p < 0.05$). This analysis was performed using statistical software Statistica, version 7.0 (StatSoft Inc., Tulsa, OK, USA). In addition, data that presented a statistical difference by Tukey test were submitted to a multivariate analysis using Principal Component Analysis (PCA) by Pirouette 3.11 software (Woodinville, USA, 2003). Before the multivariate analysis, the data matrix was autoscaled for each variable to have the same importance in the analysis.

3.1.3. Results and discussion

3.1.3.1. Decay incidence, ethylene production, and respiration rate

After eight months of storage, there was no difference among treatments for decay incidence when the chambers were opened (Fig. 2). Postharvest losses of fruit due to decay are likely affected by the ripening stage and storage environment conditions. The fruit intrinsic resistance to pathogenic microorganisms decreases as the fruit ripens and storage period takes place in unfavorable conditions (Barkai-Golan, 2001). After 7 d of shelf life, fruit stored in CA had higher decay incidence than those stored in DCA-RQ1.3. Janisiewicz et al. (2003) showed that the use of 1-MCP increase decay incidence in apples, which is possibly due to the reduced defense mechanism of the fruit against pathogen attack, since the defense mechanism of the fruit requires ethylene for its activation. This corroborates with our observation because ethylene emission was lower in fruit stored in CA (Fig 2A), resulting in higher decay incidence (Fig. 3) at 7 d of shelf life. Similar behavior was reported by Thewes et al. (2017b) when studying 'Fuji Suprema' apples stored in CA and DCA-RQ. 'Royal Gala' apples stored under DCA-RQ also had higher amounts of healthy fruit in comparison with the ones stored under CA (Both et al., 2017; Weber et al., 2015). Higher ethylene production in CA at chamber opening suggested higher general metabolism of the fruit stored in higher pO_2 (1.0 kPa), which is higher than the LOL, resulting in increased respiration rate (Fig. 3) and consequently higher decay incidence as early as 7 d (Fig 2).

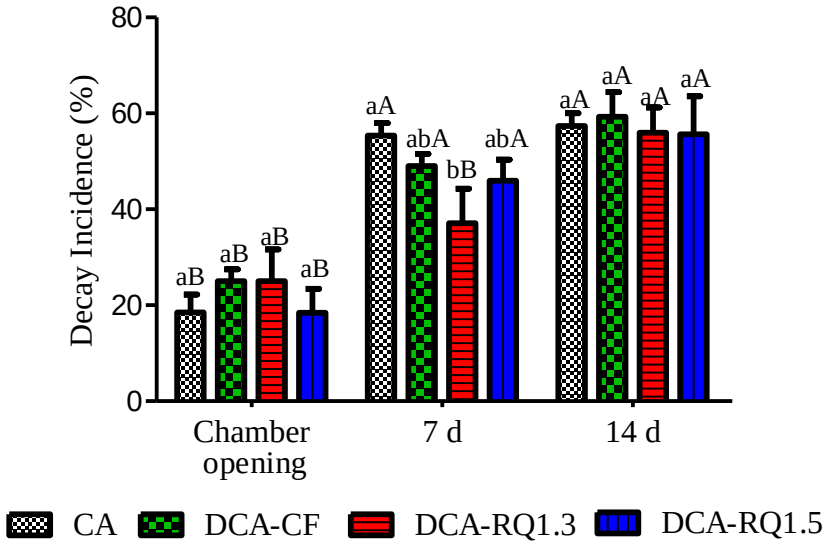

Figure 2. Decay incidence of 'Cripps Pink' apples stored under controlled atmosphere (CA) and different dynamic controlled atmosphere conditions (DCA-CF, and DCA-RQ) for 8 months, after chamber opening plus 7 and 14 d at 20 °C. Bars with the same lower case letter in each day evaluated and each bar with same upper case letter in different days of evaluation at the same treatment are not significantly differenced by Tukey's test, at 5% probability. Error bars mean standard deviation (n= 3).

There was no significant difference in decay incidence after 14 d of shelf life. Nevertheless, fewer healthy fruit were evident in relation to the 7 d period for all DCA conditions (Fig 2), consistent with an increase in ethylene production and respiration rate compared with CA (Fig 3) and the activation of the general metabolism of the fruit during this time. Furthermore, among DCA treatments, RQ1.3 had less healthy fruit compared with the 7 d period, which is possibly due to detriment of the defense mechanisms mentioned by Janisiewicz et al. (2003), and consistent again with the lower ethylene production (Fig 3A). Nevertheless, the results show that DCA storage delayed fruit senescence, especially in RQ1.3, which maintained lower ethylene production and respiration rate (Fig 3B) than the other DCA treatments. On the other hand, decay incidence was lower in all treatments at chamber opening.

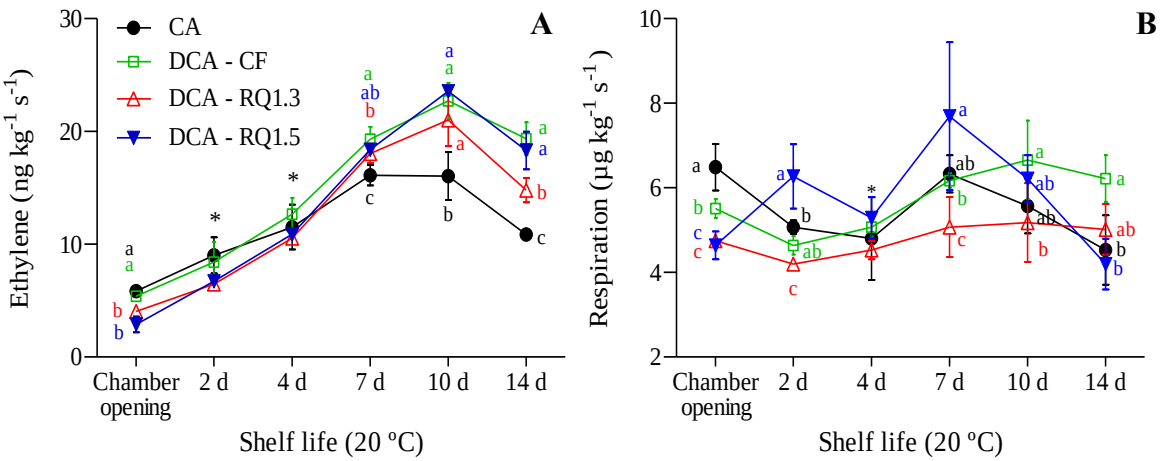

Figure 3. Ethylene production (A) and respiration rate (CO_2 release) (B) of 'Cripps Pink' apples stored under controlled atmosphere (CA) and different dynamic controlled atmosphere conditions (DCA-CF, and DCA-RQ) for 8 months, after chamber opening plus 14 d at 20 °C. *Not significant. Bars with the same lower case letter in each day evaluated, are not significantly different by Tukey's test, at 5% probability (n= 3).

There was no difference in ethylene production between DCA-RQ treatments after 7 d of shelf life. However, DCA-CF had the highest ethylene production in contrast to DCA-RQ1.3 and CA. DCA-RQ1.3 had the lowest respiration rate, evidencing lower metabolism, consistent with the highest number of healthy fruit and proving to be the best storage condition (Fig. 3B). According to Thewes et al. (2017), 'Galaxy' apples harvested at had lower respiration rates in DCA-RQ 1.3 than for CA, regardless of the maturation stage. These authors linked lower respiration rates to reduced fruit metabolism and improved quality maintenance. The respiration rate increased when the RQ level increased to 1.5, showing that RQ 1.5 promoted more anaerobic metabolism in 'Cripps Pink' apple. There was no difference between RQ 1.5 and CA.

It is usual to correlate higher ethylene production in CA with increased respiration rate due to the triggering of the fruit general metabolism and ripening (Both et al., 2017; Weber et al., 2015). As a result, higher CA respiration rates reduce fruit resistance to pathogens, which may result in higher decay incidence as reported for 'Royal Gala' apples by Weber et al. (2015) and Both et al. (2017). However, in this study, the highest decay incidence at 7 d was not correlated to higher ethylene production and, consequently, the respiration rate of 'Cripps Pink' apples stored in CA (Figs 2, 3A and 3B). During DCA storage, pO_2 changes according to fruit metabolism and maintains the minimum and safe O_2 values inside the chamber, decreasing fruit metabolism and delaying the climacteric peak and fruit senescence compared with conventional CA (Mditshwa et al., 2018). Therefore, lower ethylene production associated with higher decay incidence in CA observed in the present study may be associated with post-climacteric fruit. This period is known for decreased respiratory activity, the beginning of senescence, and consequent tissue death (Paul et al., 2012).

3.1.3.2. Apple cuticular wax content

Total cuticular wax extracted from 'Cripps Pink' apples is shown in Table 1. The mean wax concentration found for all treatments was 21.23 in 7 d, and 26.18 g m^{-2} in 14 d. The apple wax concentration was lower in DCA-RQ1.5 condition after 7 d of shelf life. Cuticular wax from 'Golden Delicious', 'Granny Smith', and 'Sturmer' cultivars ranged from 1.38 g m^{-2} to 13.17 g m^{-2} (Belding et al., 1998; Fernández et al., 2016; Lai et al., 2016), which may be a result of cultivar specificity, different growing conditions, and/or different harvest periods (Yeats and Rose, 2013). Klein et al. (2018) using this same method of sample preparation, found a total wax of 21.85 g m^{-2} in commercial 'Cripps Pink' apples while 'Royal Gala', 'Granny Smith' and 'Red Delicious' were found between 15.65 and 17.95 g m^{-2}.

Table 1. Total apple peel wax (g m^{-2}) of 'Cripps Pink' stored under controlled atmosphere (CA), dynamic controlled atmosphere based on chlorophyll fluorescence (DCA–CF) and dynamic controlled atmosphere by respiratory quotient (DCA–RQ) for 8 months plus 7 and 14 d at 20 °C.

Treatments	7 d	14 d	Mean
CA	21.77 ± 1.25*a,A	24.33 ± 2.93a,A	23.05a
DCA-CF	21.80 ± 0.40a,B	25.40 ± 1.06a,A	23.60a

DCA-RQ 1.3	21.63 ± 1.79a,B	26.47 ± 0.70a,A	24.05a
DCA-RQ 1.5	19.71 ± 0.64b,B	28.53 ± 2.04a,A	24.12a
Mean	21.23B	26.18A	

*Mean±standard deviation (SD) (n= 3) values followed by the same lower case letter in each day evaluated, and the same upper case letter in different days of evaluation for the same treatment are not significantly different by Tukey's multiple range test ($P < 0.05$).

Increased wax concentrations were observed in all DCA treatments when comparing 7 and 14 d of shelf life at 20 °C. Tessmer et al. (2012) evaluated cuticular wax of 'Gala' and 'Galaxy' apple during 120 d of storage in air and CA (1.5 % O_2, 2.5 % CO_2). These authors found longer and deeper microcracks in the cuticle, which were related to the decreased capacity of the new waxes to synthesis and cover these faults when compared with freshly harvested fruit. Notably, these changes were more evident in fruit stored under CA. Probably because of reduced respiratory process, alteration of the gas composition of the chamber, and reduced ethylene production during storage (Tessmer et al., 2012). In the present study, higher total wax content was observed in apples stored in DCA at 14 d. This increase in wax coincides with higher ethylene production observed at 7, 10 and 14 d of shelf life in DCA. Despite the higher production of ethylene in the chamber opening for apples stored in CA (Fig 3), it not increase like others treatments during the shelf life for reasons already mentioned above, such as post-climacteric period, which also reflects wax production, since there was no increase in the amount of wax in CA between 7 and 14 d (Table 1). Ethylene positively regulated the total amount of wax during the cold storage of apples (Li et al., 2017). Changes in cuticular constituents in response to ethylene was studied by Ju and Bramlage (2001) in 'Delicious' apples during fruit development and by Curry (2008) and Dong et al. (2012) in 'Royal Gala' and 'Red Fuji' apples, respectively, during cold storage. Yang et al. (2017b) reported that changes in cuticle fraction of 'Cripps Pink' apples stored at 20 °C were only observed after 25 d compared to other cultivars, such as 'Red Delicious', which showed changes after 5 d under the same conditions. Wax synthesis is a phenomenon associated with the climacteric fruit ripening (Fan et al., 1999), as it occurs after harvest in response to ethylene (Li et al., 2019). 1-Methylcyclopropene application to 'Cripps Pink' apples reduced ethylene production and wax fluid compounds, suggesting the role of ethylene as a regulator of fat synthesis in apple epidermis (Yang et al., 2017a). In our study, despite using distinct methods in the storage period, no significant changes in total wax content between treatments were found at 14 d. This

may be associated with a behavior specific to the cultivar studied, as there are drastic declines in ethylene production in ‘Gala’ apples under DCA conditions (Both et al., 2017; Thewes et al., 2017a), which was not observed in ‘Cripps Pink’ apples (Fig 3).

3.1.3.3. Apple cuticular wax composition and skin greasiness

Cuticular wax composition in apple changes during development, storage and shelf life (Bringe et al., 2006; Curry, 2008; Dong et al., 2012; Veraverbeke et al., 2001). In this study, chromatographic analysis of three fractions obtained from samples at 7d allowed the identification of 44 analytes that were comprised of 21 fatty acids (F1), 21 compounds from direct analysis of concentrated extract (F2), and 2 compounds from a derivatized fraction (F3) (Table 3). Apples develop a greasy and waxy cuticle during development and storage, therefore, studies have suggested that the development of this characteristic may be related to changes in wax composition in stored apples (Curry, 2008; Fan et al., 1999; Veraverbeke et al., 2001).

As total wax increased during shelf life at 20 °C when apples were stored in DCA (Table 1), the F1 (fatty acids) were determined after 14 d of shelf life (Table 3). Triacylglycerol fatty acids from the F1 of the apple wax were composed mainly of palmitic acid, stearic acid, oleic acid, and linoleic acid evaluated. In general, the mean value of unsaturated fatty acids for all treatments increased from 7 to 14 d of shelf life (44.62 and 98.17 g kg^{-1} of wax, respectively). This increase was highlighted by the fatty acid C20:2 which had considerably higher accumulation after 14 d of shelf life (around 8 times).

De novo synthesis of C16:0 and C18:0 fatty acids occur in plastids during fruit development. This corresponds to the first stage of cuticular wax biosynthesis (Liu et al., 2015), as well as to the unsaturated fatty acids C18:1 and C18:2 and later elongation for very long-chain fatty acids (VLCFAs) synthesis (Hernández et al., 2009), as observed for the C20:2 fatty acid. Nevertheless, it is still poorly understood how this supply of fatty acids occurs after harvest (Yang et al., 2017a). The rate of *de novo* fatty-acid synthesis is controlled by acetyl-CoA carboxylase (ACCase), which catalyzes the adenosine triphosphate (ATP)-dependent carboxylation of acetyl-CoA to malonyl-CoA (Bernard and Joubès, 2013). This reaction is used in a wide variety of pathways, including malonylation of the ethylene precursor aminocyclopropane-1-carboxylate (ACC) (Liu et al., 1983).

Therefore, low pO_2 during storage interferes in the cellular homeostasis of the fruit as the oxidative phosphorylation in the mitochondria is reduced, which leads to decreased cellular energy, consequently attenuating the metabolic processes that consume ATP, including fatty

acids synthesis (Geigenberger, 2003), although reduced compounds such as NADH and NADPH are accumulated by low oxygen storage (Ke et al., 1994). This may explain the lower concentration of palmitic acid, stearic acid and oleic acid in apples stored in DCA, especially at 14 d, where pO_2 during storage was lower.

Fatty acids are the main precursors in the biosynthesis of ester, alcohol, and aldehydes produced by the apple and are metabolized via β-oxidative enzymes and/or lipoxygenase (LOX) (Fellman et al., 2000). Thus, higher concentrations of esters in apples stored in CA were observed (Table 3), which is likely due to higher concentrations of pO_2 (1.0 kPa) in relation to the other treatments as they had greater influence in enzymatic activities of LOX or β-oxidation during storage. Apples stored in DCA-RQ1.5 produced more ethanol at both times of assessment during shelf life (Table 2), and induced anaerobic metabolism due to this storage condition that kept O_2 levels lower (0.29 kPa, on average) (Fig. 1). Ethanol may favor the formation of esters in these low O_2 conditions (Ke et al., 1994), however, in this study, ester formation did not increase under DCA conditions. Excess synthesized ethanol may suppress the activities of ACC synthase and ACC oxidase, suppressing ethylene responsiveness and, ethylene biosynthesis (Asoda et al., 2009a), and consequently other biosynthetic activities that synthesize cuticular wax compounds. Fellman et al. (2000), Holland et al. (2005) and Vogt et al. (2013) support the hypothesis that differences in ester levels and specificity found between various cultivars and tissues are not only due to the lack of production of appropriate alcohol substrates but are enzymatically limited due to alcohol acyl-transferase specificities in relation to their activity toward their precursors.

Table 2. Ethanol concentration (μ L^{-1}) of 'Cripps Pink' stored under controlled atmosphere (CA), dynamic controlled atmosphere based on chlorophyll fluorescence (DCA–CF) and dynamic controlled atmosphere by respiratory quotient (DCA–RQ) for 8 months plus 7 and 14 d at 20 °C.

Treatment	7 d	14 d	Mean
CA	42.9 ± 29.8*b,A	34.8 ± 7.0b,A	38.9b
DCA-CF	29.7 ± 6.6b,A	36.9 ± 5.9b,A	33.3b
DCA-RQ1.3	49.7 ± 14.4b,A	39.7 ± 18.7b,A	44.7b
DCA-RQ1.5	570.1 ± 359.8a,A	349.6 ± 70.7a,A	459.9a
Mean	173.1A	115.2A	

*Mean± standard deviation (SD) (n= 3) values followed by the same lower case letter in each day evaluated, and the same upper case letter in different days of evaluation for the same treatment are not significantly different by Tukey's multiple range test ($P < 0.05$).

After 7 d of shelf life, C18:1n9 and C18:2n6 had similar concentrations among treatments. However, C18:1n9 was lower in DCA conditions than CA at 14 d of storage, while C18:2n6 had higher accumulation in the condition of DCA-RQ1.3. Even at 14 d, comparison of these two fatty acids shows a higher concentration of C18:2n6 compared with C18:1n9 in all DCA treatments, an increase in linolenic acid possibly related to the increased ethylene emission of these fruit (Fig 3). Contreras et al. (2016) evaluated fatty acids from 'Jonagold' apples and found that linoleic acid increased considerably as the fruit approached the climacteric phase, ethylene either directly or via stimulating ripening being the main trigger of this change. The C18:3n3 was present in small concentrations, with no differences among treatments at 7 d of shelf life. As the shelf life period increased, the C18:3n3 accumulation was suppressed in all treatments. Contreras et al. (2016) showed similar results where the C18:3n3 level in the fatty acid fraction was extremely low for 'Jonagold' apples. Song and Bangerth (2003) found that C18:3n3 was lower than C18:1n9 or C18:2n6, which are both considered the main compound that increases with 'Golden Delicious' maturation and storage. Veraverbeke et al. (2001) reported that C16:0 and C18:0 increased during fruit maturation, suggesting that the *de novo* FA biosynthesis and that they accumulate in the cuticular wax of the apple fruit. In the present study, C16:0 had a higher concentration than C18:0 in both shelf life periods at all treatments. Due to pO_2 in CA being higher than the LOL of the fruit, this treatment is expected to increase fruit ripening compared with other storage treatments. The highest accumulation of fatty esters, such as ethyl palmitate, ethyl oleate, ethyl linolenate, hexyl palmitate, and the largest contribution of other fatty acids in the apple wax from CA treatment in the 7 d analysis support this view. Yang et al. (2017b) observed that esters and fatty acids increased continuously during the development of 'Cripps Pink' apple cuticle layer during storage (70 d at 20 °C), confirming that the accumulation of wax constituents classified as fluids may be the main contributors to disagreeable greasy sensation on fruit skin.

Skin greasiness was detected in apples stored in CA and DCA-CF, but not in that DCA-RQ treatments (Table 3). The occurrence of greasiness was highest in fruit stored in CA and DCA-CF at 14 d reaching 91 and 35 %, respectively. Curry (2008), Fan et al. (1999) and Veraverbeke et al. (2001) suggest that the development of greasiness in stored apples is related to changes in the wax lipid production. Christeller and Roughan (2016) associated the induction

of greasiness with the accumulations of novel long-chain unsaturated fatty acid esters of farnesyl. Yang et al. (2017b) classified wax fractions of 'Cripps Pink' apple according to the individual physical state of each compound at 20 °C in solids (mainly alkanes and fatty alcohols) and fluids (esters, unsaturated fatty acids, and sesquiterpenoids). The wax composition of CA-stored apples in our study were consistent with previous reports (Yang et al., 2017b, 2017a), since it was possible to identify higher concentrations of fluid fractions, including unsaturated fatty acids and fatty acid esters. Additionally, it was highlighted the higher concentration of ursolic acid and oleanolic acid in apples stored in CA at 7 d. At 14 d, C16:0 had a greater accumulation in CA (Table 3). The accumulation of these compounds seems to contribute to the skin greasiness of the apple. Christeller and Roughan (2016) and Ju and Bramlage (2001) considered α-farnesene to be associated with the greasiness. However, this compound was not well correlated with greasiness in the present study, although the product of oxidation farnesyl alcohol had better correlation (Table 3 and Fig 4).

In contrast to fluid fractions, alkanes and fatty alcohols are crystalline compounds (Ensikat et al., 2006) and determine the crystalline structure of epicuticular wax (Koch and Ensikat, 2008; Liu et al., 2012). Nonacosane ($C_{29}H_{60}$) is an important alkane found in apple wax and the most abundant component in all samples. Alkanes, mainly nonacosane and fatty alcohols, such as 10-nonacosanol, are important representatives of cuticular wax in many apple cultivars (Belding et al., 1998; Verardo et al., 2003). 10-Nonacosanol accumulated more under both DCA-RQ treatments (Table 3), which may contribute to maintaining the crystalline structure of the wax and avoiding the feeling of greasiness.

In addition to lipids derived from fatty acids pathways, cuticles are also composed of lipids derived from isoprene, mostly triterpenoids. Triterpenoid biosynthesis involves the union of six isoprene units to produce squalene as a precursor (Kuzuyama, 2002). Ursolic acid (3β-hydroxy-ursan-12-en-28-oic acid, UA) and oleanolic acid (3β-hydroxy-olean-12-en-28-oic acid, OA) as predominant triterpenic acids, were found in apple cuticular wax. Moreover, CA storage resulted in the highest amounts of these triterpenes in wax composition, differing from all the other treatments after 7 d of shelf life (Table 3).

During cold storage, apples may develop various fungal diseases, including blue mold (*Penicillium expansum*), gray mold (*Botrytis cinerea*), bitter decay (*Colletotrichum* spp.), and bull's eye decay (*Cryptosporiopsis perennans*) (Børve et al., 2013; Grantina-Ievina, 2015; Nunes et al., 2001). Pathogenic infections stimulate respiration rates and, ethylene production of fruit (Rojas et al., 2014), and the activation of defense mechanisms that act in response to the accumulation of reactive oxygen species (ROS) by pathogen attack or injury (Janisiewicz

et al., 2016). The synthesis of secondary metabolites is increased, which reflects the host cells trying to alleviate the initial effects of the infection, developing mechanisms to regulate high ROS concentrations (Žebeljan et al., 2019). The high concentrations of UA and OA in CA-stored apples that coincide with the high decay incidence (Fig. 2) may be correlated to the intensification of these defense mechanisms mentioned above, since the presence of pentacyclic triterpenoids has been reported as biologically active compounds, with antioxidant activities and antimicrobial properties (Szakiel et al., 2012). Chu et al. (2018) found ROS accumulation and reduced defense capability of the antioxidant system after removing the wax from blueberry fruit during cold storage.

Table 3. The composition of peel wax (g kg^{-1}) and skin greasiness of 'Cripps Pink' apples stored under controlled atmosphere (CA), dynamic controlled atmosphere based on chlorophyll fluorescence (DCA–CF) and dynamic controlled atmosphere by respiratory quotient (DCA–RQ) for 8 months plus 7 and 14 d at 20 °C.

Compounds	7 d			
	CA	DCA-CF	DCA-RQ1.3	DCA-RQ1.5
C14:0 (Myristic acid)	0.37 ±0.14	0.35 ±0.06	0.42 ±0.12	0.32 ±0.10
C16:0 (Palmitic acid)	17.02ab ±4.31	19.73a ±0.32	15.77ab ±1.67	12.75b ±0.50
C17:0 (Heptadecanoic acid)	0.33 ±0.02	0.27 ±0.04	0.28 ±0.01	0.26 ±0.05
C18:0 (Stearic acid)	10.76a ±1.38	9.13ab ±1.48	10.31ab ±1.07	7.18b ±1.45
C18:1n9 (Oleic acid)	15.37 ±2.20	17.06 ±1.19	14.62 ±0.89	15.29 ±1.95
C18:2n6 (Linoleic acid)	19.52 ±4.36	20.17 ±0.78	17.14 ±0.52	19.96 ±3.07
C18:3n3 (α-Linolenic acid)	0.77 ±0.11	0.77 ±0.11	0.81 ±0.10	0.82 ±0.07
C20:0 (Arachidic)	2.80 ±0.16	2.85 ±0.45	2.90 ±0.52	2.88 ±0.72
C20:2 (cis-11,14-Eicosadienoic acid)	6.67 ±0.51	8.42 ±2.64	6.94 ±1.08	7.22 ±1.48
C20:3n6 (cis-8,11,14-Eicosatrienoic acid)	0.19a ±0.03	0.14a ±0.03	nd	nd
C21:0 (Henicosanoic acid)	0.28a ±0.02	0.27a ±0.06	nd	nd
C22:0 (Behenic acid)	2.83 ±0.27	3.51 ±0.77	3.32 ±0.38	3.43 ±0.94
C22:1n9 (Erucic acid)	1.53 ±0.83	1.52 ±0.46	1.80 ±0.45	1.47 ±0.08
C24:0 (Lignoceric acid)	1.20 ±0.17	2.20 ±0.48	3.20 ±0.14	4.20 ±0.34
Σ Satured Fatty acids	35.60 ±6.37	37.59 ±2.08	34.24 ±3.62	28.38 ±3.77
Σ Unsatured Fatty acids	44.06 ±7.26	48.07 ±1.54	41.32 ±2.05	44.76 ±4.24

α-farnesene	2.92b	±1.18	4.82a	±0.36	0.62c	±0.30	3.01ab	±0.83
Ethyl palmitate	1.41a	±0.02	0.66b	±0.11	0.67b	±0.14	0.71b	±0.20
Ethyl linoleate	2.03	±0.80	1.41	±0.29	1.32	±0.58	2.37	±0.18
Ethyl Oleate	1.63a	±0.08	nd		nd		0.32b	±0.10
Hexyl palmitate	0.63a	±0.25	nd		0.31b	±0.07	0.31b	±0.15
Ethyl linolenate	2.72a	±1.81	1.81b	±0.09	1.88b	±0.21	1.89b	±0.67
Hexadecane	1.47a	±0.08	nd		0.42c	±0.09	0.26cd	±0.12
Hexacosane	5.80a	±1.33	0.94b	±0.06	1.67b	±0.30	1.41b	±0.48
Heptadecane	1.05a	±0.09	0.40b	±0.06	0.96a	±0.19	1.05a	±0.35
Octacosane	6.87	±0.48	8.01	±2.39	5.63	±0.46	7.76	±1.89
Nonacosane	54.24	±7.60	52.14	±2.37	62.86	±6.23	62.85	±8.34
2-methyloctacosane	0.67a	±0.14	nd		0.29ab	±0.01	0.63a	±0.37
2,6-Di-tert-butylphenol	1.35a	±0.29	0.94a	±0.20	0.25b	±0.01	0.42b	±0.04
Farnesyl alcohol	2.89a	±0.95	1.78ab	±0.35	1.34b	±0.14	1.38b	±0.21
Octacosanol	6.53	±5.36	nd		0.71	±0.01	1.46	±0.30
10-nonacosanol	10.66b	±1.55	9.11b	±0.48	17.86a	±3.34	19.18a	±2.61
(Z)-9-Octadecenamide	3.65a	±0.60	nd		0.30c	±0.07	0.52c	±0.26
Octadecanal	1.62a	±0.36	1.58ab	±0.41	0.63b	±0.08	1.46a	±0.09
Henicosanal	1.12	±0.02	1.50	±0.33	1.12	±0.04	1.36	±0.30
Octacosanal	3.58b	±0.14	3.89ab	±0.21	3.86ab	±0.63	4.91a	±0.81
Tetracosanal	10.88	±2.02	8.75	±0.99	11.51	±3.32	13.51	±1.15
Oleanolic acid	16.23a	±1.27	2.34b	±0.14	2.49b	±0.13	2.65b	±0.50
Ursolic acid	59.91a	±7.80	10.06b	±1.56	9.07b	±0.43	8.82b	±1.89
Greasiness**	51a	±4	9b	±5	0		0	
				14 d				
C13:0 (Tridecanoic acid)	0.32	±0.04	0.32	±0.07	0.42	±0.15	nd	
C14:0 (Myristic acid)	0.25ab	±0.04	0.18b	±0.03	0.24a	±0	nd	
C16:0 (Palmitic acid)	30.99a	±1.59	11.22b	±0.98	12.86b	±1.86	8.98b	±2.26
C16:1 (Palmitoleic acid)	nd		0.13	±0.01	nd		nd	
C17:0 (Heptadecanoic acid)	nd		nd		0.23a	±0.02	nd	
C18:0 (Stearic acid)	11.93a	±0.91	4.71b	±0.37	5.71b	±1.46	3.71b	±0.78
C18:1n9 (Oleic acid)	24.78a	±1.87	14.19bc	±1.65	16.60b	±0.08	10.55c	±1.28
C18:2n6 (Linoleic acid)	15.48b	±1.28	19.23ab	±2.9	22.01a	±0.17	15.49b	±2.93
C20:0 (Arachidic acid)	2.50a	±0.39	1.90ab	±0.14	2.29a	±0.08	1.38b	±0.20
C20:2 (cis-11,14-Eicosadienoic acid)	55.48	±1.91	61.70	±2.32	57.51	±5.25	57.61	±1.57
C20:1n9 (cis-11-Eicosenoic acid)	2.90a	±0.39	0.56b	±0.07	0.60b	±0.09	0.47b	±0.05

C21:0 (Henicosanoic acid)	nd		nd		0.59a	±0.04	0.37b	±0.10
C22:0 (Behenic acid)	3.19a	±0.17	2.47ab	±0.02	2.78ab	±0.36	2.16b	±0.18
C22:1n9 (Erucic acid)	2.13a	±0.10	0.28b	±0.04	nd		nd	
C22:2 (cis-13,16-Docosadienoic acid)	2.09a	±0.01	0.40b	±0.09	0.43b	±0.06	0.30b	±0.02
C22:6n3 (cis-4,7,10,13,16,19-Docosahexaenoic acid)	nd		2.89	±0.42	nd		nd	
C24:0 (Lignoceric acid)	1.35a	±0.10	0.97ab	±0.18	0.81b	±0.18	0.71b	±0.13
C24:1n9 (Nervonic acid)	6.20	±0.04	7.20	±0.02	8.20	±0.48	9.20	±0.16
Σ Satured Fatty acids	50.53a	±3.24	21.77bc	±0.91	25.93b	±2.92	17.31c	±2.89
Σ Unsatured Fatty acids	104.64a	±4.74	99.25ab	±6.20	99.26ab	±5.20	86.72b	±4.48
Greasiness**	91a ±9		35b ±4		0		0	

*Mean±standard deviation (SD) values followed by the same lower case letter in the same line are not significantly different by Tukey's multiple range test ($P < .05$). **Mean percentage three replicates of 25 fruit.

PCA was used to better visualize the effect of treatment discrimination by the variables, thus the matrix was composed of 12 samples of different treatments of 7 d and 42 variables (including wax compounds, total wax, ethylene production, respiration rate, decay incidence, ethanol concentration, and skin greasiness) (Fig. 4). The first two PCs together represented 53.8 % of the overall data variance. PC1 provided the discrimination of CA treatments of DCA (Fig. 4A). The wax of the samples stored in CA was positively correlated with several variables, with emphasis on UA and OA, ethyl oleate, farnesyl alcohol, hexacosane, and more skin greasiness (Fig. 4B). On the other hand, DCA treatments usually had higher loadings of 10-nonacosanol, C22:0, C24:0, octacosanal, ethylene production, and ethanol concentration. The DCA treatments were adequately discriminated in the PC2, which allocated the DCA-RQ1.5 and DCA-CF on the opposite side of the PC plot with an intermediate behavior in the DCA-RQ1.3 treatment. The discrimination between these two treatments was mainly due to 10-nonacosanol, octacosanal, and ethanol that were in higher concentrations under DCA-RQ1.5. Conversely, the main variables that discriminated the condition of DCA-CF were ethylene production, and C18:1n9 fatty acid concentrations.

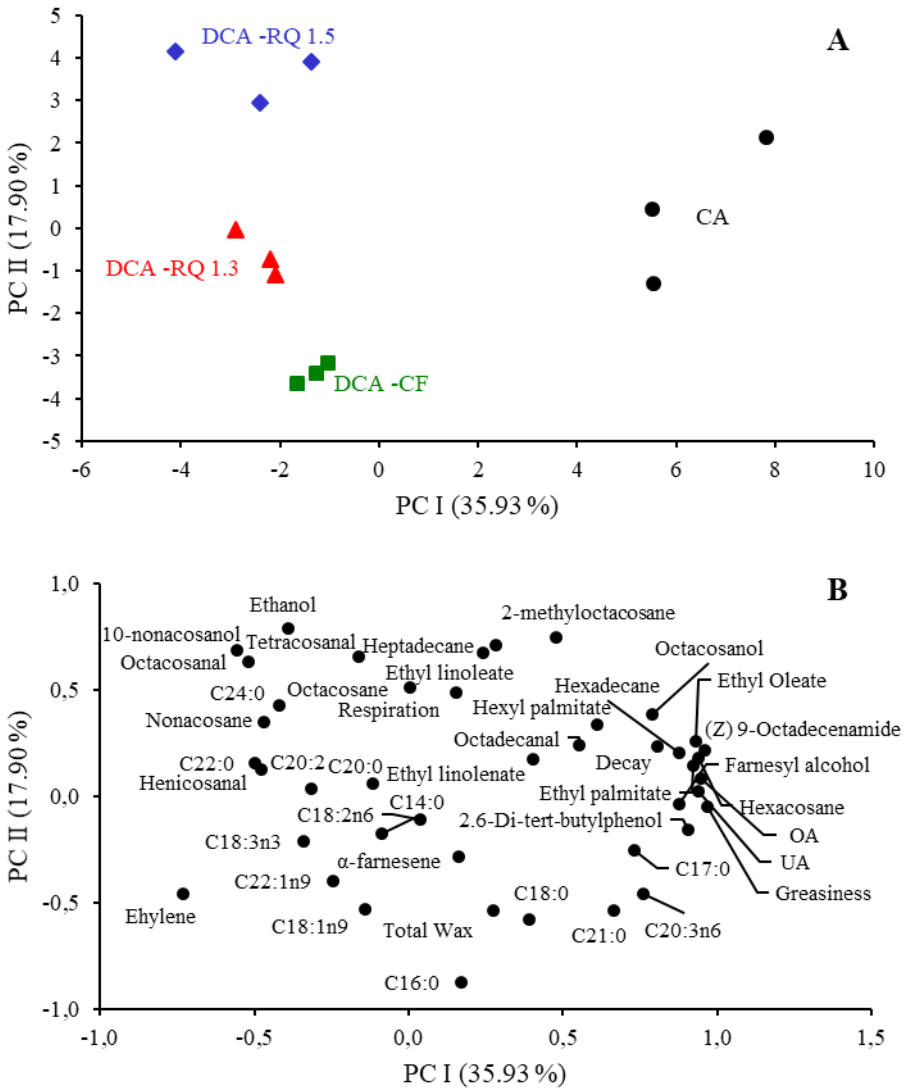

Figure 4. Scores (treatments) (A) and loadings (variables) (B) plots of the first two principal components of the identified wax compounds, total wax, ethylene production, respiration rate, decay incidence, greasiness and, ethanol concentration from apples 'Cripps Pink' under controlled atmosphere (CA), dynamic controlled atmosphere based on chlorophyll fluorescence (DCA–CF) and dynamic controlled atmosphere by respiratory quotient (DCA–RQ) for 8 months plus 7 and 14 d at 20 °C.

In plants exposed to hypoxia, changes occur in the cuticle that may result in lower wax concentrations and negative regulation of cuticle-associated genes (Kim et al., 2017). This induced change in cuticle ultrastructure promotes increased permeability of O_2 and allows the plant to adapt to O_2 deficiency (Kim et al., 2017). The results show higher concentrations in most of the compounds identified for CA-stored apples, supported by maturation and metabolic synthesis processes being more active due to higher O_2 concentrations in storage (Fig 4). However, there are also some compounds with higher concentration under DCA conditions (the lowest concentrations of O_2, i.e. those employing RQ), such as 10-nonacosanol, nonacosane,

heptadecane, and tetracosanal (Fig 4). These compounds are associated with a crystalline structure of cuticular wax, which is different from those with more fluid-like characteristics capable of sealing stomates and lenticels, making O_2 permeability difficult. Alkane synthesis, was unaffected when Arabidopsis plants were subjected to hypoxia-stress (Kim et al., 2017). However, the components of the fatty acid elongase complex involved in very long chain fatty acid synthesis were visibly unregulated under hypoxic conditions, which is possibly the mechanism capable of promoting adaptation under low pO_2 conditions.

3.1.4. Conclusions

This is the first study to evaluate the effects of long-term storage under DCA methods plus 14 d of shelf life at 20 °C on the chemical composition of apple peel wax and its relationship with the greasiness and decay incidence. Fatty acids, triterpenoids, and n-alkanes were the predominated cuticular wax compounds of 'Cripps Pink' apples. Total wax increased over the shelf life period when apples were stored in DCA, although fruit stored under DCA-RQ did not develop greasiness even after 8 months of storage plus 14 d at 20 °C. In addition, there was increased unsaturated fatty acids accumulation with longer shelf life periods, which is mainly influenced by the fatty acid C20:2. Fatty esters, ursolic acid, oleanolic acid triterpenoids and palmitic acid were the main wax chemical compounds of CA-stored apples and may be correlated with higher greasiness. The DCA-RQ1.3 showed the lowest respiration rate, evidencing a lower metabolism and corroborating with the highest number of healthy fruit and consequently being a better storage condition. Storage conditions that employ lower pO_2 DCA-RQ may have favored mechanisms of adaptation against low pO_2, inducing the formation of 10-nonacosanol and octacosanol, which confers to the cuticle a crystalline structure to the cuticle.

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3.1.5. References

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4. ARTIGO 2

4.1. THE ISOLATED OR COMBINED EFFECTS OF DYNAMIC CONTROLLED ATMOSPHERE (DCA) AND 1-MCP ON THE CHEMICAL COMPOSITION OF CUTICULAR WAX AND METABOLISM OF 'MAXI GALA' APPLES AFTER LONG-TERM STORAGE[2]

Abstract

Apples have a continuous hydrophobic layer that covers the surface of the fruit, which is called the cuticle. The effects of 1-methylcyclopropene (1-MCP) on the cuticular wax layer of apples were reported after cold storage, although the interaction between 1-MCP and dynamic controlled atmosphere (DCA) is not yet known. Therefore, this study aimed to analyze the effects of 1-MCP on the wax composition and metabolism of 'Maxi Gala' apples after storage in a controlled atmosphere (CA) and dynamic controlled atmosphere based on chlorophyll fluorescence (DCA-CF) and respiratory quotient (DCA-RQ; RQ = 1.3 and 1.5). The 1-MCP treatment effectively decreased ethylene production for CA and DCA-CF treatments, while in DCA-RQ treatments produced no effect. The average extracted cuticular wax content of 'Maxi Gala' apples was 16.65 g m^{-2} and no differences in storage conditions or 1-MCP application were observed. Alkanes, alcohols, fatty acids, aldehydes, and terpenoids were identified in the chemical composition of the cuticular waxes, being alkanes and fatty acids the predominant ones. Moreover, 1-MCP decreased fatty acid and 10-nonacosanol concentrations in the fruit. Fruit with the 1- MCP application and stored in DCA had lower α-farnesene concentrations. The wax compositions of the DCA stored apples with and without 1-MCP were similar. However, 1-MCP treatment resulted in a greater mass loss in fruit stored in DCA.

Keywords: 1-Methylcyclopropene; Cuticle; Ethylene; *Malus domestica* Borkh.

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4.1.1. Introduction

Apples are one of the most important fruit in the world and seasonally harvested, therefore, these fruit must be stored effectively to be available throughout the year (Brackmann, Weber, Pinto, Neuwald, & Steffens, 2008). Today, the main method to maintain apple quality during long-term storage is the controlled atmosphere (CA), which employs low temperatures, high relative humidity, high CO_2 partial pressure (pCO_2), and low O_2 partial pressure (pO_2) (Brackmann, Streif, & Bangerth, 1993; Brackmann, Giehl, Sestari, & Steffens, 2005). This technique reduces ethylene biosynthesis and respiration rates, consequently slowing the main biochemical processes during storage (Wright, Delong, Arul, & Prange, 2015). However, the low pO_2 (1.0-1.2 kPa) employed in CA is above the lower oxygen limit (LOL) tolerated by the apples (Bessemans, Verboven, Verlinden, & Nicolaï, 2016; Weber et al., 2015), which is the minimum pO_2 tolerated by the fruit without being damaged by anaerobic metabolism. As a result, apples began to be stored in a dynamic controlled atmosphere (DCA), which was developed to monitor and maintain pO_2 as close as possible to LOL during storage.

Three techniques can be used to monitor the LOL during DCA storage (Prange, 2018): one is based on the production of ethanol by the fruit (Veltman et al., 2003), one is based on chlorophyll fluorescence (DCA-CF) (Prange et al., 2007; Wright et al., 2012), and the other uses respiratory quotient (DCA-RQ) (Brackmann, 2015; Weber et al., 2015; Weber et al., 2017). By employing these techniques, it is possible to detect the LOL tolerated by the fruit after starting the anaerobic metabolism and adjust the pO_2 during storage to avoid excessive anaerobic metabolism (Thewes et al., 2018).

Combined with storage technologies, especially CA, 1-methylcyclopropene (1-MCP) application is widely used as an alternative for fruit quality maintenance. Its application reduces ethylene action/production and respiration rates, preventing loss of flesh firmness and reduced volatile compound production, especially after long storage (DeEll et al., 2007; Lafer, 2008; Lu et al., 2018; Thewes, Brackmann, Anese, et al., 2017; Watkins & Nock, 2012).

Apples have a continuous hydrophobic layer called the cuticle, which covers the surface of the fruit. This layer consists of both cuticular waxes and cutin (Lara et al., 2014). Currently, fruit cuticular wax synthesis is derived from saturated fatty acids with 16 or 18 carbons (C16 or C18). These fatty acids are catalyzed into very long-chain fatty acids (VLCFA, with C20 – C34 chains) (Lara et al., 2015). The VLCFA are then elongated via two biosynthetic pathways to various aliphatic wax components, such as primary and secondary alcohols, wax esters, alkanes, aldehydes, ketones, and triterpenes (Kunst & Samuels, 2003; Kunst & Samuels, 2009).

This cuticular wax composition is associated with important characteristics related to postharvest quality, including water loss and susceptibility to physical and biological stress (Lara et al., 2014). Studies on apples have shown that the composition of cuticular wax changes continuously during development and postharvest (Belding et al., 1998; Dong et al., 2012; Ju & Bramlage, 2001). Klein et al. (2020) reported changes in the wax content and composition of 'Cripps Pink' apples during shelf life after CA and DCA storage and described that DCA treatments increased the total wax content. In this work, the authors also found decreased greasiness for DCA-RQ treatments compared to CA, which was attributed to wax composition. Greasiness is a negative sensory quality attribute for consumers and causes some apple cultivars to become unpleasantly greasy on the surface during storage (Curry, 2008; Yang et al., 2017). Most of these changes in wax constituents are regulated by ethylene (Li et al., 2017; Yang et al., 2017). Therefore, storage conditions that reduce ethylene production, such as DCA and mainly DCA-RQ (Donadel et al., 2019; Schmidt et al., 2020), and also its association with ethylene action inhibitors, such as 1-MCP, may affect the chemical composition of this cuticular layer of stored apples.

Li et al. (2019) identified four key genes for the synthesis of apple wax (*MdCER4*, *MdCER6*, *MdWSD1*, and *MdMAH1*) and proved their regulation by ethylene. The authors also described that 1-MCP reduced wax density during cold storage of 'Starkrimson' apples (Li et al., 2019). Moreover, Yang et al. (2017) reported that the accumulation of liquid constituents of the wax and greasiness of the skin of 'Cripps Pink' apples were suppressed in fruit treated with 1-MCP after 70 d of storage at 20 °C. Likewise, Curry (2008) showed that 1-MCP delayed the development of wax constituents considered responsible for greasiness during the cold storage of 'Royal Gala' apples. Nevertheless, there are no results in the literature evaluating the effects of 1-MCP on the cuticular wax composition of apples stored in DCA after long-term storage.

Therefore, determining the composition of fruit cuticular wax from the interaction between DCA methods and 1-MCP may provide important information on postharvest quality in relation to characteristics not previously explored. In this context, the present work aimed to analyze the effects of 1-MCP on the wax composition and overall quality of 'Maxi Gala' apples during long-term CA and DCA storage.

4.1.2. Materials and methods

4.1.2.1. Plant material, harvest maturity, and sample preparation

'Maxi Gala' apples were harvested in a commercial orchard in Vacaria, (RS, Brazil) in the 2017 season and immediately transported to the Postharvest Research Center in Santa Maria (RS, Brazil). At a commercial harvest, 140 d after full bloom, the fruit showed an iodine-starch index of 7.20 ± 0.65, soluble solids of 12.43 ± 0.32%, titratable acidity of 0.47±0.03 mg malic acid 100 g^{-1}, respiration rate of 0.41 μg CO_2 kg^{-1} s^{-1}, ethylene production of 5.28 ng C_2H_4 kg^{-1} s^{-1}, and flesh firmness of 74.6 ± 1.17 N. Damaged and irregular fruit were discarded. Each treatment was applied to three replicates of 25 fruit in a completely randomized experimental design.

Eight storage treatments were applied: conventional CA (with static 1.2 kPa O_2 and 2.0 kPa CO_2); dynamic controlled atmosphere (DCA) monitored by chlorophyll fluorescence (DCA-CF); DCA monitored by respiratory quotient (DCA-RQ) with two levels (DCA-RQ1.3 and DCA-RQ1.5), with half of the fruit submitted to treatments with 1-MCP and the other half without 1-MCP before storage. The treatments were composed of three replicates of 25 fruit and stored for 9 months. Then, all apples were kept at 5 °C on the first day and the temperature lowered to 2 °C in the course of the following 5 days. Afterwards, the storage conditions were established within individual experimental chambers (233 L), which were all located inside a controlled-temperature storage room. Fruit temperature was monitored daily with mercury thermometers inserted inside the fruit flesh to control pulp temperature. Inside the CA storage chamber, the relative humidity was manually monitored with psychrometers and adjusted with calcium chloride (0.15 kg per treatment), which absorbed the excess humidity and maintained it at an average of 94 ± 2%.

4.1.2.2. 1-Methylcyclopropene (1-MCP) application

Before storage and after reaching 2 °C, 1-MCP (SmartFresh®, 0.14% of the active ingredient) was applied to half of the fruit. Before treatment, fruit were placed in a 230-L chamber at 2.0 ± 0.1 °C and a solution of 1-MCP was prepared according to the package instructions in order to produce a concentration of 0.625 μL L^{-1} inside the experimental chamber. This solution was placed in Petri dishes inside the chamber. The chamber was hermetically sealed and the air inside circulated with a fan. After 24 h, the fruit were removed from the chamber and stored for 9 months under CA, DCA-CF, DCA-RQ 1.3, and DCA-RQ 1.5.

4.1.2.3. CA and DCA setup and maintenance

The CA storage rooms were sealed and the storage conditions were established within 24 h. When the storage temperature reached 2.0 °C, the CA, DCA-CF and DCA-RQ were established by nitrogen flushing to reach 1.2 kPa O_2 for CA, and to 0.5 kPa O_2 for DCA conditions. Five days were required for this oxygen pull-down. The pCO_2 was established by fruit respiration.

During storage, the pO_2 was changed according to the fruit metabolism in DCA and maintained statically in CA. To control pO_2 in DCA-RQ conditions, the respiratory quotient (RQ) was determined twice a week according to the method proposed by Weber et al. (2015). Thus, the RQ was set to 1.3 and 1.5 and the pO_2 changed accordingly to maintain the RQ values (Fig. 1B). The different DCA-RQ levels (1.3 and 1.5) represent the relation of produced CO_2/consumed O_2, indicating the anaerobic metabolism rate (Weber et al., 2015). The RQ was calculated by considering an interval of 13 h of chamber closure.

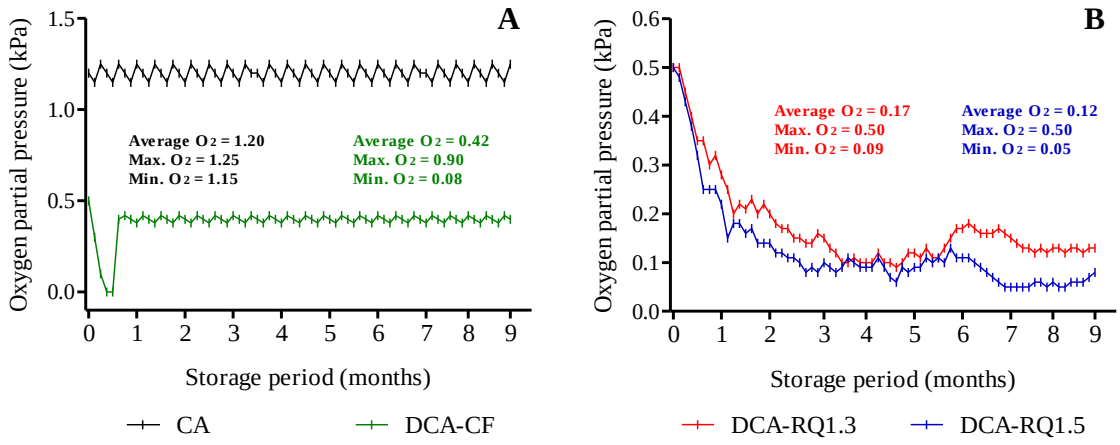

Figure 1. Oxygen variation under controlled atmosphere (CA) and dynamic controlled atmosphere with chlorophyll fluorescence (DCA-CF) (A), dynamic controlled atmosphere with quotient respiratory 1.3 (DCA-RQ1.3) and 1.5 (DCA-RQ1.5) (B) during 9 months of storage.

Furthermore, DCA-CF was monitored according to Prange et al. (2007). Chlorophyll fluorescence was monitored in six apples during exposure to low pO_2. Thus, apples cooled to 2 °C were placed in a perforated plastic container (18 cm width, 27 cm length, 25 cm height) with the fluorescence sensors installed on the top. The container was placed inside an experimental DCA-CF chamber, sealed, and covered with black plastic to protect from the light. The

fluorescence monitoring system was activated and the pO_2 reduced. Then, the respiration process reduced the pO_2 until a change in fluorescence was detected. The lowest O_2 set point was determined by identifying the pO_2 where an inflection in the fluorescence signal was detected, and then by increasing pO_2 by 0.4 kPa as a safety factor (Fig. 1A). Chlorophyll fluorescence was monitored every hour for the entire storage period.

4.1.2.4. Wax compound extraction, identification, and quantification

Sample preparation and wax compound extraction were carried out according to Klein et al. (2019). The wax was extracted from 10 cm^2 of the epidermis homogenized in 5 mL of distilled water, followed by the addition of 2 mL of extraction solvent (chloroform) plus 4 mL of dispersion solvent (acetone). The sample was then shaken for 30 min. The partition procedure was repeated twice with 1 mL of chloroform. The sample was then centrifuged for phase separation and the organic fraction reserved and transferred to a 10-mL volumetric flask, which was added with a 250-µL of a methyl tricosanoate solution (C23:0; 4.006 g L^{-1}; Sigma-Aldrich, St. Louis, USA). The extraction yield was gravimetrically obtained from the extracts (5 mL). Results were expressed in g m^{-2} of wax.

Wax compounds were determined from three fractions: fatty acids profile (F1), compounds with high boiling point (F2), and other compounds from the direct analysis of the concentrated extract (F3). The F1 was obtained by transesterification/esterification of 4 mL of the extract after drying under continuous N_2 flow at 40 °C. In this extract, the fatty acid methyl esters (FAMEs) were obtained according to Hartman and Lago (1973). The F2 were determined after derivatization with MTBSTFA (N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide; Sigma-Aldrich) according to Oms-Oliu et al. (2011). Finally, F3 compounds were acquired by drying 500 µL of the extract, reconstituting with 50 µL of chloroform, and injecting directly into the chromatographic system.

The three quantitative determinations were carried out by injecting 1µL of sample in a gas chromatograph equipped with a flame ionization detector (GC-FID; Varian Star, 3400 CX, Walnut Creek, USA) and an autosampler (Varian, model 4200, CA, USA) as described by Klein et al. (2019). The FAMEs solution (F1) were injected in splitless mode (splitter off for 1 min; 20:1) and a temperature of 250 °C. The FAMEs separation were carried out using a polar capillary column CP-Wax 52CB (Chrompack, Middelburg, 50 m × 0.32 mm × 0.20 µm). The initial column temperature was set at 50 °C, remaining for 1 min, increasing to 180 °C at 10 °C min^{-1}. Then, the temperature increase at 2 °C min^{-1} until 200 °C, and then at 10 °C min^{-1} to

230 °C, and maintained in isotherm for 5 min. The detector was maintained at 240 °C. The identification was by comparing their experimental retention times with the ones found in the FAME Mix-37 standard (P/N 47885-U, Sigma-Aldrich, St. Louis, USA).

The fractions F2 and F3 were injected in splitless mode (splitter off for 1 min; 20:1) at 300 °C. Wax compounds were separated using a capillary column of nonpolar phase, BPX-5 (SGE, Australia, 25 m × 0.22 mm; 0.25 μm of thickness film). H_2 was used as carrier gas at a constant pressure of 103 kPa. The column initial temperature was 50 °C, where it remained 1 min, increasing to 200 °C at a rate of 10 °C min^{-1} and again increasing to 320 °C at a rate of 3 °C min^{-1}, remaining isothermic for 10 min. The detector was maintained at 280 °C. The analytes with no available standard (F2 and F3) were identified by gas chromatography coupled to a mass spectrometer (GC/MS, Shimadzu QP2010 Plus, Kyoto, Japan) based on the comparison of the obtained mass spectra obtained with those available from the National Institute of Standards and Technology (NIST) and the literature. For these analyses, the same chromatographic conditions described above were used and He was used as the carrier gas. The MS operated in the electron impact ionization mode with ionization energy of 70 eV and single quadrupole type analyzer operating in scan mode in a mass range of 35−450 m/z.

The concentration of the compounds for all fractions were calculated by internal standardization (C23:0) and the results were expressed as g of compound per kg wax (Klein et al., 2019).

4.1.2.5. Metabolism and overall quality analysis

4.1.2.5.1. Ethylene production and respiration rate

A batch of approximately 1.5 kg fruit was put into a 5-L container and hermetically closed for about 2 h. Thereafter, two samples of 1 mL were taken out of the container and injected into a Varian gas chromatograph model Star CX 3400 (Varian, Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and Porapak N80/100 column to determine the ethylene concentration in the container headspace. Injector, column, and detector temperatures were 140, 90, and 200 °C, respectively. Results were expressed in ng kg^{-1} s^{-1}. The same air of the container for ethylene measurement was circulated throughout an electronic gas analyzer (Isolcell®, model Oxycarb) to determine fruit respiration by its CO_2 production. Respiration rates were expressed in μg kg^{-1} s^{-1}. These analyses were performed according to Thewes et al. (2015).

4.1.2.5.2. Soluble solids and titratable acidity

Slices of the equatorial region of the fruit were used to produce juice using a juicer (Philips Walita®). Drops of the juice were used to measure the soluble solids by refractometry (Biobrix, Model 103, Curitiba, Brazil), and the results expressed as a percentage. Then, 10 mL from the same juice were taken and diluted in 100 mL of distillated water. This solution was titrated with a 0.1 N NaOH solution until pH 8.1. The results were expressed as mEq 100 mL^{-1}.

4.1.2.5.3. Juiciness

Using 10 fruit of each replicate, 20 g of the apple pulp was used and pressed for one minute at a pressure of 150 kPa. Juiciness was calculated by weighing the fruit pulp before and after pressing according to the methodology proposed by Lunardi et al. (2004). The results were expressed as a percentage of the initial fruit pulp mass.

4.1.2.5.4. Mass loss

Mass loss was obtained by weighting the fruit before and after the storage and expressed as a percentage of the initial fruit mass (Brackmann et al., 2014).

4.1.2.5.5. Pulp cracking

Pulp cracking was determined by counting the fruit that showed any sings of cracking on the pulp compared to the total number of fruit per replicate. Results were presented as a percentage of the total fruit.

4.1.2.6. Statistical analyses

The experiment was conducted in a completely randomized design with four storage conditions with or without 1-MCP application. Analysis of variance (ANOVA) at 5% of error probability was carried out ($p < 0.05$) and means that showed significant differences were subjected to Tukey's test at 5% error probability. Additionally, data were submitted to

multivariate principal component analysis (PCA) using Pirouette 3.11 software (Woodinville, USA, 2003) to show trends in the results. Before PCA, the data matrix was autoscaled in order for each variable to have the same weight during analysis.

4.1.3. Results and Discussion

4.1.3.1. Apple cuticular wax content

The total apple peel wax of 'Maxi Gala' apple was not affected by storage treatments. The mean amount of cuticular wax isolated from 'Maxi Gala' apple was 16.65 g m^{-2} (Supplementary Material 1). Ju and Bramlage (2001) reported values of 3.1 and 14.1 g m^{-2} of total wax at the start and end of development, respectively, for 'Delicious' apples. Leide et al. (2018) described cuticular wax content of 1.13 and 0.77 g m^{-2}, respectively, for 'Florina' and 'Prima' apples shortly after harvesting. On other hand, 'Royal Gala', 'Granny Smith', 'Pink Lady', and 'Red Delicious' apples purchased locally presented cuticular wax content of 16.12, 15.65, 21.85, and 17.95 g m^{-2}, respectively (Klein et al., 2019). Klein et al. (2020) reported mean total wax content for 'Cripps Pink' apples after 8 months of storage in CA, DCA-CF, and DCA-RQ1.3 and RQ1.5 of 21.23 g m^{-2} at 7 d and 26.18 g m^{-2} at 14 d of shelf life at 20 °C.

Studies have shown that ethylene increases the accumulation of cuticular wax of fruit during cold storage, while 1-MCP inhibits this phenomenon (Cajuste et al., 2010; Dong et al., 2012; Ju and Bramlage, 2001; Li et al., 2019). This is because of the regulation of four key genes of the synthesis of apple wax (*MdCER4*, *MdCER6*, *MdWSD1*, and *MdMAH1)*, in which ethylene acts directly by increasing the expression levels of *MdCER6* (Li et al., 2019). Following the same authors, *MdCER6* is a key gene to synthesize very long-chain fatty acids (VLCFA), in addition to regulating the pathway of alcohol formation, stimulating *MdCER4* and *MdWSD1*. However, this suppression in the cuticular wax content of 1-MCP-treated apples was not observed here. This may be due to the modulation of the climacteric fruit sensitivity to 1-MCP as a function of the internal ethylene concentration (IEC) (Zhang et al., 2011). It is plausible that endogenous ethylene competes with 1-MCP to bind to receptors and, therefore, despite exogenous ethylene production being suppressed by 1-MCP, the IEC may still be enough for the expression of genes related to wax synthesis. Both et al. (2018) showed that, even with low ethylene production of 1-MCP-treated 'Galaxy' apples, the fruit still had IEC greater than CA after 9 months of storage in DCA-RQ.

4.1.3.2. Apple cuticular wax composition

Thirty-one compounds including alkanes, alcohols, fatty acids, aldehydes, and terpenoids were identified and quantified in the cuticular waxes of 'Maxi Gala' apples treated or not with 1-MCP after storage (Tables 1 and 2). Alkanes and fatty acids were the predominant constituents of cuticular waxes.

Alkanes constituted an important fraction of the total amount of wax in all treatments, which was mainly influenced by nonacosane. The concentration of this compound did not differ between atmosphere conditions when fruit were treated with 1-MCP. However, it was lower in DCA-RQ1.3 when compared to DCA-CF in fruit without 1-MCP treatment. Similar behavior was found for tetracosane (Table 2). As predominant components of fruit cuticular wax, alkanes play important roles in determining cuticle properties. In *Arabidopsis* leaves, alkanes are associated with better skin barrier properties and are produced in response to water deficits (Bourdenx et al., 2011; Kosma et al., 2009), promoting greater resistance to water movement in the membranes than other cuticular wax components (Grncarevic and Radler, 1967). This indicates that alkanes may be the most effective water-retaining components of wax and therefore essential for preventing weight loss during apple storage, as reported by Chai et al. (2020). The authors showed that the lowest alkane content in the related 'Red Star' apples was related to the highest rate of weight loss during storage at 20 °C for 49 d. Baritelle et al. (2001) related greater weight loss during storage for 'Golden Delicious', 'Red Delicious', 'Fuji', and 'Roma' untreated apples than those treated with 1-MCP. This has always been related to the fact that 1-MCP slows the respiratory rate and also possibly increases alkane content, as observed in this study after CA storage.

Fatty acids were the major constituents in fruit stored in CA, mainly CA without 1-MCP (Table 1). This fraction was lower in DCA treatments, regardless of 1-MCP application. In all storage conditions with 1-MCP, eight fatty acids were not detected, which were currently constituents of the cuticular wax composition in apples without 1-MCP. For treatments without 1-MCP, lauric, myristic, palmitic, palmitoleic, stearic, oleic, and linoleic acids had higher concentrations in the fruit stored in CA. In addition, 1-MCP had lower concentrations of palmitic acid, stearic acid, oleic acid, linoleic acid, α-linolenic acid, and cis-11-eicosenoic acid for the same condition (Table 1). (Li et al., 2019) reported that 1-MCP decreased the expression level of key genes in the synthesis of VLCFAs and consequently all other wax constituents. Therefore, these lower fatty acids concentrations in fruit treated with 1-MCP found in this study (Table 1), corroborate the previous findings (Curry, 2008; Dong et al., 2012; Li et al., 2017).

Fatty acid elongation uses C16 and C18 saturated fatty acids and the unsaturated fatty acids C18:1 and C18:2 that are esterified to Co-enzyme A (CoA) before entering the endoplasmic reticulum-bound multi-enzymatic fatty acid elongase (FAE) complexes (Bernard and Joubès, 2013). Low pO_2 during storage interferes in the cellular homeostasis of the fruit due to decreased cellular energy, consequently weakening the metabolic processes that consume ATP, including fatty acid synthesis (Geigenberger, 2003). This may explain the lower concentration of palmitic acid, stearic acid, oleic acid, and linoleic acid in DCA-stored apples, where pO_2 during storage was lower than CA (Figure 1).

Table 1. The fatty acids (F1) of peel wax (g kg^{-1}) of 'Maxi Gala' apples, without and with 1-methylcyclopropene (1-MCP) treatment, stored under controlled atmosphere (CA), dynamic controlled atmosphere based on chlorophyll fluorescence (DCA–CF) and dynamic controlled atmosphere by respiratory quotient (DCA–RQ) for 9 months plus 7 d of shelf life.

Compounds	Without 1-MCP				With 1-MCP			
	CA	DCA-CF	DCA-RQ1.3	DCA-RQ1.5	CA	DCA-CF	DCA-RQ1.3	DCA-RQ1.5
C8:0 (Caprylic acid)	nd	nd	1.70±0.20 (a)	1.70±0.31 (a,B)	7.43±0.68 (a)	2.43±0.44 (b)	3.92±1.38 (b)	3.13±0.50 (b,A)
C10:0 (Capric acid)	0.71±0.07	nd	nd	nd	nd	nd	nd	nd
C11:0 (Undecanoic acid	nd	nd	0.27±0.04 (a)	0.32±0.03 (a)	nd	nd	nd	nd
C12:0 (Lauric acid)	1.11±0.20 (a)	0.35±0.10 (b)	0.43±0.05 (b)	0.49±0.07 (b)	nd	nd	nd	nd
C14:0 (Myristic acid)	4.74±0.09 (a)	0.36±0.10 (b)	0.55±0.14 (b)	0.32±0.07 (b)	nd	nd	nd	nd
C15:0 (Pentadecanoic acid)	nd	nd	0.36±0.03 (a)	0.36±0.08 (a)	nd	nd	nd	nd
C15:1 (cis-10-Pentadecenoic acid)	nd	nd	0.52±0.04 (a)	0.58±0.07 (a)	nd	nd	nd	nd
C16:0 (Palmitic acid)	87.84±10.4 (a,A)	18.65±4.39 (b)	17.03±4.38 (b)	21.09±4.29 (b)	26.75±3.45 (a,B)	16.69±3.59 (b)	19.79±4.04 (ab)	16.41±0.93 (b)
C16:1 (Palmitoleic acid)	5.37±1.63 (a)	nd	0.27±0.04 (b)	nd	nd	nd	nd	nd
C18:0 (Stearic acid)	42.64±9.17 (a,A)	10.99±2.12 (b)	10.91±3.20 (b)	12.72±4.24 (b)	13.10±0.78 (B)	9.15±2.83	10.14±1.28	8.67±1.42
C18:1n9 (Oleic acid)	126.9±26.66 (a,A)	9.53±1.70 (b)	11.65±2.32 (b)	11.01±1.36 (b)	14.9±0.92 (a,B)	9.57±1.29 (b)	12.25±2.02 (ab)	10.24±0.81 (b)
C18:2n6 (Linoleic acid)	47.28±1.38 (a,A)	7.79±1.21 (b)	8.41±1.95 (b)	8.40±0.86 (b)	11.28±1.39 (a,B)	7.98±1.11 (b)	9.03±0.31 (ab)	8.36±0.79 (b)
C18:3n6 (γ-Linolenic acid)	0.25±0.05 (b)	nd	0.62±0.13 (a)	0.84±0.11 (a)	nd	nd	nd	nd
C18:3n3 (α-Linolenic acid)	3.40±0.40 (b,A)	0.83±0.17 (c)	nd	5.61±0.10 (a,A)	0.64±0.23 (B)	0.65±0.06	0.82±0.05	0.74±0.06 (B)
C20:0 (Arachidic acid)	7.05±0.91 (a)	5.22±0.72 (a)	5.67±0.96 (a)	0.81±0.06 (b,B)	5.86±0.35	5.22±0.62	5.76±0.45	5.83±1.10 (A)
C20:1n9 (cis-11-Eicosenoic acid)	7.39±1.06 (b,A)	0.71±0.20 (c)	0.71±0.07 (c)	12.67±0.46 (a,A)	0.72±0.27 (B)	0.70±0.11	0.84±0.05	0.82±0.04 (B)
C20:2 (cis-11,14-Eicosadienoic acid)	11.92±2.14 (a)	11.18±1.69 (a)	10.94±1.29 (a)	6.97±0.23 (b,B)	11.99±1.57	12.31±1.77	11.69±1.47	12.47±0.83 (A)
C22:0 (Behenic acid)	8.11±1.70 (a)	6.03±1.21 (a)	6.88±0.89 (a)	0.69±0.16 (b,B)	7.42±0.64	6.14±0.85	6.67±0.51	7.14±0.95 (A)
C22:2 (cis-13,16-Docosadienoic acid)	127.04±0.96	132.96±7.78	124.59±2.19	133.42±3.85	135.66±14.32	135.50±6.70	132.28±12.89	138.87±8.97
C24:0 (Lignoceric acid)	nd	nd	nd	nd	23.87±0.74 (a)	3.19±0.33 (b)	3.56±0.49 (b)	3.61±0.17 (b)

ND: Not detected. Means followed by equal uppercase letter do not differ between with and without 1-MCP application, in each storage condition. Means followed by equal lowercase letter do not differ between storage conditions in each 1-MCP strategy, by Tukey test at 5 % error probability.

During the ripening process, the composition of the cuticle is altered due to unsaturated fatty acids accumulation (Yang, Zhou, Zhang, et al., 2017), as observed in both CA groups, with and without 1-MCP. A comparison of both groups points to CA without 1-MCP as being the one with the most accumulation of unsaturated fatty acids. In DCA-RQ1.5, α-linolenic acid, γ-linolenic acid, and cis-11-eicosenoic acid had the highest concentrations in the group without 1-MCP (Table 1). Furthermore, 1-MCP application decreased α-linolenic and *cis*-11-eicosenoic acid concentrations in this storage condition.

Cis-11,14-eicosadienoic acid and behenic acid had lower concentrations in DCA-RQ1.5 storage without 1-MCP application. Regarding the other treatments with 1-MCP, DCA-RQ1.5 was greater than the same storage condition without 1-MCP (Table 1). Nevertheless, *cis*-13,16-docosadienoic is the acid that most contributed to the fatty acid fraction of the cuticular wax, as its concentration was not influenced by any of the tested variables, in addition to having a mean wax of 133 g kg^{-1} in all treatments. In 'Cripps Pink' apples stored under the same conditions of this study (after 14 d of shelf life), *cis*-11,14-eicosadienoic was the most abundant acid in this fraction of the wax (Klein et al., 2020). Lignoceric acid was only present in fruit treated with 1-MCP and the highest concentration was in CA-stored fruit.

10-nonacosanol was identified in all treatments, although 1-MCP-treated fruit had lower concentrations than untreated ones (Table 2). No influences between storage conditions was observed in 1-MCP-treated fruit, although higher 10-nonacosanol was observed in fruit without 1-MCP in CA (Table 2). Similar behavior was observed in ethylene production (Table 1), corroborating Li et al. (2017, 2019), who reported that 1-MCP decreased the expression of the *MdCER4* gene, which is the main regulator of the alcohol formation pathway in 'Starkrimson' apple in cold storage.

α-Farnesene oxidation products are involved in superficial scald development in apples (Delong and Prange, 2003). Thus, the pre-storage treatment of susceptible fruit using antioxidants, including DPA (diphenylamine), may delay α-farnesene autoxidation and reduce respiration rates and ethylene evolution (Whitaker et al., 2000). Fruit without 1-MCP application and stored in DCA had higher α-farnesene concentrations compared to those with the 1-MCP application. Among the storage conditions in fruit without 1-MCP, DCA-CF and DCA-RQ1.3 had the highest concentrations (Table 2). This influence of 1-MCP was also observed by Ding et al. (2020), who showed that 1-MCP effectively inhibited α-farnesene accumulation compared to the control group without 1-MCP during 113 d of storage at 0 °C in 'White Winter Pearmain' apples. On the other hand, Yang et al. (2016) described that 1-MCP to significantly delayed α-farnesene production in 'Golden Delicious' apples during shelf life

at 20 °C. Nevertheless, fruit treated with 1-MCP had α-farnesene production similar to untreated fruit, but this accumulation was only observed at 40 d. Therefore, this lower α-farnesene concentration in 1-MCP-treated fruit and stored in DCA from our study may only be a delay in the production. In the 1-MCP-treated group, the storage conditions that contributed to higher α-farnesene concentrations were CA and DCA-CF, which is due to the higher pO_2 levels (Fig. 1) during storage. Thus, there may be a favorable interaction between 1-MCP and DCA-RQ capable of suppressing α-farnesene production. Notably, DCA-CF was identified as a potential method of controlling superficial scald (DeLong et al., 2007; Zanella et al., 2005), which is mainly associated with low pO_2 during storage. Therefore, the present study has shown that DCA-RQ decreases pO_2 even lower (Fig. 1) and possibly improves superficial scald control.

Regarding the identified aldehydes, the storage condition did not influence the concentration of these compounds, although this was not the case for hexacosanal and heptacosanal, as 1-MCP promoted higher concentrations in fruit stored in CA and DCA-RQ1.3 (Table 2).

Ursolic acid (UA) concentrations did not differ between the storage conditions, despite 1-MCP contributing to higher UA concentrations in CA and DCA-CF (Table 2). Oleanolic acid (OA) was not influenced by any of the tested variables. Fruit without 1-MCP had no changes in squalene concentrations in relation to storage conditions, but for 1-MCP-treated fruit, CA storage promoted higher concentration. Squalene is another terpenoid with important biological functions, including protecting against bacterial and fungal infections (Becker et al., 2019). Chai et al. (2020) analyzed 10 apple cultivars and found no terpenoids before cold storage, although these compounds were present in all cultivars after storage except for ‘Red Star’, which may partially explain why this cultivar had a lower shelf life than the others.

Table 2. Other compounds (F2 and F3) of peel wax (g kg^{-1}) of 'Maxi Gala' apples, without and with 1-methylcyclopropene (1-MCP) treatment, stored under controlled atmosphere (CA), dynamic controlled atmosphere based on chlorophyll fluorescence (DCA–CF) and dynamic controlled atmosphere by respiratory quotient (DCA–RQ) for 9 months plus 7 d of shelf life.

Compounds	Without 1-MCP				With 1-MCP			
	CA	DCA-CF	DCA-RQ1.3	DCA-RQ1.5	CA	DCA-CF	DCA-RQ1.3	DCA-RQ1.5
α-farnesene	2.14±0.30 (b)	4.62±0.36 (a,A)	4.79±0.03 (a,A)	1.67±0.41 (b,A)	1.98±0.17(a)	2.09±0.16 (a,B)	0.50±0.09 (b,B)	0.61±0.20 (b,B)
10-nonacosanol	4.83±1.62 (a,A)	1.66±0.79 (b,A)	0.49±0.03 (b,A)	0.54±0.14 (b,A)	0.32±0.03 (B)	0.40±0.09 (B)	0.30±0.03 (B)	0.26±0.08 (B)
Tetracosane	9.98±0.05 (a,B)	10.06±0.54 (a)	8.34±0.01 (b)	10.67±0.15 (a)	10.61±0.10 (A)	9.99±1.03	9.84±1.08	10.23±0.68
Hexacosane	2.53±1.01	3.40±2.39	1.67±0.12 (B)	4.60±2.57	2.76±0.11	3.99±2.29	2.42±0.30 (A)	2.45±1.16
Nonacosane	104.64±3.96 (ab,B)	113.85±11.86 (a)	92.67±4.44 (b)	108.44±6.08 (ab)	112.07±0.79 (A)	116.02±2.51	102.97±9.41	113.59±11.18
Hexacosanal	5.55±0.39 (B)	6.67±0.97	4.74±1.26	5.22±2.42	7.11±0.65 (A)	8.05±0.41	7.01±0.85	8.23±1.45
Heptacosanal	5.09±0.59 (B)	6.51±0.95	3.85±1.61 (B)	4.20±2.10	6.42±0.44 (A)	8.15±1.41	6.79±0.62 (A)	7.62±1.36
Henicosanal	2.36±0.10	2.72±0.24	2.00±0.54	2.25±0.80	2.83±0.36	3.07±0.39	2.94±0.27	3.45±0.41
Squalene	2.93±0.66	2.85±0.94	2.72±0.42	2.81±0.58	3.65±0.16 (a)	2.94±0.35 (b)	2.62±0.07 (bc)	2.20±0.17 (c)
Oleanolic acid	0.61±0.10	0.53±0.11	0.73±0.34	0.41±0.03 (A)	0.62±0.43	0.31±0.20	0.33±0.06	0.27±0.10 (B)
Ursolic acid	5.45±0.68 (B)	6.01±0.44 (B)	6.25±0.17	6.09±0.46	8.92±0.58 (A)	12.57±1.48 (A)	7.09±0.80	6.60±0.60

Means followed by equal uppercase letter do not differ between with and without 1-MCP application, in each storage condition. Means followed by equal lowercase letter do not differ between storage conditions in each 1-MCP strategy, by Tukey test at 5 % error probability.

In general, it was possible to observe the important effects of 1-MCP on the wax composition of ‘Maxi Gala’ apples in CA compared to non-treated fruit. However, the same comparison employing DCA storage technologies had little to no effects. This may have occurred due to the influence of ethylene concentration on the cuticular wax synthesis, which was higher in CA-stored fruit. Hence, CA was the storage atmosphere that had the highest differences in wax composition compared to the other storage conditions treated with 1-MCP. Therefore, regarding the cuticle wax composition of ‘Maxi Gala’ apples, 1-MCP had little effect when using storage technologies that are effective in suppressing ethylene production, such as the DCA methods used in this study.

4.1.3.3. Metabolism and overall quality of fruit

The 1-MCP treatment was effective in decreasing ethylene production for CA and DCA-CF treatments, while 1-MCP had no effect on ethylene production for DCA-RQ1.3 and RQ1.5 treatments (Table 3). This is important because it shows the effectiveness of DCA-RQ storage in reducing ethylene production. This, in addition to the reduced financial cost, other studies have already shown a better volatile profile of apples stored in the DCA-RQ without 1-MCP (Thewes et al., 2017b). Without 1-MCP, DCA-CF and DCA-RQ reduced more effectively ethylene production than CA storage. This may be an effect of the higher ethanol, acetaldehyde and ethyl acetate production by fruit submitted to DCA-RQ, which affect ethylene suppression (Pesis, 2005; Weber et al., 2016) by the ACC synthase and reduced ACC oxidase enzyme activity (Asoda, Terai, Kato, & Suzuki, 2009; Weber et al., 2016), as this storage condition maintained O_2 levels low (Fig. 1B) and induced anaerobic metabolism. With the 1-MCP application, there were no differences between treatments for ethylene production.

Respiration rates were affected by storage conditions, being higher in CA compared to DCA treatments for fruit with and without 1-MCP. The 1-MCP application did not affect the respiration rate, regardless of the storage condition (Table 3). In climacteric fruit, the increased ethylene concentration stimulated the respiration rate of the fruit (Both et al., 2017; Thewes et al., 2015; Weber et al., 2015), as evidenced in the present study for ‘Maxi Gala’ apples stored in CA. However, the 1-MCP application generally reduced respiration rate compared to fruit without its application, which was not observed here.

Neither CA nor DCA conditions, with or without 1-MCP, affected soluble solid content or acidity in ‘Maxi Gala’ after storage (Table 1). DCA-RQ1.5 with 1-MCP had less juiciness when compared to the other storage conditions and also in the same condition with the 1-MCP

application. In fruit without 1-MCP, the juiciness was not affected by the atmospheric conditions. Fruit with 1-MCP application had greater weight loss during DCA storage, in particular, DCA-RQ1.5 greater mass loss coincided with less juiciness. For DCA-RQ1.5 without 1-MCP, the juiciness was greater and had less mass loss. Only fruit stored in DCA-CF without 1-MCP showed pulp cracking, which is probably correlated to the less weight loss.

Postharvest weight loss of fruit results from water loss and respiration (CO_2 loss) due to its diffusion through the fruit cuticle (Lara et al., 2014). Excessive water loss, which is the main cause of weight loss in fruit storage, promotes browning and loss of fruit texture and flavor (Lufu et al., 2020). Chai et al. (2020) evaluated the weight loss of 10 apple cultivars after 49 days of storage (25 ºC, 90% relative humidity) and reported that the highest weight loss rate of 8.37% was for 'Red Star' apples, while the lowest rate of weight loss of 3.64% was observed for the 'Mutsu' apples. The authors also correlated that the alkanes of the cuticular wax are essential to maintain the weight of the apple fruit during storage. This group stands out compared to other wax components for providing greater resistance to the movement of water in artificial membranes and experimental models with Arabidopsis (Grncarevic & Radler, 1967; Bourdenx et al., 2011). In our study, weight loss was related to fruit treated with 1-MCP (Fig 2), especially those stored in DCA (Table 3). Although alkanes did not differ significantly in fruit stored in DCA with or without 1-MCP (Table 2). However, the principal component analysis shows for treatments without 1-MCP (Fig. 3) that alkanes are inversely correlated with weight loss and juiciness, which is maintained when fruit lose less water. Further evidence that corroborates previous studies is that in fruit without 1-MCP, the condition of DCA-CF had a higher concentration of nonacosane and lower mass loss (Table 2 and 3).

Table 3. Metabolism and overall quality analysis of ‘Maxi Gala’ apples, without and with 1-methylcyclopropene (1-MCP) treatment, stored under controlled (CA) atmosphere and different dynamic controlled atmosphere (DCA-CF and RQ) for 9 months plus 7 d of shelf life at 20 °C.

	Without 1-MCP				With 1-MCP			
	CA	DCA-CF	DCA-RQ1.3	DCA-RQ1.5	CA	DCA-CF	DCA-RQ1.3	DCA-RQ1.5
Ethylene	14.82±3.06* (a,A)	2.68±1.61 (b,A)	0.52±0.54 (b,A)	0.32±0.47 (b,A)	0.05±0.00 (a,B)	0.07±0.08 (a,B)	0.02±0.01 (a,A)	0.03±0.01 (a,A)
Respiration rate	2.47±0.42 (a,A)	0.92±0.06 (b,A)	1.14±0.07 (b,A)	1.10±0.08 (b,A)	2.87±0.64 (a,A)	1.48±0.72 (b,A)	0.94±0.16 (b,A)	1.05±0.10 (b,A)
Soluble solids	13.25±0.30	13.50±0.62	13.45±0.38	13.60±0.37	13.30±0.26	13.50±0.50	13.80±0.16	13.90±0.26
Acidity	4.50±0.28	4.51±0.54	4.65±0.20	4.73±0.25	4.66±0.15	4.71±0.28	4.64±0.19	4.70±0.08
Juiciness	65.33±0.80	66.02±1.52	66.18±1.53	68.96±2.62 (A)	66.24±1.87 (a)	67.82±1.26 (a)	65.62±0.91 (ab)	63.02±1.64 (b,B)
Mass loss	2.95±0.25 (a)	1.84±0.42 (b,B)	2.88±0.61 (a,B)	2.90±0.40 (a,B)	2.60±0.40 (c)	3.17±0.39 (bc,A)	3.73±0.34 (ab,A)	4.22±0.42 (a,A)
Pulp cracking	0	4.00±1.63	0	0	0	0	0	0

*Means±SD (standard deviation) values followed by equal uppercase letter do not differ between with and without 1-MCP application, in each storage condition. Means followed by equal lowercase letter do not differ between storage conditions in each 1-MCP strategy group, by Tukey test at 5% error probability. Controlled atmosphere (CA), dynamic controlled atmosphere based on chlorophyll fluorescence (DCA–CF) and dynamic controlled atmosphere by respiratory quotient (DCA–RQ). Results expressed in: Ethylene (ng kg^{-1} s^{-1}); Respiration rate (µg kg^{-1} s^{-1}); Soluble solids (%); Acidity (mEq 100 mL^{-1}); Juiciness, mass loss and pulp cracking (%).

4.1.3.4. Multivariate analysis (PCA)

Principal component analysis (PCA) was performed to analyze the 1-MCP treatment, storage conditions, and variables determined in this experiment. All experimental data are shown in Figure 2. The first and second principal component (PC1 and PC2) defined 53.77% of the overall variation and, throughout the PC1, treatments with and without 1-MCP were discriminated. However, there was a greater difference between CA and DCA without 1-MCP than with 1-MCP-treated fruit. This occurred as a result of the higher abundance of several fatty acids, mainly important fatty acids for wax biosynthesis, such as C16:0 and C18:0, 10-nonacosanol, and ethylene production that were positively correlated with CA without 1-MCP. In addition, the fruit stored in DCA-RQ without 1-MCP were discriminated from the other treatments as observed throughout the PC2, when they were positively correlated to the presence of α-farnesene and fatty acids C15:0, C11:0, C15:1, and C18:3n6.

Regarding apples without 1-MCP, all treatments were well discriminated. Nevertheless, treatments with the 1-MCP application were grouped due to the lower difference in concentration among compounds than fruit without 1-MCP. Because of this, another PCA was performed only for the group of samples treated with 1-MCP (Figure 3). PC1 and PC2 defined 56.41% of the overall variation. It was possible to observe the discrimination of CA from DCA in PC1 (38%) due to its correlation with the fatty acids C16:0, C18 0, C18 2n6, C18:1n9, C24:0, C8:0, and C22:0, squalene, UA, and OA in addition to higher ethylene production and respiration rate. On the other hand, DCA-stored apples discriminated DCA-CF storage, which was associated mainly with compounds such as heptacosanal, hexacosanal, henicosanal, and C20:2 as observed in the PC2. The samples stored in DCA-RQ1.3 and DCA-RQ1.5 were not discriminated, but they correlated with weight loss.

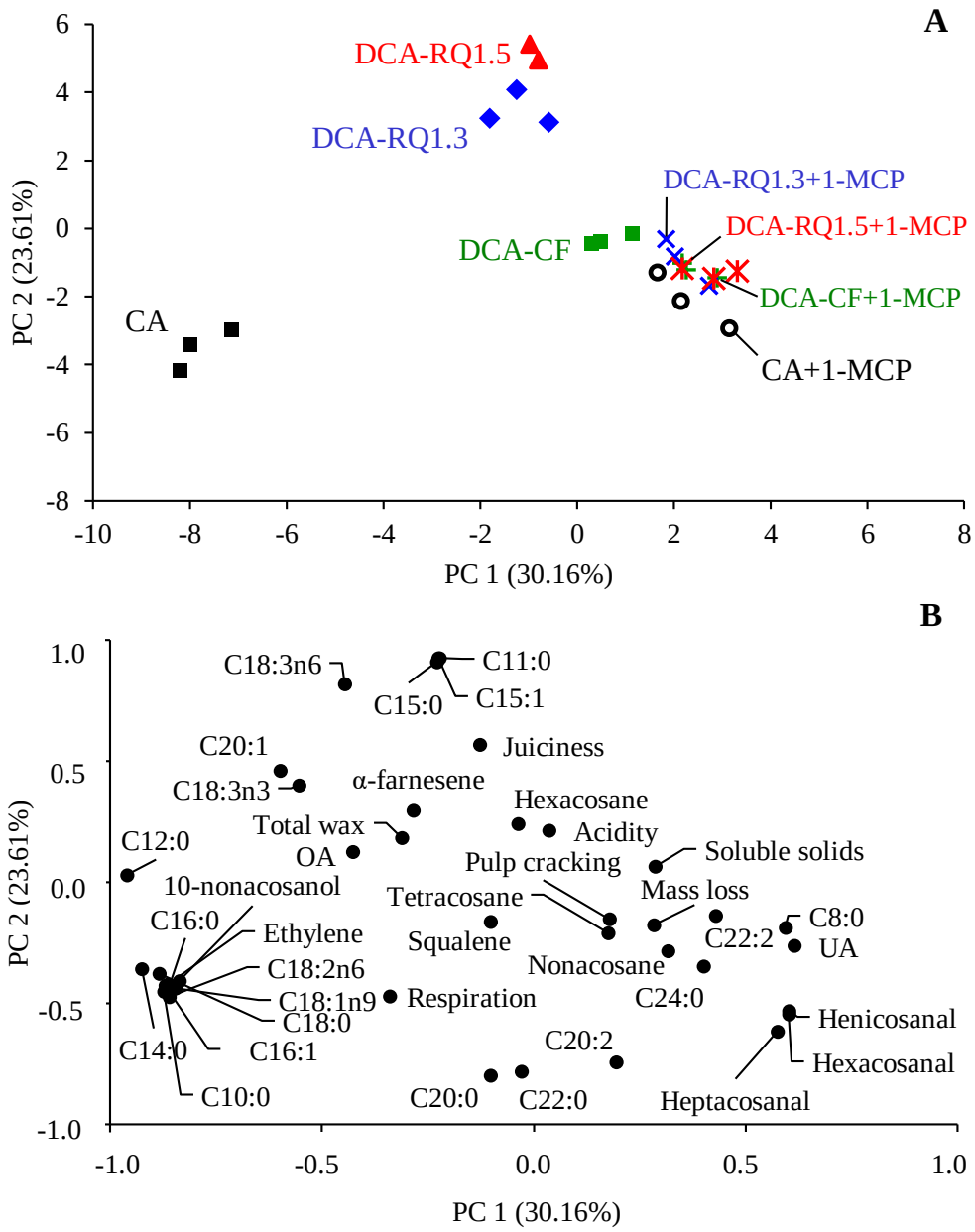

Figure 2. Scores (treatments) (A) and loadings (variables) (B) plots of the first two principal components of the identified wax compounds, total wax, ethylene production, and respiration rate of 'Maxi Gala' apples, without and with 1- methylcyclopropene (1-MCP) treatment, stored under controlled atmosphere (CA), dynamic controlled atmosphere based on chlorophyll fluorescence (DCA–CF) and dynamic controlled atmosphere by respiratory quotient (DCA–RQ) for 9 months plus 7 d of shelf life. UA: ursolic acid; OA: oleanolic acid

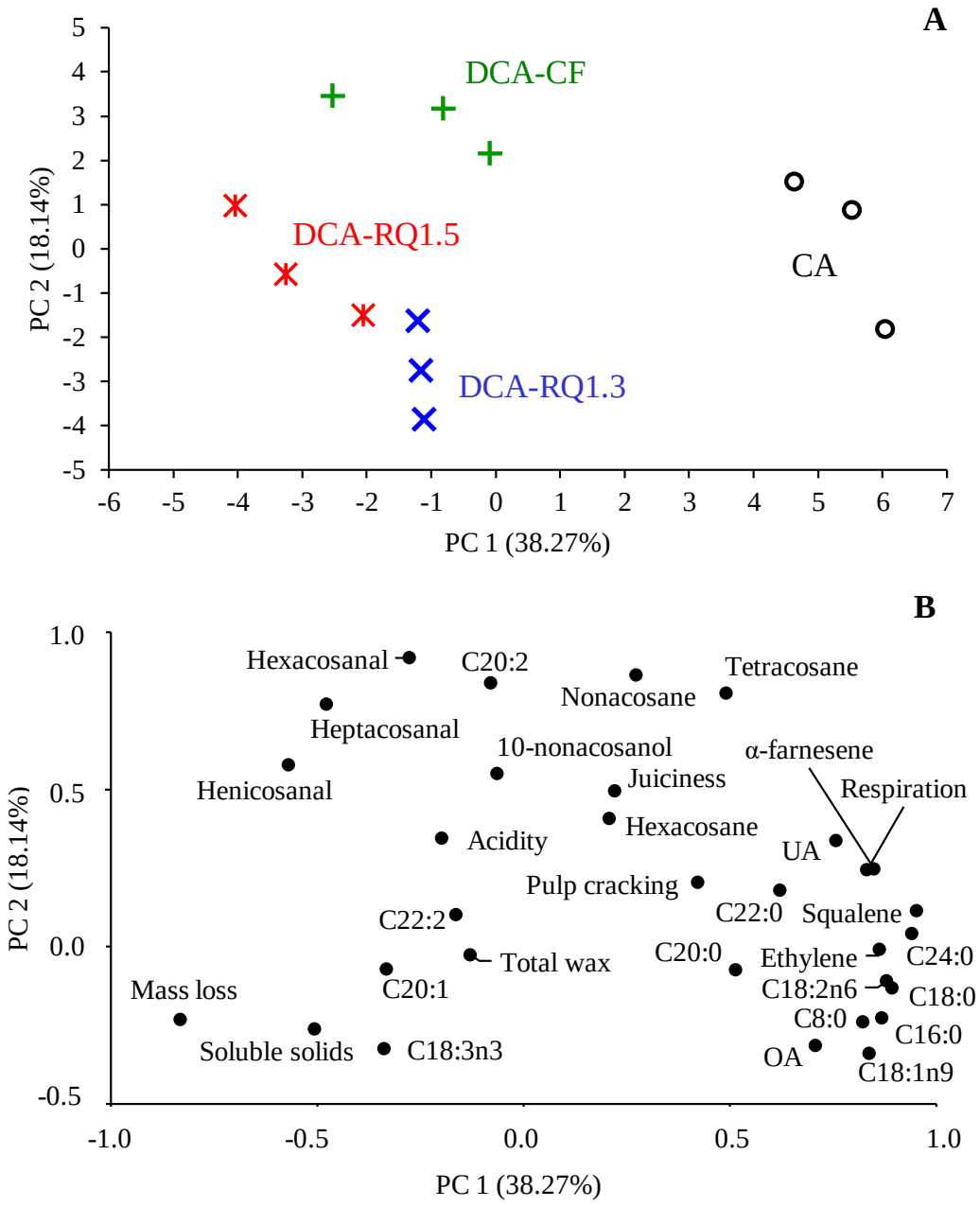

Figure 3. Scores (treatments) (A) and loadings (variables) (B) plots of the first two principal components of the identified wax compounds, total wax, ethylene production, and respiration rate of 'Maxi Gala' apples, with 1-methylcyclopropene (1-MCP) treatment, stored under controlled atmosphere (CA), dynamic controlled atmosphere based on chlorophyll fluorescence (DCA–CF) and dynamic controlled atmosphere by respiratory quotient (DCA–RQ) for 9 months plus 7 d of shelf life. UA: ursolic acid; OA: oleanolic acid.

4.1.4. Conclusion

The isolated or combined effects of 1-MCP and the dynamic controlled atmosphere on the cuticle wax composition of 'Maxi Gala' apples were studied. 1-MCP application was effective in reducing ethylene production in CA, although DCA, especially DCA-RQ, has also been shown to be efficient in reducing the overall metabolism of the fruit similarly as 1-MCP treatment in CA. The storage atmospheres and treatment with 1-MCP did not influence the total wax content. The wax compositions of the apples stored in DCA were similar when 1-MCP was applied. However, 1-MCP may be associated with a suppression in alkane biosynthesis and, consequently, greater mass loss in fruit stored in DCA.

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SUPLEMENTARY MATERIAL 1

Total apple peel wax of 'Maxi Gala' (g m^{-2}), without and with 1-methylcyclopropene (1-MCP) treatment, stored under controlled (CA) atmosphere and different dynamic controlled atmosphere (DCA-CF and DCA-RQ) for 9 months plus 7 d of shelf life.

Treatment	Without 1-MCP	With 1-MCP
CA	16.21±0.63 (a,A)	14.90±1.32 (a,A)
DCA-CF	15.37±1.57 (a,A)	16.37±1.13 (a,A)
DCA-RQ1.3	17.10±0.26 (a,A)	15.09±1.42 (a,A)
DCA-RQ1.5	15.35±0.27 (a,A)	14.81±0.69 (a,A)

*Mean±SD (standard deviation) values followed by the same lower case letter in the same column, and the same upper case letter in the same line for each of the evaluations are not significantly different by Tukey's multiple range test ($P < 0.05$). Controlled atmosphere (CA), dynamic controlled atmosphere based on chlorophyll fluorescence (DCA–CF) and dynamic controlled atmosphere by respiratory quotient (DCA–RQ).

5. ARTIGO 3

5.1. CUTICULAR WAX CHEMICAL COMPOSITION AND METABOLISM OF 'ELSTAR' APPLES DURING THE STORAGE: INFLUENCE OF THREE DIFFERENT DYNAMIC CONTROLLED ATMOSPHERE TECHNIQUES[3]

Abstract

There is little information available that relates the wax contents and compositions with the quality of the apples stored in different storage conditions, especially those technologies recently developed, such as dynamic controlled atmosphere (DCA). Therefore, the objective of this study was to evaluate the concentration and chemical composition of peel waxes of 'Elstar' apple stored under CA, either without or with 1-MCP treatment, DCA-CF, DCA-RQ, and DCA-CD and its relationship with their metabolism, decay incidence, and overall quality of fruit. The storage under DCA, mainly DCA-RQ and DCA-CD, or CA + 1-MCP blocked ethylene production and delays fruit ripening. DCA stored fruit resulted in lower decay incidence and was associated with a greater accumulation of compounds of anaerobic metabolism, especially under DCA - RQ. Storage conditions with less pO_2 resulted in less intensity of color and more opaque fruit when compared to CA, but the green color of the epidermis was better maintained by the storage under DCA – RQ and DCA – CD. DCA-RQ and DCA-DC were the treatments with the lowest wax levels. These treatments showed similarities in the chemical composition of wax. Fruit stored in CA showed higher concentrations of most of the wax compounds identified, mainly of the fatty acid fraction. The quality parameters of 'Elstar' apples after storage were not directly correlated with the wax composition, but were correlated with the effect of this storage condition on the general metabolism of the fruit.

Keywords: *Malus domestica* Borkh.; Postharvest quality; DCA-RQ; DCA-CD; Cuticle.

[3] Artigo formatado de acordo com as normas da revista Scientia Horticulturae.

5.1.1. Introduction

In Europe, one of the most produced apple cultivars belongs to the 'Elstar' group, with an estimated harvest of 312 thousand tons in 2020 (WAPA, 2020). Apple cultivation has a restricted harvest to a few months of the year, which results in periods of high and low supply of fruit. Due to its seasonality, storage is an important factor in offering quality fruit throughout the year to consumers.

Controlled atmosphere (CA) is the main technique used for apple storage. It is based on the control of temperature, relative humidity (RH), and gas composition, with a substantial reduction in O_2 partial pressure (pO_2) and an increase in CO_2 (pCO_2) partial pressure (Argenta et al., 2000; Brackmann et al., 2008; Kittemann et al., 2015). The storage condition is determined for each cultivar or mutant and kept constant throughout the storage period (Argenta; Fan; Mattheis, 2000; Brackmann et al., 2008; Kittemann; Neuwald; Streif, 2015; Weber et al., 2011). For 'Elstar' apples, pO_2 of 1.0 to 2.0 kPa are generally used (Veltman et al., 2003). The ideal pO_2 value in CA storage results in a decrease in fruit respiration, and thus, fruit have a longer shelf life. This value remains above the lower oxygen limit (LOL) tolerated by the fruit during storage, preventing damages to the fruit caused by excessive anaerobic metabolism. The 1-MCP application blocks ethylene receptors and consequently delays the fruit ripening (Watkins, 2006). With the ethylene action inhibition, the expression of several genes and enzymes activity, such as ACC oxidase (Tatsuki, Endo, & Ohkawa, 2007; Wakasa et al., 2006), ethylene receptors (Tatsuki, Endo, & Ohkawa, 2007), and enzymes of the formation of the volatile compounds (Yang et al., 2016) are downregulated.

Recent developments towards dynamic CA storage (DCA) have been able to monitor the apple responses to pO_2 by decreasing it periodically during storage to determine the lowest safe O_2 setpoint (Bessemans et al., 2016; Wright et al., 2015, 2012). In such cases, it is possible to reduce O_2 to extremely low levels (<0.5 kPa), inducing anaerobic metabolism, which causes the production of CO_2 to be greater than the consumption of O_2 by the fruit. The regularly changing gas concentrations in DCA are currently monitored by three techniques: based on ethanol production by fruit (EtOH) (Deuchande et al., 2016; Veltman et al., 2003), on chlorophyll fluorescence (CF) (Prange et al., 2007; Prange et al., 2003), and on respiratory quotient (RQ) (Bessemans et al., 2016; Brackmann, 2015; Weber et al., 2015). However, the lack of tightness of the storage chambers can interfere with the determination of the LOL, which is one of the problems faced in DCA, minimized by the installation of a mini-chamber inside the commercial chamber (Brackmann, 2015) or the use of mathematical models to set pO_2

setpoint (Bessemans et al., 2018). Recently, Thewes et al. (2020) developed a technique to monitor the anaerobic metabolism of fruit only by CO_2 production (DCA-CD, carbon dioxide), since the production of CO_2 decreases with the reduction of pO_2 in the chamber. This alternative facilitates the measurement of LOL at a commercial level without the need for additional equipment for CA chambers monitoring, in addition to the little influence of the external environment on its determination.

The literature is extensive for the evaluation of quality parameters of apples stored under the aforementioned DCA techniques, especially for DCA – CF and DCA – RQ, but is scare for DCA – CD. For example, storage in CA results in fruit with better maintenance of flesh firmness, acidity, and less ethylene production, respiration rate, and the occurrence of physiological disorders (Argenta et al., 2000; Brackmann et al., 2008; Lumpkin et al., 2015; Weber et al., 2016); DCA-CF reduces the occurrence of physiological disorders when compared to CA, mainly from superficial scald and decay (Both et al., 2017; Mditshwa et al., 2017; Prange et al., 2015; Zanella et al., 2005); DCA-RQ provides less incidence of superficial scald, flesh breakdown, greater flesh firmness, and healthy fruit and higher emission of volatile compounds (Schaefer & Bishop, 2010; Bessemans et al., 2016; Both et al., 2017; Keshri et al., 2020; Thewes et al., 2017; Thewes et al., 2020; Weber et al., 2015); and DCA - CD had a lower decay incidence and flesh breakdown in ‘Imperial Gala’ and ‘Golden Delicious’ apples and greater flesh firmness for ‘Imperial Gala’, ‘Fuji Suprema’, ‘Golden Delicious’ and ‘Cripps Pink’ apples (Thewes et al., 2020).

More recently, it is suggested that the cuticular wax that covers fruit surface may be related to the quality of fruit storage during post-harvest (Lara, 2018; Lara et al., 2014). In general, the components of cuticular waxes include very-long-chain (VLC) aliphatic compounds (e.g., esters, ketones, aldehydes, alkanes, alcohols, and fatty acids), flavonoids, sterols, and terpenoids (Lara et al., 2015). Studies have shown that changes in wax composition during the postharvest period, mainly in cold storage or in CA, were associated with changes in fruit quality. Chai et al. (2020) reported the positive effect of alkanes in the total wax of apple fruit peel, being essential for storage and quality control, mainly by decreasing weight loss. Yang et al. (2017) showed that during fruit storage, ‘Jonagold’ and ‘Cripps Pink’ apple wax composition changed and wax morphology leads to peel greasiness Klein et al. (2020) found decreased greasiness for ‘Cripps Pink’ apples stored in DCA-RQ, when compared to CA, which was attributed to variation in wax composition, mainly in compounds such as esters, ursolic and oleanolic acids, and C16:0 fatty acid. Nevertheless, still little information is available to relate the wax contents and compositions with the quality of the apples stored under different storage

conditions, mainly to those technologies developed recently, such as DCA, and no studies have evaluated the effect of DCA-CD, proposed recently by Thewes et al. (2020).

Therefore, the objective of this study was to evaluate the concentration and chemical composition of peel waxes of 'Elstar' apples stored under CA, either without or with 1-MCP treatment, DCA-CF, DCA-RQ, and DCA-CD for 9 months of storage plus 7 d shelf life at 20 °C. Additionally, these treatments also were evaluated in relation to their metabolism, decay incidence, and overall quality of fruit and correlated with their wax content and composition.

5.1.2. Materials and methods

5.1.2.1. Sample preparation and storage conditions

'Elstar' apples were harvested in 2017, at the optimal maturity from the determination of the index starch pattern index: 2.8 (SPI, scale: 1 maximal starch to 10 fully hydrolyzed starch) from commercial orchards in the Constance Lake region of Southwest Germany. Immediately after harvest, fruit were transported to storage the Competence Centre for Fruit Growing at Lake Constance (KOB), Germany, and randomly allocated into replicates of 20 fruit with each treatment having 4 replicates, for each evaluation after 9 months of storage. Samples were placed in 250-L experimental gas-tight chambers. Each chamber was connected to an automatic CA system (Isollcel®, Bolzano Italy), to continuously control the gas partial pressure setpoints. The pCO_2 was 2.0 kPa for CA stored 'Elstar' apples. For DCA, the pCO_2 was 1.2 kPa. The experimental chambers were allocated inside a cold room at temperature of 1.0 ± 0.2 °C. The pO_2 variation for storage conditions are shown in Figure 1. Throughout storage, the temperature was monitored daily with a mercury thermometer inserted in fruit flesh, to determine the pulp temperature. The relative humidity was set and maintained at 94 ± 1% with the aid of a psychrometer.

Fruit treated with 1-MCP and subsequently stored in CA were put into the 250-L chamber together with a solution containing 0.650 μL L^{-1} 1-MCP (SmartFresh®, 0.14% of active ingredient the relation to calculate the dose is 1 g for 1000 nL L^{-1} for 1m^3). Thereafter, the chamber was hermetically closed for 24 hours. During this period, the air inside the chamber was homogenized with a fan. After the fruit were removed from the 1-MCP application chamber, the atmospheric conditions were installed.

The atmosphere conditions evaluated were: [1] CA - 1.2 kPa O_2 + 2.0 kPa CO_2; [2] CA+1-MCP - 1.2 kPa O_2 + 2.0 kPa CO_2; [3] DCA-CF + 1.2 kPa CO_2; [4] DCA-RQ + 1.2 kPa CO_2; [5] DCA-CD + 1.2 kPa CO_2.

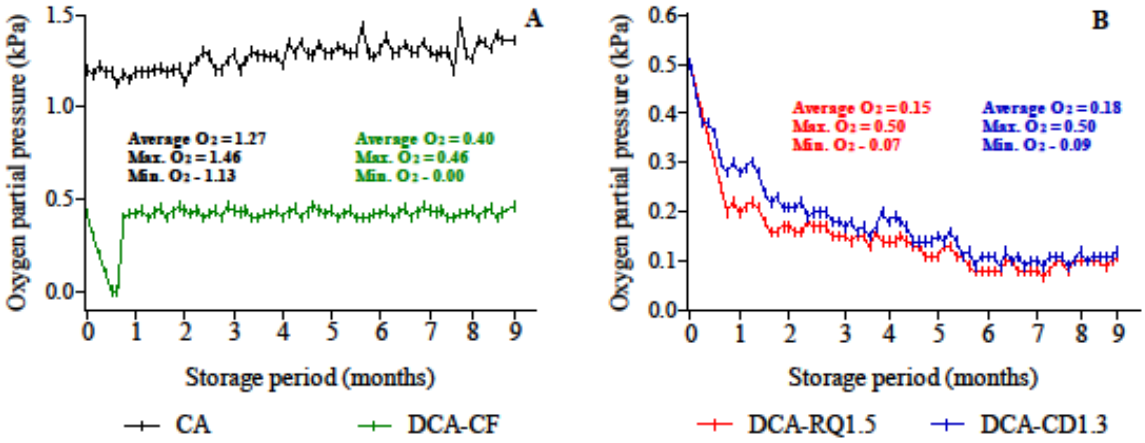

Figure 1. Oxygen setpoint variation of 'Elstar' apples stored under CA and DCAs at 1 ºC over 9 months.

5.1.2.1.1. DCA-CF

DCA-CF was monitored according to Prange et al. (2007). Chlorophyll fluorescence was monitored in six apples during exposure to low pO_2. The fluorescence monitoring system was activated and the pO_2 reduced. Afterwards, the respiration process reduced the pO_2 until a change in fluorescence was detected. The lowest O_2 set point was determined by identifying the pO_2 where an inflection in the fluorescence signal was detected, and then by increasing pO_2 by 0.4 kPa as a safety factor. Chlorophyll fluorescence was monitored every hour for the entire storage period.

5.1.2.1.2. DCA-RQ

For DCA-RQ, the The experimental chamber was closed for 13 to 14 h, and the pO_2 and pCO_2 were measured before and after this time. The ratio between pCO_2 release and pO_2 uptake was calculated to give the RQ (Weber et al., 2015). In this experiment, RQ treatment tested was 1.5, and was calculated twice a week. When the RQ was below the setpoint, the pO_2 was decreased, and if the RQ was above the setpoint, the pO_2 was increased.

5.1.2.1.3. DCA-CD

The pO_2 setpoint was based only on CO_2 production, according to the method described by Thewes et al. (2020). In brief, to determine the CO_2 production rate the storage chambers were closed over a period of 13 h, without scrubbing the CO_2 produced by fruit, and the pCO_2 was measured and the value recorded. Immediately after this period, the pCO_2 was measured again. This procedure was performed twice a week. The pCO_2 before and after 13 h of chamber closing was used as inputs in the system FruitAtmo® (Frigotec, Germany) that estimated the novel oxygen setpoint based on CO_2 production only. This pO_2 was set in the storage room and maintained constantly over a period of 3 - 4 d and thereafter the CO_2 production rate was repeated to verify if the pO_2 should be adjusted.

5.1.2.2. Fruit metabolism, quality and anaerobic metabolism metabolites accumulation

After 9 months of storage plus 7 d of shelf life, fruit were assessed for metabolism, quality characteristics, and volatile compounds of anaerobic metabolism, in order to show the effect of each storage condition, as described below.

5.1.2.2.1. Ethylene production and respiration rate

Measured by placing a sample of about 0.8 to 1 kg of intact fruit into a 4.25 L jar. To measure ethylene, the jar was closed hermetically for 2 h. 1 mL of headspace gas from the jar was injected into a Carlo Erba, Fractovap Series 2150 gas chromatograph, equipped with a flame ionization detector (FID) and a stainless steel column, 0.9 m × 3.17 mm filled with 60-mesh activated alumina. Injector and oven temperatures were 175 °C and 100 °C, respectively, and data were expressed in pg kg^{-1} s^{-1}. To determine the respiration rate, the air of the same jar was circulated throughout a gas analyzer (Hartmann and Braun GmbH, Germany) to measure CO_2 and results were expressed in μg kg^{-1} s^{-1}.

5.1.2.2.2. Decay incidence

The method proposed by Thewes et al. (2015) was used. Decay incidence was evaluated by counting the fruit with fungal injury higher than 5 mm, in relation to the total number of fruit per replicate (20 fruit each replicate). The results were expressed in percentage.

5.1.2.2.3. Fruit peel background color

The background color of the epidermis of apples was measured at one point in the equatorial region of four samples per treatment after 9 months of storage plus 7 d at 20 °C according to Kasampalis et al. (2020) with modifications. A Minolta CR-400 colorimeter (Minolta Sensing Inc. Konica, Japan), equipped with an 8-mm measuring head, illuminant C, and an observation angle of 10°, was used. The colorimeter was calibrated using the manufacturer's standard white plate. Color changes were quantified in the L*, a*, and b* color space. Hue angle [(Hue = 180 tan−1 (b*/a*)] and chroma values [C*= $(a*2 + b*2)^{1/2}$] were calculated from a* and b* values. L* refers to lightness, ranging from 0= black to 100= white; a*, intensity of the red color; b*, intensity of the yellow color; hue angle (Hue) value is defined as a color wheel, with red-purple color at an angle of 0°, yellow color at 90°, bluish-green color at 180° and blue color at 270°, and chroma (C*) represents color saturation, which varies from dull (low values) to vivid (high values).

5.1.2.2.4. Determination anaerobic metabolism metabolites accumulation

After storage plus 7 d of shelf life at 20 °C, apples were cooled to 0.5 °C and the juice prepared and extracted according to Both et al. (2014). The juice was immediately stored at −30 °C until GC analyses. The ethanol extraction was carried out by HS-SPME. A 10 mL aliquot of this juice was placed inside a 20 mL vial following the addition of 3 g of NaCl and 10.0 µL of a 3-octanol standard solution (0.0822 g L^{-1}). The vial was sealed with a screw cap and septum coated with PTFE/silicone, and submerged in a water bath at 40 °C for 15 min. The fiber was then exposed to the headspace of the sample for 60 min under constant stirring at the same temperature for sorption and submitted to GC analysis. Divinylbenzene/carboxen/polydimethylsiloxane (DVB/Car/PDMS) fiber (Supelco, 50/30µm × 20 mm) has used for extraction. The ethanol was identified and quantified on a GC coupled to a mass spectrometer (Shimadzu QP2010 Plus - GC/MS, Shimadzu Corporation, Kyoto, Japan). The chromatographic conditions employed were according to Thewes et al. (2017b) with slight modifications. The fiber was desorbed at 250 °C for 10 min and the injection split ratio was 1:5. The compounds were separated on a ZBWAX polar phase capillary column (Zebron, Phenomenex, USA; 60 m × 0.25 mm; 0.25 µm of thickness film). Helium was used as carrier gas at a constant pressure. The column temperature began at 35 °C, kept for 3 min, and increased

at 2 °C min^{-1} to 80 °C and then increased at 5 °C min^{-1} to 230 °C. The column temperature remained isothermal for 5 min. The GC/MS interface and MS ion source temperature were maintained at 230 °C. The detector operated in the electron impact ionization mode with ionization energy of +70 eV and scan mass range from 35 to 350 m/z. A series of homologous n-alkanes was analyzed under the same conditions to calculate the linear retention index (LRI). Acetaldehyde, ethanol, and ethyl acetate were identified by comparing mass spectra with the standard. Quantification was performed with the use of 3-octanol as internal standardization (Both et al., 2014).

5.1.2.3. Determination of wax composition

Sample preparation and wax compounds extraction were carried out according to Klein et al. (2019). The wax was extracted from 10 cm^2 of the epidermis homogenized in 5 mL of distilled water, followed by the addition of 2 mL of extraction solvent (chloroform) plus 4 mL of dispersion solvent (acetone). The sample was then shaken for 30 min. The partition procedure was repeated twice with 1 mL of chloroform. Afterwards, the sample was centrifuged for phase separation and the organic fraction reserved and transferred to a 10 mL volumetric flask, which was added of 250 µL of a methyl tricosanoate solution (C23:0; 4.006 g L^{-1}; Sigma-Aldrich, St. Louis, USA). The extraction yield was gravimetrically obtained from the extracts (5 mL). Results were expressed in g m^{-2} of wax.

Wax compounds were determined from three fractions: fatty acids profile (F1), compounds with high boiling point (F2), and other compounds from the direct analysis of the concentrated extract (F3). The F1 was obtained by transesterification/esterification of 4 mL of the extract after drying under continuous N_2 flow at 40 °C. In this extract, the fatty acid methyl esters (FAMEs) were obtained according to Hartman and Lago (1973). The F2 were determined after derivatization with MTBSTFA (N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide; Sigma-Aldrich) according to Oms-Oliu et al. (2011). Finally, F3 compounds were acquired by drying 500 µL of the extract, reconstituting with 50 µL of chloroform, and injecting directly into the chromatographic system.

The three quantitative determinations were carried out in a gas chromatograph equipped with a flame ionization detector (GC-FID; Varian Star, 3400 CX, Walnut Creek, USA) as described by Klein et al. (2019). The FAMEs (F1) were identified by comparing their experimental retention times with the ones found in the FAME Mix-37 standard (P/N 47885-U, Sigma-Aldrich, St. Louis, USA). The analytes with no available standard (F2 and F3) were

identified by gas chromatography coupled to a mass spectrometer (GC/MS, Shimadzu QP2010 Plus, Kyoto, Japan) based on the comparison of the obtained mass spectra obtained with those available from the National Institute of Standards and Technology (NIST) and the literature. The results were expressed as g of compound per kg wax.

5.1.2.4. Statistical analysis

Results were subjected to analysis of variance (ANOVA) for all the parameters evaluated. ANOVA significant ($p < 0.05$) parameters were compared through the Tukey test. This analysis was performed using statistical software Statistica, version 7.0 (StatSoft Inc., Tulsa, OK, USA). In addition, data were submitted to a multivariate analysis using Principal Component Analysis (PCA) by Pirouette 3.11 software (Woodinville, USA, 2003). Additionally, a heatmap analysis and hierarchical cluster analysis (HCA) were carried out in the Metaboanalyst platform (Xia Lab @McGill, Quebec, Canada) to group variables and samples. The data matrix was preprocessed by auto-scaling for each variable to obtain the same weight for all variables (mean = 0 and variance = 1) before each multivariate analysis.

5.1.3. Results

5.1.3.1. Fruit metabolism, quality and anaerobic metabolites accumulation

Apples stored in CA had the highest production of ethylene, but the treatment with 1-MCP was effective in decreasing the ethylene production using this storage technique (Table 1). Without 1-MCP, DCA treatments were more effective than CA storage. In addition, there was no difference among DCA methods, with the exception of DCA-CF (Table 1), which produced more ethylene but still less compared to apples stored in CA. The respiration rate was not affected by the storage conditions in DCA. However, in CA, the respiration rate was higher than CA+1-MCP and also than all DCA treatments.

Corroborating the higher ethylene production and respiration rate, apples stored in CA had the highest decay incidence. Among treatments employed, DCA-RQ was the condition that had less decay incidence after storage and shelf life, without differing from fruit stored under DCA – CD and DCA – CF.

Table 1. Ethylene production, respiration rate, and decay incidence of 'Elstar' apples after 9 months of storage under controlled atmosphere (CA), either without or with 1-MCP treatment (0.650 μL L^{-1}), and dynamic controlled atmosphere monitored by chlorophyll fluorescence (DCA-CF), by respiratory quotient (DCA-RQ) and by carbon dioxide (DCA-CD), plus 7 d of shelf life at 20 °C.

Treatment	Ethylene (pg kg^{-1} s^{-1})	Respiration (μg kg^{-1} s^{-1})	Decay (%)
CA	86.33±5.23* (a)	1.82±0.13 (a)	26.67±2.36 (a)
CA+1-MCP	nd	1.60±0.13 (b)	22.5±2.89 (ab)
DCA-CF	33.67±4.29 (b)	1.56±0.08 (b)	18.6±4.54 (bc)
DCA-RQ	nd	1.44±0.01 (b)	13.75±4.79 (c)
DCA-CD	nd	1.44±0.09 (b)	17.50±2.89 (bc)

*Mean±standard deviation (n = 4) values in the same columns followed by the same letter did not differ significantly by Tukey's multiple range test ($P<0.05$). nd.: not detected.

The color parameters L* and a* were not influenced by the storage conditions (Table 2) and the apples were characterized by colorations with high lightness and green color tendency. The parameter of b* was lower in the storage conditions DCA-RQ and DCA-CD, higher in apples stored in CA, and did not differ in the others, showing that fruit stored under DCA – RQ and DCA – RQ were less yellow than CA stored one. Similar behavior was observed for C* (Table 2). Apparently, lower pO_2 used during the storage of 'Elstar' apples in DCA, mainly in RQ and CD (Fig. 1) maintained low the intensity of the yellow color of the peel compared to the other storage conditions studied. Finally, the Hue angle was greater in DCA-RQ and DCA-CD and did not differ in the other conditions.

Table 2. Color parameters of 'Elstar' apples after 9 months of storage under controlled atmosphere (CA), either without or with 1-MCP treatment (0.650 μL L^{-1}), and dynamic controlled atmosphere monitored by chlorophyll fluorescence (DCA - CF), by respiratory quotient (DCA - RQ) and by carbon dioxide (DCA - CD), plus 7 d of shelf life at 20°C.

Treatment	L	a	b	C	Hue
CA	75.16±1.10	-13.11±0.36	46.16±0.09 (a)	47.99±0.13 (a)	105.85±0.41 (b)
CA+1-MCP	75.56±0.74	-13.20±0.35	45.39±0.28 (b)	47.27±0.37 (b)	106.21±0.31 (b)
DCA-CF	76.23±0.67	-12.85±0.36	45.22±0.4 (b)	47.02±0.31 (b)	105.86±0.53 (b)
DCA-RQ	76.69±0.97	-13.80±0.73	43.18±0.16 (c)	45.33±0.32 (c)	107.72±0.86 (a)

DCA-CD	76.07±0.61	-13.75±0.51	43.54±0.28 (c)	45.66±0.29 (c)	107.53±0.64 (a)

*Mean±standard deviation (n = 3) values in the same columns followed by the same letter did not differ significantly by Tukey's multiple range test (P<0.05).

DCA-RQ was the storage condition that most accumulated the products of the anaerobic metabolism acetaldehyde, ethanol, and ethyl acetate (Table 3). For ethanol, there was no difference between the other conditions. DCA-CD, DCA-CF, and CA did not differ regarding the accumulation of acetaldehyde, but the application of 1-MCP favored greater accumulation when compared to the same storage condition. CA either without or with 1-MCP and DCA-CF did not differ in relation to ethyl acetate, but they differ from DCA-CD and DCA-RQ, being the latter had greater accumulation.

Table 3. Anaerobic metabolism of 'Elstar' apples showing the concentration of the metabolites after 9 months of storage under controlled atmosphere (CA), either without or with 1-MCP treatment (0.650 μL L^{-1}), and dynamic controlled atmosphere monitored by chlorophyll fluorescence (DCA - CF), by respiratory quotient (DCA - RQ) and by carbon dioxide (DCA - CD), plus 7 d of shelf life at 20°C.

Treatment	Acetaldehyde (μg L^{-1})	Ethanol (μg L^{-1})	Ethyl acetate (μg L^{-1})
CA	0.64±0.07 (d)	0.37±0.06 (b)	6.01±1.52 (c)
CA+1-MCP	5.85±0.72 (b)	1.02±0.47 (b)	6.79±1.44 (c)
DCA-CF	2.01±0.39 (cd)	0.42±0.21 (b)	6.14±0.44 (c)
DCA-RQ	9.10±1.56 (a)	74.41±20.22 (a)	393.26±31 (a)
DCA-CD	2.65±0.30 (c)	5.03±0.85 (b)	66.69±8.79 (b)

*Mean±standard deviation (n = 3) values in the same columns followed by the same letter did not differ significantly by Tukey's multiple range test (P<0.05).

5.1.3.2. Wax content and composition of apple peel

The cuticular wax content and composition obtained from apples stored under different techniques were shown in Table 4. The results showed that the apples stored in DCA-RQ and DCA-CD had the lowest accumulation of total wax and they differ in amount from CA treatments (without and with 1-MCP application) and DCA-CF.

The apple peel wax content and composition were determined after 9 months of storage plus 7 d of shelf life at 20 °C (Table 4). Among three fractions evaluated from peel extracts, 27 compounds were identified in samples, and were grouped in fatty acids (16), alcohols (4), aldehydes (2), hydrocarbons (3), and triterpenic acids (2).

Fatty acids accounted for the largest proportion of the total wax content among the compounds. The fatty acids C12:0, C18:1n9, and C18:3n3 had no influence of the storage condition. The C17:0 was present only in apples stored in DCA-RQ. Higher levels of the C14:0 and C15:0 were accumulated in fruit stored under CA+1-MCP and did not differ among other treatments of storage, for C14:0. The C16:0 was the majority compound in all storage conditions. DCA-CF and DCA-CD had the lowest concentrations of this compound, while CA+1-MCP presented the highest concentration. The similar behavior was observed for C18:0. Both fatty acids C16: 0 and C18:0 were also determined in their free form, different from those esterified. These compounds were in higher concentrations in apples stored in CA, with and without 1-MCP and they were different from each other, whereas for DCA treatments, was not detected for C16:0 or did not differ for C18:0. In DCA-RQ, C18:2n6 was higher and differed only from CA. Apples stored in DCA-RQ and DCA-CD accumulated more C20:0, C20:2 fatty acids. The C22:0 fatty acid was higher in all DCA treatments, and differed only from CA+1-MCP. On the other hand, C20:1 fatty acid was not detected in DCA-CF and accumulated more in the condition of CA, without the influence of the application of 1-MCP. The C22:2 fatty acid was higher in DCA-CD and differed only from DCA-CF and CA+1-MCP.

For alcohols, 1-hexacosanol and phytol did not differ between treatments. 10-nonacosanol showed higher levels in apples stored in CA, either without or with 1-MCP, which were different from each other while the lowest levels were in apples in DCA-CD. Unlike 1-octacosanol, which accumulated more in DCA-CD and less in CA without 1-MCP.

The identified aldehydes, heptadecanal and octadecanal, had the same behavior among treatments, the largest accumulations were identified in apples in CA compared to DCA-CD, without differing from the others.

The alkanes responsible for the structural formation of wax crystals, identified in the samples as docosane, nonacosane and 2-methylhexacosane were influenced by the storage conditions. For nonacosane and 2-methylhexacosane, apples stored in CA, had higher accumulations of these compounds, but the application of 1-MCP decreased their concentration. DCA-CD did not differ from CA without 1-MCP. Docosane accumulated more in apples stored in DCA-RQ and DCA-CD, than CA treatments.

Ursolic acid (3β-hydroxy-ursan-12-en-28-oic acid, UA) and oleanolic acid (3β-hydroxy-olean-12-en-28-oic acid, OA) were triterpenic acids found in apple cuticular wax. UA had the highest concentration levels compared to OA, but was not influenced by the storage conditions tested. DCA-CD accumulated more OA, followed by CA+1-MCP when CA presented the lowest concentration of this compound.

Table 4. The composition of peel wax and total wax of 'Elstar' apples after 9 months of storage under controlled atmosphere (CA), either without or with 1-MCP treatment (0.650 μL L^{-1}), and dynamic controlled atmosphere monitored by chlorophyll fluorescence (DCA - CF), by respiratory quotient (DCA - RQ) and by carbon dioxide (DCA - CD), plus 7 d of shelf life at 20 °C.

	CA	CA+1-MCP	DCA-CF	DCA-RQ	DCA-CD
Total wax (g m^{-2})	14.38±0.47 (a)	14.65±0.18 (a)	13.98±0.41 (a)	12.93±0.08 (b)	12.75±0.43 (b)
Compounds (g kg^{-1})			*Fatty acids*		
C12:0	1.34±0.25*	1.18±0.09	1.09±0.33	1.16±0.02	1.28±0.27
C14:0	4.12±1.18 (b)	6.47±0.27 (a)	2.66±0.78 (b)	3.00±0.52 (b)	2.92±0.61 (b)
C15:0	1.39±0.54 (b)	3.02±0.04 (a)	nd	1.07±0.09 (b)	nd
C16:0	122.37±17.82 (ab)	146.64±5.52 (a)	76.61±19.63 (c)	93.72±35.43 (bc)	65.34±8.88 (c)
C17:0	nd	nd	nd	0.64±0.08	nd
C18:0	63.50±7.33 (ab)	74.47±6.74 (a)	40.6±10.39 (c)	46.42±16.79 (bc)	29.20±1.13 (c)
C18:1n9	27.17±9.00	21.14±1.73	26.69±1.70	25.65±1.33	25.36±1.79
C18:2n6	26.18±2.42 (b)	27.32±1.42 (ab)	30.35±3.99 (ab)	31.99±1.79 (a)	30.19±1.28 (ab)
C18:3n3	6.25±0.75	7.60±1.67	6.91±1.48	7.25±0.16	7.30±0.66
C20:0	10.70±0.32 (ab)	9.87±1.25 (b)	11.95±0.55 (ab)	12.37±1.34 (a)	12.56±0.91 (a)
C20:1	2.15±0.58 (a)	2.47±0.31 (a)	nd	1.77±0.13 (b)	1.71±0.06 (b)
C20:2	19.57±0.68 (b)	20.5±2.88 (ab)	20.28±1.01 (ab)	22.90±0.06 (a)	23.07±0.93 (a)
C22:0	3.24±0.14 (ab)	2.98±0.27 (b)	3.70±0.18 (a)	3.61±0.43 (a)	3.68±0.26 (a)
C22:2	76.43±4.03 (ab)	73.9±9.32 (b)	75.3±0.68 (b)	84.32±3.29 (ab)	86.46±3.7 (a)
C16:0**	6.42±0.68 (a)	1.03±0.22 (b)	nd	nd	nd

(*continuation*)

Compounds	CA	CA+1-MCP	DCA-CF	DCA-RQ1.5	DCA-CD1.3
C18:0**	4.04±0.70 (a)	2.77±0.18 (b)	1.90±0.14 (bc)	1.7±0.55 (c)	1.04±0.10 (c)
			Alcohols		
1-Hexacosanol	4.75±1.63	5.15±1.16	6.20±0.35	6.72±0.88	6.62±0.51
1-Octacosanol	11.03±5.24 (b)	14.89±2.33 (ab)	17.69±0.41 (ab)	14.27±5.52 (ab)	19.02±0.27 (a)
10-Nonacosanol	1.20±0.14 (a)	0.86±0.06 (b)	0.81±0.24 (bc)	0.52±0.07 (cd)	0.33±0.04 (d)
Phytol	13.21±0.47	11.32±1.70	13.07±0.32	10.92±3.72	14.33±0.24
			Aldehydes		
Heptadecanal	3.37±0.2 (a)	2.84±0.17 (ab)	2.83±0.42 (ab)	2.88±0.18 (ab)	2.69±0.26 (b)
Octadecanal	0.27±0.02 (a)	0.21±0.03 (ab)	0.20±0.05 (ab)	0.25±0.05 (ab)	0.19±0.01 (b)
			Hydrocarbons		
Docosane	20.58±0.94 (bc)	20.03±1.05 (c)	22.21±0.61 (ab)	23.92±0.54 (a)	23.65±0.76 (a)
Nonacosane	117.69±5.98 (a)	100.39±2.57 (c)	103,11±5.95 (bc)	108.10±5.64 (abc)	111.48±4.39 (ab)
2-Methylhexacosane	2.15±0.09 (a)	1.89±0.10 (b)	2.1±0.17 (ab)	2.13±0.06 (ab)	2.23±0.11 (a)
			Triterpenic acids		
Oleanolic Acid	0.69±0.08 (c)	1.68±0.31 (b)	1.76±0.46 (ab)	0.96±0.26 (bc)	2.64±0.73 (a)
Ursolic Acid	4.41±0.34	4.52±0.74	5.36±0.13	4.20±1.62	5.71±0.06

*Mean±standard deviation values followed by the same lower case letter in the same line did not differ significantly by Tukey's multiple range test ($P<0.05$). **Free fatty acid. nd.: not detected.

5.1.3.3. Chemometric analysis

The correlation between treatments and variables was explored by chemometrics analysis by a principal component analysis (PCA), hierarchical cluster analysis (HCA) and, heatmap (Fig. 2), According to PCA analysis, it was possible to separate along PC 1 the fruit stored under CA, either without or with 1-MCP treatment from those under DCA, manly, DCA-RQ, and DCA-CD (Fig. 2A). This different response of fruit stored under CA and DCA is closely correlated to higher ethylene production, respiration rate, decay incidence, total wax, and the highest values of color parameters a*, b*, and C, and this information is observed also in HCA and heatmap (Fig. 2C). Fatty acids C18:0 and C16:0, free and esterified and the alcohol 10-nonaconasol were also correlated with apples stored in CA (Fig. 2B and 2C). On the other hand, fruit stored under DCA-RQ and DCA-CD were correlated to a higher concentration of the volatile metabolites of anaerobic metabolism, fatty acids C20:0, C20:2, C22:2, and C18:2n6, alcohols 1-octacosanol and 1-hexacosanol, ursolic acid and oleanolic acid triterpenoids, and the highest values of color parameters L* and Hue (Fig 2B and 2C). The PC 2 was able to discriminate DCA-RQ from others DCA, as it was highly correlated with volatile metabolites from anaerobic metabolism. Additionally, it was possible to discriminate the CA treatments (with and without 1-MCP application), when CA were highly correlated with the higher ethylene production and respiration rate, as well as the higher decay incidence. Apples stored in CA+1-MCP had higher accumulation of esterified fatty acids C18:0, C16:0, C14:0, C15:0 and, C20:1.

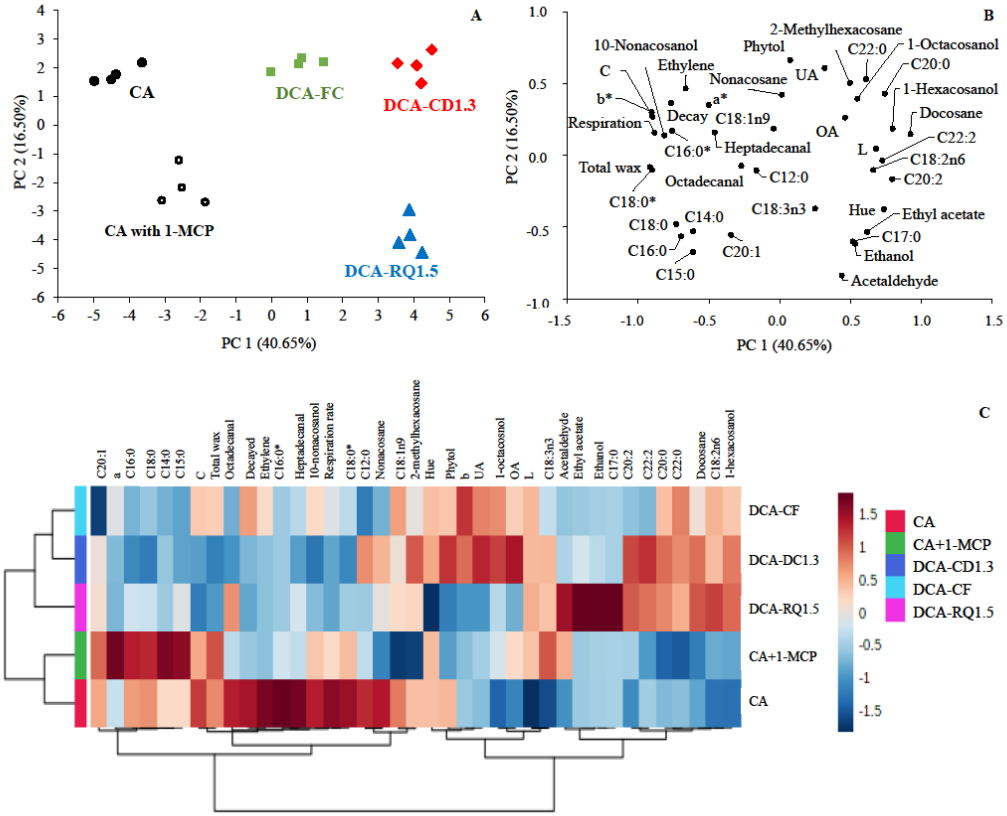

Figure 2. Principal component analysis (PCA), hierarchical cluster analysis (HCA) and, heatmap of the metabolism, overall quality, anaerobic metabolites accumulation, total wax, and wax compounds of 'Elstar' apples after 9 months of storage under controlled atmosphere (CA), either without or with 1-MCP treatment (0.650 µL L^{-1}), and dynamic controlled atmosphere monitored by chlorophyll fluorescence (DCA-CF), by respiratory quotient (DCA-RQ) and by carbon dioxide (DCA-CD), plus 7 d of shelf life at 20°C. Abbreviation: UA: ursolic acid; OA: oleanolic acid.

5.1.4. Discussion

Low-oxygen treatments have been linked to reduced ethylene levels by limiting 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase activity (Yip et al., 1988) or by promoting the production of volatiles compounds from fermentation (Bai et al., 2004; Pesis, 2005). The accumulation of the anaerobic metabolism compounds is associated with the inhibition of the electron transport chain by the low pO_2, which is insufficient for the aerobic metabolism of the fruit, thus, there is an accumulation of pyruvate that is rapidly converted, mainly, to acetaldehyde, as an alternative to the production of energy (Ke et al., 1994). In this case, the incidence of decay was higher in fruit that did not accumulate acetaldehyde, ethanol, and ethyl acetate, so the low pO_2 (0.15 kPa) promoted by DCA-RQ may have influenced the maintenance of quality. The products of anaerobic metabolism, are able to reduce ethylene synthesis, which delays ripening and in turn results in less decay incidence (Pesis et al., 2010; Weber et al., 2020, 2016). Thewes et al. (2021) when storing 'Royal Gala' apples at 0.15 kPa static O_2, showed whereas this condition contributed to increasing the decay incidence after storage. They results do not corroborate those obtained in this work, evidencing that dynamic storage benefits the accumulation of compounds of anaerobic metabolism that is not harmful to the fruit (Table 1 and 2). In addition, the low pO_2 suppressed ethylene production, corroborating previous studies (Schmidt et al., 2020; Thewes et al., 2015; Thewes et al., 2021), which is associated with low oxygen-dependent ACC oxidase activity, a key enzyme of ethylene metabolism (Asoda et al., 2009). The ethylene reduction was also efficient with the application of 1-MCP in CA (Table 1), equaling the effect with the DCA treatments, behavior also observed by Both et al. (2018) for Galaxy apples. In the case of 1-MCP, it affects ACC oxidase activity by reducing the expression of key genes, such as *MdACO1* and *MdACS1* (Tatsuki et al., 2007; Wakasa et al., 2006; Yang et al., 2013). A high rate of ethylene production generally induces a high rate of respiration during ripening (Pre-Aymard et al., 2003). The gas concentration of the storage atmosphere in CA (1.2 kPa O_2 + 2.0 kPa CO_2) for 'Elstar' apples was not able to suppress the general metabolism of the fruit, and consequently had a higher decay incidence (Table 1).

In the process of fruit ripening, one of the main phenomena is characterized by degreening, which is due to the degradation of chlorophyll (Chl) (Roongruangsri, Rattanapanone et al., 2013) triggered by a multistep enzymatic process (Christ & Hortensteiner, 2014). The ethylene could affect Chl degradation during fruit senescence (Feng et al., 2000), and it plays an essential role in regulating climacteric fruit ripening (Yang et al., 2013).

Furthermore, data have shown that 1-methylcyclopropene (1-MCP), reduces the chlorophyllase activity and causes retention of the green peel colour in vegetables and fruit (Gong and Mattheis, 2003, Hershkovitz et al., 2005). In this study, of the color parameters that were influenced by the storage conditions, b*, C and Hue^{o}, it is possible to make a relationship with a delay in the degreening in the conditions of DCA-CD and RQ, without differences between them. This may be related to an undetectable ethylene production of these treatments that would be crucial to trigger the enzymatic process involved in the degradation of Chl, according to the evidence cited above. The CA condition presented more yellow fruit, with the color angle dangling to yellow with more vivid saturation (Table 2), corroborating with the highest levels of ethylene and activation the general metabolism of the fruit. Degreening of 'Fuji' apples was inhibited by 1-MCP (Fan et al., 1999), and 1-MCP-treated 'Red Chief' apples had a greener background color than untreated fruit, corroborating with the results of this study (Table 2). Although the fruits treated with 1-MCP did not show any difference in relation to the DCA-CF, the other tested DCA had a greener background color. Therefore, DCA-RQ and CD are more efficient in delaying the senescence of stored fruits than those treated with 1-MCP. In this case, both had undetectable ethylene production, thus, there may be a relationship between compounds of anaerobic metabolism with the delay in the chlorophyll degradation process or these, more efficiently inhibiting ethylene production.

The content of cuticular wax in the apple increases during fruit development and storage (Baker, 1963; Lara et al., 2019). Changes in wax development were associated with ethylene production during the ripening of 'Delicious' apples, indicating that the increase in wax production coincided with the climacteric peak, with the apex of ethylene production (Ju and Bramlage, 2001). Tessmer et al., (2012) observed more evident changes in the fruit stored in CA. When evaluating the cuticle of 'Gala' and 'Galaxy' apples after 120 days of storage in a regular atmosphere (0 ºC and 90% RH) and CA (1.5% O_2, 2.5% CO_2, 0 ºC and 90 % RH), these authors associated with the reduction of the respiratory process, by changes in the chamber gas composition and reducing ethylene production during storage. The lowest wax content found in this study were in apples stored in DCA-RQ and DCA-CD (Table 4), where ethylene production was not detected, corroborating with the aforementioned statements. In addition, CA fruit treated with 1-MCP were equal in relation to the wax content of untreated fruit, contrary to what has been demonstrated on the effect of 1-MCP on cuticular wax. 'Autumn Gold' and 'Royal Gala' apples treated with 1-MCP and stored under refrigeration for 6 months had a delay in the development of some constituents of the wax (Curry, 2008). Dong et al. (2012) pointed to a decrease in nonacosane and an increase in 10-nonacosanol levels during the

storage of ‘Red Fuji’ apples treated with 1-MCP compared to untreated controls. In ‘Starkrimson’ apples, ethephon (synthetic product precursor of ethylene) and 1-MCP, increased and delayed the accumulation of cuticular waxes, respectively, during cold storage (Li et al., 2017). Klein et al. (2020b) showed that ethylene directly influences the alcohol synthesis of cuticular wax when assessing cuticular wax changes in ‘Maxi Gala’ apples stored in DCA with 1-MCP. However, 1-MCP had a low effect on the wax composition of apples when associated with DCA, with very specific changes such as a lower accumulation of α-farnesene (Klien et al., 2020b). Apparently, different cultivars, even when submitted to the same storage conditions, do not show common behavior for changes in the composition of wax.

Wax biosynthesis and accumulation are influenced by several abiotic factors, such as light, temperature, heat, and humidity (Weng et al., 2010). The synthesis of wax compounds occurs, firstly, by fatty acid synthase (FAS) complex that generates pool of malonyl CoA and fatty acids (C16-C18). The multienzyme fatty acid elongase (FAE) complex elongates the C16-C18 fatty acids to C24-C36, the very-long chain fatty acids (VLCFAs). Thus, the derivatives of VLCFA, are synthesized by two main pathways: alcohol formation pathway and alkane formation pathway (Kunst and Samuels, 2003). The main key genes involved in the biosynthesis of apple wax have been described, namely *MdCER4*, *MdWSD1* (both involved in the formation of alcohol), *MdCER6* (important for the synthesis of many long-chain fatty acids), and in some apple cultivars may be under ethylene control (Li et al., 2019). Based on this, the majority of wax compounds (Fig. 2), mainly fatty acids free or esterified may be related to the variables of higher ethylene production, respiration rate, in general, with the activation of the fruit metabolism after storage in CA. Veraverbeke et al. (2001) suggested that when biosynthesis of fatty acids occurs, C16:0 and C18:0 accumulate in the apple cuticular wax, especially during fruit ripening. Hence, this may explain the higher concentration of these precursor compounds, because the pO_2 in the CA is greater than the LOL of the fruit, this treatment accelerates the ripening of the fruit compared to other treatments.

Regarding DCA, mainly DCA-CD, which is the most innovative treatment in this study, it was not possible to relate the wax compounds directly with any of the quality parameters evaluated. The merit of the DCA-RQ and DCA - CD treatments for maintaining the quality of apples was more strongly linked to suppressing the general metabolism of the fruit and, consequently, reducing the decay incidence (Table 1). Among them, the wax composition was quite similar (Table 4). While for Klein et al. (2020a), triterpenoids, such as ursolic acid and oleanolic acid, were higher in CA, alcohols like 10-nonacosanol were higher in DCA-RQ for ‘Cripps Pink’ apples, the opposite was determined in this study. Thus, once again we can infer

these differences to the specificities of each cultivar. The difference between these two methods was more strongly correlated to the compounds of anaerobic metabolism (Table 3 and Fig. 2), which was significantly greater for DCA-RQ with a very small difference in relation to pO_2 (0.03 kPa) (Fig. 1). In this case, it is noted that DCA-CD was able to accurately measure the LOL, configuring a safe alternative to DCA-RQ. DCA-CF was an intermediate treatment with regard to quality, it was effective in reducing the overall metabolism of the fruit but still less effective in maintaining quality than the other DCA and 1-MCP treatments.

5.1.5. Conclusion

The storage under DCA, mainly DCA-RQ and DCA-CD, or CA + 1-MCP blocks ethylene production and delays fruit ripening, maintaining better quality of fruit. Lower overall metabolism in DCA stored fruit resulted in lower decay incidence and was associated with a greater accumulation of anaerobic metabolism compounds, especially under DCA – RQ and DCA – CD. Regarding color parameters, storage under DCA – RQ and DCA – CD maintained greener skin color when compared to all other storage conditions, showing that these conditions were able to delay ripening.

DCA-RQ and DCA-CD were the treatments with the lowest wax levels. These treatments showed similarity in the chemical composition of wax. Fruit stored in CA showed higher concentrations of most of the wax compounds identified, mainly of the fatty acid fraction. The quality parameters of ‘Elstar’ apples after storage were not directly correlated with the wax composition, but with the effect of this storage on the general metabolism of the fruit.

This and other works that relate the cuticular wax to the general quality of the fruit are important to provide information about the specificities of a cultivar against certain storage conditions. However, it is not yet clear whether there is a direct relationship of wax compounds on the quality of stored fruit, especially under low pO_2 conditions. Investigations in which enzymatic responses or specific genes to wax biosynthesis could be explored possibly would further clarify the functional significance of this cuticular layer.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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6. DISCUSSÃO GERAL

A maçã (*Malus* x *domestica* Borkh.), além da sua perecibilidade relativamente alta, é um produto suscetível a distúrbios de armazenamento, principalmente durante o armazenamento à longo prazo. Para evitar perdas pós-colheita e aumentar a comercialização durante o período entressafra, a fruta é mantida sob diferentes condições de armazenamento, principalmente AC. Com o passar do tempo, novas tecnologias foram desenvolvidas para manter e estender a qualidade de maçãs durante o período pós-colheita. Baseadas no ajuste das condições de armazenamento em função do metabolismo da própria fruta, as técnicas para o monitoramento desse armazenamento, como ACD monitorada pela detecção da produção de etanol, ACD-FC, ACD-QR, têm sido investigadas e aprimoradas. Além disso, torna-se possível o desenvolvimento de novas tecnologias, como ACD-DC, capazes de minimizar algumas das falhas das técnicas já existentes.

Parâmetros de qualidade como firmeza de polpa, metabolismo geral da fruta, acidez total titulável, sólidos solúveis totais, perfil de compostos voláteis, incidência à podridão, distúrbios fisiológicos entre outros, são comumente medidos e avaliados em maçãs submetidas ao armazenamento com as tecnologias mencionadas anteriormente. Os efeitos das condições desse armazenamento, principalmente a concentração gasosa do meio, sobre esses parâmetros avaliados ditam a eficácia do armazenamento na manutenção da qualidade de maçãs. Entretanto, algumas características das frutas, até então negligenciadas, têm ganhado espaço nas investigações científicas. Desta forma, um tópico que desperta interesse é o papel da cutícula na qualidade pós-colheita das frutas. Cada vez mais, um volume crescente de dados de pesquisas mostra a influência moduladora dessa camada cuticular sobre uma série de características importantes que determinam a vida útil e o potencial de armazenamento de frutas.

O conhecimento sobre o armazenamento de maçãs, especialmente em atmosfera controlada dinâmica, tem sido extensivamente explorado no que diz respeito à manutenção da qualidade, especialmente de maçãs. Embora haja diversos estudos sobre este campo, nenhum avaliou os efeitos desse armazenamento dinâmico sobre a cutícula da fruta e sua relação com outros parâmetros de qualidade. Diante disso, nesta tese estão apresentados os três estudos que objetivaram preencher as lacunas do conhecimento sobre as alterações dessa camada cuticular de maçãs armazenadas em sistemas de atmosfera controlada dinâmica e sua relação com a manutenção da qualidade durante o período pós-colheita.

No primeiro artigo foram avaliados os efeitos da AC, ACD-FC e ACD-QR em dois níveis – 1,3 e 1,5 – no metabolismo, incidência de podridão, concentração e composição química da cera da casca de maçãs ‘Cripps Pink’ após o armazenamento, durante a vida de prateleira. A cultivar ‘Cripps Pink’ é caracterizada por desenvolver uma superfície bastante oleosa durante o amadurecimento e especialmente, durante o armazenamento (YANG et al., 2017b, 2017a). Esse trabalho comprovou que o armazenamento em ACD–-QR, empregando níveis extremamente baixos de O_2 (0,13 – 0,21 kPa), diminuiu a incidência de oleosidade após armazenamento de longo prazo apesar do aumento do conteúdo total de cera durante a vida de prateleira. Além disso, a oleosidade desenvolvida em maçãs armazenadas em AC ou ACD-FC foi relacionada com uma composição de cera mais rica de ésteres, ácidos triterpênicos ursólico e oleanólico e ácido palmítico. Também foi comprovado que armazenamento de maçã em AC e ACD por longo prazo afeta a composição da cera da casca. Estudos, mostram que a redução do processo respiratório, pela redução dos níveis de O_2 no armazenamento implicam na redução da produção de etileno (CURRY, 2008; LI et al., 2017; TESSMER; ANTONIOLLI; APPEZZATO-DA-GLÓRIA, 2012). A biossíntese de cera é um processo dependente do etileno e a síntese dos principais componentes da cera pode ser suprimida pela menor atividade respiratória, ocasionando redução na formação de ceras. Portanto, condições de armazenamento que alterem essa dinâmica do processo respiratório e produção de etileno auto catalítico de frutas, terão influência na formação e composição de cera.

Baseado nesses resultados, o segundo artigo buscou explicar a interação entre o armazenamento dinâmico com o uso do 1-MCP em maçãs ‘Maxi Gala’ armazenadas em AC, ACD-FC e ACD-QR em dois níveis – 1,3 e 1,5. A influência do 1-MCP sobre a camada cuticular já tem sido relatada. Maçãs ‘Autumn Gold’, ‘Royal Gala’ e ‘Starkrimson’ tratadas com 1-MCP e posteriormente submetidas à refrigeração tiveram atraso no desenvolvimento de alguns constituintes da cera (CURRY, 2008; LI et al., 2017). 1-MCP inibiu a expressão dos principais genes envolvidos na biossíntese de cera (LI et al., 2019). Entretanto, os efeitos da interação da ACD com 1-MCP sobre a composição de cera e sua relação com a qualidade de maçãs, ainda não havia sido relatado. Os resultados mostraram que o 1-MCP foi eficaz na redução da produção de etileno para os tratamentos AC e ACD-FC, enquanto que não teve efeito na produção de etileno para os tratamentos ACD-QR1.3 e QR1.5. Em relação a composição de cera, o 1-MCP teve um baixo efeito na composição da cera quando associado à ACD e não teve influência sobre o teor de cera para nenhum dos tratamentos. Questiona-se sobre a possibilidade de que, apesar da produção exógena de etileno ser suprimida pelo 1-MCP, e isso foi observado em nosso estudo, o etileno endógeno ainda pode ser suficiente para a

expressão de genes relacionados à síntese de cera. BOTH et al. (2018) mostraram que, mesmo com baixa produção de etileno de maçãs ‘Galaxy’ tratadas com 1-MCP, a fruta ainda tinha IEC maior do que AC após 9 meses de armazenamento em DCA-RQ. Ou ainda, algumas cultivares de maçã parecem ser capazes de regenerar sítios ou que a ligação com o 1-MCP é incompleta. Maçãs ‘McIntosh’, por exemplo, podem precisar de maiores concentrações de 1-MCP, talvez porque esta cultivar produz grandes quantidades de etileno (WATKINS; NOCK; WHITAKER, 2000).

Alguns autores correlacionaram que os alcanos da cera cuticular são essenciais para manter o peso da maçã durante o armazenamento (CHAI et al., 2020). Em nosso estudo, a perda de peso foi relacionada às frutas tratadas com 1-MCP, especialmente aquelas armazenadas em DCA, e nas frutas desse tratamento os alcanos foram inversamente correlacionados com a perda de peso e suculência, corroborando com as afirmações anteriores. AC apresentou as maiores diferenças na composição da cera em comparação com as outras condições de armazenamento tratadas com 1-MCP e isso pode estar correlacionado diretamente com a influência do etileno na síntese da cera cuticular.

Por fim, o terceiro artigo apresentado nesta tese comparou os efeitos da nova tecnologia de armazenamento, ACD-DC com outras técnicas de ACD já consolidadas, como ACD-FC e ACD-QR além de AC e AC+1-MCP para o armazenamento de maçãs ‘Elstar’. O novo método de ACD apresentou resultados similares de concentração e composição de cera ao armazenamento em ACD-QR. Foi eficiente em reduzir o metabolismo da fruta, sendo observado pelos parâmetros de qualidade, que mais uma vez, foram correlacionados fortemente com a ação do etileno, ou a ativação do metabolismo anaeróbico da fruta pela baixa pO_2 empregada do que sobre um composto de cera especificamente.

A partir dos resultados apresentados nesta tese, é possível verificar a influência das diferentes técnicas de ACD na concentração e na composição química da cera da casca de maçãs ‘Cripps Pink’, ‘Maxi Gala’ e ‘Elstar’, bem como a sua relação com a manutenção da qualidade de maçãs após longos períodos de armazenamento. Apesar das especificidades de cada um dos estudos, existe uma similaridade quanto ao perfil qualitativo da composição das ceras, sendo distribuídos os compostos entre ácidos graxos (presentes majoritariamente esterificados), os alcanos, especialmente nonacosano e os ácidos triterpênicos.

A maior parte dos estudos publicados sobre cera cuticular de frutas indicam que as cutículas das frutas continuam evoluindo após a colheita. E os resultados apresentados nesta tese corroboram com essa informação. No entanto, não é possível estabelecer um padrão de alteração comum esperado para diferentes cultivares submetidas à mesma condição de

armazenamento. Assim, torna-se necessário mais investigações sobre as especificidades dessa camada cuticular capazes de levar a uma melhor compreensão dos mecanismos subjacentes à grande variação do potencial pós-colheita de maçãs frente às novas tecnologias de armazenamento.

7. CONSIDERAÇÕES FINAIS

Os efeitos do armazenamento de longo prazo sob métodos ACD na composição química da cera de casca de maçã e sua relação com a qualidade pós colheita foram avaliado.

Em maçãs ‘Cripps Pink’ armazenadas em AC, ACD-FC e ACD-QR e avaliadas após 8 meses mais 14 dias de vida de prateleira, os ácidos graxos, triterpenóides e n-alcanos foram os compostos de cera cuticular predominantes.

A concentração de cera aumentou durante a vida de prateleira quando as maçãs ‘Cripps Pink’ foram armazenadas em ACD, embora frutas armazenadas sob ACD-QR não desenvolveram oleosidade na epiderme mesmo após 8 meses de armazenamento mais 14 dias a 20 °C.

Compostos como ésteres graxos, ácido ursólico, ácido oleanólico e ácido palmítico foram os principais compostos químicos da cera de maçãs ‘Cripps Pink’ armazenadas em AC e que podem estar correlacionados com o desenvolvimento da oleosidade na epiderme.

ACD-QR1,3 para ‘Cripps Pink’ apresentou a menor taxa respiratória, evidenciando um menor metabolismo e corroborando com o maior número de frutos sadios e consequentemente uma melhor condição de armazenamento.

Condições de armazenamento de maçãs ‘Cripps Pink’ sob ACD, principalmente o ACD-QR pode ter favorecido mecanismos de adaptação à baixa pO_2, induzindo a formação de compostos de cera específicos como 10-nonacosanol e octacosanol, que conferem à cutícula uma estrutura cristalina.

A interação do uso do 1-MCP e da ACD na composição da cera cuticular de maçãs ‘Maxi Gala’ mostrou que o 1-MCP foi eficaz na redução da produção de etileno em AC, embora ACD, especialmente ACD-QR, também tenha se mostrado eficiente na redução do metabolismo geral da fruta. As atmosferas de armazenamento e o tratamento com 1-MCP, para essa cultivar, não influenciaram o teor total de cera. As composições de cera das maçãs armazenadas em ACD foram semelhantes quando o 1-MCP foi aplicado. No entanto, o tratamento com 1-MCP resultou em uma maior perda de massa nos frutos armazenados em ACD.

Para maçãs ‘Elstar’, o armazenamento sob ACD-QR e ACD-DC, ou AC + 1-MCP reduziu a produção de etileno e retardou o amadurecimento dos frutos. O metabolismo geral mais baixo nas frutas armazenadas com ACD resultou menor incidência à podridão e foi associado a um maior acúmulo de compostos do metabolismo anaeróbio, especialmente em

ACD – QR e ACD - DC. As condições de armazenamento com menos pO_2 resultaram em frutos coloração da epiderme mais verde quando comparados ao CA.

ACD-QR e ACD-DC foram os tratamentos com os teores de cera mais baixos. Esses tratamentos apresentaram similaridade na composição química da cera. Frutas armazenadas em AC apresentaram maiores concentrações da maioria dos compostos de cera identificados, principalmente da fração de ácidos graxos.

Os parâmetros de qualidade das maçãs ‘Elstar’ após o armazenamento não foram diretamente correlacionados com a composição da cera, mas sim com o efeito deste armazenamento sobre metabolismo geral da fruta.

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