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Fabio Rodrigo Thewes

ATMOSFERA CONTROLADA DINÂMICA MONITORADA PELO QUOCIENTE RESPIRATÓRIO E SUA INTERAÇÃO COM ESTÁDIOS DE MATURAÇÃO SOBRE A CONSERVAÇÃO DA QUALIDADE E O PERFIL VOLÁTIL DE MAÇÃS 'GALAXY'

Santa Maria, RS
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Dissertação apresentada ao Curso de Pós-Graduação em Agronomia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestre em Agronomia.**

Orientador: Prof. Dr. Auri Brackmann

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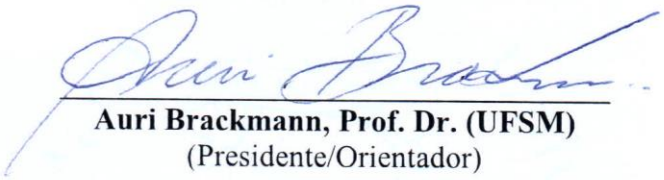

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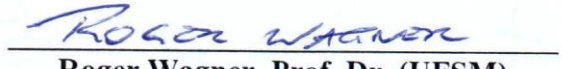

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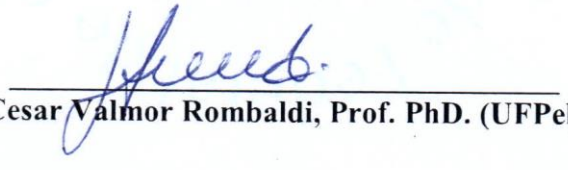

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em especial aos meus pais Roque e Marieta Thewes pela educação, apoio e exemplo de vida e
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RESUMO

ATMOSFERA CONTROLADA DINÂMICA MONITORADA PELO QUOCIENTE RESPIRATÓRIO E SUA INTERAÇÃO COM ESTÁDIOS DE MATURAÇÃO SOBRE A CONSERVAÇÃO DA QUALIDADE E O PERFIL VOLÁTIL DE MAÇÃS 'GALAXY'

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ORIETADOR: Auri Brackmann

A colheita de maçãs no estádio de maturação correto para longos períodos de armazenamento é uma tarefa cada vez mais complicada, especialmente em função da falta de mão-de-obra. Em função disso, o desenvolvimento de técnicas de armazenamento que possibilitem o armazenamento de frutas colhidas fora do estádio de maturação adequado é necessário. Nesse sentido, na presente dissertação, buscou-se avaliar uma nova técnica de armazenamento em atmosfera controlada dinâmica baseada no monitoramento do limite mínimo de O_2 (LMO) pelo quociente respiratório (ACD-QR) e comparar com a da atmosfera controlada convencional (AC) e a combinação de AC com aplicação de 1-metilciclopropeno (AC + 1-MCP) sobre a qualidade física, química e o perfil volátil de maçãs 'Galaxy' colhidas em três estádios de maturação (verde, madura e sobremadura). A dissertação é composta de dois capítulos (artigos): o primeiro direcionado à avaliação do metabolismo e da qualidade física e química e o segundo ao metabolismo e à composição volátil. Frutos dos três estádios de maturação armazenados ACD-QR apresentaram menor atividade da enzima ACC oxidase, etileno interno, produção de etileno e respiração em comparação aos frutos armazenados em AC. O armazenamento em ACD-QR 1,3 resultou em manutenção da qualidade similar ao armazenamento em AC + 1-MCP, independentemente do estádio de maturação. Menor incidência de polpa farinácea e degenerescência de polpa foi verificada em frutos armazenados em ACD-QR e AC + 1-MCP. A firmeza de polpa foi maior em frutos armazenados em ACD-QR 1,3, quando colhidos verdes, maior em AC + 1-MCP, ACD-QR 1,3 e ACD-QR 1,5 em frutos maduros e maior em AC + 1-MCP em frutos colhidos sobremaduros. O armazenamento em ACD-QR 1,5 resultou em acúmulo de acetaldeído, etanol e acetato de etila, porém numa concentração que não causa aumento no extravasamento de eletrólitos e distúrbios fisiológicos relacionados à fermentação, como a degenerescência de polpa. A redução do O_2 a pressões parciais extremamente baixas, pelo armazenamento em ACD-QR 1,5, resultou em frutos com maior concentração de compostos voláteis que compõe o aroma, independentemente do estádio de maturação, em comparação aos frutos armazenados em AC. Adicionalmente, esse incremento na produção de compostos voláteis não ocorreu apenas em forma de acetatos de etila, mas também na forma de ésteres característicos de maçãs 'Galaxy', como o acetato de butila, acetato de hexila e acetato de 2-metilbutila. Com a aplicação de 1-MCP houve redução na produção de aroma em comparação a AC sem uso do produto, também não houve incremento na produção de ésteres com o avanço da maturação nos frutos tratados com 1-MCP, resultando em frutos com concentração de ésteres similar entre os três estádios de maturação. Levando em consideração a qualidade física, química e a produção de compostos voláteis, as melhores condições de armazenamento para todos os estádios de maturação segue a seguinte ordem: ACD-QR 1,5 > ACD-QR 1,3 > AC + 1-MCP > AC.

Palavras-chave: *Malus domestica*, etileno, desordens fisiológicas, firmeza de polpa, metabolismo anaeróbico.

ABSTRACT

DYNAMIC CONTROLLED ATMOSPHERE MONITORED BY RESPIRATORY QUOTIENT AND ITS INTERACTION WITH THE MATURITY STAGES ON QUALITY AND VOLATILE PROFILE CONSERVATION IN 'GALAXY' APPLE

AUTHOR: Fabio Rodrigo Thewes
ADVISOR: Auri Brackmann

Harvesting the apple at the correct maturity stage for long-term storage is becoming more and more difficult, especially due to the lack of manual labor. Thereby, the development of storage techniques that allows the storage of fruit harvested outside the correct maturity is necessary. Thus, on the present dissertation we aimed to evaluate a new dynamic controlled atmosphere storage technique based on the lower oxygen limit (LOL) monitoring by the respiratory quotient (DCA-RQ) and compare it with controlled atmosphere (CA) and the combination of CA with 1-methylcyclopropene application (CA + 1-MCP) on the physical, chemical quality and volatile profile of 'Galaxy' apple harvested in three maturity stages (unripe, ripe and overripe). Two chapters composed the dissertation: the first is directed to evaluate the metabolism, physical and chemical quality and the second to evaluate the metabolism and volatile profile. Fruit of the three maturity stages stored under DCA-RQ showed lower ACC oxidase enzyme activity, internal ethylene, ethylene production and respiration rate as compared to fruit stored under CA. The storage under DCA-RQ 1.3 resulted in similar quality keeping as compared to CA + 1-MCP. Lower mealiness incidence and flesh breakdown were observed in DCA-RQ and CA + 1-MCP stored fruit. Higher flesh firmness was verified in DCA-RQ 1.3, if fruit were harvested unripe, higher in CA + 1-MCP, DCA-RQ 1.3 and DCA-RQ 1.5 in ripe harvested fruit and higher in CA + 1-MCP overripe harvested apple. The storage under DCA-RQ 1.5 resulted in acetaldehyde, ethanol and ethyl acetate accumulation, but at a concentration that did not increase the electrolyte leakage and physiological disorders related to fermentation, like flesh breakdown. Oxygen lowering down to extremely low partial pressure, by the storage under DCA-RQ 1.5, resulted in fruit with higher volatile compounds concentration that composed the aroma, regardless the maturity stage, as compared to fruit stored under CA. Additionally, the volatile compounds increase was not only observed for ethyl esters, but also in characteristic 'Galaxy' apple esters, like butyl acetate, hexyl acetate and 2-methylbutyl acetate. With the 1-MCP application was observed a reduction in the aroma production as compared to CA and not allowed the increment of esters concentration with the maturity advance, resulting in fruit with similar ester concentration on the three maturity stages. Taken in account the physical, chemical quality and the volatile compounds, the best storage conditions for all maturity stages follow this order: DCA-RQ 1.5 > DCA-RQ 1.3 > CA + 1-MCP > CA.

Keywords: *Malus domestica*, ethylene, physiological disorders, flesh firmness, anaerobic metabolism.

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LISTA DE ABREVIATURAS

1-MCP	1-metilciclopropeno/1-methylcylclopropene
2-OG	2-Oxoglutarate
AAT	Álcool Acetil Transferase
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
ACR	Advanced Control Respiration
ACD-FC	Atmosfera controlada dinâmica monitorada pela fluorescência de clorofilas
ACD-QR	Atmosfera controlada dinâmica monitorada pelo quociente respiratório
ADH	Álcool desidrogenase
ANOVA	Análise de Variância
ATP	Adenosina trifosfato
BCAT	Branched-chain amino acid aminotransferase
CA	Controlled atmosphere
C_2H_4	Fórmula molecular do Etileno
C_4H_6	Fórmula molecular do 1-MCP
CO_2	Dióxido de carbono (gás carbônico)
DCA	Dynamic controlled atmosphere
DCS	Dynamic Control System
DCA-CF	Dynamic controlled atmosphere – chlorophyll fluorescence
DCA-RQ	Dynamic controlled atmosphere – respiratory quotient
DNA	Deoxyribonucleic acid
FA	Fatty acids
FAD^+/FADH	Flavina adenina dinucleotídeo oxidada/ reduzida
FID	Flame ionization detector
FIRM	Fluorescence Interactive Response Monitor
FS	Fotossistema
G-6-P	Glucose-6-phosphate
GC-FID	Cromatógrafo a gás com detector por ionização em chama

GC-MS	Cromatógrafo a gás acoplado à espectrômetro de massa
GTP	Guanosine triphosphate
ha	Hectare
HS-SPME	Solid phase microextraction
IEC	Internal Ethylene Concentration
ILOs	Initila Low Oxygen Stress
kg	Quilograma
kPa	Kilopascal
LRI	Linear Retention index
LED	Light Emitting Diode
LMO/LOL	Limite mínimo de oxigênio/Lower oxygen limit
LOX	Lipoxigenase
$\mu L\ L^{-1}$	Microlitro por litro
µg	Micrograma
mL	Mililitro
MdACO1	Gene para ACC oxidase em maçã
MdACS1	Gene para ACC sintase em maçã
MdERS1/MdERS2	Genes para receptores de etileno em maçã
MpAAT1	Proteína da AAT1 em *Malus pumila*
mg	Miligrama
mL	Mililitro
mm	Milímetro
N	Newton ou Normal
N_2	Nitrogênio
NaCl	Cloreto de sódio
NAD^+/ NADH	Nicotinamida adenina dinucleotídeo oxidada/ reduzida
NADPH	Nicotinamida adenina dinucleotídeo fosfato reduzida
NaOH	Hidróxido de sódio
O_2	Oxigênio
OAA	Oxaloacetate
ºC	Temperatura em graus Celsius
PC	Principal Component
PCA	Ponto de compensação anaeróbica e Principal Component Analysis

PDC	Enzima piruvato descarboxilase
PDH	Piruvato desidrogenase
pH	Potencial hidrogeniônico
PQ	Plastoquinona
QR	Quociente respiratório
R-5-P	Ribulose-5-phosphate
RNA	Ribonucleic acid
RQ	Respiratory quotient
ROS	Espécies Reativas de Oxigênios
s	Segundo
TCA	Tricarboxylic acids cycle
ULO	Ultralow oxygen (ultrabaixo oxigênio)

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1. INTRODUÇÃO E JUSTIFICATIVA

A produção nacional de maçãs é concentrada em duas cultivares, Gala e Fuji, e suas respectivas mutantes (AGAPOMI, 2015). As mutantes apresentam qualidade visual superior às cultivares de origem, principalmente, em função do maior recobrimento da epiderme com coloração vermelha (BRACKMANN et al., 2009; WEBER et al.,2013). Entretanto, o uso de poucas cultivares e fatores intrínsecos da espécie, culminam em um curto período de colheita (janela de colheita) dos frutos.

O período ideal de colheita, para longos períodos de armazenamento, ocorre quando os frutos atingem níveis de iodo-amido entre 4 a 7 para 'Gala' e mutantes e 5 a 6 para 'Fuji' e mutantes, numa escala de 0 a 10 (STREIF, 1984). Porém, os frutos permanecem neste estádio de maturação por um período muito curto (de uma a no máximo duas semanas), o que torna praticamente inviável a colheita de todos os frutos no ponto ideal de maturação para longos períodos de armazenagem. Outro entrave da colheita é que a mesma é realizada manualmente, dificultando ainda mais a colheita no estádio de maturação correto. Em vista disso, a colheita é iniciada antes dos frutos atingirem o ponto de maturação ideal e vai até estádios de maturação avançados. Portanto, é de fundamental importância o desenvolvimento de técnicas de armazenamento para cada estádio de maturação.

Atualmente, a forma de armazenamento mais utilizada para o armazenamento de maçãs, independente do estádio de maturação, é a atmosfera controlada (AC) (BRACKMANN et al., 2012; WEBER et al., 2013), que consiste na redução da pressão parcial de O_2 e no aumento da de CO_2. Porém, nesse método de armazenamento não é possível monitorar o metabolismo dos frutos, em tempo real, para estabelecer a pressão parcial de O_2 e, por segurança, o oxigênio é mantido em um nível bem acima do limite mínimo tolerado pelos frutos (LMO) durante o armazenamento, o que não permite a máxima redução do metabolismo e, por consequência, a máxima manutenção da qualidade. No armazenamento em AC ocorrem significativas perdas em função da ocorrência de distúrbios fisiológicos, podridões, perda firmeza de polpa e outros atributos de qualidade. Assim, é necessário o desenvolvimento de uma técnica de armazenamento que detecte o LMO e assim possibilite o armazenamento em menores pressões parciais de O_2, adequando o nível de oxigênio a cada estádio de maturação para manter a qualidade dos frutos após o armazenamento.

Estudos realizados que avaliaram a interação de diferentes estádios de maturação e condições de armazenamento em AC verificaram que maçãs colhidas antes do estádio ideal apresentavam melhor manutenção da firmeza de polpa (ARGENTA; MONDARDO, 1994;

BRACKMANN et al., 2002, 2004), menor incidência de degenerescência de polpa, polpa farinácea (BRACKMANN et al., 2002) e suscetibilidade à ocorrência de podridões (ARGENTA; MONDARDO, 1994; BRACKMANN et al., 2002; VILANOVA et al., 2012, 2014). Entretanto, os frutos são mais suscetíveis à ocorrência de escaldadura (ARGENTA; MONDARDO, 1994), além de produzirem menos compostos voláteis (SONG; BANGERTH, 1996; BANGERTH; SONG; STREIF, 2012) e possuírem menor tamanho e, por consequência, menor produtividade.

Em contrapartida, quando os frutos são colhidos em estádios de maturação avançados ocorre rápida perda de firmeza de polpa (BRACKMANN et al., 2004), maior suscetibilidade a podridões (VILANOVA et al., 2014) e polpa farinácea (BRACKMANN et al., 2002) em função da maior produção de etileno (ARGENTA; MONDARDO, 1994; FELLMAN et al., 2003) e maior taxa respiratória durante armazenamento em AC (STEFFENS et al., 2007). Porém, os frutos mais maduros produzem mais compostos voláteis (SONG; BANGERTH, 1996; FELLMAN et al., 2003; BANGERTH; SONG; STREIF, 2012) e ocorre um aumento no recobrimento da epiderme com coloração vermelha (BRACKMANN et al., 2004), melhorando a qualidade organoléptica e visual dos frutos em relação àqueles colhidos antes do estádio ideal. Em função disso, torna-se necessário o desenvolvimento de tecnologias que permitem o armazenamento de maçãs colhidas em diferentes estádios de maturação reduzindo as perdas de qualidade ocasionadas pela redução da firmeza da polpa, ocorrência de podridões e distúrbios fisiológicos e manutenção do perfil volátil similar ao de entrada da câmara de armazenamento nos frutos colhidos em maturação avançada e aumento dos voláteis naqueles colhidos antes do período ideal.

Uma tecnologia passível de ser utilizadas para contornar esses problemas é o armazenamento dos frutos em atmosfera controlada dinâmica (ACD). Esse novo método de armazenamento, permite o monitoramento do limite mínimo de oxigênio tolerado pelos frutos durante o armazenamento e, assim, a redução dos níveis de oxigênio o mais baixo possível para aquele estado metabólico do fruto. Com isso, é possível a adequação das pressões parciais de O_2 durante o armazenamento para cada estádio de maturação ajustando a atmosfera da câmara ao metabolismo dos frutos. Atualmente, existem três métodos que permitem monitorar o LMO durante o armazenamento, baseados na produção de compostos relacionados à fermentação (etanol) (MARVIL, 2015; VELTMANN et al., 2003), fluorescência de clorofilas (DeELL et al., 1999; PRANGE et al., 2007; WRIGHT et al., 2010, 2012) e no quociente respiratório (ACD-QR) (BRACKMANN; WEBER; BOTH, 2015; GASSER et al., 2008; WEBER et al., 2015; WRIGHT et al., 2012). Dentre estes métodos de ACD, o mais eficiente na manutenção da

qualidade é o método baseado no quociente respiratório (DCA-QR) (BRACKMANN; WEBER; BOTH, 2015; WEBER, 2014), uma vez que este método permite induzir a fermentação dos frutos em níveis seguros, aumentando a produção de etanol e, consequentemente, reduzindo a produção de etileno e os eventos desencadeados pelo mesmo. De acordo com vários estudos, a aplicação de etanol reduz a produção de etileno (ASODA et al., 2009; JIN et al., 2013; LIU et al., 2012). Assim, é provável que para cada estádio de maturação haja um nível de quociente respiratório adequado, tornando necessárias pesquisas nesse sentido.

Tecnologias complementares à AC e a sua comparação ao armazenamento em ACD também necessitam de mais estudos para verificar sua eficiência nos diversos estádios de maturação dos frutos. Uma destas tecnologias complementares à AC é a aplicação de 1-MCP, que é um composto que inibe a ação do etileno (BLANKENSHIP; DOLE, 2003; WATKINS, 2006). Entretanto, atualmente há poucas informações na literatura da interação deste composto com diferentes estádios de maturação na colheita e a comparação de sua eficiência com a de uma tecnologia limpa sem uso de produtos químicos, como a ACD. Trabalhos realizados avaliado o retardo entre a colheita e a aplicação do 1-MCP, verificaram que a sua eficiência diminui com o avanço da maturação dos frutos (AMARANTE et al., 2010; THEWES, 2013; WATKINS; NOCK, 2005). A aplicação de 1-MCP em maçãs 'Royal Gala' sobremaduras (índice de iodo amido 8,53) não trouxe benefícios sobre a manutenção da qualidade após o armazenamento em AC e ultra baixo oxigênio (ULO) (BOTH et al., 2014), demonstrando que a aplicação de 1-MCP tem pouco efeito em frutos sobremaduros. Em vista disso, é importante avaliar o efeito do 1-MCP em frutos colhidos em diferentes estádios de maturação e compara-lo ao armazenamento em ACD, no qual as pressões parciais de O_2 são extremamente baixas e se adaptam ao metabolismo dos frutos reduzindo assim ao máximo a atividade metabólica.

A pesquisa de novas técnicas de armazenamento que possibilitem a manutenção da qualidade dos frutos após o armazenamento similar a colheita, independente do estádio de maturação dos frutos, necessitam ser testadas e comparadas a tecnologias já consolidadas em nível mundial como a AC e a sua combinação ao 1-MCP. Uma alternativa seria o armazenamento em ACD-QR, que é uma tecnologia limpa com amplo leque de aplicabilidade no armazenamento de maçãs**,** em função do monitoramento do LMO tolerado pelos frutos. Esta técnica permite aos armazenadores a determinação do metabolismo dos frutos em tempo real, adaptando o nível de O_2 à necessidade dos frutos durante o armazenamento em vez de usar um nível fixo de O_2, como utilizado no armazenamento em AC.

1.1. HIPÓTESE

Há interação entre o armazenamento em AC, AC + 1-MCP e ACD-QR com os estádios de maturação sobre a qualidade física e química de maçãs ‘Galaxy’;

Existe um nível de QR adequado para cada estádio de maturação de maçãs ‘Galaxy’;

A ACD pelo método QR mantém uma melhor qualidade dos frutos, em relação à AC, em todos os estádios de maturação;

A ACD-QR mantém a qualidade dos frutos similar ao armazenamento em AC + 1-MCP, tornando dispensável o uso de 1-MCP.

O armazenamento em ACD-QR permite manter a qualidade durante o armazenamento em maçãs colhidas em diferentes estádios de maturação.

Frutos armazenados em ACD-QR mantém maior concentração de compostos voláteis em comparação a AC e AC + 1-MCP, independentemente do estádio de maturação.

1.2. OBJETIVOS

Verificar a interação entre estádios de maturação, AC, AC + 1-MCP, ACD-QR 1.3 e ACD-QR 1.5 sobre o metabolismo e a manutenção da qualidade física e química de maçãs ‘Galaxy’ após 9 meses de armazenamento mais 7 dias de vida de prateleira a 20°C;

Estabelecer o melhor QR para cada estádio de maturação para o armazenamento de maçãs ‘Galaxy’ por longos períodos (9 meses);

Avaliar a eficiência do armazenamento em ACD-QR em comparação a AC e AC + 1-MCP em maçãs armazenadas sobre a produção de compostos aromáticos voláteis em maçãs ‘Galaxy’ colhidas em diferentes estádios de maturação.

2. REVISÃO DE LITERATURA

2.1. PRODUÇÃO BRASILEIRA DE MAÇÃS

O Brasil é um país autossuficiente na produção maçãs, realizando exportações para países da Europa no período da entressafra europeia (REENTZ et al., 2009). Porém, no ano de 2014 as exportações caíram 5,46% em função de problemas climáticos ocorridos no Brasil (REENTZ et al., 2015). A produção nacional de maçãs está localizada nos três estados do sul em função das necessidades climáticas. A produção somada destes estados corresponde a mais de 98% da produção nacional de maçãs, que é de 1,1 milhões de toneladas (AGAPOMI, 2015; REENTZ et al., 2015).

Desse montante, quase que a totalidade são das cultivares Gala e Fuji, com suas respectivas mutantes. Dentre esses dois grupos de cultivares, a 'Gala' e suas mutantes perfazem aproximadamente 60% da produção nacional (PETRI; HAWERROTH; LEITE, 2010). 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 torna mais atrativas ao consumidor. Atualmente, a instalação de pomares novos e a substituição de antigos é realizada com essas mutantes, mais especificamente em torno de 70 a 80% dos pomares com a mutante 'Galaxy' (*Informação pessoal*), o que a torna a mutante de 'Gala' mais produzida comercialmente no Brasil.

O uso de praticamente uma única cultivar em todos os pomares, resulta em problemas no manejo e na colheita dos frutos, uma vez que o período ideal de colheita ocorre em um curto período de tempo. Em função disso, a colheita dos frutos inicia antes do período ideal e se prolonga até os frutos atingirem maturação avançada, prejudicando o armazenamento destes frutos.

2.2. ESTÁDIOS DE MATURAÇÃO E SUA RELAÇÃO COM O ARMAZENAMENTO

O ponto de colheita é um dos fatores mais importantes na determinação da qualidade e do potencial de armazenamento de maçãs (ARGENTA; MONDARDO, 1994; BEAUDRY et al., 1993; BRACKMANN et al., 2002; LU et al., 2012). Entretanto, a realização da colheita no momento correto não é uma tarefa fácil para o fruticultor, uma vez que, os frutos permanecem nesse estádio de maturação por apenas um curto período de tempo e há desuniformidade de maturação entre frutos numa mesma planta. Outro fator que dificulta a colheita no estádio

correto é que a mesma é realizada manualmente, exigindo muita mão-de-obra num curto espaço de tempo.

Diversos estudos têm correlacionado o estádio de maturação do fruto na colheita e seu potencial de armazenamento em atmosfera controlada. Brackmann et al. (2004) verificaram que quando a colheita de maçãs 'Fuji' foi realizada após o estádio ideal, os frutos apresentavam elevada produção de etileno e taxa respiratória, o que pode ocasionar problemas durante o armazenamento. Dentre os principais problemas ocasionados pela colheita tardia está a elevada incidência de podridões e distúrbios fisiológicos, como degenerescência de polpa, polpa farinácea e rachaduras (ARGENTA; MONDARDO, 1994; BRACKMANN et al., 2002). Os frutos colhidos em estádios de maturação mais avançados possuem maior susceptibilidade à ocorrência de podridões pela menor capacidade de produção de metabólitos secundários como a lignina (CHÁVEZ et al., 2014; VILANOVA et al., 2012, 2014) e flavonoides (SAVIKIN et al., 2014), que conferem aos frutos resistência contra patógenos.

Apesar dos efeitos negativos do atraso na colheita sobre o potencial de armazenamento, ocorre um significativo aumento na produção de compostos voláteis, melhorando assim o aroma dos frutos (BANGERTH; SONG; STREIF, 2012; SONG; BANGERTH, 1996). Estes mesmos autores atribuem a maior produção de compostos voláteis à maior produção de etileno e taxa respiratória. Com o aumento da taxa respiratória ocorre um significativo aumento na produção de precursores de compostos voláteis, como os ácidos graxos (BRACKMANN; STREIF; BANGERTH, 1993; SONG; BANGERTH, 2003). Assim, frutos com maior taxa respiratória apresentam maior potencial de produção de precursores de compostos voláteis e, consequentemente, maior produção de aroma. Em contrapartida, frutos colhidos antes do estádio de maturação ideal, apresentam baixa capacidade de produção de compostos voláteis, em condições normóxicas. Porém, não foram encontrados estudos na literatura avaliando a emissão de compostos voláteis em maçãs de diferentes estádios de maturação armazenadas em ACD-QR, onde é possível a indução do metabolismo fermentativo dos frutos através do armazenamento em QR superior a 1,0.

Por outro lado, a colheita de frutos antes do estádio ideal resulta em frutos com menor tamanho, ou seja, menor produtividade e rentabilidade aos produtores, além de menor recobrimento da epiderme com coloração vermelha, reduzindo assim o seu potencial de comercialização (DE CASTRO; BIASI; MITCHAM, 2007; STANGER et al., 2013; WATKINS et al., 2005). Esses frutos de colheita precoce ainda não atingiram o pico climatérico e, por consequência, o amadurecimento é retardado durante o armazenamento, facilitando o armazenamento em AC. Porém, são mais suscetíveis à ocorrência de escaldadura superficial

(ARGENTA; MONDARDO, 1994; BEAUDRY et al., 1993; KADER, 1999; LU et al., 2012; SABBAN-AMIN et al., 2011). A escaldadura é um distúrbio fisiológico relacionado à produção de espécies reativas de oxigênios (ROS) que aumentam a oxidação do composto α-farneseno, causando manchas escuras na epiderme dos frutos e diminuindo a qualidade visual dos mesmos (SABBAN-AMIN et al., 2011). Assim, o uso da ACD-QR pode controlar este distúrbio, através da redução das pressões parciais de O_2 durante o armazenamento, inibindo a oxidação do α-farneseno e, consequentemente, a incidência deste distúrbio em frutos colhidos antes do ponto de maturação ideal. Frutos colhidos antes do momento correto também apresentam menor produção de compostos voláteis (BANGERTH; SONG; STREIF, 2012; FELLMAN et al., 2003; SONG; BANGERTH, 1996).

De acordo com o acima exposto, o ideal seria colher todos os frutos no momento em que apresentam uma maturação correta (BEAUDRY et al., 1993; KADER, 1996) e armazena-los até a comercialização. Entretanto, isso não é possível, tornando necessária a adaptação das tecnologias de armazenamento para os diferentes estádios de maturação dos frutos afim de minimizar ao máximo as perdas no período pós-colheita e ofertar aos consumidores produtos de alta qualidade. Nesse sentido o uso da ACD-QR pode ser uma aliada dos armazenadores de maçãs, em função do seu princípio de monitoramento do LMO, que permite adequar a atmosfera da câmara ao estado metabólico dos frutos de diferentes estádios de maturação.

2.3. FORMAS DE ARMAZENAMENTO

2.3.1. Atmosfera controlada (AC) e ultra baixo O_2 (ULO)

A AC é uma das tecnologias de armazenamento mais utilizadas em nível comercial, conjuntamente com a atmosfera refrigerada. De acordo com a Agapomi (2015), em torno de 66% das maçãs são armazenadas em AC no Brasil. Nessa técnica de armazenamento, além da redução da temperatura e controle da umidade relativa, a composição gasosa da atmosfera é alterada e controlada (BRACKMANN et al., 2005, 2008; KE; SALTVEIT, 1989; LUMPKIN et al., 2014, 2015). A AC é uma importante técnica para prolongar a vida pós-colheita de frutas e hortaliças (KE; SALTVEIT, 1989). Entretanto, para cada espécie de fruta e hortaliça há uma combinação de gases que melhor mantém a qualidade.

Além da variação entre espécies, a concentração ótima de gases pode variar de acordo com as diferentes mutantes originadas de uma única cultivar, como a ‘Gala’ e suas mutantes (BRACKMANN et al., 2008, 2009; WEBER et al., 2013). De acordo com esses mesmos

autores, há um valor específico de O_2 e CO_2 para cada uma das mutantes de 'Gala', como a 'Galaxy'. Brackmann et al. (2008, 2009) verificaram que maçãs 'Galaxy' apresentavam melhor manutenção de qualidade quando os frutos eram armazenados com pressões parciais de O_2 variando de 0,8 a 1,0 kPa e de CO_2 não ultrapassando 2,5 kPa, já para a cultivar 'Royal Gala' esses mesmo autores verificaram uma resposta diferenciada, com melhor manutenção da qualidade com 1,0 kPa O_2 + 2,5 kPa CO_2.

A redução das pressões parciais de O_2 e o aumento das de CO_2 resulta em diversas modificações no metabolismo dos frutos, havendo diversas formas de ação. A baixa pressão parcial de O_2 diminui a respiração dos frutos em função do O_2 ser substrato final na cadeia transportadora de elétrons (CHITARRA; CHITARRA, 2005; STEFFENS et al., 2007; TAIZ; ZEIGER, 2013; WRIGHT; ARUL; PRANGE, 2015). Além disso, as baixas pressões parciais de O_2 diminuem a produção de etileno pelos frutos, uma vez que, para a conversão do ACC (Ácido 1-aminociclopropano-1-carboxílico) em etileno pela enzima ACC oxidase é necessária a presença de oxigênio molecular (ASODA et al., 2009; YANG; HOFFMAN, 1984). Com a redução da produção de etileno ocorre um retardamento na senescência dos frutos, pois o etileno é responsável por ativar a atividade das enzimas de parede celular (PAYASI et al., 2009; PRASSANA; PRABHA; THARANATHAN, 2007) e, consequentemente, a redução da firmeza de polpa e outros eventos relacionados ao amadurecimento.

O uso de alta pressão parcial de CO_2 empregada durante o armazenamento dos frutos em AC também atua na redução do metabolismo dos frutos (LIU et al., 2004; MATHOOKO, 1996; WILD; WOLTERING; PEPPELENBOS, 1999). Como o CO_2 é um dos produtos da respiração, o aumento de sua concentração na atmosfera resulta em diminuição da atividade metabólica dos frutos. Estudos verificaram que com o uso de altas pressões parciais de CO_2 ocorre a inibição de várias enzimas da rota glicolítica e do ciclo dos ácidos tricarboxílicos, mais especificamente na fosfofrutoquinase, succinato desidrogenase e isocitrato desidrogenase (KE et al., 1995; LIU et al., 2004). Altas pressões parciais de CO_2 também têm efeito na biossíntese de etileno, reduzindo a atividade da enzima ACC sintase e competindo com o etileno pelos receptores de etileno (MATHOOKO, 1996).

Quando as condições de AC utilizadas durante o armazenamento são inadequadas para uma determinada cultivar, podem ocorrer perdas significativas em função da ocorrência de distúrbios fisiológicos, como a degenerescência de polpa (FRANCK et al., 2007; HO et al., 2013; WEBER et al., 2011, 2013). Esses distúrbios fisiológicos podem ser ocasionados tanto pelo uso de pressões parciais de CO_2 acima das toleradas pela cultivar (ARGENTA et al., 2002; CORRÊA et al., 2010; LUMPKIN et al., 2015) quanto em função de pressões parciais de O_2

demasiadamente baixas, que resultam em restrições na produção de ATP (SAQUET; STREIF; BANGERTH, 2003; FRANCK et al., 2007; HO et al., 2013) ou ainda na ocorrência de metabolismo anaeróbico, resultando no acúmulo de acetaldeído, etanol e acetato de etila, que, quando em altas concentrações, podem causar distúrbios fisiológicos (SAQUET; STREIF; BANGERTH, 2003; SAQUET; STREIF, 2008; WRIGHT; ARUL; PRANGE, 2015). Esse problema pode ocorrer quando o armazenador lança mão de uma variação da AC, que é o uso do ULO, onde a pressão parcial de oxigênio é reduzida a níveis bem abaixo dos recomendados para AC (THEWES et al., 2015a; WEBER et al., 2011, 2013; ZANELLA, 2003).

O armazenamento de maçãs em ULO é baseado no princípio da redução do O_2 até níveis próximos (um pouco acima) do ponto de compensação anaeróbica (PCA) a fim de reduzir ao máximo a respiração dos frutos e manter a qualidade dos mesmos (GRAN; BEAUDRY, 1993; YEARSLEY et al., 1996; THEWES et al., 2015a; WEBER et al., 2011). Para tanto, é necessária a determinação do PCA para cada cultivar de maçã (GRAN; BEAUDRY, 1993). Entretanto, nessa tecnologia de armazenamento, o PCA é mensurado apenas uma única vez, antes (no início) do armazenamento e o O_2 mantido um pouco acima desse ponto, não levando em consideração que ele (PCA) pode mudar durante o armazenamento, devido ao estádio de maturação dos frutos, entre outros fatores. Esse fato pode resultar em problemas durante o armazenamento, pois, podem ocorrer períodos em que o O_2 permanecerá abaixo do ponto de compensação anaeróbico, o que pode ocasionar o desenvolvimento de distúrbios fisiológicos e, quando acima do PCA, resulta em perda de qualidade em função de respiração demasiada. Em função disso, o armazenamento de maçãs em ACD-QR pode ser uma ferramenta muito importante no acompanhamento do LMO e, assim, evitar perdas de qualidade mais pronunciadas. Cabe aqui ressaltar que o LMO e o PCA não são sinônimos, pois em muitos estudos são apresentados como sinônimos, podendo ocasionar perdas de qualidade durante ao armazenamento quando os dois são considerados sinônimos (WRIGHT; ARUL; PRANGE, 2015). O ponto de compensação anaeróbica é a pressão parcial de O_2 em que a produção de CO_2 é mínima (BOERSING; KADER; ROMANI, 1988), já o LMO é a menor pressão parcial de O_2 em que os frutos podem ser armazenados com segurança, mantendo a qualidade após o armazenamento (WRIGHT; ARUL; PRANGE, 2015), sendo que o LMO pode ser maior ou menor do que o PCA, dependendo da espécie, estádio de maturação, cultivar, entre outros fatores.

Em função do acima exposto, o armazenamento de maçãs em pressões parciais de O_2 extremamente baixas deve ser realizado somente com o emprego de tecnologias que permitem monitorar o LMO durante o armazenamento. Durante o armazenamento, o LMO tolerado pelos

frutos varia em função da temperatura (WRIGHT et al., 2010), cultivar (GASSER et al., 2008), estádio de maturação, além de outros fatores.

2.3.2. Atmosfera controlada dinâmica (ACD)

Durante os últimos anos, ocorreu uma profunda transformação na área de pós-colheita de frutas com o surgimento de novas tecnologias de armazenamento, como o uso de estresse inicial por baixo O_2 (ILOs) (BOTH et al., 2014; ZANNELA, 2003), ULO (THEWES et al., 2015a; WEBER et al., 2011, 2013) e mais recentemente a ACD (PRANGE et al., 2007; VELTMAN et al., 2003; WEBER et al., 2015). A ACD é uma técnica de armazenamento que permite o monitoramento do metabolismo dos frutos, de modo não destrutivo, durante todo o período de armazenamento, através do monitoramento de metabólitos (etanol), fluorescência de clorofilas e quociente respiratório. Atualmente, esses três métodos de ACD estão em uso comercial (WRIGHT; ARUL; PRANGE, 2015) e serão descritos abaixo.

2.3.2.1. ACD monitorada pela produção de etanol pelos frutos

Esse método de monitoramento do LMO se baseia na determinação da concentração de etanol no ar da câmara de armazenagem (*Dynamic Control System* - DCS®) ou na polpa dos frutos (ILOS-Plus®). O princípio do método DCS®, desenvolvido na Holanda, baseia-se no monitoramento contínuo da concentração de etanol no *"headspace"* da câmara enquanto a pressão parcial de O_2 é reduzida até o momento em que a concentração de etanol no *"headspace"* da câmara aumenta significativamente, momento em que a pressão parcial O_2 é aumentada para que a concentração de etanol volte ao normal (VELTMAN et al., 2003), sendo feito o monitoramento da câmara durante todo período de armazenamento.

Como o etanol é um metabólito intermediário e precursor de vários outros compostos, a determinação deste composto no ar da câmara comercial é bastante complicada em função de sua alta variabilidade e necessidade de equipamentos de alto custo e difícil manejo, como o cromatógrafo. A aplicação de etanol ou o etanol produzido pelos frutos é rapidamente convertido em ésteres etílicos, como o acetato de etila (BRACKMANN; STREIF; BANGERTH, 1993; JIN et al., 2013; LIU et al., 2012). Esse fato, tornou essa tecnologia de ACD pouco difundida em nível comercial, sendo apenas utilizada em algumas poucas câmaras comerciais. Entretanto, mesmo com os problemas expostos acima, o armazenamento de maçãs 'Elstar' em DCS® resultou em melhor manutenção da firmeza de polpa, reduziu a incidência de

escaldadura e manteve a coloração verde de fundo mais intensa em relação aos frutos armazenados em AC (SCHOUTEN et al., 1997; VELTMAN et al., 2003).

Além do método de mediação de etanol no ar da câmara, há uma outra tecnologia comercial baseada em um método destrutivo para determinação da concentração de etanol no suco. Esse método é comercializado pela empresa Marvil da Itália e é denominado ILOS-Plus® (MARVIL, 2015). De acordo com as informações fornecidas pela empresa, o método possui a vantagem de uma rápida determinação da concentração de etanol nos frutos e a mão-de-obra não necessita ser especializada em função do fácil manuseio do sensor de etanol. A determinação do etanol é feita por um biossensor enzimático que imobiliza algumas biomoléculas na superfície do sensor, possibilitando a determinação do etanol (MARVIL, 2015). Entretanto, esse método possui o mesmo problema observado no método DCS®, pois o etanol produzido pelos frutos pode ser convertido em outros compostos causando erros na determinação do LMO durante o armazenamento, além de necessitar a abertura da câmara para retirada de amostras para a determinação de etanol, o que torna o método pouco prático em nível comercial.

2.3.2.2. ACD monitorada pela fluorescência de clorofilas (ACD-FC)

O método de determinação da fluorescência de clorofilas foi o primeiro método de monitoramento do LMO adotado em larga escala em nível comercial. Essa técnica se baseia no princípio da emissão de fluorescência pelas clorofilas presentes na epiderme dos frutos. A fluorescência das clorofilas ocorre em maior intensidade em situações de estresse, que impossibilitam a transferência da energia luminosa captada pela clorofila para os centros de reação (TAIZ; ZEIGER, 2013). Esses estresses podem ser de várias naturezas, como secas, danos por herbicidas, excesso de luz, temperatura (TAIZ; ZEIGER, 2013), além de estresse por baixo oxigênio (DeELL et al., 1999; PRANGE et al., 2007).

O efeito de baixas pressões parciais de O_2 sobre a ocorrência da fluorescência de clorofilas ainda não está bem elucidado, porém algumas hipóteses têm sido formuladas sobre o efeito do baixo O_2 sobre a fluorescência. As principais hipóteses são: [1] com a redução das pressões parciais de O_2 a níveis extremamente baixos ocorre a produção de acetaldeído, etanol e acetato de etila, que podem causar danos na membrana celular e suas organelas, como os cloroplastos, danificando os fotossistemas (FS), dificultando a transferência da energia luminosa e acarretando no aumento da fluorescência (MAXWELL; JOHNSON, 2000); [2] níveis extremamente baixos de O_2 provocam a acidose celular e suas organelas, o que também

pode danificar os FS aumentando a fluorescência de clorofilas (PRANGE et al., 2005); [3] a adoção de pressões parciais de O_2 extremamente baixas resulta em um acúmulo de compostos reduzidos no citosol e na mitocôndria que podem ser transportados para o cloroplasto e 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; ARUL; PRANGE, 2015), gerando um sinal do LMO tolerado pelos frutos e [4] o processo de dissipação da energia lumínica na forma não fotoquímica (fluorescência) tem ligação com o ciclo das xantofilas, mais especificamente a zeaxantina (WRIGHT et al., 2011), onde, na transformação de zeaxantina para anteraxantina, é necessário O_2 (pela enzima zeaxantina epoxidase), assim a redução da concentração de O_2 pode resultar no acúmulo de zeaxantina, aumentando a fluorescência de clorofilas.

O monitoramento da fluorescência de clorofilas é realizado durante todo o período de armazenamento através da alocação, de uma amostra representativa de 6 frutos, em uma caixa de plástico que está equipada com um sensor de fluorescência FIRM (*Fluorescence Interactive Response Monitor*). Essa caixa é alocada no interior da câmara comercial, para ficar com a atmosfera da câmara (geralmente são alocados 6 desses sensores em cada câmara comercial). Para análise da fluorescência essa amostra deve ser mantida no escuro. Juntamente com o FIRM estão localizadas quatro lâmpadas LED (*Light Emitting Diode*), que servem como fonte de luz para medir o nível de estresse dos frutos (LMO). O sinal captado pelo sensor é transferido para um *software* específico que registra a intensidade da fluorescência enquanto a pressão parcial de O_2 é reduzida durante o início do armazenamento. A pressão parcial de O_2 é reduzida até o momento em que é detectado um pico na emissão de fluorescência. A partir desse momento, o O_2 é incrementado em 0,2 kPa, porém nunca a pressão parcial da câmara deverá ser menor que 0.4 kPa, e este nível é mantido 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 (PRANGE et al., 2007; TRAN et al., 2015).

A emissão da fluorescência pela clorofila é um método amplamente utilizado em nível comercial, porém os sensores para medição da fluorescência são de alto custo, o que dificulta o aumento de sua utilização no Brasil. Por outro lado, em maçãs ‘Royal Gala’ e ‘Galaxy’ armazenadas em ULO e ACD-FC, não houve diferença significativa entre as duas formas de armazenamento na manutenção da qualidade (THEWES et al., 2015a). Esses autores atribuem esse resultado ao estresse inicial muito intenso (próximo a 0,0 kPa O_2), necessário para que ocorra o pico na florescência e, com isso, o O_2 seja incrementado na câmara. Esse fato, longo período com o O_2 próximo a 0.0 (em torno de 4 a 5 dias) até que ocorra o pico de fluorescência,

deve ocorrer em função da fluorescência de clorofilas não ser uma resposta direta do efeito do baixo oxigênio no metabolismo do fruto, mas sim uma resposta da mudança do metabolismo e seus produtos na célula, como acidose citoplasmática, redução da PQ, produção de etanol, que ocorre bem depois do momento em que o O_2 reduzido, podendo o O_2 ter ficado em concentrações abaixo do LMO por tempo demasiado, resultando em distúrbios fisiológicos.

Na literatura vários trabalhos têm sido conduzidos comparando o uso da ACD-FC e AC sobre a ocorrência de distúrbios fisiológicos, produção de etileno, compostos voláteis e qualidade em geral (DEUCHANDE et al., 2016; GABIOUND REBEAUND; GASSER, 2015; GASSER et al., 2008; KÖPCKE, 2015; PRANGE et al., 2015; RAFFO et al., 2009; THEWES et al., 2015a; TRAN et al., 2015). Em um estudo com maçãs 'Golden Delicious', a ACD-FC resultou em melhor manutenção da qualidade em comparação a AC isolada, porém, quando comparado a AC + 1-MCP a manutenção da qualidade foi geralmente inferior (GABIOUND REBEAUND; GASSER, 2015), entretanto, o uso da ACD-FC resultou em melhor manutenção da produção de ésteres em comparação a AC + 1-MCP (RAFFO et al., 2009).

Atualmente, pesquisas estão sendo realizadas para o aprimoramento da técnica da ACD-FC, especialmente para o uso em cultivares altamente suscetíveis a distúrbios fisiológicos, como a escaldadura. Esse aprimoramento da fluorescência possui o nome de ACD-FC 'Extra', ou seja, algum tratamento adicional é realizado para o controle de algum distúrbio específico. A ACD-FC 'Extra' apresentou ótimos resultados no controle da escaldadura superficial em cultivares altamente suscetíveis, como a Cortland (PRANGE et al., 2015), mostrando que essa tecnologia ainda não está pronta e há margem para evolução na manutenção da qualidade de maçãs. Esse é um resultado importante para maçãs orgânicas, pois nestas não é permitida a aplicação de nenhum produto em pós-colheita para controle desse distúrbio, podendo a ACD-FC 'Extra' surgir como uma ótima ferramenta para os armazenadores.

2.3.2.3. ACD monitorada pelo quociente respiratório (ACD-QR)

Essa tecnologia é baseada no princípio de igualdade entre consumo de O_2 e produção de CO_2 em condições de respiração aeróbica, ou seja, QR próximo a 1,0 em condições normóxicas. O QR mesmo em condições normais de O_2 pode variar um pouco acima ou abaixo de 1,0, dependendo dos substratos utilizado na respiração, mais especificamente da quantidade de oxigênio na composição do substrato. Assim, de acordo com Goyette et al. (2012), o QR é igual a 1,0 quando açúcares estão sendo consumidos na respiração. Entretanto, não é apenas o tipo

de substrato que influência o QR dos frutos durante o armazenamento, mas a pressão parcial de O_2 e CO_2 também.

Estudos realizando o monitoramento do QR durante a redução da pressão parcial de O_2 no interior da câmara verificaram que a partir de um certo nível de O_2, o QR tende a aumentar, o que é um indicativo de fermentação nos frutos (BOERSING; KADER; ROMANI, 1988; GOYETTE et al., 2012). Assim, com o monitoramento do QR pode-se determinar o ponto de compensação anaeróbica dos frutos, ou seja, a pressão parcial de O_2 na qual a produção de CO_2 é mínima (GASSER et al., 2010). Esses autores monitoraram o ponto de compensação anaeróbica durante o armazenamento de maçãs 'Idared', 'Maigold', 'Elstar' e 'Braeburn', sendo os valores de 0,2 a 0,3 para as três primeiras cultivares e 0,4 kPa para a 'Braeburn', porém esses valores de O_2 não necessariamente indicam o LMO tolerado pelos frutos durante o armazenamento, uma vez que um pouco de fermentação pode ser interessante pela produção de etanol, que atua sobre várias rotas metabólicas.

A partir dessas constatações, estudos foram realizados tentando aplicar essa técnica em nível comercial, com a medição do QR em câmaras comerciais, porém ocorreram problemas em função da entrada de O_2 nas câmaras, ou seja, as câmaras não estavam completamente vedadas. Com a entrada de O_2 durante a medição do QR ocorre uma superestimação do mesmo, ou seja, o LMO é superestimado por causa de erros na determinação do QR durante o armazenamento. Atualmente, uma empresa holandesa (Van Amerongen CA Technology B.V.) comercializa a tecnologia de medição do QR em câmara comercial, denominada Advanced Control Respiration - ACR®. Para tanto, a empresa realiza o desligamento de todo o sistema de ventilação, refrigeração e absorção de CO_2 por um período de aproximadamente seis horas, para evitar mudanças de pressão no interior da câmara e permitir o acúmulo de CO_2 e consumo do O_2 pelos frutos. Além disso, a câmara é equipada com um sensor de pressão que mantém a câmara sempre sob pressão através da injeção de nitrogênio puro durante as 6 horas em que é determinado o QR. Com a manutenção da câmara sob pressão, caso a mesma não esteja completamente estanque, a tendência é sair gás da câmara e não entrar ar, resultando assim numa determinação de QR mais confiável. Um estudo utilizando essa metodologia para determinação de QR foi realizado na Holanda, numa câmara comercial de maçãs 'Elstar' com resultados promissores (VAN SCHAIK et al., 2015), sendo que o incremento nos valores de QR aconteciam conjuntamente com os de etanol, indicando que através do uso da ACD-QR era possível a indução de um pouco de fermentação.

Tentando contornar o problema da vedação da câmara e diminuir os custos com sensores de pressão e gás nitrogênio, a empresa norte americana Storage Control System Inc. (Sparta,

MI, USA) desenvolveu um dispositivo alocado no interior da câmara comercial (Safepod), que permite fechamento hermético automatizado. No interior desse recipiente são colocadas amostras de frutos para realizar a determinação do QR. Assim, durante o período em que não é determinado o QR o dispositivo permanece aberto dentro da câmara de AC, permitindo que a composição gasosa seja similar à da câmara, sendo fechado apenas quando se deseja determinar o QR (WRIGHT; ARUL; PRANGE, 2015). Com esse dispositivo reduz-se muito as chances de erro na determinação do QR e, consequentemente, na estimação do LMO durante o armazenamento. Entretanto, nesse sistema e naquele em que o QR é determinado em toda câmara, são necessários analisadores de O_2 muito precisos e sem muita variação temporal, pois, a relação entre produção de CO_2 e consumo de O_2 é feita através da leitura em um determinado período de tempo de fechamento do dispositivo com os frutos.

Com o intuito de solucionar os problemas em função do erro dos analisadores e a variação temporal dos mesmos, foi desenvolvido um dispositivo no Núcleo de Pesquisa em Pós-colheita da Universidade Federal de Santa Maria (NPP-UFSM) (BRACKMANN, 2015). Esse método possui um dispositivo similar ao método da empresa Storage Control System Inc. (Sparta, MI, USA), onde são alocadas amostras de frutos para determinação do QR. Porém, o diferencial desse método de determinação do QR é o uso, além do dispositivo com amostra de frutos, de um reservatório de gás com a concentração de gases igual ao da atmosfera da câmara no momento de fechamento do dispositivo com os frutos, para servir de reserva de gases para leitura das pressões parciais de O_2 e CO_2 inicial. Esse reservatório é vedado no momento do fechamento do dispositivo de medição do QR e é usado para determinação dos níveis de O_2 e CO_2 inicial, no momento da leitura da concentração gasosa no dispositivo com os frutos. Dessa maneira evita-se erros no cálculo do QR em função dos analisadores, além de possibilitar várias determinações do QR e, assim, ter um valor mais confiável. Essa tecnologia de armazenamento será comercializada pela empresa italiana ISOCELL, com o nome comercial de RQ-StoreFresh®, após a adaptação da técnica para uso comercial.

O método da ACD-QR tem como principal vantagem, em relação a DCS e ACD-FC, a detecção do LMO dos frutos diretamente (pela relação entre produção de CO_2 e consumo de O_2), enquanto que os métodos apresentados anteriormente apresentam como mecanismo detecção o monitoramento de um evento que é resultante de períodos em baixo O_2, como descrito acima. Além dessa vantagem, o método de armazenamento em ACD-QR permite a indução de uma pequena fermentação, o que resulta em um leve incremento na produção de etanol, que por sua vez reduz a produção de etileno retardando o amadurecimento (ASODA et al., 2009; JIN et al., 2013; LIU et al., 2012). Estudos realizados com maçãs ‘Royal Gala’

verificaram que o uso da ACD-QR foi mais eficiente na redução da produção de etileno e degenerescência de polpa em comparação ao armazenamento em ACD-FC e AC (BOTH, 2015; WEBER et al., 2015). Em maçãs 'Galaxy' também foi observada melhor manutenção da qualidade após o armazenamento quando os frutos foram armazenados em ACD-QR em comparação a ACD-FC e AC (BRACKMANN; WEBER, BOTH, 2015). Um estudo desenvolvido na Bélgica com maçãs 'Granny Smith', verificou controle da escaldadura superficial e maior manutenção da firmeza de polpa em frutos armazenados em ACD-QR (BESSEMANS et al., 2016). Entretanto, o estudo realizado por esses autores demonstra o quão importante é a estanqueidade da câmara de armazenamento (da câmara onde é determinado o QR), pois, nesse estudo ocorreu entrada de ar na câmara de determinação do QR resultado em erros graves na determinação do mesmo, resultando inclusive em QR negativo, o que não é possível em termos fisiológicos. Assim, percebe-se que essa é uma tecnologia muito promissora para o armazenamento de maçãs por longos períodos, mantendo a qualidade dos frutos similar após o armazenamento em comparação a colheita. Entretanto, os estudos ainda são escassos e ainda não foram realizados para determinar a sua eficiência na manutenção da qualidade de maçãs colhidas em diferentes estádios de maturação.

2.4. APLICAÇÃO DE 1-METILCICLOPROPENO (1-MCP)

O uso do 1-MCP é amplamente praticado em todo o mundo, seja em associação com o armazenamento refrigerado ou em AC. Ele é um composto na forma de pó que, quando dissolvido em água, libera o 1-MCP (C_4H_6) na forma gasosa. O 1-MCP se liga de maneira irreversível nos receptores de etileno, impedindo assim a ligação do etileno e, consequentemente, os eventos desencadeados pelo mesmo (BLANKENSHIP; DOLE, 2003; SISLER; SEREK, 1997; WATKINS, 2006). O 1-MCP se liga aos receptores de maneira competitiva com o etileno, porém ele possui uma afinidade muito maior que o próprio etileno com os receptores de etileno (SISLER; SEREK, 1997).

O efeito do 1-MCP, além de inibir a ação do etileno, também apresenta influência na expressão gênica dos frutos. O seu efeito sobre a expressão gênica está relacionado a genes que codificam as enzimas ACC oxidase (*MdACO1*), ACC sintase (*MdACS1*) (WAKASA et al., 2006; TATSUKI et al., 2007) e receptores de etileno (*MdERS1* e *MdERS2*) (TATSUKI; ENDO, 2006; TATSUKI et al., 2007). Assim, com a aplicação de 1-MCP ocorre um bloqueio de genes relacionados a enzimas produtoras e receptoras de etileno, resultando no retardamento da senescência dos frutos, com maior manutenção da firmeza de polpa dos frutos (BRACKMANN

et al., 2013; WATKINS, 2006; WATKINS; NOCK, 2012), menor taxa respiratória (PRÉ-AYMARD et al., 2003; WATKINS, 2006; WATKINS; NOCK, 2012) e menor incidência de polpa farinácea (BRACKMANN et al., 2014; STEFFENS et al., 2008).

Desta maneira, o 1-MCP poderia ser uma ótima alternativa para o bloqueio do amadurecimento de frutos colhidos em estádios de maturação avançados e, assim, reduzir as perdas ocasionadas pela rápida senescência destes frutos. Em maçãs 'Royal Gala', colhidas tardiamente, a aplicação de 1-MCP não apresentou muito benefício sobre a manutenção da qualidade pós-armazenamento, porém, houve significativa redução da atividade da enzima ACC oxidase, produção de etileno e respiração (BOTH et al., 2014; BRACKMANN et al., 2010; THEWES et al., 2015b). Entretanto, é necessário a realização de estudos avaliando o efeito do 1-MCP e sua comparação a ACD, pois não há estudos comparando a sua eficiência com o uso da ACD para bloquear o amadurecimento de maçãs colhidas em diferentes estádios de maturação. Em frutos colhidos tardiamente a aplicação de 1-MCP potencializou a ocorrência de distúrbios fisiológicos (WATKINS, 2006; ARGENTA et al., 2010; JUNG; WATKINS, 2011; NOCK; WATKINS, 2013), fato esse que pode ser contornado pelo armazenamento em ACD.

Em frutos colhidos antes do período ideal de maturação há maior suscetibilidade à ocorrência de escaldadura. Vários estudos demonstram que a aplicação de 1-MCP apresenta benefícios sobre a redução na incidência deste distúrbio (ZANELLA, 2003; TSANTILI et al., 2007). Esse efeito do 1-MCP ocorre devido à atividade inibitória sobre a produção de α-farneseno (LURIE et al., 2002; MOGGIA et al., 2010). Assim, o seu uso em maçãs colhidas antes da maturação ideal poderia prevenir a ocorrência deste distúrbio, entretanto, pode resultar em frutos com menor produção de compostos voláteis. Assim, a ACD por utilizar níveis de O_2 extremamente baixos, poderia contornar o problema da escaldadura, dispensando a aplicação de 1-MCP e evitando a redução na emissão de compostos voláteis.

Os compostos voláteis emitidos pela maçã possuem fundamental importância na comercialização dos frutos, pois o aroma é um atributo de qualidade. Estes compostos possuem uma correlação positiva com a produção de etileno (LURIE et al., 2002; DEFILIPPI et al., 2004; DEFILIPPI et al., 2005; BANGERTH; SONG; STREIF, 2012), tão logo, o 1-MCP possui um efeito direto no perfil volátil dos frutos tratados. Além do efeito direto do 1-MCP sobre a emissão de compostos voláteis, existe um efeito indireto sobre a formação de ácidos graxos insaturados, pela diminuição da taxa respiratória dos frutos (BRACKMANN; STREIF; BANGERTH, 1993; SONG; BANGERTH, 2003), resultando em menor formação de

precursores de compostos voláteis, especialmente precursores de ésteres e, consequentemente, reduzindo o montante de compostos voláteis produzidos pelos frutos.

2.5. PRODUÇÃO DE COMPOSTOS VOLÁTEIS E SUA RELAÇÃO COM O AROMA

O aroma de maçãs é uma complexa interação entre mais de 400 compostos de diferentes grupos orgânicos, como ésteres, álcoois, aldeídos, cetonas, terpenos, ácidos, entre outros. Porém, destes 400 compostos em torno de 40 a 50 são de fundamental importância no odor de maçãs, sendo a grande maioria ésteres (SALAZAR; OROZCO, 2011). Entre os ésteres, os mais importantes variam de cultivar para cultivar, sendo que para maçãs do grupo 'Gala' os mais importantes são: acetato de butila, acetato de hexila e acetato de 2-metilbutila (SALAZAR; OROZCO, 2011; YOUNG et al., 1996).

Os ésteres são formados através da ação da enzima álcool acil CoA tranferase (AAT), que realiza a combinação de um álcool com o ácido acético (acetato) do AcetilCoa (DEFILIPPI et al., 2005; YANG et al., 2016). Dentre os álcoois produzidos pela maçã, o mais abundante é o 1-butanol (MEHINAGIC et al., 2006), que é originário da degradação de ácidos graxos. Esses ácidos graxos podem ser obtidos de diversas maneiras no metabolismo dos frutos, degradação de membrana (CONTRERAS; TJELLSTRÖM; BEAUDRY, 2016; CONTRERAS; BEAUDRY, 2013; SONG; BANGERTH, 2003), síntese *de novo* de ácidos graxos (CONTRERAS; TJELLSTRÖM; BEAUDRY, 2016; SONG; BANGERTH, 2003), ácidos graxos livres, além de outras.

Os estudos avaliando o efeito de condições de atmosfera controlada reportam que a redução nas pressões parciais de O_2 resulta em drástica redução na produção de compostos voláteis, mais especificamente na produção de ésteres (BOTH et al., 2014; BRACKMANN; STREIF; BANGERTH, 1993; ECHEVERRÍA et al., 2004; 2008; LUMPKINS et al., 2014, 2015; RAFFO et al., 2009). Com a redução do oxigênio no interior da câmara ocorre redução na β-oxidação e lipoxigenase (LOX), reduzindo a produção de precursores de ésteres, como álcoois e AcetilCoA (ECHEVERRÍA et al., 2004; SALAZAR; OROZCO, 2011). Por outro lado, o baixo O_2 reduz a produção de etileno e a respiração, o que também resulta em uma significativa redução na expressão e atividade de enzimas responsáveis pela produção de ésteres (DEFILIPPI et al., 2005; SONG; BANGERTH, 1996, 2003; YANG et al., 2016).

O aumento da concentração de CO_2 na câmara de armazenamento também influencia negativamente a produção de compostos voláteis (BRACKMANN; STREIF; BANGERTH, 1993; LUMPKIN et al., 2015). Em maçãs 'Golden Delicious' o uso de 3,0 kPa de CO_2

combinado com 1,0 kPa de O_2 resultou em redução na produção de ésteres de cadeia linear, porém não foi observado efeito na emissão de compostos voláteis de cadeia ramificada (BRACKMANN; STREIF; BANGERTH, 1993), ou seja, o CO_2 teve efeito na degradação de ácidos graxos e na de aminoácidos não. Entretanto, em maçãs 'Fuji' armazenadas em altas pressões parciais de CO_2 (5,0 kPa) a concentração de ésteres de butila, proprila e hexila foi significativamente reduzida em comparação a condições normais de CO_2, por outro lado, a concentração de ésteres etílicos e metílicos foi significativamente incrementada pelo CO_2 (LUMPKIN et al., 2015). Esses resultados demonstram que o efeito do CO_2 sobre o perfil volátil dos frutos é influenciado pela pressão parcial de O_2 associada.

A tendência de uso de condições com níveis de O_2 cada vez mais baixos durante o armazenamento de maçãs torna necessário o estudo do impacto desse manejo sobre a composição volátil de maçãs após o armazenamento, especialmente em condições de ACD, onde os estudos ainda são raros. Em maçãs 'Pinova' o armazenamento em ACD-FC resultou em significativa redução na produção dos principais ésteres em relação à AC, porém com maior concentração do que os frutos tratados com 1-MCP (AC + 1-MCP) (RAFFO et al., 2009). Por outro lado, o armazenamento de maçãs 'Royal Gala' em ACD-QR resultou em maior manutenção dos principais ésteres em comparação a ACD-FC (BOTH, 2015), mostrando que a ACD-QR é uma tecnologia promissora no armazenamento de maçãs. Entretanto, ainda não foram realizados estudos avaliando o armazenamento de maçãs colhidas em diferentes estádios de maturação em ACD-QR. Por outro lado, o uso de pressões parciais de O_2 extremamente baixas pode resultar em uma demasiada indução do metabolismo fermentativo, resultado no acúmulo de acetaldeído, etanol e acetato de etila, compostos estes relacionados com a formação de *off-flavors* em maçãs (FORNEY et al., 2000; WRIGHT; ARUL; PRANGE, 2015), porém, a presença desse compostos é de fundamental importância para conferir aos frutos o aroma típico de maçã (ECHEVERRÍA et al., 2008). Entretanto, não há estudos na literatura avaliando o efeito do metabolismo anaeróbico sobre a composição volátil, especialmente, sobre os ésteres de cadeia linear, como acetato de butila e hexila. Em melões a aplicação de etanol na forma de vapor ou sua injeção na cavidade seminal resultou em um significativo aumento dos ésteres, não apenas ésteres de etila, mas ésteres característicos da fruta como acetato de butila e hexila (LIU et al., 2012). Assim, a indução do metabolismo anaeróbico, de forma segura através do armazenamento em ACD-QR com níveis de QR um pouco acima de 1.0, poderá resultar em redução na produção de etileno e taxa respiratória e produzir os precursores de compostos voláteis, mantendo frutos com boas características físico-químicas e com aroma pronunciado.

Com a redução da pressão parcial de O_2, ocorre um acúmulo de compostos reduzidos nas células (TAIZ; ZEIGER, 2013; WRIGHT; ARUL; PRANGE, 2015), compostos estes que podem ser utilizados pela fermentação, resultando na produção de acetaldeído, etanol e acetato de etila. Por outro lado, esses compostos reduzidos podem ser utilizados para outras rotas metabólicas, como redução da plastoquinona. Entretanto, como em pressões parciais de O_2 extremamente baixas ocorre supressão do catabolismo de ácidos graxos, reduzindo a produção de precursores de voláteis, os compostos reduzidos presentes na célula podem ser transportados para os cloroplastos permitindo a síntese de precursores de voláteis através da *de novo* biossíntese de ácidos graxos ao invés da sua oxidação pela fermentação. Um estudo recente, adotando concentrações estáticas de O_2 extremamente baixa (0.3 kPa) não observou redução na concentração de acetato de hexila, porém, os acetatos de butila e propila foram significativamente reduzidos pelo baixo O_2 em maçãs 'Scarlett Spur Red Delicious' (LUMPKIN et al., 2014). Porém, atualmente há poucos relatos na literatura avaliando o armazenamento em ACD-QR sobre a produção de compostos voláteis, demonstrando a necessidade de pesquisas estudando o efeito dessa técnica de armazenamento sobre produção de compostos voláteis.

3. ARTIGO 1

3.1. DYNAMIC CONTROLLED ATMOSPHERE, CONTROLLED ATMOSPHERE, 1-METHYLCYCLOPROPENE AND ITS INTERACTION WITH MATURITY AT HARVEST ON 'GALAXY' APPLE QUALITY[1]

Abstract

The objective of this study was to evaluate the interaction among controlled atmosphere (CA), CA + 1-methylcyclopropene (1-MCP) and dynamic controlled atmosphere monitored by respiratory quotient (DCA-RQ) with three maturity stages at harvest (unripe, ripe and overripe) on 'Galaxy' apple metabolism and quality after harvest and 9 months storage plus 7 days of shelf life at 20 °C. The treatments evaluated were: [1] CA (1.2 kPa O_2 + 2.0 kPa CO_2); [2] CA + 1-MCP application (0.625 µL L^{-1}); [3] DCA-RQ 1.3 + 1.2 kPa CO_2 and [4] DCA-RQ 1.5 + 1.2 kPa CO_2. These 4 treatments were evaluated in each maturity stage. Fruit stored under DCA-RQ 1.3 showed lower ethylene production rate, respiration rate, mealiness and higher flesh firmness as compared to CA stored fruit, but not differed from those treated with 1-MCP. DCA-RQ 1.5 increased the acetaldehyde, ethanol and ethyl acetate concentration, regardless the fruit maturity at harvest. The storage of 'Galaxy' apple under DCA-RQ is efficient in quality keeping regardless the maturity stage at harvest, resulting in low ethylene production, mealiness incidence, high flesh firmness and juiciness. DCA-RQ 1.3 show similar quality keeping as compared to 1-MCP application, being a promising storage technology, even for organic apple storage.

Keywords: *Malus domestica*, respiratory quotient, ethylene, physiological disorders, flesh firmness, anaerobic metabolism.

3.1.1. Introduction

Harvest the fruits at the correct maturity stage is a key factor to obtain high fruit quality during the postharvest handling period. However, this is a very hard task, especially due to the short harvest window and the lack of manual labor. Thus, the harvest begin before the correct fruit maturity (unripe) and extends until advanced maturity stages (overripe). Unripe apple are more susceptible to superficial scald (Beaudry et al., 1993; Lu et al., 2012), has lower red skin

[1] Artigo formatado de acordo com as normas da revista Postharvest Biology and Technology.

coloration (Brackmann et al., 2004) and sugar accumulation (Brackmann et al., 2004; Lu et al., 2012). On the other hand, overripe apple are more susceptible to decay incidence (Vilanova et al., 2012; Vilanova et al., 2014), physiological disorders (Beaudry et al., 1993; Fan et al., 2011) and increase the volatile compounds biosynthesis (Song and Bangerth, 1996; Bangerth et al., 2012). Thereby, the development of a storage technology to mitigate the postharvest losses due to the incorrect harvest is necessary.

The storage of apple in dynamic controlled atmosphere (DCA) may be a promising technology to avoid postharvest losses. This technology allows to detect the lower oxygen limit (LOL) tolerate by fruit in real time throughout the storage and change oxygen according to fruit metabolism. Nowadays, there are three methodologies to detect the LOL during apple storage: monitoring the ethanol production by fruit (Veltman et al., 2003), chlorophyll fluorescence (DCA-CF) (DeEll et al., 1999; Prange et al., 2007; Wright et al., 2010; Wright et al., 2012) and respiratory quotient (DCA-RQ) (Brackmann et al., 2015; Weber et al., 2015). Among these three methodologies to monitor the LOL during storage, DCA-RQ maintain higher fruit quality of 'Royal Gala' (Weber et al., 2015) and 'Galaxy' (Brackmann et al., 2015) apple harvested at the correct maturity stage, as compared to CA and DCA-CF. Thereby, the DCA-RQ may be a key storage technology for apple harvested at the incorrect maturity stage, i.e. unripe and overripe apple, avoiding significant quality losses during storage and shelf life.

The new DCA-RQ storage method, which is a clean technology without chemical use, need to be compared to a worldwide used technology to check its efficiency, such as the 1-methylcyclopropene (1-MCP) application and its interaction with maturity stages. 1-MCP application suppresses ethylene production, maintain higher flesh firmness (Corrent et al., 2004; Watkins, 2006; Lu et al., 2012; Watkins and Nock, 2012; Thewes et al., 2015b), titratable acidity (Corrent et al., 2004; Lu et al., 2012), reduces physiological disorders (Steffens et al., 2008; Lu et al., 2011; Thewes et al., 2015b), among other benefits. In cold stored 'Fuji' and 'Royal Gala' apples, the 1-MCP application maintained higher flesh firmness and titratable acidity, independently of the harvest maturity, as compared to fruit without 1-MCP (Corrent et al., 2004; Lu et al., 2012). Nevertheless, there are little information about the interaction of 1-MCP application and maturity stages of apple stored under CA. Additionally, no reports were found in literature comparing the 1-MCP application to DCA-RQ and its interaction with maturity at harvest.

In this context, the objective of this study was to evaluate the interaction among CA, CA + 1-MCP, DCA-RQ 1.3 and DCA-RQ 1.5 with three maturity stages at harvest (unripe, ripe

and overripe) on ‘Galaxy’ apple metabolism and quality after harvest and 9 months storage plus 7 days of shelf life at 20 °C.

3.1.2. Material and methods

3.1.2.1. Experimental material, harvest maturity, CA and DCA-RQ conditions

The experiment was carried out at the Postharvest Research Center of the Federal University of Santa Maria, Brazil, during the year of 2014. The experimental material was composed by apple of the cultivar Galaxy, harvested at three maturity stages (unripe – 02/08/2014, ripe (commercial harvest peak) – 02/15/2014 and overripe – 02/22/2014) in a commercial orchard located at Vacaria-RS, Brazil.

In order to determine the maturity stages at harvest, the iodine-starch index was measured (0 – unripe and 10 – overripe), as proposed by Streif (1984). Apple with iodine-starch lower to 3.5 where considered unripe, between 3.5 – 7.0 where considered ripe and iodine-starch higher than 7.0 overripe apple. The iodine-starch was determined in four replicates of 20 fruit each replicate.

In each maturity stage, the fruit were harvest at the morning and transported to the Postharvest Research Center at the afternoon. At the morning of the next day, fruit were selected to remove fruit with any type of mechanical damage due to the transportation. Thereafter, the experimental samples were randomly performed, 4 samples of 25 fruit per treatment. The treatments evaluated were: [1] controlled atmosphere (CA) (1.2 kPa O_2 + 2.0 kPa CO_2) (control); [2] CA + 1-MCP application (0.625 µL L^{-1}); [3] DCA-RQ 1.3 + 1.2 kPa CO_2 and [4] DCA-RQ 1.5 + 1.2 kPa CO_2.

3.1.2.2. 1-MCP treatment

In order to 1-MCP application fruit were put into a 230-liter chamber, located inside a cold room (1.5 ± 0.1 °C). Afterward a solution containing 0.625 µL L^{-1} 1-MCP was prepared (SmartFresh®, 0.14% of active ingredient) and allocated into Petry discs, thereafter, the chamber was hermetically closed during 24 hours. During the 24 hours, the air contained inside the chamber was bustling with a fan to homogenize the air. After this period, the fruit were

removed from the 1-MCP application chamber and stored in CA according to above reported condition.

3.1.2.3. CA and DCA-RQ setup and maintenance

The CA and DCA-RQ conditions were installed inside experimental mini-chambers of 230 liters, where the 4 samples of each treatment were stowed. On the first storage day the temperature was reduced down to 5.0 °C and thereafter reduced gradually to the desired storage temperature in 5 days (final temperature: 1.5 °C for all treatments and maturity stages). After this, the CA and DCA storage conditions were setup. To obtain the desired atmospheric condition the CA and DCA-RQ chambers were flushed with nitrogen until to the oxygen partial pressure pre-established for CA (1.2 kPa) and reduced down to 0.5 kPa for DCA-RQ condition, this process was also carried out in 5 days. The carbon dioxide partial pressure was obtained by its accumulation in the storage chamber by fruit respiration.

During all the storage period, the oxygen and carbon dioxide partial pressure were determined and corrected 4 time every day with aid of automatic CA and DCA control system (Valis®, Lajeado, RS, Brazil). The equipment compared the oxygen and carbon dioxide measured to a set point. If the oxygen was lower to the set point, air was injected up to the desired partial pressure. The same manner was used for carbon dioxide correction, but generally the CO_2 was above the desired concentration and the excess of CO_2 in the chamber was automatically absorbed with a lime scrubber. During the storage, the gas analyzer was weekly calibrated with a standard gas, in order to ensure a correct measurement of the oxygen and carbon dioxide partial pressures.

The LOL was determined according to the methodology proposed by Brackmann et al., (2015) and Weber et al., (2015). Two RQ levels were tested: DCA-RQ 1.3 and DCA-RQ 1.5. The RQ was calculated every three days, with a chamber closure of 13 hours between the first and second determination of O_2 and CO_2 partial pressure inside the chamber. The O_2 and CO_2 partial pressure were determined before and after the 13 hours of chamber closure. The RQ was calculated by the reason between CO_2 production and O_2 uptake in this time. Whenever the RQ level was outside the desired, the automatic DCA control system changed the O_2 set point (Figure 1a and b), as proposed by the methodology afford mentioned.

3.1.2.4. Temperature and relative humidity

The storage temperature was seated and maintained at 1.5 ± 0.1 °C during the entirely period, with the exception of the onset of storage, where a conditioning to low temperature was carried out to simulate the commercial storage temperature reduction. Throughout the storage, the temperature was monitored daily with a mercury thermometer inserted in fruit flesh, to determine the pulp temperature. The relative humidity was seated and maintained at 94 ± 1% with aid of a psychrometer.

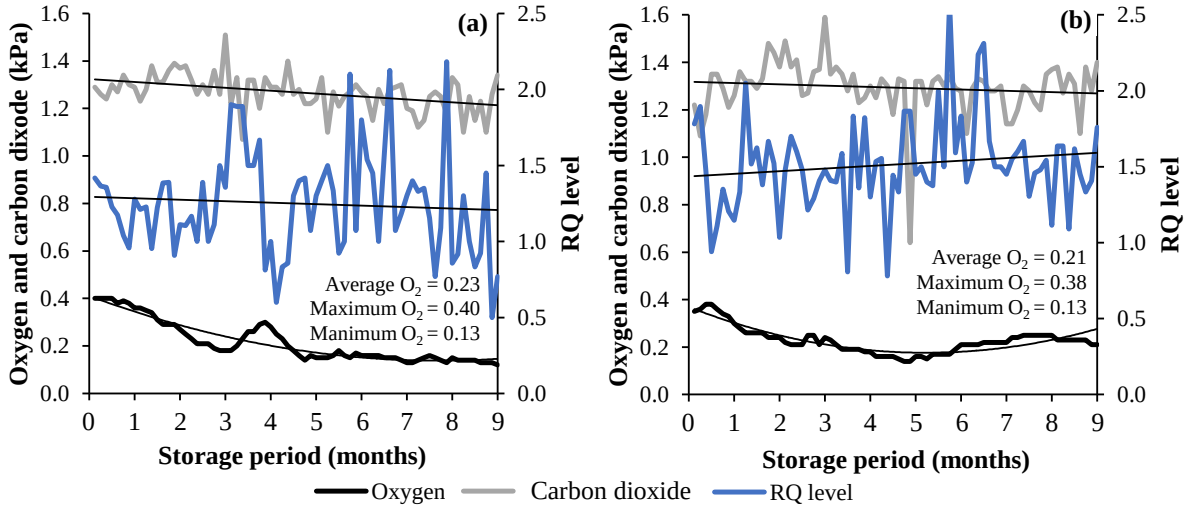

Figure 1 - Oxygen, carbon dioxide and respiratory quotient (RQ) level variation in DCA-RQ 1.3 (a) and DCA-RQ 1.5 (b) during 9 months of storage. Santa Maria, Brazil, 2015.

3.1.2.5. Metabolism and quality analyses

3.1.2.5.1. ACC oxidase enzyme activity

This enzyme was evaluated according to the methodology proposed by Bufler (1986). Results were expressed in ng kg^{-1} s^{-1} of ethylene.

3.1.2.5.2. Ethylene production and respiration rate

Determined by the stowage of 1.5 kg fruit inside a 5-liter container and thereafter hermetically sealed during about 2 hours. During these 2 hours, the fruit were held inside a

chamber at 20 ± 1 °C. Thereafter, 2 aliquots of 1 mL were taken 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 a Porapak N80/100 column to determine the ethylene concentration in the container headspace. The temperature of the injector, column and detector were: 140, 90 and 200 °C, respectively. The ethylene concentration was positively quantified by a standard gas injection. Results were expressed as ng kg^{-1} s^{-1}. The respiration rate was determined by CO_2 accumulation inside the same container to ethylene production. The CO_2 inside the container was quantified with an electronic gas analyzer (Schele®, model KB7). Respiration rate was expressed as ug kg^{-1} s^{-1}.

3.1.2.5.3. Gas diffusion rate

Determined according to the method proposed by Schotsmans et al. (2003), with modifications. Thus, 5 mm thickness pulp samples were taken of 10 fruit, per replicate, theses samples were put in an apparatus with two hermetically sealed chambers separated by the 5 mm apple pulp slice with 3.69 cm^2 of area as the modification proposed by Anese et al., (2016). Results were expressed as ug m^{-2} s^{-1}.

3.1.2.5.4. Acetaldehyde, ethanol and ethyl acetate concentration

Anaerobic metabolism products were evaluated according to methodology proposed by Saquet and Streif (2008). Thus, 10 mL of juice were put into 20 mL vial flasks and hermetically closed, warmed up to 70 °C in a water bath during 30 min. Thereafter, 100 µL of the headspace air were injected into a Dani gas chromatograph (Dani Intruments Spa., Viale Brianza, Cologno Monzese, Italygas) fitted with a capillary DN-WAX column at 60 °C and a flame ionization detector (FID) at 250 °C. Concentrations were positively identified and quantified from the peak area of standard solutions of each compound, with results expressed in mg L^{-1}.

3.1.2.5.5. Electrolyte leakage

From each replicate were taken 10 discs (5 mm thickness and diameter) from 10 different fruit. These discs were stowed into 20 mL 0.4 M mannitol solution during 1 hour (20 ± 1 °C), afterward the conductivity of the suspension was measured. The suspension was then

placed for 30 min at 120 °C into a water bath and thereafter allowed to cool into a -30 °C freezer down to 20°C, then conductivity was measured again and taken as total leakage. Results expressed as percentage.

3.1.2.5.6. Mealiness

Evaluated by slicing the fruits on the equatorial region and visualization of any symptom of mealy pulp. Results expressed as percentage of total fruit.

3.1.2.5.7. Juiciness

Determined according to methodology proposed by Lunardi et al. (2004). Thus, 20 g of the apple pulp, obtained from 10 fruit from each replicate, were pressed during one minute at a pressure of 10 kg cm^{-1} in a pneumatic press, developed at Postharvest Research Center, Santa Maria, RS, Brazil. By weighting the fruit pulp before and after pressing, the juiciness was calculated and results expressed as percentage.

3.1.2.5.8. Flesh breakdown

Evaluated by slicing the fruit on the equatorial region and visualization of any symptom of pulp browning. Results expressed as percentage of total fruit.

3.1.2.5.9. Decay and pulp cracking

Evaluated by counting the fruit with fungal infection higher 5 mm and pulp cracking in relation to the total number of fruit per replicate (25 fruit each replicate). Results expressed as percentage of total fruit.

3.1.2.5.10. Healthy fruit

This was quantified taken in account the total number of fruit per replicate (25 fruit) minus fruit with any symptom of decay, pulp cracking, mealiness and flesh breakdown incidence. Results were expressed as percentage of healthy fruit.

3.1.2.5.11. Flesh firmness

Determined in two opposite sides of the equatorial region of fruit flesh, where previously the skin was removed, with the aid of 11 mm tip penetrometer. Results expressed as Newton (N).

3.1.2.5.12. Soluble solids

Slices of the equatorial region of the 25 fruit of each replicate were taken of and make a juice with a juicer (Philips Walita®). From this juice was measured the soluble solids with a refractometer, results expressed as %.

3.1.2.5.13. Titratable acidity

From the same sample of juice to determine soluble solids, was taken an aliquot of 10 mL and diluted in 100 mL of distillated water. This solution was titrated with a 0.1 N NaOH solution up to pH 8.1. Results expressed as % of malic acid.

3.1.2.6. Statistical analysis

The experiment was conducted in a completely randomized scheme with a factorial arrangement (4 storage conditions × 3 maturity stages at harvest). Results were submitted to a Principal Component Analysis (PCA) using The Unscrambler® X software (version 9.7, CAMO A/S, Trondheim, Norway) to show an overview of the results. Before the PCA performing the data matrix was auto scaled for each variable in order to obtain the same weight for all variables (mean = 0 and variance = 1). Additionally, a variance analysis (ANOVA) at 5% of error probability was carried out. Data that showed significant difference by ANOVA were subjected to the Tukey's test at 5% error probability.

3.1.3. Results and discussion

3.1.3.1. Quality analyses at harvest

After harvest, the fruit were transported to the postharvest research center and submitted to an initial analysis to gain an insight of the initial quality (Table 1). Higher ACC oxidase enzyme activity was verified in overripe apple, but ethylene production was higher in ripe and overripe apple as compared to unripe apple. Analyzing the ACC oxidase enzyme activity and ethylene production together, is noteworthy that the limiting factor for ethylene production in unripe apple is the low ACC (1-aminocyclopropane-1-carboxilate) content and not the ACC oxidase enzyme activity. The respiration rate was higher in ripe harvested apple, intermediary in overripe apple and lowest in unripe harvested apple. Brackmann et al. (2004) also found lower respiration rate in overripe apple as compared to ripe apple, they explained overripe apple stay over the climacteric peak, and therefore the respiration declined. Fruit harvested at ripe stage showed higher gas diffusion rate as compared to unripe and overripe apple. Ripe and overripe apple had higher juiciness, soluble solids, fruit weight, and lower flesh firmness and acidity.

Table 1 - Metabolism and quality of 'Galaxy' apple harvested in 3 maturity stages plus 1 day at 20°C after harvest (initial analyses). Santa Maria, Brazil, 2015.

Variable	Units	Maturity stages			Mean
		Unripe	Ripe	Overripe	
Iodine-starch index	0 - 10	3.38 C*	6.46 B	9.66 A	6.50
Fruit weight	g	116.1 B	122.7 A	123.2 A	120.7
ACC oxidase	ng kg^{-1} s^{-1}	8.99 B	8.44 B	15.8 A	11.1
Ethylene production	ng kg^{-1} s^{-1}	0.34 B	0.60 A	0.59 A	0.51
Respiration rate	µg kg^{-1} s^{-1}	5.39 C	7.86 A	6.72 B	6.66
Gas diffusion rate	µg m^{-2} s^{-1}	2.81 B	3.42 A	2.69 B	2.97
Juiciness	%	57.6 B	70.7 A	72.6 A	66.9
Flesh firmness	Newton	85.8 A	76.9 B	72.9 C	78.5
Soluble solids	%	12.0 C	13.4 B	14.6 A	13.4
Titratable acidity	% malic acid	0.37 A	0.34 B	0.33 B	0.35

*Means followed by equal uppercase letters in the lines do not differ by Tukey's test, at 5% probability.

3.1.3.2. Quality analyses after storage

No difference among storage conditions and maturity stages were observed for decay incidence and pulp cracking (data not showed). The average decay incidence was 23.9% and pulp cracking of 2.1% (mean of all storage conditions and maturity stages).

As expected, the PCA was efficient in discriminate the fruit response to the different treatments and show an overview of the results (Figure 2a and b). According to Figure 2a, the PC I was important in separate the CA stored fruit, either ripe or overripe, from those unripe CA, DCA-RQ 1.3, DCA-RQ 1.5 and of the three maturity stages stored under CA + 1-MCP. Correlated to ripe and overripe CA stored apple are high ACC oxidase enzyme activity, ethylene production, respiration rate, physiological disorders and soluble solids (Figure 2b). On the other hand, unripe CA + 1-MCP, DCA-RQ 1.3 and DCA-RQ1.5 stored apple, ripe apple stored in CA + 1-MCP and DCA-RQ 1.3 and overripe apple stored under CA + 1-MCP are correlated to high flesh firmness, healthy fruit, gas diffusion rate and titratable acidity (Figure 2b). According to the results, DCA-RQ 1.3 maintain similar quality as compared to fruit stored under CA + 1-MCP and higher quality as compared to CA only, independently of the maturity stage at harvest. This is an important practical result due to the high cost of 1-MCP application for the storers and the replacement of 1-MCP to a clean technology, as such DCA-RQ 1.3.

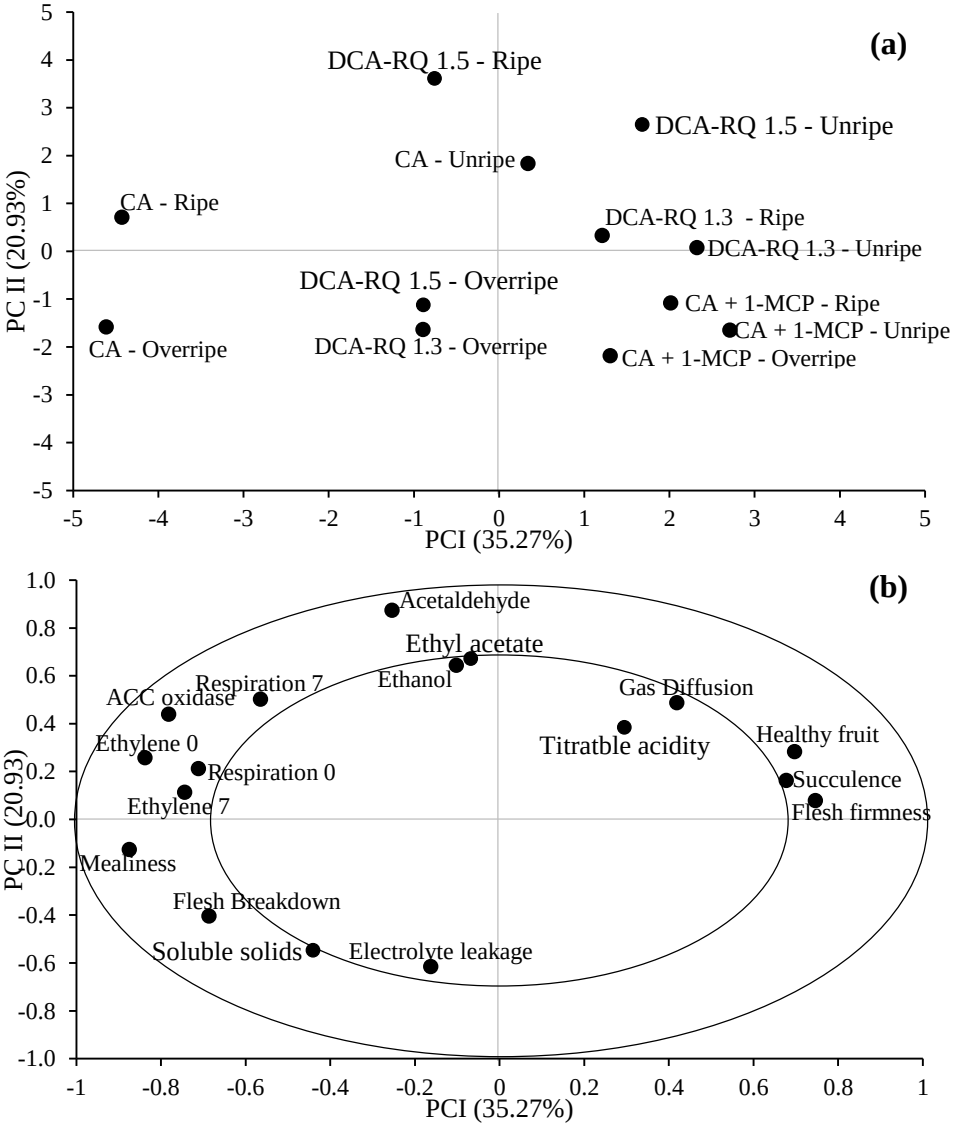

Figure 2 - (a) - scores (treatments) and (b) - correlation loadings (variables) plots showing the two major principal components of 'Galaxy' apple harvested in three maturity stages and its interaction with controlled atmosphere (CA) and dynamic controlled atmosphere (DCA-RQ) after nine months of storage plus 7 days shelf life at 20°C. Santa Maria, Brazil, 2015.

Concerning to the PC II, it was important to show the distinct response of apple harvested at ripe stage and stored under DCA-RQ 1.5 from those stored in the other conditions (Figure 2a). This different response of ripe DCA-RQ 1.5 stored apple is related to the high acetaldehyde, ethanol and ethyl acetate production by these fruit (Figure 2b). The extremely low oxygen partial pressure (0.21 kPa in average) employed during DCA-RQ 1.5 (Figure 1b) induced the anaerobic metabolism leading in acetaldehyde, ethanol and ethyl acetate accumulation in apple pulp. The PC II also show that gas diffusion rate, healthy fruit, flesh

firmness and titratable acidity are more correlated to fruit stored in DCA, especially DCA-RQ 1.3 as compared to those stored in CA + 1-MCP (Figure 2b).

The ACC oxidase is a key enzyme in the ethylene biosynthesis pathway, so the control of its activity is extremely important to control fruit ripening. There was a significant interaction between storage conditions and maturity stages for ACC oxidase enzyme activity (Figure 3a). Fruit of the three maturity stages stored under CA showed the highest ACC oxidase enzyme activity, but if the fruit were treated with 1-MCP (CA + 1-MCP) the ACC oxidase enzyme activity was drastically reduced at the three maturity stages. The lower ACC oxidase enzyme activity by fruit treated with 1-MCP is related to the lower gene expression which codify this enzyme by 1-MCP application (Wakasa et al., 2006; Tatsuki et al., 2007; Yang et al., 2013). Thewes et al. (2015b) also found a lower ACC oxidase enzyme activity by 1-MCP application in CA stored 'Royal Gala' apple. On the other hand, the storage under DCA-RQ 1.3 and DCA-RQ 1.5 was also efficient in suppressing the ACC oxidase enzyme activity, regardless the maturity stage at harvest. Additionally, fruit stored under DCA-RQ 1.3 showed similar ACC oxidase enzyme activity as compared to fruit stored in CA + 1-MCP, showing that the 1-MCP may be replaced by the storage in DCA-RQ 1.3. Perhaps, the low ACC oxidase enzyme activity, by fruit stored under DCA-RQ 1.3, is a result of the extremely low oxygen employed during storage (Figure 1a), as soon as oxygen is necessary to transform ACC to ethylene by the ACC oxidase. Weber et al. (2015) observed a similar response for ACC oxidase activity in 'Royal Gala' apple stored under DCA-RQ, with RQ ranging from 2.0 up to 6.0, corroborating our results.

Comparing the three maturity stages, unripe fruit stored in CA and DCA-RQ 1.5 showed the highest ACC oxidase enzyme activity, differing from ripe harvested fruit. This result shows that overripe fruit stay over the climacteric peak, and after storage the unripe CA stored fruit stay near the climacteric and ripe fruit at the climacteric peak. Nevertheless, if the fruit were treated with 1-MCP, a different response among maturity stages is verified, with higher ACC oxidase activity in ripe and overripe apple, but no difference among maturity stages is verified in DCA-RQ 1.3 stored apple, showing that the storage under DCA-RQ 1.3 is more efficient to avoid the rise in ACC oxidase enzyme activity with maturity advance.

Ethylene has a key importance in apple metabolism and quality changing before, during and after storage. At either chamber opening or 7 days of shelf life there was an interaction between storage conditions and maturity stages for ethylene production (Figure 3b and c). Independently of the harvest maturity, fruit stored under CA showed the highest ethylene production both at chamber opening and after 7 days of shelf life. Thewes et al. (2015a) also

found higher ethylene production in CA stored ‘Royal Gala’ and ‘Galaxy’ apple as compared to DCA-CF and ultralow oxygen storage (0.40 kPa O_2). The high ethylene production by fruit stored under CA is related to the high oxygen partial pressure during storage, which allowed high ACC oxidase enzyme activity (Figure 3a) leading in high ethylene biosynthesis rate (Yang and Hoffman, 1984). Fruit stored in DCA-RQ 1.3 and CA + 1-MCP showed the lowest ethylene production at either chamber opening or 7 days of shelf life in unripe and ripe harvested apple, but in overripe apple, only after 7 days of shelf life CA + 1-MCP and DCA-RQ1.3 showed lower ethylene production rate as compared to fruit stored in DCA-RQ 1.5. Some early studies reported that the ethylene biosynthesis is significantly suppressed as the oxygen partial pressure is reduced in ‘Royal Gala’ (Both et al., 2014a; Weber et al., 2015; Thewes et al., 2015a) and ‘Galaxy’ apple (Brackmann et al., 2015; Thewes et al., 2015a), but these studies were carried out in only one maturity stage (ripe).

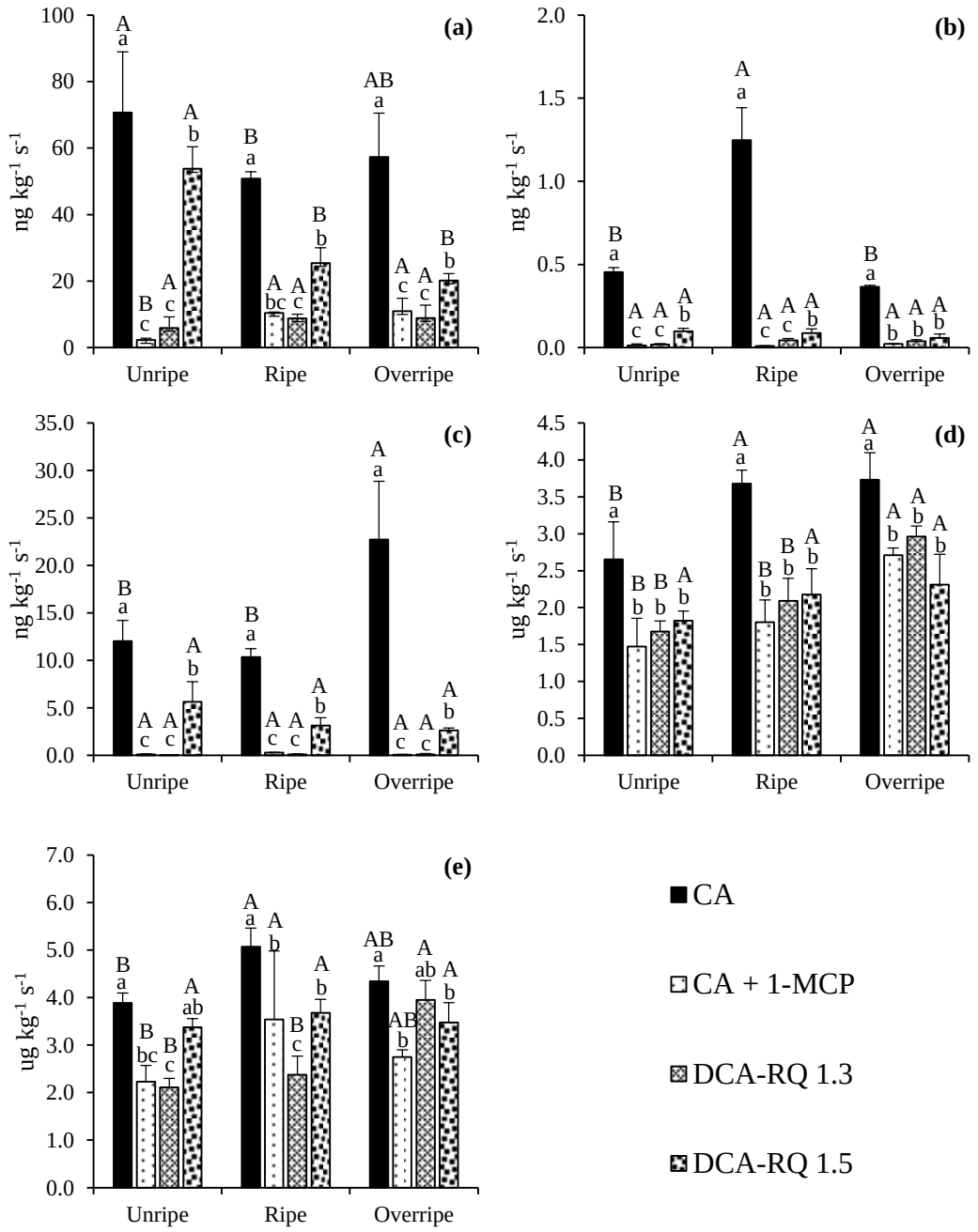

Figure 3 - ACC oxidase enzyme activity after 7 days of shelf life (a), ethylene production at chamber opening (b), and after 7 days of shelf life at 20°C (c), respiration rate at chamber opening (d), and after 7 days of shelf life at 20°C (e) of 'Galaxy' apple harvested in three maturity stages stored in CA, CA + 1-MCP and DCA-RQ. Bars with the same lower case letter in the same maturity stage, and each bar with the same upper case letter in different maturity stages are not significantly different by Tukey's test, at 5% probability.

Evaluating the three maturity stages in CA stored apple, higher ethylene production was verified in ripe fruit at chamber opening, but after 7 days of shelf life, overripe fruit showed

higher ethylene production (Figure 3b and c). If the fruit were stored in CA + 1-MCP, DCA-RQ 1.3 and DCA-RQ 1.5 the ethylene production was similar at the three maturity stages, at either chamber opening or after 7 days of shelf life. This result shows that the DCA and 1-MCP are efficient in ethylene biosynthesis suppression, maintaining similar ethylene production at the three maturity stages. Analyzing the ACC oxidase enzyme activity and ethylene production together, it is noteworthy that the low ethylene production in fruit stored under DCA-RQ 1.5 is due to the low ACC concentration, showing that the storage in DCA-RQ 1.5 may be inhibited the ACC synthase enzyme activity (Figure 3a, b and c). In 'Golden Delicious' apple harvested in different maturity stages and cold stored, the 1-MCP suppressed the ethylene production, independently of the harvest maturity (Gabiound Rebeaund and Gasser, 2015; Gago et al., 2015), corroborating our results. However, our result showed at the first time that apple storage in DCA-RQ also maintain low ethylene production at the three maturity stages and that DCA-RQ 1.3 has similar effect as compared to 1-MCP application.

The high ethylene production by fruit stored in CA resulted in higher respiration rate, at chamber opening and after 7 days of shelf life, independently of the maturity stage at harvest (Figure 3d and e). At chamber opening, fruit stored under CA + 1-MCP, DCA-RQ 1.3 and DCA-RQ 1.5 showed similar respiration rate at the 3 maturity stages, but after 7 days of shelf life, DCA-RQ 1.3 was more effective in respiration suppression in unripe and ripe harvested fruit, as compared to the other storage treatments. In 'Royal Gala' apple, the storage in DCA-RQ reduced the respiration rate of optimal harvested (ripe) fruit and was correlated to ethylene production (Weber et al., 2015), corroborating the result of the present study. The lower respiration rate at the three maturity stages by DCA-RQ 1.3 show that the entirely fruit metabolism is reduced and fruit quality may be maintained (Steffens et al., 2007; Thewes et al., 2015a), as exhibited by the opposite response of respiration rate and fruit quality in the PCA (Figure 2b).

Ripe and overripe apple stored under CA showed the highest respiration rate at chamber opening, but after 7 days of shelf life there was higher respiration in ripe harvested fruit as compared to unripe fruit (Figure 3d and e), showing that overripe apple stay over the climacteric peak. The respiration rate at chamber opening had more relation to the fruit metabolism throughout the storage period, as reported in an early study with 'Royal Gala' apple stored under ULO conditions (Both et al., 2014a), so ripe and overripe apple had higher metabolism during storage. However, if the fruit were stored under CA + 1-MCP and DCA-RQ 1.3 only overripe fruit showed higher respiration rate and fruit of the three maturity stages showed similar respiration rate if stored under DCA-RQ 1.5. These results together showed that the DCA-RQ,

regardless of the RQ level, significantly reduced the metabolism during storage, as compared to CA stored fruit. This is a significant practical result because apple harvest is carried out at different maturity stages and generally are stored together in the same storage chamber, showing these apples should be stored under DCA-RQ, with RQ ranging between 1.3 – 1.5, due to the similar respiration rate in the different maturity stages at harvest. The effect of DCA-RQ on respiration rate is also extended up to 7 days of shelf life, where only overripe fruit showed higher respiration rate, if fruit were stored in DCA-RQ 1.3 and no difference among maturity stages if stored in DCA-RQ 1.5. The low respiration rate delayed ripening and physiological disorders incidence in ripe and overripe apple stored under DCA-RQ (Figure 2b).

Throughout the storage and shelf life fruit need to maintain its respiration for energy supply, so the O_2 should be diffused from the external atmosphere to the inner cells and the CO_2 from the inner cells to the external atmosphere. Thereby, the gas diffusion rate plays a fundamental role in fruit quality maintenance. The gas diffusion rate was significantly affected by the maturity stages and storage conditions (Figure 4a). DCA-RQ 1.5 showed higher gas diffusion rate in unripe apple if compared to CA + 1-MCP. In ripe harvested apple, CA stored fruit showed the lowest gas diffusion rate and was no difference among CA + 1-MCP, DCA-RQ 1.3 and DCA-RQ 1.5, but in overripe fruit, the storage under DCA-RQ 1.3 promoted the highest gas diffusion rate as compared to CA and DCA-RQ 1.5. Fruit with higher gas diffusion rate had higher cell integrity and the intercellular space are free, allowing the gas diffuse through these spaces (Schotsmans et al., 2004; Brackmann et al., 2014). The lower gas diffusion in CA stored fruit is related to the higher mealiness incidence in these fruit, leading in cell adhesion loss and interruption of the intercellular spaces (Brackmann et al., 2014).

There was no significant difference for gas diffusion rate among the three maturity stages if the apple were stored under CA (Figure 4a). However, if the fruit were stored under CA + 1-MCP and DCA-RQ 1.3, higher gas diffusion was verified in ripe harvested apple as compared to unripe harvested fruit, however, overripe DCA-RQ 1.5 stored apple showed lower gas diffusion rate in relation to unripe and ripe harvested apple. These results showed that if the ripe apple were stored under CA, gas diffusion decreased during storage as compared to harvest (Table 1), but in CA + 1-MCP and DCA-RQ 1.3 the high gas diffusion rate in ripe harvested apple is maintained, showing that this conditions maintained higher cell integrity. The lower gas diffusion rate in unripe harvested apple is related to the pulp compaction of early harvested apple, but in overripe apple, it is reduced due to the advanced ripening stage that these fruit stay. According to Schotsmans et al. (2004), the gas diffusion in fruit flesh changes with the maturity stage at harvest, corroborating results of our research. In ‘Brookfield’ apple stored

under CA, the gas diffusion in flesh was reduced with ripening advance and was correlated with mealiness incidence (Brackmann et al., 2014).

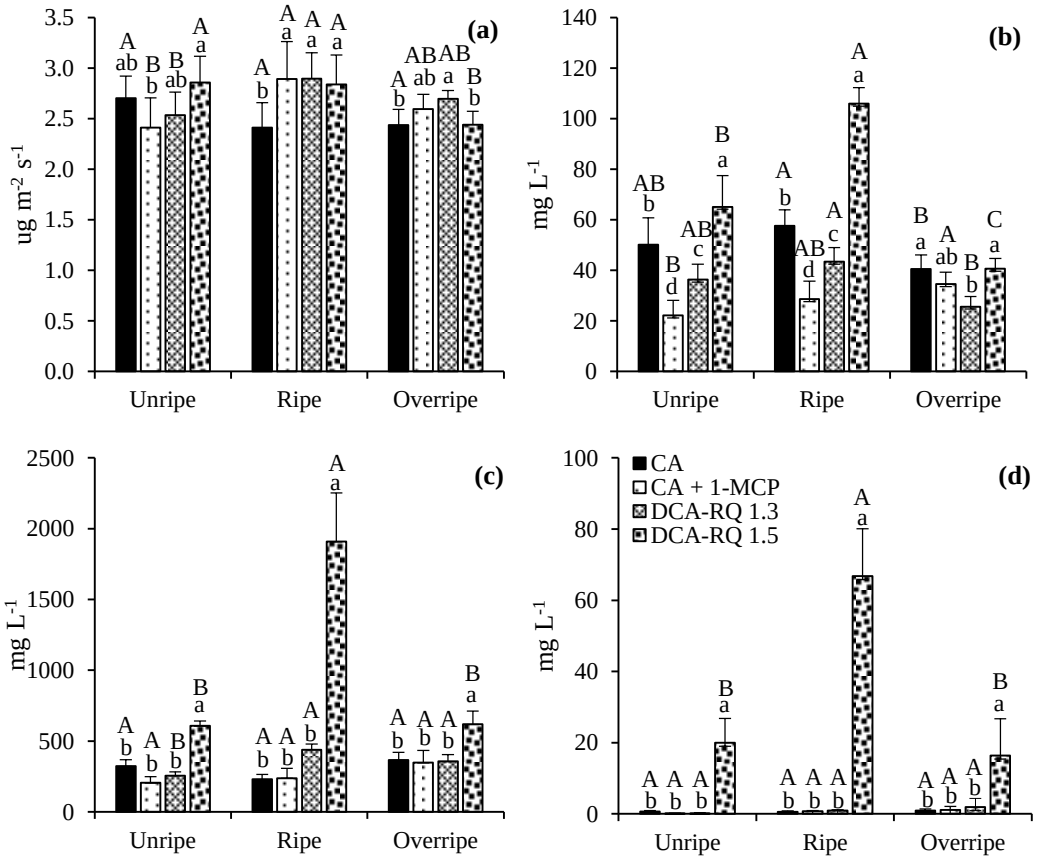

Figure 4 - Gas diffusion rate (a), acetaldehyde (b), ethanol (c) and ethyl acetate (d) concentration of 'Galaxy' apple harvested in three maturity stages stored in CA, CA + 1-MCP and DCA-RQ. Bars with the same lower case letter in the same maturity stage, and each bar with the same upper case letter in different maturity stages are not significantly different by Tukey's test, at 5% probability.

Storage the apple under extremely low oxygen partial pressure, as reported on the present study (Figure 1), resulted in anaerobic metabolism products accumulation, as well as acetaldehyde, ethanol and ethyl acetate (Pesis, 2005). Unripe and ripe harvested apple showed the same response to the treatments, with higher acetaldehyde concentration in DCA-RQ 1.5, intermediary concentration in CA and DCA-RQ 1.3 and lowest concentration in CA + 1-MCP stored apple (Figure 4b). A noteworthy fact is that fruit stored under CA + 1-MCP showed lower acetaldehyde production as compared to CA storage only. Lee et al. (2012) also verified a significant lower acetaldehyde concentration in apple treated with 1-MCP as compared to

untreated apple. This fact may be related to the effect of 1-MCP on the pyruvate decarboxylase (PDC) activity and expression (Harb et al. 2010; Ortiz et al. 2010; Harb et al. 2011). The 1-MCP application reduced the ethylene production (Figure 3b and c) and thereby the PDC expression and activity, as well as ethylene modulate the PDC expression (Harb et al. 2010; Harb et al. 2011). Perhaps, the lower ethylene production by fruit stored under DCA-RQ 1.3 (Figure 3b and c) also resulted in lower PDC expression and activity, explaining the lower acetaldehyde production by apple stored under this condition (Figure 3b).

According to fruit ripening advance, is expected higher fermentation potential, at either CA or DCA storage (Ke et al., 1994; Wright et al., 2015). At the present study, higher acetaldehyde accumulation was verified in ripe harvested fruit as compared to overripe fruit, with the exception to CA + 1-MCP stored apple (Figure 4b). However, an important result is that overripe harvested apple stored under CA showed the same acetaldehyde concentration as compared to DCA-RQ 1.5. The high acetaldehyde concentration in CA storage fruit is related to advanced ripening stage, resulting in cell compartmentalization loss, reducing the gas diffusion in flesh (Figure 4a), which resulted in anaerobic metabolism even under high oxygen partial pressure (1.2 kPa) accumulating acetaldehyde in flesh as a response to senescence (Ke et al., 1994; Wright et al., 2015). On the other hand, overripe DCA-RQ 1.5 stored apple also showed low gas diffusion rate (Figure 4a), but they were not in an advanced ripening stage, due to the low respiration rate (Figure 3d and e) and low mealiness incidence (Figure 5b), showing that in this case the acetaldehyde accumulation is due to the low oxygen employed during storage (Figure 1b), 0.21 kPa in average.

Acetaldehyde is the ethanol precursor, so is expected high ethanol concentration in apple with high acetaldehyde, if the alcohol dehydrogenase (ADH) is not the limiting factor, as soon as acetaldehyde is a dangerous compound to apple cell in high concentration and therefore is quickly transformed in ethanol (Pesis, 2005; Lee et al., 2012). Regardless the maturity stage at harvest, fruit stored under DCA-RQ 1.5 showed higher ethanol production (Figure 4c). The higher ethanol production by these fruit is a result of the extremely low oxygen employed during storage (Figure 1b) resulting in acetaldehyde concentration, allowing ethanol production by the ADH (Ke et al., 1994; Saquet and Streif, 2008; Harb et al. 2010; Harb et al. 2011; Lee et al., 2012). Nevertheless, it appears that fruit stored under CA, CA + 1-MCP and DCA-RQ 1.3 showed similar ADH enzyme activity, due to the same ethanol concentration and differential acetaldehyde concentration, but fruit stored under DCA-RQ 1.5 showed higher ADH activity culminating in high ethanol production. The extremely low oxygen partial pressure (Figure 1b) induced the ADH enzyme activity during storage, as reported in an early paper with ‘Bartlett’

pears (Ke et al., 1994). Fruit harvested at ripe stage showed the highest ethanol concentration, if stored under DCA-RQ 1.5, ripe and overripe apple higher concentration if stored in DCA-RQ 1.3 and no difference among maturity stages if the fruit were stored under CA and CA + 1-MCP (Figure 4c). In 'Granny Smith' apple, the storage under DCA-RQ 2.0, the ethanol concentration did not increase as compared to CA storage only (Bessemans et al., 2016). The differences between their and our study is a result of an error in RQ determination by Bessemans et al. (2016), because the chamber gas leakage, overestimating the LOL during storage. Probably, due to this those apples did not show an increment in ethanol concentration by storage under DCA-RQ 2.0. Additionally, this difference may be a result of the different cultivars studied in our and their study.

The highest ethanol production by fruit stored under DCA-RQ 1.5 resulted in higher ethyl acetate production by the alcohol acyltransferase (AAT), independently of the maturity stage at harvest (Figure 4d). The ethyl acetate is an important volatile compound to the apple flavor, if it is produced in low concentration it contributes significantly to apple flavor (Dixon and Hewett, 2000; Echeverría et al., 2008; Wright et al., 2015), but in high concentration it is associated to off-flavor formation (Wright et al., 2015). Similarly to the ethanol production, fruit stored under CA, CA + 1-MCP and DCA-RQ 1.3 showed the same ethyl acetate production and there are no difference among the three maturity stages at harvest. According to Echeverría et al. (2004), the precursor concentration (ethanol in this case) is more important for the ester formation as compared to the enzyme activity. On the other hand, the 1-MCP application increases the AAT expression in apple (Harb et al., 2010; Harb et al., 2011) and peaches (Ortiz et al., 2010). Thus, the differential ethyl acetate production by fruit stored under DCA-RQ 1.5 is due to the higher ethanol concentration.

Fruit with high anaerobic metabolism accumulated acetaldehyde, ethanol and ethyl acetate, which acts on the membrane integrity resulting in electrolyte leakage (Saquet et al., 2000). However, in the present study the ethanol production did not result in higher electrolyte leakage, regardless the maturity stage (Figure 5a), showing that at this concentration the anaerobic metabolism product did not result in cell membrane damage. Additionally, the electrolyte leakage stay in an opposite side as compared to anaerobic metabolism products along the PC II, showing that fruit with low anaerobic metabolism products showed higher electrolyte leakage (Figure 2b). Fruit treated with 1-MCP showed the highest electrolyte leakage at the three maturity stages (Figure 5a). Otherwise in 'Golden Delicious' apple, the 1-MCP application reduced significantly the electrolyte leakage, independently of the maturity at harvest (Gago et al., 2015). The electrolyte leakage is a good indicator of membrane integrity

(Wade, 1995; Gago et al., 2015), so 1-MCP treated fruit showed lower membrane integrity at the present study. Perhaps, the higher electrolyte leakage by fruit with 1-MCP is a result of lower respiration (Figure 3d and e), resulting in lower membrane reparation due to the low energy production (Saquet et al., 2000) or due to any damage of the 1-MCP directly on the cell membrane. In loquat fruit the 1-MCP application change the membrane lipids composition, increasing the unsaturated/saturated fatty acids ratio (Cao et al., 2009), so 1-MCP application may caused similar effect at the present study, increasing the membrane permeability. The membrane permeability was higher in overripe harvested fruit, independently of the storage condition, showing overripe apple had lower membrane integrity. This result agree with some previous works, which also found high electrolyte leakage with the ripening advance (Wade, 1995; Antunes and Sfakiotakis, 2008). These same authors attributed the high electrolyte leakage due to the higher membrane fluidity by the change in fatty acid composition (increase in unsaturated/saturated fatty acid ratio).

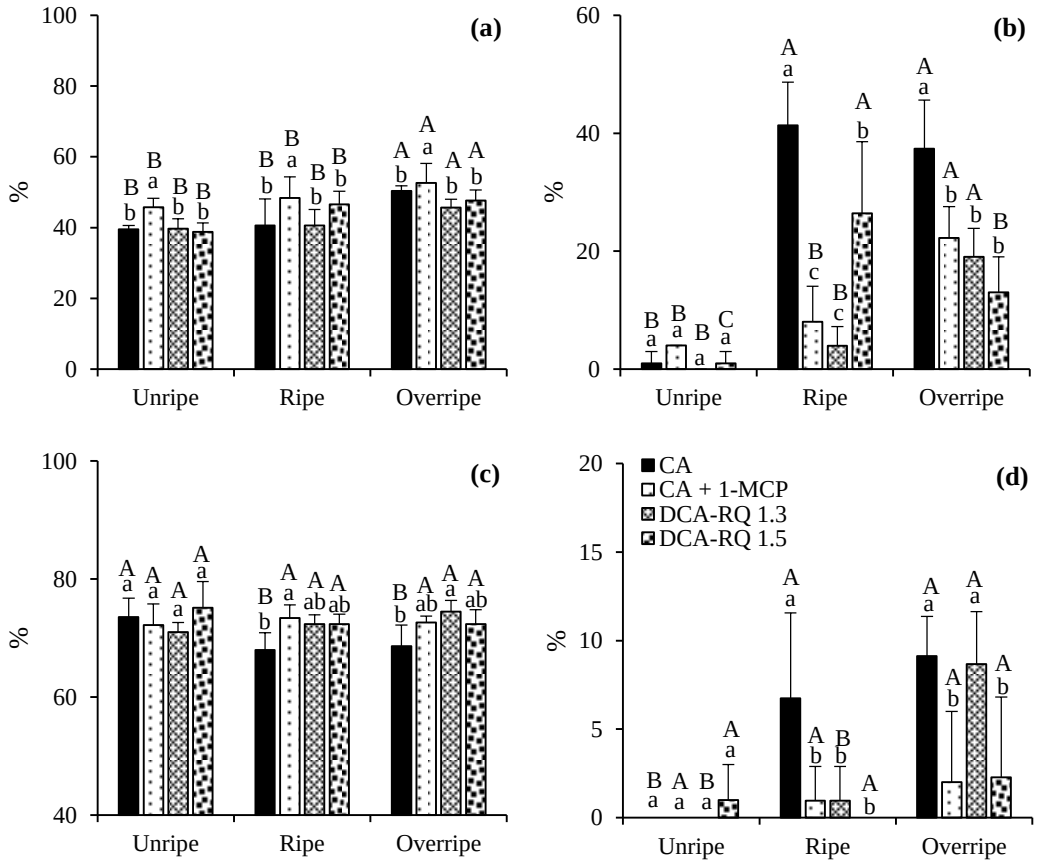

Figure 5 - Electrolyte leakage (a), mealiness (b), juiciness (c) and flesh breakdown (d) of 'Galaxy' apple harvested in three maturity stages stored in CA, CA + 1-MCP and DCA-

RQ. Bars with the same lower case letter in the same maturity stage, and each bar with the same upper case letter in different maturity stages are not significantly different by Tukey's test, at 5% probability.

One of the main problems of harvested the fruit after the optimal maturity is the mealiness incidence. At the present study, there was a significant interaction between maturity stages and storage condition for mealiness incidence (Figure 5b). If the fruit were harvested unripe, the mealiness incidence was very low, without difference among storage condition. However, in ripe and overripe harvested apple there was a significant difference among storage conditions, with higher mealiness incidence in CA stored fruit as compared to the other storage conditions. In overripe harvested apple, CA + 1-MCP, DCA-RQ 1.3 and DCA-RQ 1.5 showed similar mealiness incidence, but in ripe harvested fruit, CA + 1-MCP and DCA-RQ 1.3 showed lower mealiness incidence compared to CA and DCA-RQ 1.5. These results shows that the DCA and 1-MCP are efficient in ripening control, decreasing the mealiness incidence as compared to CA storage only. The higher mealiness incidence by fruit stored in CA is related to the higher ethylene production and respiration rate (Figure 3b, c, d and e). The relationship among these variables is confirmed by their closeness in the PCA (Figure 2b). The higher ethylene production in CA stored fruit started the cell wall enzymes (Prassana et al., 2007; Nishiyama et al., 2007; Goulao and Oliveira, 2008; Payasi et al., 2009), which degraded the middle lamella, reducing the cell adhesion, resulting in flesh with mealy aspect. Mealiness is an unwanted characteristic for apple marketing (Moshou et al., 2003), showing that the CA stored fruit had reduced market quality as compared to DCA and CA + 1-MCP stored apple.

Regarding the maturity stages, low mealiness incidence was verified in unripe harvested apple, nevertheless, if the fruit were stored in CA + 1-MCP and DCA-RQ 1.3, no increment in mealiness incidence was observed in fruit harvested at ripe stage as compared to unripe harvested apple (Figure 5b). Additionally, a noteworthy fact is that overripe harvested fruit stored in CA + 1-MCP, DCA-RQ 1.3 and DCA-RQ 1.5 showed lower mealiness incidence as compared to ripe harvested fruit stored under CA (Figure 2a and b). This is an important practical result because we can store overripe harvested apple with lower mealiness incidence as compared to ripe harvested stored under CA. The lower mealiness in these fruit is related to the lower ethylene production, resulting in lower cell wall enzymes activity, maintaining a higher cell adhesion, even in overripe harvested apple.

As higher the mealiness incidence, lower is the apple juiciness (Arefi et al., 2016). The juiciness together with crispness shows a key importance in consumer apple acceptability

(Harker et al., 2008). The storage conditions showed similar juiciness if the apple were harvested before the optimal maturity (unripe) (Figure 5d). However, ripe harvested fruit stored under CA + 1-MCP showed higher juiciness as compared to CA storage, but there was no difference as compared to those fruit stored under DCA. In overripe apple, the storage under DCA-RQ 1.3 showed the highest juiciness compared to CA, without difference from those stored under CA + 1-MCP and DCA-RQ 1.5. These results showed that DCA and 1-MCP had a similar effect on fruit juiciness maintenance. Another important result is that if the fruit were stored under CA the juiciness decreased with the ripening advance, but in CA + 1-MCP and DCA the juiciness was similar in the three maturity stages. The lower juiciness in ripe and overripe CA stored fruit is related to the higher mealiness incidence in this fruit (Figure 5b), the inverse relationship between juiciness and mealiness is confirmed by the opposite response in the PCA (Figure 2b). Additionally, the lower juiciness, in CA stored fruit, is a result of the middle lamella degradation, which allows the cell separation instead of cell rupture, resulting in flesh with a mealy aspect (Both et al., 2014b; Arefi et al., 2016).

As well as mealiness, flesh breakdown is another important physiological disorder of the Gala group apple. It is characterized as the cell compartmentalization loss, resulting in cell dead and flesh browning due to phenolic oxidation. At the present study, there was no significant difference among storage conditions if fruit were harvested before the optimal harvest period (unripe) (Figure 5d). However, if the fruit were harvested at the ripe and overripe stage, CA stored fruit showed higher flesh breakdown in relation to all other treatments, with the exception of overripe apple, where CA did not differ from those stored under DCA-RQ 1.3. This is an important practical result, because the storage under DCA-RQ maintained similar quality as compared to CA + 1-MCP, showing the 1-MCP may be replaced by the storage under DCA, reducing the storage cost. The effect of 1-MCP on flesh breakdown is widely studied and its effect is related to the cultivar, maturity stage at harvest, time between harvest and 1-MCP application, among some other factors (Watkins, 2006). In some cases its application reduced flesh breakdown (Watkins, 2006) or increased flesh breakdown (DeEll et al., 2008; Jung and Watkins, 2011; Lee et al., 2012; Watkins and Nock, 2012; Nock and Watkins 2013). The flesh breakdown is also related to anaerobic metabolism during storage (Franck et al., 2007; Fan et al., 2011) that provides insufficient energy to supply cell membrane reparation (Ho et al., 2013). Nevertheless, at the present study, the storage under DCA-RQ 1.5 did not result in higher flesh breakdown, showing that at this oxygen partial pressure (0.21 kPa O_2 in average) (Figure 1b), the energy supply is enough to maintain cell compartmentalization and the anaerobic metabolism products accumulation did not increase the flesh breakdown.

The higher flesh breakdown in apple stored under CA is related to the higher ethylene production and respiration rate, as showed by the closeness among these parameters in the PCA (Figure 2b). In 'Royal Gala' and 'Galaxy' apple, the ethylene production and respiration rate also had a close relationship with flesh breakdown incidence (Thewes et al., 2015a). Comparing the maturity stage in each storage condition, ripe and overripe CA stored apple showed higher flesh breakdown as compared to unripe CA stored apple (Figure 5d), but if fruit were treated with 1-MCP or stored under DCA-RQ 1.5, no increment in flesh breakdown was observed with the maturity stage advance. Overripe DCA-RQ 1.3 stored apple showed higher flesh breakdown as compared to unripe and ripe apple. Some early studies observed an increment in flesh breakdown with the maturity advance in apples stored under normal atmosphere and CA (Beaudry et al., 1993; Fan et al., 2011; Lu et al., 2012; Kweon et al., 2013; Moggia et al., 2015). The results of the present study showed that the 1-MCP application and DCA-RQ 1.5 storage did not allow the flesh breakdown increasing with the maturity stage advance.

Taken in account all physiological disorders, decay incidence and pulp cracking it is possible to obtain the total percentage of fruit without any damage, which are considered healthy fruit (Figure 6a). As expected unripe harvested fruit had higher healthy fruit amount as compared to the other maturity stages. However, there is no reduction in healthy fruit amount between ripe and overripe harvested apple. The lower amount of healthy fruit by ripe and overripe apple is a result of the higher metabolism (Figure 3b – e) related to the higher ethylene production (Figure 2b), which advanced the ripening and physiological disorders resulting in lower healthy fruit amount (Thewes et al., 2015a). The lower healthy fruit amount by ripe and overripe apple can be related to the lower flesh firmness (Figure 6b), which is related to the high mealiness and flesh breakdown in these fruit, as showed by PCA between healthy fruit and these variables (Figure 2b).

Evaluating the effect of storage conditions in each maturity stage, no difference were verified in unripe and overripe harvested apple (Figure 6a). Nevertheless, in ripe harvested fruit, higher healthy fruit amount is observed in CA + 1-MCP and DCA-RQ 1.3 as compared to CA. Once again, the storage under DCA showed similar effect as compared to 1-MCP application in CA stored fruit. The higher healthy fruit by CA + 1-MCP and DCA-RQ 1.3 stored apple is a result of lower mealiness (Figure 5b) and flesh breakdown incidence (Figure 5d), especially CA + 1-MCP. In 'Royal Gala' apple stored under CA (1.2 kPa O_2 + 1.2 kPa CO_2) the 1-MCP application resulted in higher healthy fruit amount and was correlated to the lower mealiness incidence (Thewes et al., 2015b). The storage of 'Galaxy' apple under DCA-RQ 1.5 resulted in higher healthy fruit amount as compared to the storage in CA (Brackmann et al., 2015).

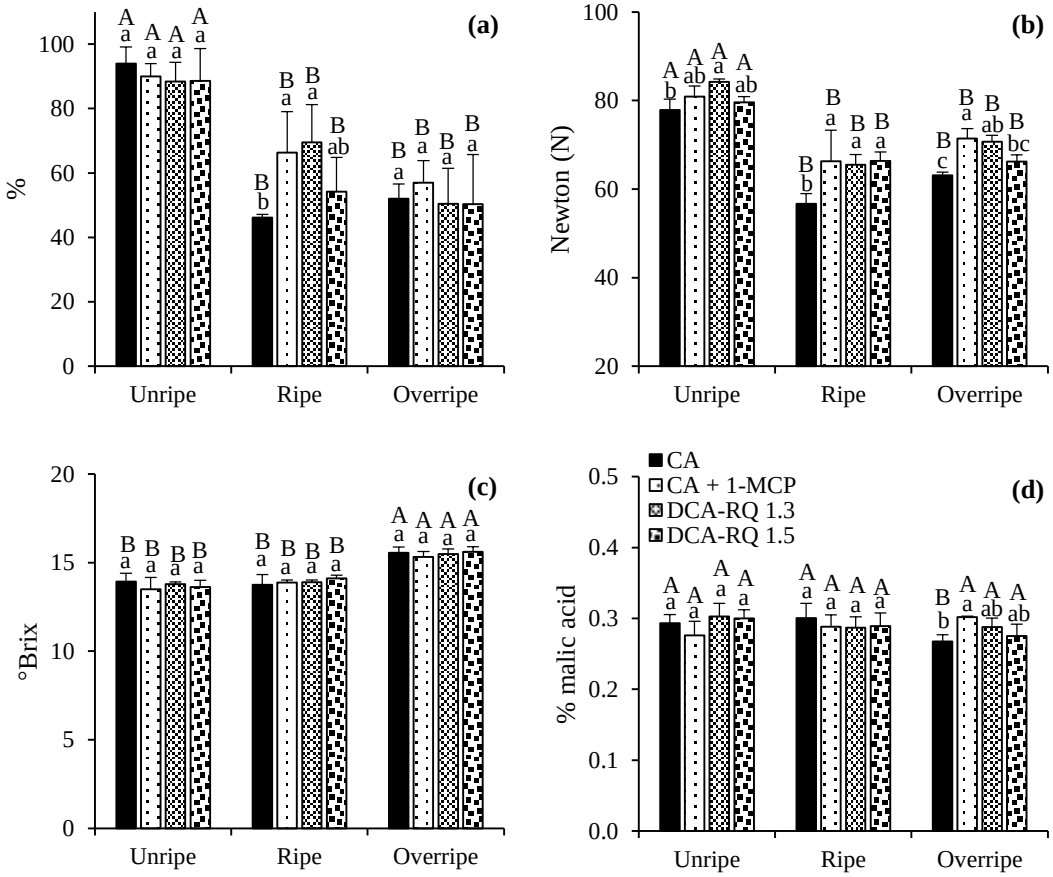

Figure 6 - Healthy fruit (a), flesh firmness (b), soluble solids (c) and titratable acidity (d) of 'Galaxy' apple harvested in three maturity stages stored in CA, CA + 1-MCP and DCA-RQ. Bars with the same lower case letter in the same maturity stage, and each bar with the same upper case letter in different maturity stages are not significantly different by Tukey's test, at 5% probability.

There was a significant interaction between maturity stages and storage conditions for flesh firmness (Figure 6b). Unripe DCA-RQ 1.3 stored apple showed higher flesh firmness as compared to CA, but no difference were verified if compared to CA + 1-MCP and DCA-RQ 1.5. Ripe harvested apple stored under CA + 1-MCP, DCA-RQ 1.3 and DCA-RQ 1.5 resulted in higher flesh firmness as compared to CA storage. Similarly, in overripe apple, the storage under CA + 1-MCP resulted in higher flesh firmness as compared to fruit stored under CA and DCA-RQ 1.5, but there was no difference between CA + 1-MCP and DCA-RQ 1.3. The higher flesh firmness, in fruit treated with 1-MCP or stored under DCA-RQ 1.3, are a result of lower ethylene production and respiration rate (Figure 2a – b), as well as ethylene is important to signalize the cell wall enzymes, starting its expression and activity (Nishiyama et al., 2007; Prasanna et al., 2007; Goulao and Oliveira, 2008; Payasi et al., 2009). Brackmann et al. (2015)

found higher flesh firmness in ‘Galaxy’ apple stored under DCA-RQ 1.5 as compared to DCA-CF and CA, corroborating our results. Additionally, the higher flesh firmness in 1-MCP treated apple is a worldwide know result (Corrent et al., 2004; Lu et al., 2012; Gago et al., 2015; Thewes et al., 2015b), but our result showed that the 1-MCP can be replaced to DCA-RQ 1.3 storage, which is a clean technology.

Regarding the maturity stages in each storage condition, higher flesh firmness was verified in unripe harvested fruit as compared to ripe and overripe apple (Figure 6b). On the other hand, there was no difference between ripe and overripe apple, regardless the storage conditions. In ‘Golden Delicious’ apple there was also no difference between ripe and overripe apple after cold storage (Gago et al., 2015), corroborating our study. The higher flesh firmness in unripe apple after storage is related to the higher firmness at harvest and lower ethylene production (Table 1). This behavior of flesh firmness is normal, being confirmed in some early works (Beaudry et al., 1993; Brackmann et al., 2004; Fan et al., 2011; Gago et al., 2015).

Fruit harvested at overripe maturity stage showed higher soluble solids, regardless the storage condition (Figure 6c). Concerning the storage conditions in each maturity stage, there was no difference among treatments. The higher soluble solids in overripe apple is in accordance with literature (Brackmann et al., 2004; Lu et al., 2012; Gago et al., 2015). Together with soluble solids, titratable acidity is a key factor in consumer apple acceptably (Harker et al., 2008) and there is a relationship between these two variables. In the present study, there was a significant interaction between storage conditions and maturity stages for titratable acidity (Figure 6d). No difference was verified among storage conditions in unripe and ripe harvested apple, but in overripe harvested apple, CA + 1-MCP stored apple showed higher titratable acidity as compared to CA only. At this maturity stage, CA + 1-MCP did not differ from those fruit stored under DCA-RQ. This result showed that 1-MCP reduced acidity loss during storage when applied to fruit stored in CA. Corrent et al. (2004) also found higher titratable acidity by 1-MCP treatment, independently of the maturity at harvest. Brackmann et al. (2015) and Weber et al. (2015) found higher acidity in apple stored under DCA-RQ as compared to CA, but in the present research, there was no difference between CA and DCA-RQ, independently of the maturity stage.

In relation to the maturity stages, lower acidity is observed in overripe apple stored under CA, as compared to unripe and ripe harvested apple. If fruit were stored under CA + 1-MCP, DCA-RQ 1.3 and DCA-RQ 1.5 no reduction in acidity was observed as the maturity advanced (Figure 6d). These results showed that CA was not able to prevent acidity loss in overripe apple, but CA + 1-MCP, DCA-RQ 1.3 and DCA-RQ 1.5 prevented acidity loss during storage. The

low acidity in overripe CA stored apple is related to higher respiration rate, due to the high oxygen during storage (1.2 kPa O_2). Some early paper also found lower acidity in overripe harvested apple as compared to unripe and ripe harvested apple (Brackmann et al., 2004; Fan et al., 2011; Lu et al., 2012).

3.1.4. Conclusions

DCA-RQ 1.3 and DCA-RQ 1.5 are efficient in quality keeping of 'Galaxy' apple resulting in low ethylene production, mealiness incidence, and high flesh firmness and juiciness, regardless the maturity stage. DCA-RQ 1.3 show similar quality keeping as compared to 1-MCP application in CA stored fruit, being a promising storage technology, such as for organic apple.

DCA-RQ 1.5 result in apple with high acetaldehyde, ethanol and ethyl acetate concentration, but its concentration is not enough to increases the electrolyte leakage and physiological disorder related to fermentation, especially flesh breakdown.

Unripe harvested apple are lesser responsive to the storage conditions and are easier to store as compared to ripe and overripe apple, because do develop low mealiness, flesh breakdown and maintain high flesh firmness, regardless the storage condition. Moreover, DCA-RQ storage suppresses the mealiness in overripe apple, showing overripe apple should be stored under DCA-RQ, regardless the RQ level.

Fruit stored under DCA-RQ 1.5 reach the lowest oxygen partial pressure during storage before the ones stored under DCA-RQ 1.3, showing that the fruit stored under DCA-RQ 1.5 have higher anaerobic metabolism before fruit stored under DCA-RQ 1.3.

3.1.5. References

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4. ARTIGO 2

4.1. DYNAMIC CONTROLLED ATMOSPHERE STORAGE SUPPRESSES METABOLISM AND ENHANCES THE VOLATILE PRODUCTION OF 'GALAXY' APPLE HARVESTED IN THREE MATURITY STAGES[2]

Abstract

The objective of this study was to assess the interaction between storage conditions and three maturity stages at harvest (unripe, ripe and overripe) on the metabolism and volatile compounds production of 'Galaxy' apple after harvest and 9 months of storage plus 7 days of shelf life at 20°C. The following treatments were evaluated in each maturity stage: [1] Controlled atmosphere (CA) (1.2 kPa O_2 + 2.0 kPa CO_2); [2] CA + 1-methylcyclopropene (1-MCP) application (0.625 µL L^{-1}); [3] Dynamic controlled atmosphere based on respiratory quotient 1.3 (DCA-RQ 1.3) + 1.2 kPa CO_2 and [4] DCA-RQ 1.5 + 1.2 kPa CO_2. Fruit stored under DCA-RQ 1.5 showed higher total ester concentration and higher concentration of characteristic aroma volatile compounds of 'Galaxy' apple. The largest amount of esters occurred in overripe apple stored under DCA-RQ 1.3 and DCA-RQ 1.5. 1-MCP application suppressed the volatile compounds production, not allowing its increment with the advance of maturity stages and reduced the main esters after storage in relation to harvest. The DCA-RQ suppressed significantly the internal ethylene, ethylene production and respiration rate, but the metabolism reduction in DCA-RQ 1.5 stored fruit did not result in volatile compounds suppression.

Keywords: *Malus domestica*, respiratory quotient, aroma, anaerobic metabolism, ethylene, respiration rate.

4.1.1. Introduction

Volatile compounds show a fundamental importance in apple acceptance by the consumers (López et al., 2007). These compounds change significantly according to the maturity of fruit at harvest and the storage conditions (Song and Bangerth, 1996; Fellman et al., 2003; Bangerth et al., 2012). Among the volatile compound produced by apple, esters show

[2] Artigo formatado de acordo com as normas da revista Postharvest Biology and Technology.

major impact in fruit aroma. Apple of the 'Gala' group, such as 'Galaxy', show relatively high ester production, especially butyl acetate, 2-methylbutyl acetate and hexyl acetate (Young et al., 1996; Salazar and Orozco, 2011; Both et al., 2014), which are the most important esters of this cultivar. These compounds increase with the maturity advance at harvest, but if the fruit are harvested in advanced maturity stages some other quality parameters, such as firmness and acidity, are reduced, showing the necessity to develop a storage technology that allow harvest the fruit in different maturity stages without significant volatile compounds and physical quality losses.

Nowadays, almost the total apple are stored under controlled atmosphere (CA), especially during long-term. Nevertheless, the storage under CA strongly reduced the ester formation (Brackmann et al., 1993; Fellman et al., 2003; López et al., 2007; Raffo et al., 2009; Lumpkin et al., 2014; Lumpkin et al., 2015). The low oxygen partial pressure employed during CA storage, suppresses the ethylene biosynthesis, which is important to start the ester forming enzymes (Yang et al., 2016). Additionally, the low oxygen during CA storage suppresses the volatile precursor production via β-oxidation and lipoxygenase (LOX) pathway (Brackmann et al., 1993 Song and Bangerth, 2003) because both routs require oxygen to run (Echeverría et al., 2004). The storage under CA maintain fruit quality during a long-term storage (6 up to 7 months), but to maintain quality throughout longer periods and avoid significant quality losses, additional technologies should be employed, such as 1-methylcyclopropene (1-MCP) treatment.

The 1-MCP application is a worldwide used technology. Its application extend the storage period due to the ethylene action blocking (Blankenship and Dole, 2003; Watkins, 2006; Lee et al., 2012), delaying the fruit ripening. 1-MCP treated apple maintain higher flesh firmness (Fawbush et al., 2009; Moggia et al., 2010; Brackmann et al., 2013), titratable acidity, soluble solids (Blankenship and Dole, 2003; Watkins, 2006), lower superficial scald (Tsantili et al., 2007; Moggia et al., 2010) and mealiness (Brackmann et al., 2014), despite some other benefits on fruit quality. On the other hand, the ethylene action blocking has a negative impact in fruit volatile biosynthesis, especially alcohols and esters (Lurie et al., 2002; Kondo et al., 2005; Lee et al., 2012; Thewes et al., 2015; Yang et al., 2016). The lower ethylene production and action in fruit treated with 1-MCP suppresses the precursor and volatile compounds biosynthesis, reducing the characteristically apple aroma. Additionally, the 1-MCP application has high cost for the storers and is not allowed for organic apple storage. Therefore, happen the necessity to develop a storage technology that reduces the fruit metabolism similarly to 1-MCP application without significant volatile compounds loss, to maintain the characteristic apple variety aroma.

During the last few years, a new storage technology based on the lower oxygen limit (LOL) tolerated by the fruit throughout the storage has been develop and adopted in commercial storage rooms. This new storage technology is called dynamic controlled atmosphere (DCA) and now are three methodologies to detect the LOL in real time during storage. Techniques based on the ethanol production by fruit (Veltmann et al., 2003), fruit chlorophyll fluorescence emission (DeEll et al., 1999; Prange et al., 2007; Wright et al., 2010; Wright et al., 2012) and respiratory quotient (Gasser et al., 2008; Wright et al., 2012; Brackmann, 2015; Weber et al., 2015). According to Raffo et al. (2009), the storage of 'Pinova' apple under DCA based on chlorophyll fluorescence (DCA-CF) reduced the main esters as compared to CA storage (1.5 kPa O_2 + 1.3 kPa CO_2), but if compared to CA + 1-MCP higher ester amount are maintained. Nevertheless, there are no results in the literature evaluating the effect of DCA based on respiratory quotient (DCA-RQ) on the volatile profile of apple. This technology allows to induce a little fermentation by fruit (Brackmann et al., 2015; Weber et al., 2015), which can supply the volatile compounds precursors to the enzymes, resulting in a significant increment in volatile compound biosynthesis as compared to CA and CA + 1-MCP. In oriental sweet melons, the ethanol application increased the volatile compound production, like ethyl esters, butyl acetate and hexyl acetate (Liu et al., 2012).

In view of the above exposed, the objective of this study was to assess the interaction between storage conditions and three maturity stages at harvest (unripe, ripe and overripe) on the metabolism and volatile compounds production of 'Galaxy' apple after harvest and 9 months of storage plus 7 days of shelf life at 20°C.

4.1.2. Material and methods

4.1.2.1. Plant material, orchard location, harvest maturity and sample preparation

Apple of the cultivar Galaxy, a 'Gala' strain, were harvested in a commercial orchard located at Vacaria, RS, Brazil. The 'Galaxy' apple were grafted on M9 rootstocks and a density of 3,575 plants ha^{-1} was used in the orchard. During the growing season, the following fertilization were carried out: 80 kg ha^{-1} of nitrogen and 120 kg ha^{-1} of potassium.

The 'Galaxy' apple were harvested in three maturity stages, according to the iodine-starch index (Streif, 1984). Unripe apple had at harvest and iodine starch lower to 3.5, ripe iodine-starch index between 3.5 – 7.0 and overripe with iodine-starch index over 7.0. In each harvest, the iodine-starch index was determined in 3 replicates of 20 fruit each. At this analysis,

unripe apple had iodine-starch index in average = 3.42; ripe apple = 6.48 and overripe apple = 9.67.

Immediately after harvest, the fruit were transported to the Postharvest Research Center of the Federal University of Santa Maria, RS, Brazil. At the Postharvest Research Center, the fruit were submitted to a selection process, aiming to eliminate fruit with any damage and homogenize fruit samples. Thereafter samples of 25 fruit each, were performed, 3 samples per treatment.

4.1.2.2. Storage conditions

After the sample preparation, fruit were placed into 233 l experimental CA chambers to install the following treatments: [1] controlled atmosphere - CA (1.2 kPa O_2 + 2.0 kPa CO_2); [2] CA + 1-methylcyclopropene application (0.625 µL L^{-1}); [3] DCA-RQ 1.3 + 1.2 kPa CO_2 and [4] DCA-RQ 1.5 + 1.2 kPa CO_2. Each treatment was composed by 3 replicates of 25 fruit each, totalizing 75 fruit per treatment.

The storage temperature was seated at 1.5 ± 0.1 °C and monitored daily during the 9 months of storage with the aid of mercury thermometers inserted inside the fruit flesh to determine the pulp temperature. Inside the storage chamber, the relative humidity was monitored manually with psycrometers and controlled by the allocation of calcium chloride, which absorbed the excess of humidity inside the chamber, maintaining the average at 94 ± 2% of relative humidity.

4.1.2.3. 1-MCP treatment

Fruit of the treatment with 1-MCP were stowed inside a experimental chamber of 233 l and thereafter treated with 1-MCP. A solution containing 0.625 µL L^{-1} 1-MCP was prepared (SmartFresh®, 0.14% of active ingredient) and allocated into Petry discs inside the chamber, immediately after, the chamber was hermetically closed during 24 hours. During the 24 hours, the air inside the chamber was bustling with a fan. This process was carried out at the storage temperature (1.5 ± 0.1 °C). After the 1-MCP treatment period the fruit were stored according to the conditions above described.

4.1.2.4. CA and DCA-RQ setup and maintenance

The experimental chambers were hermetically closed and the CA and DCA-RQ conditions installed. At the first storage day, the temperature was reduced down to 5.0°C and thereafter gradually down to 1.5°C in 5 days. At the day that the temperature reached the pre-established level (1.5°C), the CA and DCA-RQ were setup. To obtain the desired atmospheric condition the chambers were flushed with nitrogen until to the oxygen pre-established partial pressure for CA (1.2 kPa) and reduced down to 0.5 kPa for DCA-RQ conditions, this process was also carried out in 5 days. The carbon dioxide partial pressure was obtained by its accumulation in the storage chamber by fruit respiration. Thus, during the first 5 days of storage only the temperature was reduced, from the fifth day up to the tenth day the CA and DCA-RQ conditions were installed. This procedure was carried out to simulate the commercial CA and DCA storage condition.

Throughout the storage period the oxygen partial pressure were changed according to fruit metabolism in DCA and maintained constant for CA. To measure the LOL in real time during storage period the respiratory quotient (RQ) was measured two times a week, according to the method proposed by Brackmann (2015). Thus, the RQ was seated at 1.3 and 1.5, and the oxygen partial pressure changed accordingly to maintain this RQ level. The RQ was calculated with a chamber closure of 13 hours between the first and second reading. The RQ was calculated by the reason between CO_2 production and O_2 uptake. In relation to the CA conditions, they were maintained according to the method proposed by Brackmann et al. (2014).

4.1.2.5. Metabolism and volatile compounds analyses

These analyses were carried out at harvest, for the three maturity stages, and after 9 months of storage under CA, CA + 1-MCP, DCA-RQ 1.3 and DCA-RQ 1.5 plus 7 days of shelf life at 20 ± 2°C and relative humidity 80 ± 2%.

4.1.2.5.1. Internal ethylene concentration

Determined according to the methodology proposed by Mannapperuma et al. (1991). Thus, the internal air of fruit was withdrew and two samples (1 mL) injected into a gas chromatograph (Varian®, model Star 3400CX) equipped with a flame ionization detector (FID) and a Porapak N80/100 column. The temperatures of the column, injector and the detector were 90, 140 and 200°C, respectively. Results were expressed in ug L^{-1}.

4.1.2.5.2. Ethylene production and respiration rate

To determine the ethylene biosynthesis rate and respiration rate, the fruit were stowed inside 5-liter flask and hermetically closed during about 2 hours. After, 2 samples of 1 mL were taken of the flask and injected in the same gas chromatograph used to determine the internal ethylene. Taken in account the flask volume, fruit weight, ethylene concentration inside flask and time of closure the ethylene production rate was calculated and expressed in ng kg^{-1} s^{-1}. Immediately after the ethylene determination, the internal air of the same flask was circulated through an electronic gas analyzer (Schele®, model KB7), which determined the CO_2 concentration inside the flask. Respiration rate was expressed as µg CO_2 kg^{-1} s^{-1}.

4.1.2.5.3. Volatile compounds analysis

4.1.2.5.3.1. Sample preparation

In order to prepare the samples for volatile compounds analysis, the fruit pulp was cooled down to 0°C. Immediately after the pulp cooling, horizontal slices of the equatorial region of fruit were taken, discharged the seeds, and centrifuged under low temperature, to avoid the chemical and enzymatic oxidation of samples (the maximum juice temperature during sample preparation was 5°C). The juice was placed inside 100 mL amber flash and immediately frozen down to -30°C up to the volatile compounds analysis.

4.1.2.5.3.2. Volatile compounds quantification

Samples were stored under -30°C up to the day of the analysis, at the day of analysis the samples were thawed in ambient temperature up to the juice was liquid (temperature 2°C). An aliquot of 10 mL of this juice was taken, mixed with 3g NaCl and 10 µL of 3-octanol standard solution (10 µg mL^{-1}) inside a 20 mL vial that allowed hermetically sealing with a PTFE-coated silicone lid.

From this solution, the volatile compounds were extracted from the headspace via solid phase microextraction (HS-SPME). A Divinylbenzene/carboxen/polydimethylsiloxane (DVB/Car/PDMS) fibre (Supelco, 50/30 µm × 20 mm) was preconditioned following the manufacturer protocol. Before the fiber exposing, the vial was submerged in a water bath at 35

°C during 5 min. After 5 min, the fiber was exposed to the headspace of the sample during 60 min under constant stirring at the same temperature.

The volatile compounds were quantified with a DANI® (Dani Intruments Spa., Viale Brianza, Cologno Monzese, Italygas) gas chromatograph equipped with a flame ionization detector (FID). The fiber was thermally desorbed into the injection port during 10 min at a temperature of 250 °C in a split less mode. A capillary column DN-WAX (60 m × 0.25 mm × 0.25 µm) allowed separating the volatile compounds. As carrier gas nitrogen at a constant flow of 1.0 mL min^{-1} was used. The temperature ramp used during the analysis was: initial temperature 35 °C held during 3 min, then a ramp of temperature of 2 °C min^{-1} up to 80 °C, thereafter, another ramp of 5 °C min^{-1} up to 230 °C and held at this temperature during 5 min. The temperature of FID detector was 230 °C. To calculate the linear retention index was analyzed a series of n-alkanes in the same chromatographic conditions used to analyze the volatile compounds. This analysis was carried out according to method proposed by Both et al. (2014).

4.1.2.5.3.3. Volatile compounds identification

The volatile compounds were identified using a Shimadzu QP2010 Plus gas chromatography coupled to mass spectrometry (GC/MS; Shimadzu Corporation, Kyoto, Japan). Thus, the same chromatographic conditions described to quantify the volatile compounds were used, with helium as the carrier gas. The detector was operated in the electron impact ionization, ionization energy of +70 eV and a scan mass from 35 up to 350 m/z. The mass spectra of each compound was compared with mass spectra available in the National Institute of Standards and Technology (NIST) library and by comparing the linear retention index (LRI) with those available in the scientific literature.

4.1.2.6. Statistical analysis

All data were submitted to a Principal Component Analysis (PCA) using The Unscrambler® X software (version 9.7, CAMO A/S, Trondheim, Norway) to show an overview of the results. Before the PCA the data matrix was auto scaled for each variable in order to obtain the same weight for all variables (mean = 0 and variance = 1). A Pearson correlation among internal ethylene, ethylene production, respiration rate and volatile compounds was carried out to show the linear relationship among the volatile compounds, ethylene and

respiration rate. Additionally, a variance analysis (ANOVA) at 5% of error probability was carried out. Data that showed significant difference by ANOVA were subjected to the Tukey's test at 5% error probability. The experiment was conducted in a completely randomized scheme with a factorial arrangement (4 storage conditions × 3 maturity stages at harvest).

4.1.3. Results and discussion

4.1.3.1. Metabolism and volatile profile at harvest

Before storage, the fruit were submitted to an initial analysis in order to gain insights about its metabolism and volatile profile at chamber entering and the results are summarized in Table 1. Fruit harvested at ripe and overripe maturity stages showed higher internal ethylene and ethylene production, but higher respiration rate was only observed in ripe harvested fruit as compared to unripe and overripe fruit, which is in accordance with the results of literature (Beaudry et al., 1993; Brackmann et al., 1993; Fellman et al., 2003). Perhaps, the fruit harvested at ripe stage were at the climacteric and overripe apple over the climacteric respiration peak, explaining the declined respiration in overripe apple in relation to ripe fruit.

High ethylene in ripe and overripe harvested fruit resulted in higher ester concentration, especially butyl acetate, 2-methylbutyl acetate and hexyl acetate (Table 1). The higher ester concentration with maturity advance stay in accordance with the literature, which observed a close relationship between ester production, ethylene and respiration rate (Song and Bangerth, 1996; Fellman et al., 2003; Bangerth et al., 2012) (Figure 1). Additionally, overripe apple also showed higher ester precursor concentration, it means 1-butanol, 2-methyl-1-butanol and 1-hexanol, which are precursors of the above-mentioned esters. These precursors are originated from fatty acids via β-oxidation and LOX, with the exception 2-methyl-1-butanol that is derived from amino acids, (Brackmann et al., 1993; Echeverría et al., 2008; Yang et al., 2016), which provided acyl CoA moiety and carbon skeletons to the ester formation.

Table 1 - Internal ethylene (µg L^{-1}), ethylene production (ng kg^{-1} s^{-1}), respiration rate (µg kg^{-1} s^{-1}) and volatile compounds production (µg L^{-1}) of 'Galaxy' apple harvested in three maturity stages at harvest (before storage).

(Continue)

		Maturity stages		
Compounds	LRI	Unripe	Ripe	Overripe
Matabolism*				
Internal ethylene	-	0.57 ± 0.19 b	2.26 ± 0.61 a	2.27 ± 0.15 a
Ethylene production	-	0.34 ± 0.12 b	0.60 ± 0.15 a	0.59 ± 0.18 a
Respiration rate	-	5.39 ± 1.02 c	7.86 ± 0.15 a	6.72 ± 1.03 b
Esters**				
Methyl acetate	839	0.05 ± 0.01 b	0.09 ± 0.02 b	1.39 ± 0.11 a
Ethyl acetate	897	0.51 ±0.08 b	1.01 ±0.14 b	5.70 ± 1.88 a
Ethyl propanoate	962	0.23 ± 0.24 a	0.01 ± 0.01 a	ND
Ethyl isobutanoate	968	ND	0.02 ± 0.01 a	ND
Propyl acetate	983	0.95 ± 0.53 b	1.73 ± 0.26 b	55.98 ± 16.80 a
Methyl 2-methyl butanoate	1015	ND	0.36 ± 0.34 b	2.50 ± 0.13 a
Isobutyl acetate	1018	2.34 ± 0.45 b	3.08 ± 0.22 b	35.95 ± 10.33 a
Ethyl butanoate	1042	ND	0.02 ± 0.01 a	ND
Ethyl 2-methyl butanoate	1057	43.62 ± 22.14 b	19.71 ± 0.29 b	112.28 ± 30.69 a
Butyl acetate	1083	91.12 ± 21.01 c	388.19 ± 9.76 b	4374.08 ± 848.58 a
2-Methyl butyl acetate	1128	20.06 ± 8.89 c	159.37 ± 12.53 b	1757.62 ± 292.90 a
Butyl Propanoate	1137	0.14 ± 0.10 b	0.08 ± 0.04 b	1.95 ± 0.83 a
3-Methyl butyl acetate	1168	35.05 ± 15.60 b	39.22 ± 4.10 b	530.47 ± 216.97 a
4-Pentenyl acetate	1192	0.25 ± 0.28 a	0.06 ± 0.02 a	ND
Butyl butanoate	1202	1.86 ± 1.41 a	2.40 ± 0.18 a	7.43 ± 12.87 a
Z-2-Pentenyl-acetate	1241	0.35 ± 0.19 b	1.16 ± 0.13 b	14.78 ± 2.82 a
Hexyl acetate	1262	40.75 ± 7.33 c	420.93 ± 19.29 b	4331.64 ± 1422.8 a
Z-2-Hexenyl acetate	1286	3.39 ± 3.43 ab	0.44 ± 0.05 b	6.27 ± 0.18 a
Z-3-Hexenyl acetate	1290	1.35 ± 0.80 b	3.42 ± 0.31 b	13.12 ± 2.37 a
E-3-Hexenyl acetate	1294	5.41 ± 4.38 b	0.17 ± 0.06 b	53.98 ± 12.34 a
5-Hexenyl-acetate	1316	1.49 ± 0.98 b	5.23 ± 0.37 b	105.05 ± 24.37 a
E-2-Hexenyl acetate	1321	0.21 ± 0.11 b	9.43 ± 0.72 a	11.78 ± 3.78 a
Heptyl acetate	1364	0.36 ± 0.40 a	0.09 ± 0.02 a	1.84 ± 3.18 a
Butyl hexanoate	1394	0.59 ± 0.37 b	0.07 ± 0.01 b	5.95 ± 4.46 a
Benzyl acetate	1726	0.42 ± 0.24 b	1.66 ± 0.11 b	9.16 ± 4.13 a
Alcohols				
2-Propanol	936	0.03 ± 0.03 a	0.03 ± 0.01 a	ND
Ethanol	945	1.14 ± 0.74 b	0.32 ± 0.05 b	9.81 ± 1.35 a
1-Butanol	1162	7.85 ± 2.91 b	33.75 ± 8.64 b	311.42 ± 52.25 a
4-Methyl-2-pentanol	1176	0.67 ± 0.62 b	0.31 ± 0.10 b	12.72 ± 4.53 a
3-Hexanol	1201	0.06 ± 0.11 a	0.06 ± 0.02 a	3.80 ± 3.28 a
2-Methyl-1-butanol	1211	ND	7.17 ± 2.34 b	211.14 ± 73.32 a

(Ends)

1-Pentanol	1249	ND	2.03 ± 0.35 a	0.39 ± 0.67 b
2-Methyl-2-buten-1-ol	1297	2.17 ± 1.67 a	ND	ND
1-Hexanol	1352	94.80 ± 19.11 b	93.09 ± 17.60 b	1112.85 ± 531.68 a
E-3-Hexen-1-ol	1361	1.46 ± 0.70 a	0.46 ± 0.06 a	15.49 ± 15.92 a
Z-3-Hexen-1-ol	1380	ND	0.17 ± 0.05 b	7.10 ± 2.44 a
E-2-Hexen-1-ol	1399	22.24 ± 5.77 a	9.26 ± 2.24 a	28.79 ± 18.80 a
E-5-Hexen-1-ol	1407	0.78 ± 0.54 a	1.57 ± 0.32 a	10.74 ± 9.39 a
E-1-Octen-3-ol	1445	0.27 ± 0.19 b	0.21 ± 0.01 b	3.19 ± 0.51 a
1-Heptanol	1454	0.85 ± 0.29 b	1.42 ± 0.12 b	23.57 ± 7.92 a
6-Methyl-5-hepten-2-ol	1465	0.34 ± 0.30 b	0.21 ± 0.04 b	5.68 ± 1.68 a
2-Ethyl 1-hexanol	1485	0.85 ± 0.36 b	0.47 ± 0.14 b	6.06 ± 1.11 a
1-Octanol	1554	0.25 ± 0.10 b	0.65 ± 0.06 b	2.44 ± 0.85 a
Aldehydes				
Acetaldehyde	644	0.44 ± 0.07 b	0.08 ± 0.01 b	5.42 ± 1.59 a
Butanal	890	0.38 ± 0.05 b	0.22 ± 0.05 b	5.01 ± 1.67 a
Hexanal	1099	1.25 ± 0.27 b	0.08 ± 0.02 b	3.58 ± 1.53 a
Z-3-Hexenal	1148	0.97 ± 0.23 b	3.08 ± 0.40 b	51.63 ± 8.50 a
Z-2-Hexenal	1205	0.14 ± 0.12 b	ND	28.66 ± 8.91 a
E-2-Hexenal	1222	163.73 ± 8.28 a	75.08 ± 8.52 a	146.59 ± 82.03 a
Ketones				
2-Propanone	831	0.74 ± 0.29 b	0.20 ± 0.05 b	6.10 ±2.75 a
6-Methyl-5-heptene-2-one	1334	0.50 ± 0.27 b	0.02 ± 0.001 b	5.46 ± 0.85 a

* Means followed by equal letters in the line do not differ by Tukey's test at 5% of error probability.
**Concentrations were calculated relative to an internal standard (3-octanol).
LRI: Linear retention index; ND: not detected.

4.1.3.2. Metabolism and volatile profile after storage plus shelf life

After 9 months of storage plus 7 days of shelf life at 20°C, there was a significant interaction between maturity stage and storage conditions for internal ethylene, ethylene production and respiration rate (Figure 2a – c). Fruit stored in CA showed higher internal ethylene and ethylene production as compared to the other storage conditions, independently of the maturity stage. Additionally, in unripe apple, the storage under DCA-RQ 1.5 resulted in higher internal ethylene and ethylene production as compared to CA + 1-MCP and DCA-RQ 1.3, but in ripe and overripe apple, there was no difference among the storage under CA + 1-MCP, DCA-RQ 1.3 and DCA-RQ 1.5 for these two variables (Figure 2a and b). The higher internal ethylene concentration and production by fruit stored under CA is a result of the high oxygen partial pressure employed during storage (1.2 kPa), which allowed the ACC

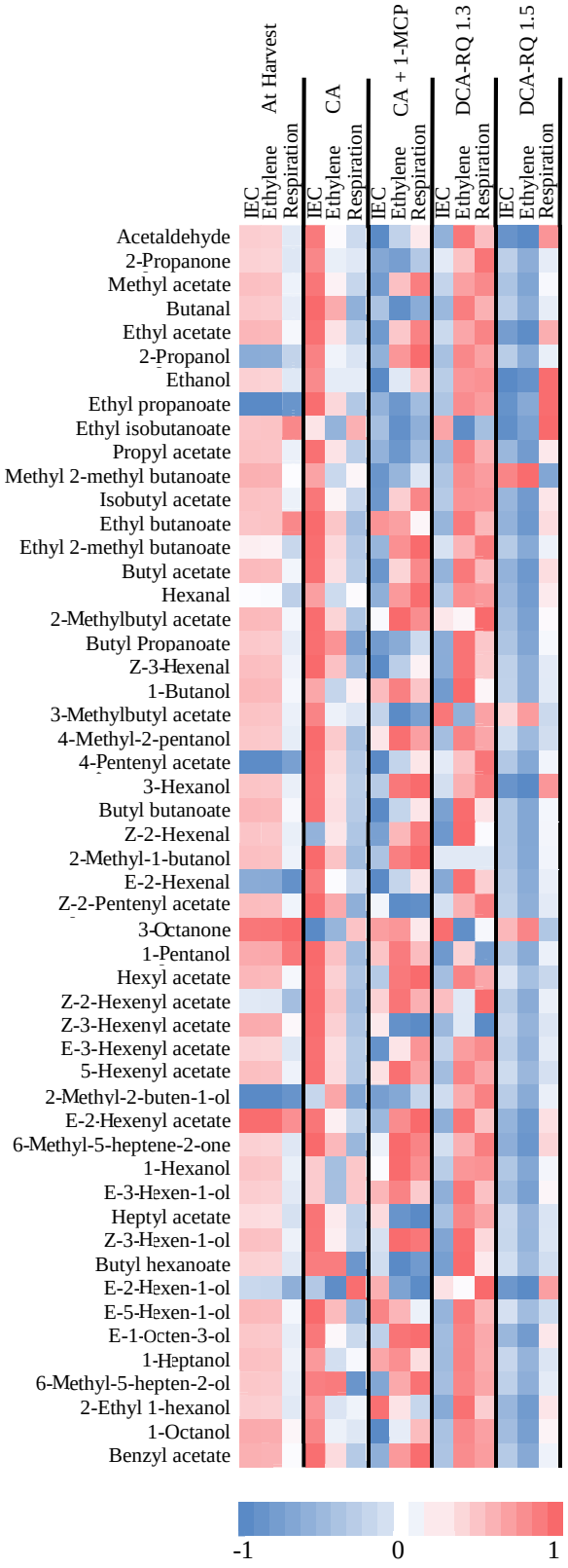

Figure 1 - Heat map showing the Pearson correlation, at harvest and after nine months of storage under CA, CA + 1-MCP, DCA-RQ 1.3 and DCA-RQ 1.5 plus 7 days of shelf life, among internal ethylene concentration (IEC), ethylene production (Ethylene),

respiration rate (Respiration) and the volatile compounds production of 'Galaxy' apple harvested in three maturity stages.

(1-aminocyclopropane-1-carboxilate) oxidation, by ACC oxidase, resulting in higher ethylene (Yang and Hoffman, 1984). Early works also found high ethylene in CA stored fruit as compared to CA + 1-MCP (Lee et al., 2012; Bekele et al., 2015; Thewes et al., 2015) and DCA-RQ (Brackmann et al., 2015; Weber et al., 2015).

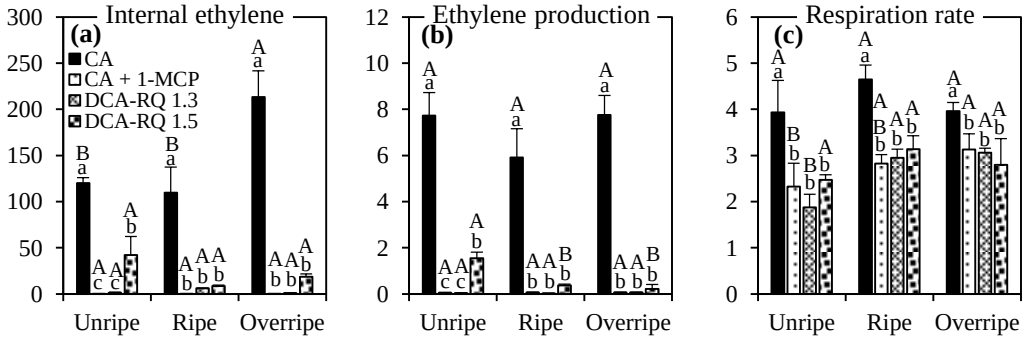

Figure 2 - Internal ethylene (µg L^{-1}) (a), ethylene production (ng kg^{-1} s^{-1}) (b) and respiration rate (µg kg^{-1} s^{-1}) (c) after 7 days of shelf life at 20°C of 'Galaxy' apple harvested in three maturity stages and its interaction with CA, CA + 1-MCP and DCA-RQ. Bars with the same lower case letter in the same maturity stage, and each bar with the same upper case letter in different maturity stages are not significantly different by Tukey's test, at 5% probability.

Regarding the maturity stages in each storage condition, higher internal ethylene was observed in overripe apple if fruit were stored under CA, but no difference among maturity stages was identified if fruit stored under CA + 1-MCP, DCA-RQ 1.3 and DCA-RQ 1.5 (Figure 2a). However, the ethylene production showed a different response to the maturity stages, with no difference in CA, CA + 1-MCP and DCA-RQ 1.3, but unripe fruit stored under DCA-RQ 1.5 showed higher ethylene production rate as compared to ripe and overripe fruit (Figure 2b). Analyzing these two variables (internal ethylene and ethylene production) together, is noteworthy that the ethylene biosynthesis rate can be the same among maturity stage, but its concentration around the cell can be change according to some other factors, such as ethylene diffusion rate from inside to outside flesh. In this manner, the internal ethylene showed a higher Pearson correlation with the volatile compounds as compared to ethylene production in CA stored apple (Figure 1). This shows that the ethylene concentration around cells is more important as compared to its production rate to start the volatile compounds biosynthesis under

aerobic conditions and that its concentration around the cells did not change only in function to its production rate.

The higher internal ethylene and ethylene production by CA stored fruit resulted in higher respiration rate, independently of maturity stage (Figure 2c). Fruit stored under CA + 1-MCP, DCA-RQ 1.3 and DCA-RQ 1.5 did not differ in its respiration rate, regardless the maturity stage. Comparing the maturity stages in each storage condition, no difference was observed if fruit were stored under CA and DCA-RQ 1.5. In contrast, overripe CA + 1-MCP and DCA-RQ 1.3 stored fruit showed higher respiration rate as compared to unripe fruit, with no difference from those harvested at ripe maturity stage. Reducing the respiration rate is important to extend storage and shelf life period, but, it is extremely important to supply energy for cell maintenance and volatile compounds biosynthesis (Song and Bangerth, 2003; Bangerth et al., 2012). Thus, is important reducing the partial pressure of oxygen to partial pressures that allows long-term storage and did not suppress the volatile compounds biosynthesis.

The GC-FID detected more than 100 volatile compounds, from which 51 were identified by the GC/MS. The 52 volatile compounds comprised some types: 25 esters, 17 alcohols, 6 aldehydes and 2 ketones. These compounds were identified in all samples before (with some exceptions) and after storage, independently of the maturity stage and storage condition. For all these compounds was identified a significant interaction between maturity stage and storage conditions.

Due to the large amount of compounds quantified and identified, an exploratory multivariate analysis was carried out to show an overview of the effect of maturity stages and storage conditions on the volatile profile (Figure 3). Together the first principal component (PC I) and second (PC II) explained 73.64 % of the overall variable variance (Figure 3). According to this analysis, the treatments (combination of maturity stages and storage conditions) were separated in different groups, either along PC I or PC II (Figure 3a). The PC I was important to separate the fruit stored under CA + 1-MCP, independently of the maturity, unripe and ripe DCA-RQ 1.3 from overripe apple stored under DCA-RQ 1.3 and DCA-RQ 1.5 (Figure 3a). The major of the volatile compounds were correlated to overripe apple stored under DCA-RQ 1.3 and DCA-RQ 1.5, and no compounds were correlated to the storage under CA + 1-MCP (fruit of the three maturity stages) and DCA-RQ 1.3 (unripe and ripe fruit) (Figure 3b).

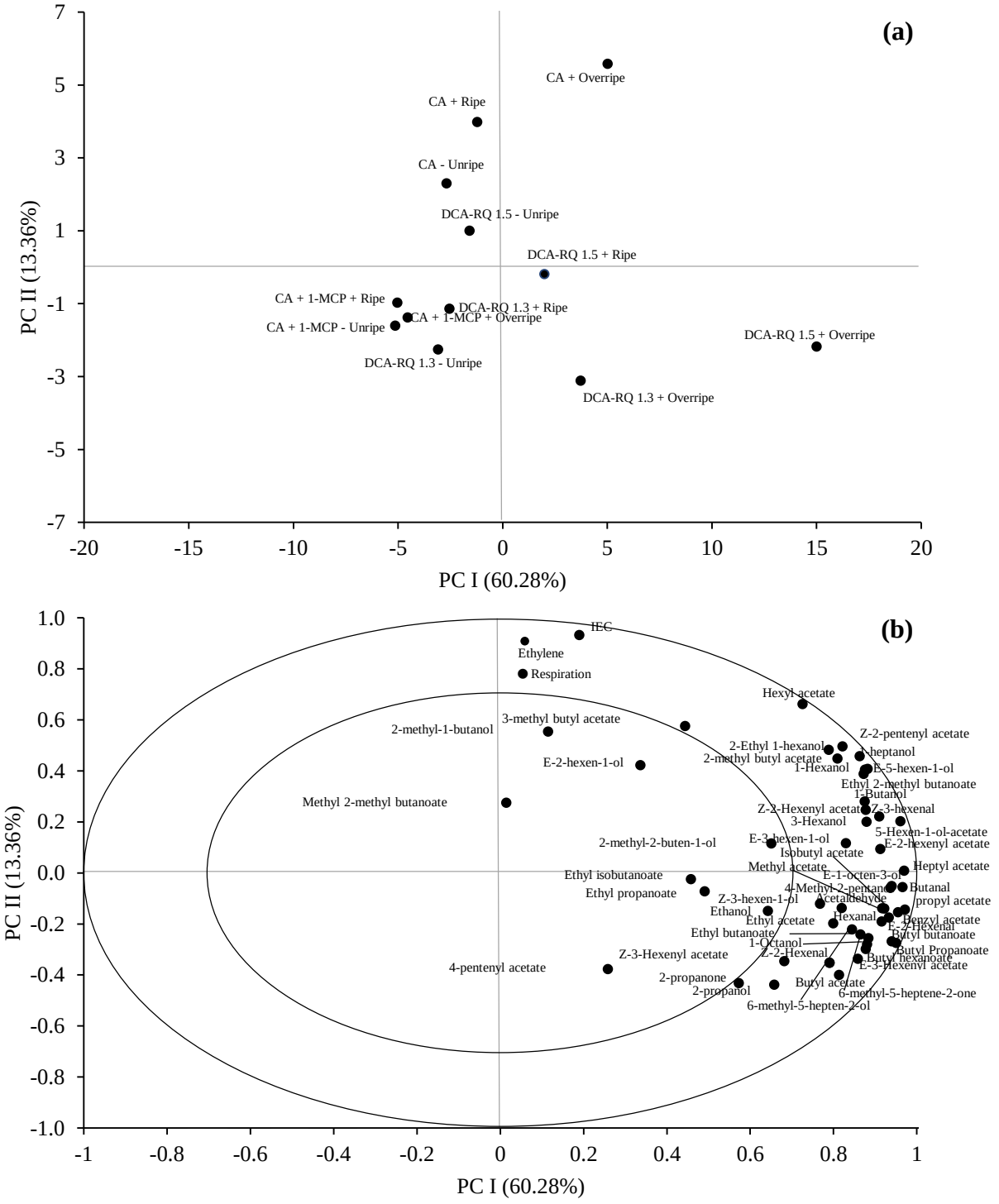

Figure 3 - (a) - scores (treatments) and (b) correlation loadings (variables) plots showing the two major principal components of the volatile profile in 'Galaxy' apple harvested in three maturity stages and its interaction with controlled atmosphere (CA) and dynamic controlled atmosphere (DCA-RQ) after 9 months of storage plus 7 days shelf life at 20°C.

Concerning the PC II, it was important to separate the fruit stored under CA, regardless the maturity stage, and unripe DCA-RQ 1.5 stored fruit from those stored under other conditions (Figure 3a). Correlated to the fruit stored in these conditions are the internal ethylene, ethylene production rate, respiration rate, 3-methylbutyl acetate, E-2-hexen-1-ol, 2-methyl-1-butanol and methyl 2-methyl butanoate (Figure 3b). These result shows that fruit stored under CA showed higher metabolism (Figure 2a – c) and only low amount of important volatile compounds were correlated to CA stored fruit. A noteworthy fact is that the largest amount of volatile compounds are not correlated to the ethylene and respiration rate (Figure 3b), with the exception if the fruit were stored under CA, as showed by the Pearson correlation (Figure 1). These results go against to the ones reported in the literature, which informed that the volatile compounds biosynthesis is closely related to the ethylene presence and respiratory activity (Brackmann et al., 1993; Song and Bangerth, 1996; Fellman et al., 2003; Kondo et al., 2005; Bangerth et al., 2012; Both et al., 2014; Thewes et al., 2015; Yang et al., 2016). However, the results reported in the literature were all conducted in aerobic conditions, it means in air, CA and ULO conditions, where the anaerobic metabolism of fruit is not significant. The extremely low oxygen employed during DCA-RQ 1.3 (0.23 kPa O_2 in average) and DCA-RQ 1.5 (0.21 kPa O_2 in average) resulted in a significant increment in anaerobic metabolism, especially in DCA-RQ 1.5. This metabolism could provide the volatile compounds precursors, according to the hypothesized action mode of low oxygen on the *de novo* fatty acid biosynthesis (Figure 4), providing fatty acid for ester biosynthesis (Song and Bangerth, 2003) and therefore improve the main esters of this cultivar and not only ethyl esters as observed by ethanol application (Jin et al., 2013).

The esters show a key importance in the apple characteristic fruity and floral flavor (Plotto et al., 1999; Plotto and McDaniel, 2001; Komthong et al., 2006; Mehinagic et al., 2006). Fruit stored under DCA-RQ 1.5 showed higher total ester amount as compared to the other storage conditions, independently of the maturity stage (Figure 5a). On the other hand, fruit stored under CA + 1-MCP application showed the lowest ester concentration, regardless the maturity stage. The fruit stored under CA and DCA-RQ 1.3 showed an intermediary ester concentration between CA + 1-MCP and DCA-RQ 1.5, but no difference was observed between CA and DCA-RQ 1.3, with the exception in overripe apple, where CA stored fruit showed higher total ester amount. The negative effect of 1-MCP on volatile compounds biosynthesis is widely reported in literature (Kondo et al., 2005; Raffo et al., 2009; Thewes et al., 2015; Yang et al., 2016). Nevertheless, the present results showed at the first time that the oxygen lowering, at a partial pressure that allows a little fermentation, improved significantly the total ester

biosynthesis of ‘Galaxy’ apple. A study carried out with anaerobic metabolism products application also resulted in a significant increment in total ester amount in melons (Liu et al., 2012; Jin et al., 2013).

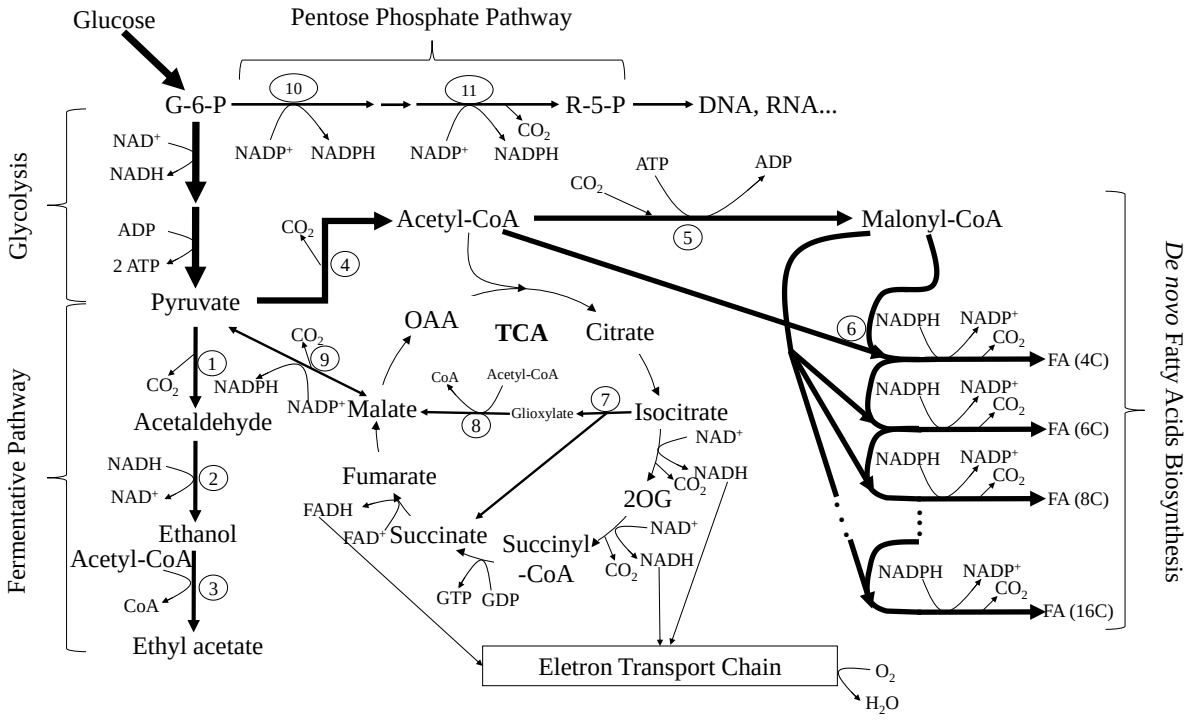

Figure 4 - Hypothesized mode of action of extremely low oxygen partial pressure during storage on the fermentative metabolism, tricarboxylic acids cycle and *de novo* fatty acids biosynthesis in ‘Galaxy’ apple stored under DCA-RQ 1.3 and DCA-RQ 1.5. More large arrows show the direction of metabolites to this pathway by the storage under extremely low oxygen. The numbers indicate the enzymes responsible for the reactions: 1: Pyruvate decarboxylase; 2: alcohol dehydrogenase; 3: alcohol acyltransferase; 4: pyruvate dehydrogenase; 5: complex of enzymes (biotin carboxylase; biotin carboxylase carrier protein and carboxyltransferase); 6: complex of enzymes (Ketoacyl-ACP Synthase; Ketoacyl-ACP reductase; hydroxyacyl-ACP dehydrase; Enoyl-ACP reductase); 7: isocitrate lyase; 8: Malate synthase; 9: NADP-malic enzyme; 10: Glucose-6-phosphate dehydrogenase and 11: 6-phosphogluconate dehydrogenase. G-6-P: glucose-6-phosphate; R-5-P: ribulose-5-phosphate; TCA: tricarboxylic acids cycle; 2OG: 2-oxoglutarate; OAA: oxaloacetate. FA (4C)…FA (16C): fatty acids from 4 up to 16 carbons. The reactions and enzymes of this scheme were based on some studies of the literature (Ke et al., 1994; Lara et al., 2011; Li-Beisson et al., 2013; Bekele et al., 2015) and the results obtained on the present study.

Regarding the summation of the most important esters of ‘Gala’ group apple, according to the literature, it means butyl acetate, 2-methylbutyl acetate and hexyl acetate (Young et al., 1996; Plotto et al., 2000; Salazar and Orozco, 2011) and the most abundant ester of the cultivar

Galaxy, a similar result to the total esters was observed (Figure 5b and c). Again, fruit stored under DCA-RQ 1.5 showed higher characteristic apple esters concentration, independently of the maturity stage, showing that the fruit stored under extremely low oxygen partial pressure (mean 0.21 kPa O_2) maintained better the main ester as compared to fruit stored under CA (mean 1.2 kPa O_2). This is an important practical result because the oxygen lowering to extremely low partial pressures allowed to maintain the physical qualities, as reported in the first chapter, such as flesh firmness, acidity and soluble solids (Brackmann et al., 2015; Weber et al., 2015), and higher characteristic esters as reported on the present study. Probably, the extremely low oxygen induced the anaerobic metabolism moving the Acetyl-CoA production to the *de novo* fatty acid biosynthesis, as proposed by the scheme in the figure 4. Concerning the maturity stages, an increment of the esters were observed with maturity advance if the fruit were stored under CA, DCA-RQ 1.3 and DCA-RQ 1.5, but if the fruit were treated with 1-MCP, no increment was observed (Figure 5b and c).

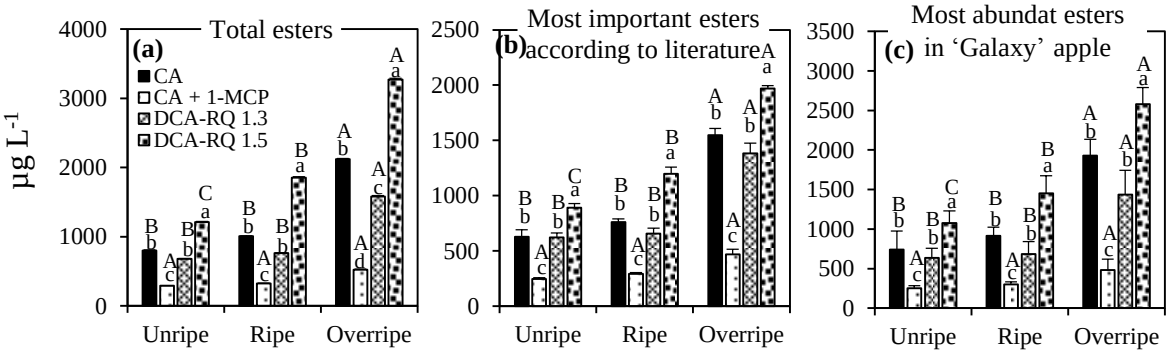

Figure 5 - Total esters (a), most important ester according to the literature (butyl acetate, 2-methylbutyl acetate and hexyl acetate) (b) and most abundant esters in 'Galaxy' apple (butyl acetate, 2-methylbutyl acetate, hexyl acetate, isobutyl acetate and ethyl 2-methyl butanoate) (c) (µg L^{-1}) after 7 days of shelf life at 20°C in three maturity stages and its interaction with CA, CA + 1-MCP and DCA-RQ. Bars with the same lower case letter in the same maturity stage, and each bar with the same upper case letter in different maturity stages are not significantly different by Tukey's test, at 5% probability.

Analyzing individually the esters produced by 'Galaxy' apple, more differences among maturity stages and storage conditions were observed (Figure 6a – z). Butyl acetate, one of the main straight-chain esters, showed higher concentration in DCA-RQ 1.3 as compared to CA and CA + 1-MCP, if the fruit were harvested in unripe maturity stage (Figure 6j). However, in ripe and overripe apple the storage under DCA-RQ 1.5 resulted in higher butyl acetate

concentration as compared to CA and CA + 1-MCP, without difference from those stored under DCA-RQ 1.3. These results go against to the ones found by Raffo et al. (2009), Both et al. (2014) and Lumpkin et al. (2014), which reported a significant reduction in the butyl acetate concentration in 'Pinova', 'Royal Gala' and 'Scartlett Spur Red Delicious' apples, respectively, stored under extremely low oxygen as compared to CA stored fruit. An interesting fact is that the 1-MCP application, in CA stored fruit, did not suppress the butyl acetate production, independently of the maturity of fruit (Figure 6j). In 'Royal Gala' apple, a similar result was obtained in CA (1.2 kPa O_2 + 1.2 kPa CO_2) stored apple, where also the 1-MCP not suppressed the butyl acetate formation (Thewes et al., 2015).

Comparing the maturity stages in each storage condition, higher butyl acetate was observed in overripe apple, regardless the storage condition, but ripe DCA-RQ 1.5 stored fruit showed higher butyl acetate as compared to unripe apple and under the other storage conditions there was no difference between unripe and ripe harvested apple (Figure 6j). These results agree with the ones of literature, which affirmed that the butyl acetate emission by apple increased with maturity advance (Brackmann et al., 1993; Song and Bangerth, 1996; Song and Bangerth, 2003; Bangerth et al., 2012). Additionally, it appear that occurs a synergism between storage under DCA-RQ, independently of the RQ level, and maturity advance on butyl acetate production by 'Galaxy' apple (Figure 6j). The straight-chain esters, such as butyl acetate, need constant ethylene supply to allow its biosynthesis, according to the results reported in literature (Brackmann et al., 1993; Song and Bangerth, 1996; Fellman et al., 2003; Bangerth et al., 2012; Both et al., 2014; Thewes et al., 2015; Yang et al., 2016), but in the present study is proved that under extremely low oxygen partial pressure, especially under the highest RQ level (DCA-RQ 1.5), the ethylene had a negative Pearson correlation with the volatile compounds production by 'Galaxy' apple (Figure 1), showing that under these conditions the ethylene is not a limiting factor to butyl acetate production.

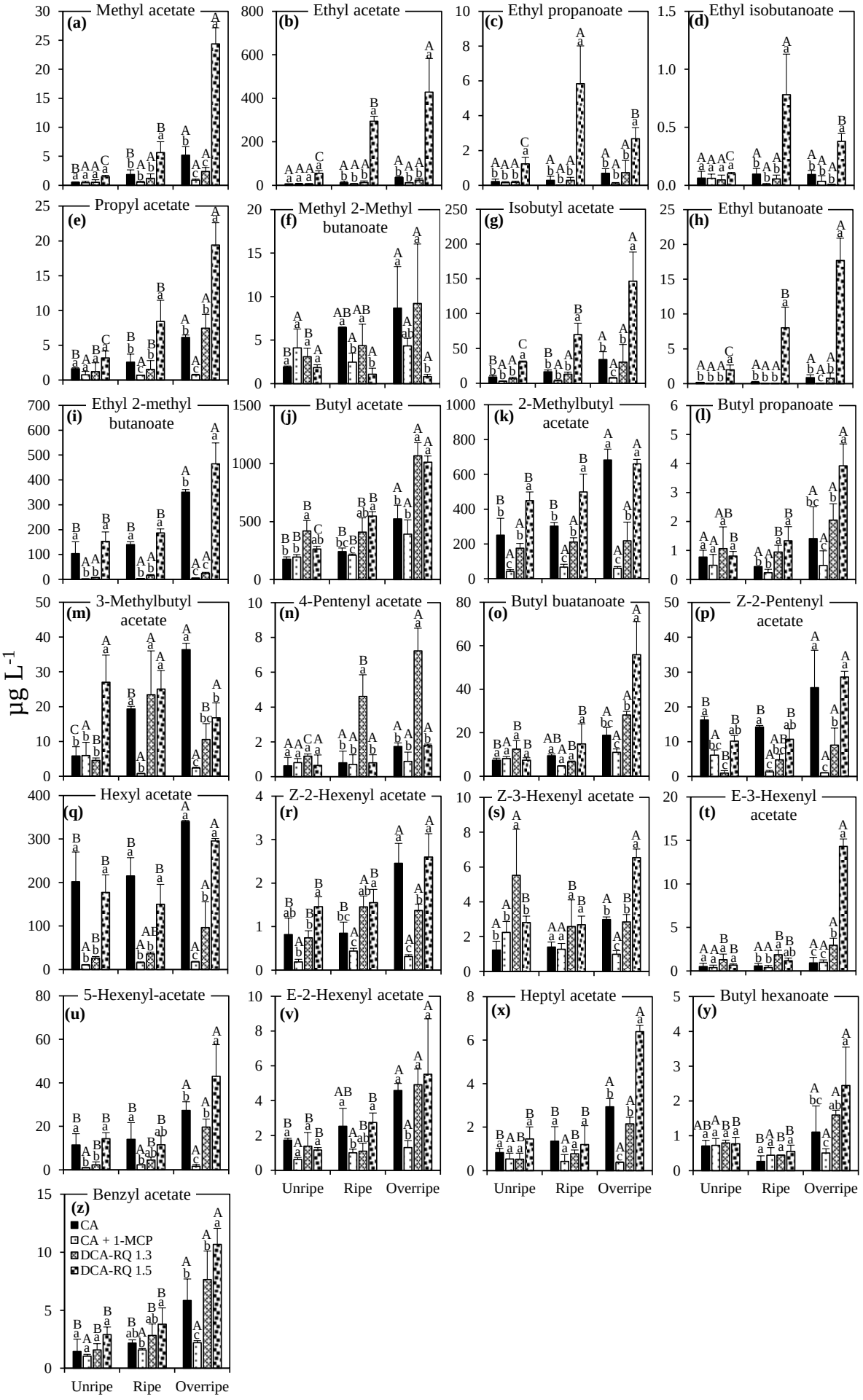
(a) Methyl acetate
(b) Ethyl acetate
(c) Ethyl propanoate
(d) Ethyl isobutanoate
(e) Propyl acetate
(f) Methyl 2-Methyl butanoate
(g) Isobutyl acetate
(h) Ethyl butanoate
(i) Ethyl 2-methyl butanoate
(j) Butyl acetate
(k) 2-Methylbutyl acetate
(l) Butyl propanoate
(m) 3-Methylbutyl acetate
(n) 4-Pentenyl acetate
(o) Butyl buatanoate
(p) Z-2-Pentenyl acetate
(q) Hexyl acetate
(r) Z-2-Hexenyl acetate
(s) Z-3-Hexenyl acetate
(t) E-3-Hexenyl acetate
(u) 5-Hexenyl-acetate
(v) E-2-Hexenyl acetate
(x) Heptyl acetate
(y) Butyl hexanoate
(z) Benzyl acetate
µg L-1
CA
CA + 1-MCP
DCA-RQ 1.3
DCA-RQ 1.5
Unripe
Ripe
Overripe

Figure 6 - Esters concentration after 7 days of shelf life at 20°C of 'Galaxy' apple harvested in three maturity stages and its interaction with CA, CA + 1-MCP and DCA-RQ. Bars with the same lower case letter in the same maturity stage, and each bar with the same upper case letter in different maturity stages are not significantly different by Tukey's test, at 5% probability.

Hexyl acetate is another ester originated from fatty acids metabolism and has a fundamental importance in 'Gala' apple flavor (Plotto and McDaniel, 2001). Fruit stored under CA and DCA-RQ 1.5 showed higher hexyl acetate concentration as compared to the ones stored under CA + 1-MCP and DCA-RQ 1.3, regardless the maturity stage at harvest (Figure 6q). Additionally, overripe fruit stored under DCA-RQ 1.3 resulted in higher hexyl acetate production as compared to CA + 1-MCP. The higher hexyl acetate production in CA and DCA-RQ 1.5 stored fruit is a result of higher 1-hexanol concentration (Figure 7h), which is a hexyl ester precursor (Holland et al., 2005; Souleyre et al., 2005). An interesting fact is that again the extremely low oxygen (0.21 kPa in average for DCA-RQ 1.5) resulted in an increment in the hexyl acetate and its precursor, going against to the knowledge in literature about this straight-chain ester biosynthesis in apple during CA storage. Up to date the literature showed that the hexyl acetate production is closely related to ethylene production, β-oxidation and LOX (Brackmann et al., 1993; Song and Bangerth, 1996; Bangerth et al., 2012; Both et al., 2014; Yang et al., 2016). This results show that under extremely low oxygen, where the anaerobic metabolism take place, the hexyl acetate precursor are provided by another pathway, like the *de novo* fatty acids biosynthesis.

The hexyl acetate concentration was higher if the fruit were harvested in overripe maturity stage and stored under CA, DCA-RQ 1.3 and DCA-RQ 1.5 as compared to unripe apple (Figure 6q). Nevertheless, if the fruit were treated with 1-MCP there was no increment in hexyl acetate with the maturity advance. This result may be related to the lower precursor concentration (Figure 7h), as showed by Lara et al. (2006), or/and because the 1-MCP application reduced the alcohol acyltransferase (AAT) enzyme expression and activity, reducing hexyl acetate production with the maturity advance (Defilippi et al., 2005; Yang et al., 2016). The absence of increment in hexyl acetate with maturity advance is adverse to the overall apple quality for the consumers, as soon as hexyl ester show a key importance in customer apple acceptance (López et al., 2007).

According to the literature, the branched-chain esters also shown a fundamental importance in apple flavor, especially the 2-methylbutyl acetate (Brackmann et al., 1993; Both et al., 2014). Fruit stored under DCA-RQ 1.5 showed the highest 2-methylbutyl acetate as

compared to the other storage conditions, with the exception in overripe harvested fruit, where DCA-RQ 1.5 did not differ from CA stored fruit (Figure 6k). According to Brackmann et al. (1993) and Echeverría et al. (2008) the branched-chain esters are lesser affected by the oxygen lowering as compared to the straight-chain ester. These same authors reported that the low oxygen had a lower effect on the amino acids metabolism and consequently on the branched-chain esters as compared to fatty acid metabolism. The storage under DCA-RQ 1.3 showed little difference from those fruit stored under CA, with the exception in overripe apple, wherever the DCA-RQ 1.3 showed lower 2-methylbutyl acetate in relation to CA, but with higher concentration as compared to CA + 1-MCP. Differently to butyl acetate, the 2-methylbutyl acetate was significantly reduced by the 1-MCP application, regardless the maturity stage of fruit. In 'Golden Delicious' and 'Royal Gala' apple, the 1-MCP application also reduced drastically the 2-methylbutyl acetate production after cold storage and controlled atmosphere, attributing this result to the lower ethylene production by 1-MCP treated fruit (Thewes et al., 2015; Yang et al., 2016). Analyzing the internal ethylene, ethylene production and 2-methylbutyl acetate concentration together, there is an inconsistence with the literature, once all fruit stored under DCA-RQ 1.3, DCA-RQ 1.5 and CA + 1-MCP showed low ethylene concentration, but fruit stored under DCA showed higher 2-methylbutyl acetate concentration as compared to CA + 1-MCP. This result shows that under extremely low oxygen the 2-methylbutyl acetate production is not under ethylene regulation (Figure 1). Overripe apple showed higher 2-methylbutyl acetate concentration if the fruit were stored under CA and DCA-RQ 1.5, but if the fruit were stored under CA + 1-MCP and DCA-RQ 1.3 no difference among maturity stages was observed (Figure 6k).

The extremely low oxygen partial pressure significantly increased the amino acids content in 'Jonagold' apple (Bekele et al., 2015), which are branched-chain ester precursors (Kochevenko et al., 2012), explaining the high 2-methylbutyl acetate and 3-methylbutyl acetate concentration in DCA-RQ 1.5 stored fruit (Figure 6k and m). Unripe apple stored under DCA-RQ 1.5 showed higher 3-methylbutyl acetate concentration as compared to the other storage conditions (Figure 6m). In ripe fruit, the storage under CA, DCA-RQ 1.3 and DCA-RQ 1.5 showed higher 3-methylbutyl acetate as compared to CA + 1-MCP, but in overripe apple, the CA stored fruit showed the highest concentration, DCA-RQ an intermediary and CA + 1-MCP the lowest 3-methylbutyl acetate concentration (Figure 6m). Both et al. (2014) found a significant reduction in 3-methylbutyl acetate concentration by oxygen lowering down to 0.5 kPa, but our study showed that oxygen lowering (0.21 kPa in average) increased the 3-methylbutyl acetate production by 'Galaxy' apple. This result agree with the literature, which

reported that 1-MCP application suppressed significantly the 3-methylbutyl acetate production by apple (Yang et al., 2016). This is an effect of 1-MCP on amino acids degradation enzyme activity (Bekele et al., 2015; Yang et al., 2016), reducing the initial conversion of free amino acids to aroma compounds in 1-MCP treated apple.

With oxygen lowering pyruvate accumulation occur in fruit flesh, which can be directed to amino acid production, like valine (Bekele et al., 2015) that is the isobutyl acetate precursor (Holland et al., 2005; Kochevenko et al., 2012). Fruit stored in DCA-RQ 1.5 showed higher isobutyl acetate concentration as compared to the other storage conditions, regardless the maturity stage (Figure 6g). Additionally, in overripe apple the 1-MCP application resulted in lower isobutyl acetate as compared to the other storage conditions. According to the literature, the 1-MCP application increased the valine concentration in apple (Lee et al., 2012; Bekele et al., 2015) and it had little effect on the branched-chain amino acid aminotransferase (BCAT) (Yang et al., 2016), showing that the lower isobutyl acetate concentration can be related to the lower alcohols precursor (Lee et al., 2012) and/or due to the lower AAT enzyme activity (Defilippi et al., 2005; Yang et al., 2016). Regarding to the maturity stages, higher isobutyl acetate were observed in overripe CA and DCA-RQ 1.5 stored apple, but no difference among maturity stages occurred if fruit were stored in CA + 1-MCP and DCA-RQ 1.3.

In relation to the remaining branched-chain esters identified in the present study, such as methyl 2-methyl butanoate and ethyl 2-methyl butanoate there was also a significant difference between storage conditions and maturity stages (Figure 6f, and i). Between these two esters, the ethyl 2-methyl butanoate is one of the most abundant ester of ‘Galaxy’ apple (Figure 6i). Regardless the maturity stage, fruit stored under CA and DCA-RQ 1.5 showed higher ethyl 2-methyl butanoate concentration as compared to fruit stored under CA + 1-MCP and DCA-RQ 1.3. This ester attributes to the fruit a sweet apple-like flavor and is characteristic to apple of ‘Gala’ and ‘Fuji’ group (Echeverría et al., 2004; Mehinagic et al., 2006; Khomthong et al., 2006; Echeverría et al., 2008). Concerning methyl 2-methyl butanoate, which also has a sweet-fruity odor and is intensely in apple of the ‘Gala’ group (Plotto and McDaniel, 2001), no difference among storage conditions were observed in unripe harvested fruit, but in ripe and overripe apple, CA and DCA-RQ 1.3 resulted in higher concentration as compared to CA + 1-MCP and DCA-RQ 1.5 (Figure 6f). This compound was higher in overripe apple, if fruit were stored under CA and DCA-RQ 1.3, but in CA + 1-MCP and DCA-RQ 1.5 stored apple, there was no difference among maturity stages.

With the oxygen lowering reported in the present study (0.23 kPa for DCA-RQ 1.3 and 0.21 kPa for DCA-RQ 1.5) it is expected an increment in the volatile compounds related to the

anaerobic metabolism, such as ethyl acetate (Wright et al., 2015), which are related to off-flavors formation if it is present in large amounts. Nevertheless, this compound is also a fundamental contributor to apple flavor if in low amount (Dixon and Hewett, 2000; Wright et al., 2015). There was no difference among storage conditions in unripe harvested fruit for ethyl acetate, but in ripe and overripe apple, the storage under DCA-RQ 1.5 resulted in higher ethyl acetate concentration as compared to the other storage conditions (Figure 6b). This is a result of higher ethanol concentration in DCA-RQ 1.5 stored apple (Figure 7a). The high ethanol concentration was substrate to the AAT enzyme for ethyl acetate production (Defilippi et al., 2005; Holland et al., 2005; Souleyre et al., 2005; Yang et al., 2016). Comparing the three maturity stages in each storage condition, no increment in ethyl acetate was observed if the fruit were stored under CA, CA + 1-MCP and DCA-RQ 1.3, but in DCA-RQ 1.5, the ethyl acetate concentration increased with the maturity advance (Figure 6b).

Concerning the remaining esters, a similar behavior to the above reported results can be observed, but the concentration of these esters are lower. Fruit treated with 1-MCP generally showed the lowest ester concentration and no increment with maturity advance occurred, if the fruit were treated with 1-MCP. Additionally, the storage under CA and DCA-RQ 1.3 and DCA-RQ 1.5 resulted the highest esters concentration and there is an increment with the maturity advance (Figure 6).

The most abundant alcohol detected in ‘Galaxy’ apple after storage was 1-hexanol (Figure 7h). Both et al. (2014) also found 1-hexanol as the most abundant alcohol of ‘Royal Gala’ apple stored in ultralow oxygen (ULO). Fruit stored under CA and DCA-RQ 1.5 showed the highest 1-hexanol concentration as compared to the ones stored under CA + 1-MCP and DCA-RQ 1.3 regardless the maturity stage. This low 1-hexanol concentration in 1-MCP treated fruit is a result of the low hexanal concentration in these fruit (Figure 8c). This result is because the 1-MCP suppressed the fatty acid metabolism reducing the β-oxidation and consequently the 1-hexanol production (Song and Bangerth, 2003; Contreras et al., 2016; Yang et al., 2016). Additionally, the high 1-hexanol concentration in DCA-RQ 1.5 stored fruit go against to the results of literature, which reported that its concentration reduced as the oxygen partial pressure are lowered down to 1.0 kPa (Echeverría et al., 2008), 0.4 – 0.6 kPa (Raffo et al., 2009) and 0.5 kPa (Both et al., 2014). Nevertheless, our work is the first in literature evaluating the effect of a little fermentation induction on volatile profile, showing that under these conditions the 1-hexanol may be produced by another way, as such the *de novo* fatty acid biosynthesis stimulated by low oxygen (Figure 4). In ‘Scarlett Spur Red Delicious’ the storage under constant 0.3 kPa

O_2 also not resulted in a significant reduction of hexyl esters and 1-hexanol after long-term storage (Lumpkin et al., 2014), corroborating our findings.

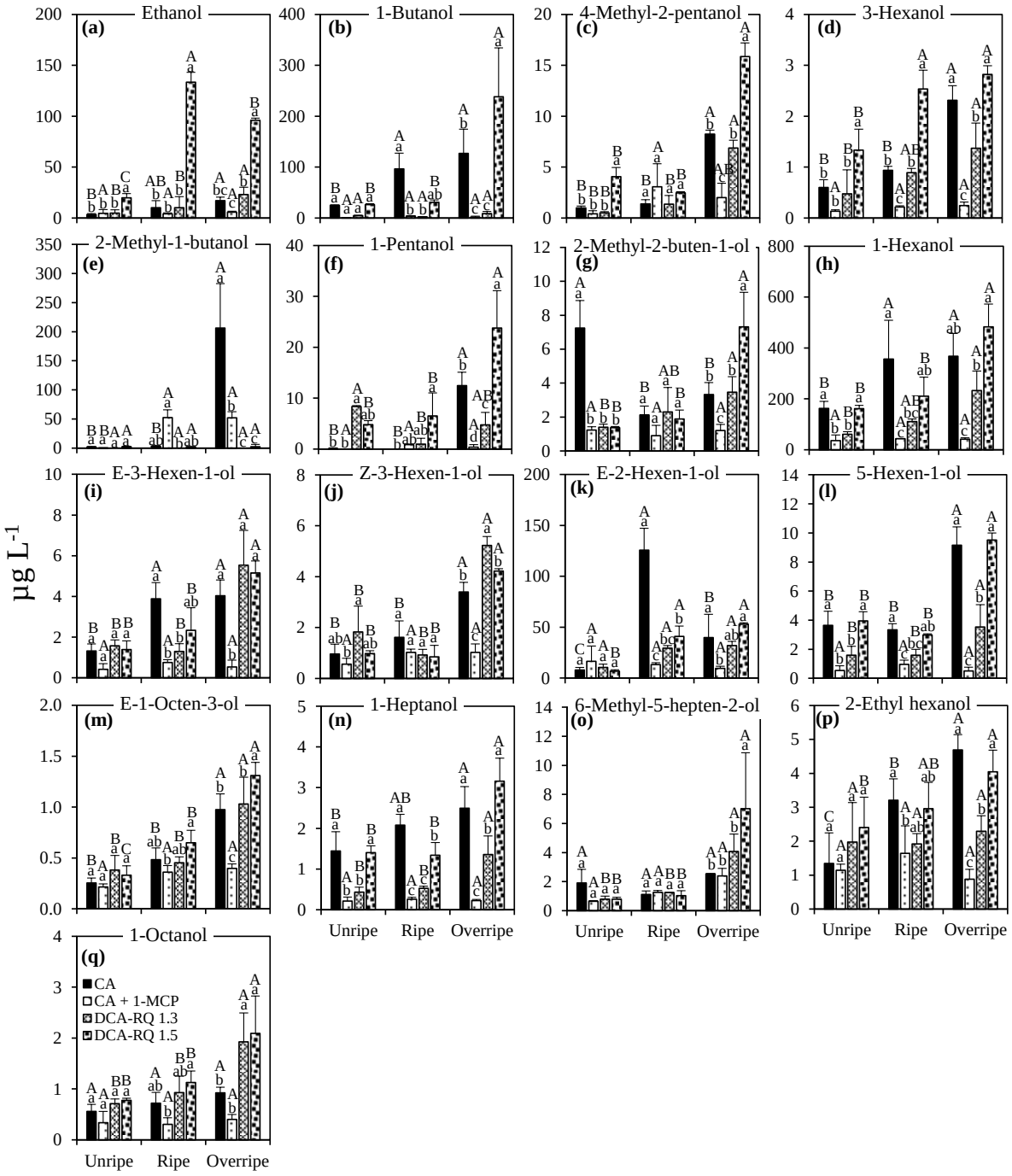

Figure 7 - Alcohols concentration after 7 days of shelf life at 20°C of 'Galaxy' apple harvested in three maturity stages and its interaction with CA, CA + 1-MCP and DCA-RQ. Bars with the same lower case letter in the same maturity stage, and each bar with the same upper case letter in different maturity stages are not significantly different by Tukey's test, at 5% probability.

Higher 1-hexanol concentration were observed in ripe and overripe apple, if the fruit were stored under CA, but if the fruit were stored under DCA-RQ 1.3 and DCA-RQ 1.5 only overripe apple showed higher 1-hexanol as compared to unripe apple and if the fruit were treated with 1-MCP no difference among maturity stages was observed (Figure 7h). This result explained the absence of any increment in hexyl acetate by 1-MCP treated fruit and the highest hexyl acetate production by overripe apple stored under CA, DCA-RQ 1.3 and DCA-RQ 1.5 (Figure 6q). Raffo et al. (2009) also found an increment in 1-hexanol concentration if the fruit were harvested in advanced maturity stages as compared to early harvested apple. The higher 1-hexanol in overripe apple probably is a result of higher free fatty acids content, especially linoleic acid (18:2), that had high Person correlation with the hexanal and 1-hexanol production by apple (Contreras et al., 2016). These authors also reported that this free fatty acid could be provided by the *de novo* fatty acid biosynthesis, fact that may be induced by the extremely low oxygen in DCA-RQ 1.5 stored fruit on the present study.

Concerning the 1-butanol, a butyl ester precursor, no difference among storage conditions was verified in unripe harvested apple, but if the fruit were harvested at ripe and overripe maturity stage, higher 1-butanol was observed in CA and DCA-RQ 1.5 stored fruit, respectively (Figure 7b). This result is related to the 1-butanal concentration in DCA-RQ 1.5, especially at overripe maturity stage (Figure 8b). This is in contradiction with the literature, which reported the oxygen lowering suppressed the 1-butanol formation in 'Pinova' (Raffo et al., 2009), 'Royal Gala' (Both et al., 2014), 'Scarlett Spur Red Delicious' (Lumpkin et al., 2014), despite some other cultivars. Similarly to 1-hexanol, higher 1-butanol were observed in ripe and overripe fruit, if stored under CA, and in overripe fruit, if stored under DCA-RQ 1.5. On the other hand, no increment in 1-butanol concentration with maturity advance was verified if the fruit were stored under CA + 1-MCP and DCA-RQ 1.3. Analyzing the 1-butanol and butyl acetate concentration together is noteworthy that the AAT enzyme activity show a distinct activity among the storage conditions, resulting in a differential butyl acetate production. The AAT enzyme show high dynamically activity, which varies according to the tissue, cultivar, precursor, fruit ripening, despite some other factors (Holland et al., 2005; Souleyre et al., 2005).

A suchlike response was observed for 2-methyl-1-butanol and 2-methylbutyl acetate concentration, where also 2-methylbutyl acetate concentration change much more with the storage conditions as compared to its precursor (2-methyl-1-butanol) (Figures 6k and 7e). Once again, this result can be related to the AAT enzyme activity, which change its affinity to the precursor concentration (Souleyre et al., 2005). According to these authors, the MpAAT1 enzyme affinity to the precursor has distinct response in low alcohol concentration (2-methyl-

1-butanol, 1-hexanol and 1-butanol, respectively) and in high alcohol concentration (1-hexanol, 1-butanol and 2-methyl-1-butanol, respectively). Thereby, the MpAAT1 affinity may be changed according to the treatment and maturity stage, resulting in a dissimilar 2-methylbutyl acetate production in relation to the precursor concentration. Regarding the maturity stages, higher 2-methyl-1-butanol was observed in overripe apple if the fruit were stored under CA, but if the fruit were treated with 1-MCP, ripe and overripe fruit showed higher 2-methyl-1-butanol as compared to unripe apple. Nevertheless, in DCA-RQ 1.3 and DCA-RQ 1.5 there was no increment in 2-methyl-1-butanol concentration with the maturity advance.

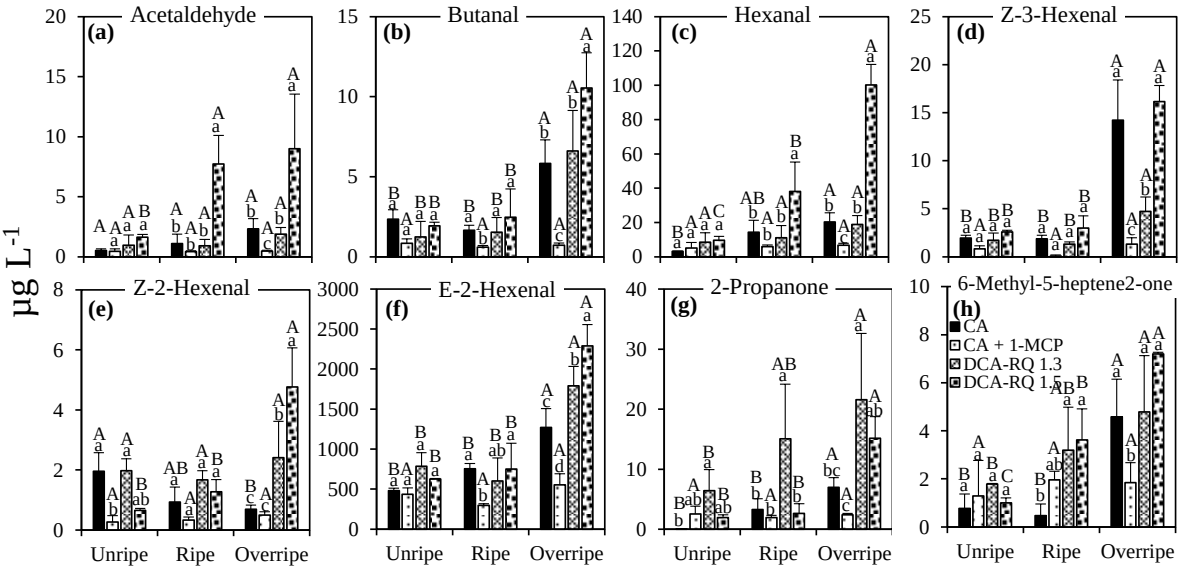

Figure 8 - Aldehydes (a – f) and ketones (g – h) concentration after 7 days of shelf life at 20°C of 'Galaxy' apple harvested in three maturity stages and its interaction with CA, CA + 1-MCP and DCA-RQ. Bars with the same lower case letter in the same maturity stage, and each bar with the same upper case letter in different maturity stages are not significantly different by Tukey's test, at 5% probability.

The action of LOX on the linolenic acid (18:3) result in E-2-hexenal formation (Figure 8f), which is the E-2-henex-1-ol precursor (Contreras et al., 2016). E-2-hexen-1-ol was not affected by the storage conditions in unripe apple, but in ripe and overripe apple, the storage under CA resulted in the highest E-2-henex-1-ol concentration, without difference from fruit stored under DCA-RQ 1.5 in overripe apple (Figure 7k). Fruit treated with 1-MCP again showed low E-2-henex-1-ol in ripe and overripe apple, which is a result of lower E-2-hexanal concentration in these fruit (Figure 8f). Ripe fruit stored under CA showed the highest E-2-henex-1-ol concentration, but if the fruit were stored under DCA-RQ 1.5, ripe and overripe

apple showed high E-2-henex-1-ol concentration, as compared to unripe apple. Additionally, no changes with maturity were observed if the fruit were stored under CA + 1-MCP and DCA-RQ 1.3.

On the present study, the ethanol concentration only was significantly increased if the fruit were stored under DCA-RQ 1.5, regardless the maturity stage (Figure 7a). The high ethanol production is a result of higher acetaldehyde concentration, especially in ripe and overripe apple (Figure 8a). In overripe apple, the 1-MCP application suppressed the acetaldehyde (Figure 8a) and ethanol (Figure 7a) production by apple. Similar results were observed in some other apple cultivars, where the 1-MCP application also reduced the formation of acetaldehyde and ethanol (Lee et al., 2012; Thewes et al., 2015). A noteworthy fact is that ripe fruit stored under DCA-RQ 1.5 showed the highest ethanol production, as compared to unripe and overripe harvested apple, but in CA and DCA-RQ 1.3 overripe apple showed the highest ethanol concentration as compared to unripe apple.

Concerning the aldehydes and ketones, higher concentration was observed in ripe and overripe apple if the fruit without 1-MCP in all storage conditions (Figure 8). However, if the fruit were stored under CA + 1-MCP there was no increment with the maturity advance. These results shows that the 1-MCP application suppressed the LOX and β-oxidation (Yang et al., 2016).

4.1.3.3. Changes in volatile compounds profile after storage in relation to harvest

The main challenge of the researchers and the storers is to maintain the volatile compounds of fruit after long-term storage under CA and DCA similar to harvest. Thereby, we performed a calculation to verify if the volatile compounds production increased or decreased during storage in each storage condition, to show the best storage condition to maintain the volatile compounds similar or higher as compared to the harvest. The results of this analysis were reported in Figure 9.

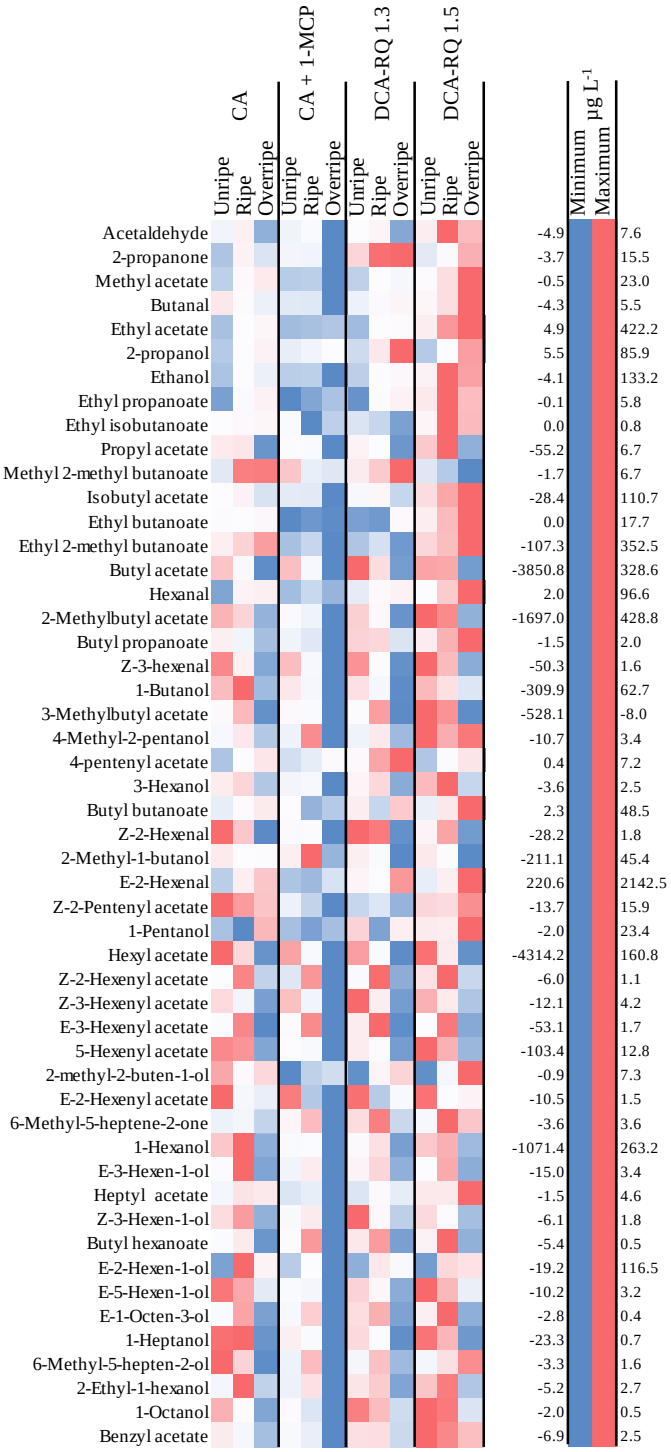

Figure 9 – Heat map showing the difference between harvest and after storage of the volatile compounds (µg L^{-1}) of 'Galaxy' apple harvested in three maturity stages and its interaction with CA, CA + 1-MCP and DCA-RQ. Positive values (in red) show increment in the concentration after storage in relation to harvest and negative values (in blue) show reduction in the concentration after storage in relation to harvest.

Concerning the main esters of the cultivar Galaxy, it mean ethyl 2-methyl butanoate, isobutyl acetate, butyl actetate, 2-methylbutyl acetate and hexyl acetate, they showed a distinct response between harvest and after storage (Figure 9). Isobutyl acetate showed and increment in its concentration in unripe and ripe CA, CA + 1-MCP and DCA-RQ 1.3 stored fruit and a reduction in overripe apple stored in the same conditions. On the other hand, in DCA-RQ 1.5 storage, there was an increment in the three maturity stages and this increment was significantly higher as compared to the other storage conditions (Figure 9). The highest increment in isobutyl acetate by fruit stored under DCA-RQ 1.5 is a result of glycolysis induction by oxygen lowering resulting in higher amount of pyruvate formation, which could provide higher valine biosynthesis (Bekele et al., 2015) and, consequently, isobutyl acetate formation.

Another important result was obtained for ethyl 2-methyl butanoate, which showed an increment in its concentration after storage as compared to harvest, if the fruit were stored under CA and DCA-RQ 1.5, however, if the fruit were treated with 1-MCP or stored under DCA-RQ 1.3 there was a decrease of this compound after storage, regardless maturity stage (Figure 9). Regarding to the butyl acetate, all overripe apple loss it during storage, regardless the storage condition. Nevertheless, unripe and ripe apple stored under DCA-RQ 1.3 and DCA-RQ 1.5 showed an increment in butyl acetate during storage. On the other hand, if the fruit were stored under CA and CA + 1-MCP there was only verified an increment of this compound in unripe apple (Figure 9). Perhaps, the extremely low oxygen used during DCA-RQ 1.3 (0.23 kPa in average) and DCA-RQ 1.5 (0.21 kPa in average) allowed the biosynthesis of butyl acetate precursors, according to the proposed scheme in figure 4, and consequently improving its production by apple stored in these condition.

Likewise the above mentioned esters, the 2-methylbutyl acetate showed an increment in all storage conditions throughout the storage if fruit were harvested unripe and a reduction if fruit were harvested at overripe maturity (Figure 9). Nevertheless, in ripe harvested fruit, only the CA + 1-MCP stored fruit showed a reduction after storage in relation to harvest. Hexyl acetate is another ester that suffer a higher reduction in unripe CA + 1-MCP stored fruit. Additionally, the lowest reduction after storage as compared to harvest was observed in unripe and ripe CA and DCA-RQ 1.5 stored fruit. Additionally, it is important to highlight that fruit treated with 1-MCP, independently of the volatile compound, showed the lowest increment, and generally a reduction in volatile compounds as compared to harvest (Figure 9).

The anaerobic metabolism, represented by the acetaldehyde, ethanol and ethyl acetate, had the highest increment during storage in DCA-RQ 1.5, independently of the fruit maturity stage at harvest, with the exception acetaldehyde in unripe apple (Figure 9). Moreover, the

ethanol concentration was only reduced in overripe CA + 1-MCP stored fruit, but the acetaldehyde concentration was reduced in overripe apple stored under CA, CA + 1-MCP and DCA-RQ 1.3. Analyzing these results is noteworthy that overripe apple showed a significant increment in fermentation at harvest (Table 1) and it is reduced during storage (Figure 9), showing that the storage under DCA-RQ 1.3 did not result in increment of anaerobic metabolism products accumulation.

4.1.4. Conclusions

In summary, the storage of 'Galaxy' apple under DCA-RQ 1.5 result in higher total ester amount and characteristic esters of 'Galaxy' apple, such as butyl acetate, ethyl 2-methyl butanoate, isobutyl acetate, 2-methylbutyl acetate and hexyl acetate in relation of conventional CA storage. DCA-RQ 1.3 shows comparable characteristic 'Galaxy' esters to fruit stored under CA. The largest amount of esters are related to overripe apple stored under DCA-RQ 1.3 and DCA-RQ 1.5. Moreover, the volatile compounds biosynthesis has no correlation with the ethylene production if the fruit were stored under DCA-RQ 1.5, differing from CA storage that show positive relationship between ethylene and volatile concentration. DCA-RQ 1.5 increase the ethanolic fermentation resulting in higher acetaldehyde, ethanol and ethyl acetate.

The 1-MCP application suppresses the main volatile compounds production, even in advanced maturity stage and reduces the main 'Galaxy' esters after storage in relation to harvest.

Either 1-MCP treatment or DCA suppresses significantly the internal ethylene, ethylene production and respiration rate, but the metabolism reduction in DCA-RQ 1.5 stored fruit not result in a volatile compounds suppression.

4.1.5. References

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5. DISCUSSÃO GERAL

Um dos maiores desafios da pomicultura nacional e mundial é a produção e distribuição de maçãs de qualidade durante todo o ano. Entretanto, essa não tem sido uma tarefa fácil em função de problemas climáticos, falta de mão-de-obra para o manejo dos pomares, colheita, doenças, pragas, condições de armazenamento inadequados, entre outros problemas. De acordo com a FAO, em torno de 15 a 30% das perdas pós-colheita de frutas ocorrem em função da colheita ser realizada no momento inadequado. Em função disso, a fundamental importância de testar novas tecnologias de armazenamento de maçãs por longos períodos.

Essas perdas, em função da colheita no momento inadequado, ocorrem devido ao armazenamento em condições inadequadas, geralmente uma mesma condição para frutos colhidos em diferentes estádios de maturação. A adoção de uma mesma condição de AC para os diferentes estádios de maturação é resultado da falta de mecanismos de detecção do LMO dos frutos nos diversos estádios de maturação em câmaras comerciais. De acordo com Argenta; Mondardo (1994) a maior parte dessas perdas é em função da ocorrência de podridões e distúrbios fisiológicos. Entretanto, no presente estudo, a incidência de podridões não foi alterada significativamente pelas condições de armazenamento e estádios de maturação, porém o armazenamento dos frutos em ACD-QR reduziu significativamente a incidência de distúrbios fisiológicos, demonstrando que o uso de uma tecnologia que permite o monitoramento do LMO mantém a qualidade de maçãs 'Galaxy' mesmo em condições de maturação inadequadas.

Um dos produtos mais utilizados durante o armazenamento de maçãs, para o controle do amadurecimento, é o 1-MCP. Contudo, nos últimos anos o seu uso está sendo proibido em países da Europa e não é permitido para frutos orgânicos (REBEAUD; GASSER, 2015), tornando necessário o desenvolvimento de tecnologias de armazenamento que permitem a substituição do 1-MCP. No presente estudo foi comprovado o potencial do armazenamento em ACD-QR na substituição do uso do 1-MCP, proporcionando frutos com qualidade físico-química similar aos frutos tratados com 1-MCP, porém com uma concentração de compostos voláteis, muito maior em comparação aos frutos tratados com 1-MCP. Esse é um resultado de grande importância prática, pelo do alto custo para a aplicação de 1-MCP e por proporcionar a oferta de frutos de uma qualidade organoléptica superior aos consumidores. Desta maneira, a adoção da ACD-QR beneficia tanto aos armazenadores, pela redução de custos e a possibilidade armazenar maçãs produzidas organicamente, quanto os consumidores, pela oferta de frutas com aroma mais pronunciado e sem a aplicação de compostos químicos. Um estudo realizado com maçã 'Pinova' verificou que o armazenamento em ACD-FC manteve maior concentração de

compostos voláteis em comparação com frutos tratados com 1-MCP (RAFFO et al., 2009). Adicionalmente, o armazenamento de maçãs 'Royal Gala' em ACD-QR 2,0 proporcionou melhor manutenção do perfil volátil em comparação ao armazenamento em ACD-FC (BOTH, 2015), demostrando o potencial de manutenção da qualidade da ACD-QR em comparação à ACD-FC e aplicação de 1-MCP.

O uso da ACD-QR ainda é uma tecnologia recente, com poucos estudos realizados até o momento. Entretanto, em maçãs 'Royal Gala' e 'Galaxy', colhidas no momento adequado, o uso da ACD-QR resultou em melhor manutenção da qualidade em comparação com frutos armazenados em AC e ACD-FC (BOTH, 2015, BRACKMANN; WEBER; BOTH, 2015; WEBER, 2014; WEBER et al., 2015). O presente estudo foi o primeiro a demostrar que a ACD-QR tem potencial de reduzir a ocorrência de distúrbios fisiológicos, perda de firmeza, acidez, entre outros, em maçãs colhidas fora do estádio de maturação ideal, ou seja antes ou após o ponto ideal de colheita. Este fato está relacionado à drástica redução na produção de etileno pelos frutos armazenados em ACD-QR, independentemente do estádio de maturação. Both (2015) também verificou uma grande redução na produção de etileno pelos frutos armazenados em ACD-QR, corroborando o presente estudo. O interessante é que os frutos tratados com 1-MCP também apresentavam uma baixa produção de etileno, o que resultou em baixa produção de compostos voláteis, fato que não ocorreu nos frutos armazenados em ACD-QR. Possivelmente, o uso de pressões parciais de O_2 extremamente baixas, que induziu um pouco de fermentação, resultou na produção dos precursores de compostos voláteis nos frutos armazenados em ACD-QR, permitido assim, a manutenção da qualidade física dos frutos similar aos tratados com 1-MCP, porém com uma maior concentração de compostos voláteis.

O armazenamento dos frutos em ACD-QR 1,5 resultou em um aumento significativo na produção de compostos originados da fermentação, acetaldeído, etanol e acetato de etila. Entretanto, a produção destes compostos não foi danosa aos frutos, pois não ocorreu aumento significativo no extravasamento de eletrólitos e distúrbios fisiológicos, discordando dos resultados observados na literatura (SAQUET et al., 2000). WEBER (2013) observou que a aplicação de etanol aos frutos somente tem efeitos danosos quando a concentração é maior que 500 $\mu L\ L^{-1}$, corroborando os resultados observados no presente estudo. Adicionalmente, a indução de uma pequena fermentação pelos frutos resultou num aumento significativo dos compostos aromáticos dos frutos, mesmo em pressões parciais de O_2 extremamente baixas (0,21 kPa em média). Possivelmente, o uso de pressões parciais de O_2 extremamente baixas resultou no acúmulo de compostos reduzidos na célula, possibilitando a biossíntese *de novo* de ácidos

graxos e, assim, dos precursores de ésteres característicos de aroma de maçãs ‘Galaxy’, como acetato de butila, hexila e 2-metilbulila.

Com os resultados dos dois artigos científicos, é possível verificar o potencial da ACD-QR na manutenção da qualidade de maçãs ‘Galaxy’ colhidas nos diferentes estádios de maturação. Ela é uma tecnologia de fácil adoção em nível comercial em função do baixo custo de sua instalação e a segurança que ela oferece aos armazenadores, em função da melhor manutenção da qualidade e do monitoramento, em tempo real, do LMO, que possibilita a certeza de que os frutos estão em condições de O_2 seguras e o mais próximo possível do limite mínimo de O_2. Além disso, ela é uma técnica que detecta o estresse dos frutos bem antes das técnicas já utilizadas comercialmente, o que proporciona uma rápida adequação do O_2 ao nível tolerado pelos frutos naquele momento. Adicionalmente, com o armazenamento em QR superior a 1,0, é induzido o metabolismo anaeróbico produzindo etanol que possui efeito em diversas rotas metabólicas dos frutos e incrementa a produção de aromas característicos de maçãs ‘Galaxy’.

6. CONSIDERAÇÕES FINAIS

O armazenamento de maçãs em ACD-QR é eficiente na manutenção da qualidade, resultando em baixa produção de etileno, menor ocorrência de polpa farinácea e alta firmeza de polpa e suculência, independentemente do estádio de maturação na colheita. O armazenamento em ACD-QR 1,3 mantém a qualidade física e química similar aos frutos tratados com 1-MCP e armazenados em AC.

Maçãs colhidas antes do ponto ideal de maturação são menos responsivas às condições de armazenamento, sendo mais fáceis de armazenar em comparação a maçã sobremadura, pela menor incidência de polpa farinácea, degenerescência de polpa e maior firmeza de polpa em todas as condições de armazenamento. Frutas sobremaduras devem ser armazenadas em ACD-QR, pela menor incidência de polpa farinácea.

Maçãs colhidas antes do ponto ideal de maturação e armazenadas em ACD-QR 1,5 apresentam significativo aumento de compostos voláteis em comparação à AC, sendo que para alguns compostos é observado até maior concentração que em maçãs colhidas no ponto ideal e armazenadas em AC.

O armazenamento em ACD-QR 1,5 resulta em acúmulo de compostos oriundos da fermentação, como acetaldeído, etanol e acetato de etila, porém sem aumentar o extravasamento de eletrólitos e distúrbios fisiológicos relacionados à fermentação, como a degenerescência de polpa.

Frutos armazenados em ACD-QR 1,5 apresentam a maior concentração de compostos voláteis totais característicos de maçãs ‘Galaxy’, como acetato de butila, butanoato de 2-metil etila, acetato de isobutila, acetato de 2-metilbutila e acetato de hexila, sendo que o armazenamento de maçãs ‘Galaxy’ em ACD-QR 1,3 mantém a concentração destes ésteres em nível similar aos frutos armazenados em AC.

Maior concentração de compostos voláteis de maçãs ‘Galaxy’ é observada em frutos colhidos sobremaduros e armazenados em ACD-QR 1,3 e ACD-QR 1,5.

A produção de compostos voláteis em condições de O_2 extremamente baixas (0,21 kPa) é desvinculada com a produção de etileno dos frutos, evidenciando a existência de uma outra rota de produção de precursores e compostos voláteis diferente nestas condições de O_2 tão baixo.

A aplicação de 1-MCP em maçãs armazenadas em AC reduz significativamente a produção de compostos voláteis, não permitindo o incremento na produção destes compostos

nos frutos colhidos sobremaduros, reduzindo os principais ésteres após o armazenamento em comparação a colheita.

Tanto a aplicação de 1-MCP quanto o armazenamento em ACD-QR suprimem o metabolismo dos frutos, porém o armazenamento em ACD-QR 1,5 além de reduzir o metabolismo e manter a qualidade físico-química mantém maior concentração de compostos voláteis após 9 meses de armazenamento mais 7 dias de vida de prateleira.

Levando em consideração a qualidade física, química e a produção de compostos voláteis, as melhores condições de armazenamento para todos os estádios de maturação segue a seguinte ordem: ACD-QR 1,5 > ACD-QR 1,3 > AC + 1-MCP > AC.

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