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Rogerio de Oliveira Anese

CONSERVAÇÃO DA QUALIDADE DE MAÇÃS MUTANTES DE 'GALA' TRATADAS COM FITORREGULADORES E ARMAZENADAS EM ATMOSFERA CONTROLADA DINÂMICA

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

Orientador: Prof. Dr. Auri Brackmann

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Aprovado em 20 de abril de 2017:

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Santa Maria, RS
2017

Treine enquanto eles dormem, estude enquanto eles se divertem, persista enquanto eles descansam, e então, viva o que eles sonham. (Provérbio Japonês)

DEDICATÓRIA

A minha família, Oneide, Evani, Daniel e a minha noiva Juliana Ribas. Dedico também a todos aqueles que trabalham por um Brasil melhor.

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RESUMO

CONSERVAÇÃO DA QUALIDADE DE MAÇÃS MUTANTES DE 'GALA' TRATADAS COM FITORREGULADORES E ARMAZENADAS EM ATMOSFERA CONTROLADA DINÂMICA

AUTOR: Rogerio de Oliveira Anese
ORIENTADOR: Auri Brackmann

Devido à colheita de maçãs ser concentrada em curto período, faz-se necessário o armazenamento de uma grande parte dos frutos para regulação do mercado. Existem tecnologias de armazenamento em desenvolvimento, como a atmosfera controlada dinâmica com quociente respiratório (ACD-QR), a qual precisa ser avaliada em diferentes temperaturas e em frutos com aplicação de fitorreguladores como o 1-metilciclopropeno (1-MCP), a aminoetoxivinilglicina (AVG) e o ácido naftaleno acético (ANA). Esta tese é composta por quatro artigos científicos que resultaram de trabalhos com os seguintes objetivos: Avaliar a produção de compostos voláteis em maçãs 'Galaxy' e 'Royal Gala' armazenadas em ACD-QR, atmosfera controlada dinâmica com fluorescência de clorofilas (ACD-FC), atmosfera controlada (AC) convencional e com aplicação de 1-MCP; avaliar se a ACD-QR e a ACD-FC permitem utilizar temperaturas mais elevadas para o armazenamento das maçãs; avaliar o efeito da aplicação da AVG (0,83 kg ha^{-1}) em combinação com o ANA (40g ha^{-1}), bem como o efeito do 1-MCP na produção de compostos voláteis e qualidade físico-química dos frutos armazenados em ACD monitorada com fluorescência de clorofilas e com quociente respiratório; identificar se o estresse por baixa pressão parcial de O_2 durante o período de cálculo do quociente respiratório possui efeito na conservação da qualidade de maçãs. Foram utilizadas maçãs 'Galaxy' e 'Royal Gala', armazenadas por nove meses mais sete dias de exposição à temperatura de 20 °C. Pode-se destacar que a maçã 'Galaxy' quando armazenada em ACD com QR 1,5 (ACD-QR1,5) manteve melhor qualidade, do que o armazenamento em AC convencional com pressões parciais de O_2 ultrabaixas (ULO – 0,4 kPa CO_2) e em ACD-FC, além de aumentar a produção de ésteres responsáveis pela formação do aroma. Além disso, a aplicação de 0,625 µL L^{-1} de 1-MCP reduziu a concentração de compostos voláteis, mesmo em frutos armazenados em ACD-QR1,5. A ACD-FC teve efeito similar ao armazenamento em ULO com 0,4 kPa de O_2. A ACD-QR1,3 e a ACD-FC permitiram armazenar a maçã 'Galaxy' em temperatura mais elevada (2,0 e 2,5 °C) do que a normalmente recomendada (1,5 °C). Além disso, a aplicação de 1-MCP não teve efeito adicional em maçãs armazenadas com temperatura mais elevada quando em ACD. A alta temperatura (2,0 e 2,5 °C) pôde ser usada em ACD-QR1,3 sem perda na produção de compostos voláteis importantes. No armazenamento em ACD-QR, o estresse causado pelas baixas pressões parciais de O_2 a que os frutos são submetidos durante o período de cálculo do QR (13 h), que pode atingir 0,0 kPa, possuiu efeito benéfico, pois manteve firmeza da polpa mais elevada, menor incidência de polpa farinácea e podridões, comparado aos frutos que não sofreram esse estresse por baixo O_2. O armazenamento de maçãs 'Royal Gala' em ACD-QR1,3 e ACD-FC tratadas em pré-colheita com AVG e ANA não aumentaram a concentração de compostos voláteis. Entretanto, a aplicação associada de AVG e ANA e o armazenamento em ACD-QR1,3 ou ACD-FC foram excelentes alternativas para a conservação dos frutos, pois mantiveram a firmeza da polpa, reduziram os distúrbios fisiológicos, proporcionando maior volume de frutos sadios.

Palavras-chave: desordens fisiológicas, firmeza de polpa, fluorescência de clorofilas, *Malus domestica*, oxigênio, quociente respiratório.

ABSTRACT

QUALITY CONSERVATION OF 'GALA' MUTANTS APPLES TREATED WITH GROWTH REGULATORS AND STORED UNDER DYNAMIC CONTROLLED ATMOSPHERE

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Being the harvest time of apples concentrated in a short period, fruit storage is needed for market regulation. New storage technologies are being developed, such as dynamic controlled atmosphere with respiratory quotient (DCA-RQ), that needs to be evaluated under different temperatures and with the application of growth regulators such as aminoethoxyvinylglycine (AVG), naphthalene acetic acid (NAA) and 1-methylcyclopropene (1-MCP). This thesis is composed of four papers, which resulted from studies with the following objectives: to evaluate volatile compounds concentration in 'Galaxy' and 'Royal Gala' apples stored under DCA-RQ, DCA with chlorophyll fluorescence (DCA-CF), controlled atmosphere (CA) with and without 0,625 µL L^{-1} of 1-MCP application; to evaluate if DCA-RQ and DCA-CF allow employing higher temperatures during apple storage; to evaluate the effect of the application of AVG (0.83 kg ha^{-1}) in combination with NAA (40g ha^{-1}), as well as the effects of 1-MCP in the production of volatile compounds and physical-chemical quality of apples stored under DCA monitored with chlorophyll fluorescence and respiratory quotient; identify if the stress by low O_2 partial pressure during RQ measurement has an effect on apple quality conservation. 'Galaxy' and 'Royal Gala' apples were stored for 9 months plus 7 days at 20 °C. It is worth noting that 'Galaxy' apples stored under DCA-RQ1.5 maintained better quality when compared to CA, ultralow oxygen (ULO – 0.4kPa) and DCA-CF, also increasing the production of esters responsible for the aroma. Besides, 1-MCP reduced volatile compounds concentration, even in apples stored under DCA-RQ1.5. Likewise, DCA-CF had a similar effect to ULO with 0.4 kPa of O_2. The DCA-RQ1.3 and DCA-CF allowed storing 'Galaxy' apples under temperatures higher (2.0 and 2.5 °C) than recommended (1.5 °C). In addition, 1-MCP application did not have additional effects when fruit were stored under DCA and higher temperatures. Higher temperatures (2.0 and 2.5 °C) could be used without reducing the production of important volatile compounds. In DCA-RQ, the stress by low O_2 employed during RQ measurement has presented a beneficial effects, with fruit maintaining higher flesh firmness, lower mealiness and decay incidence compared to fruit without RQ calculation. 'Royal Gala' apples stored under DCA-RQ1.3 and DCA-CF, submitted to the application of AVG and NAA during prehavest did show an increase in the concentration of volatile compounds. However, the combination of AVG plus NAA and the storage under DCA-RQ1.3 or DCA-CF presents an excellent alternative for storing apples, because maintains higher flesh firmness, reduce physiological disorders and maintain higher percentage of healthy fruit.

Keywords: *Malus domestica*, respiratory quotient, physiological disorders, flesh firmness, chlorophyll fluorescence, oxygen.

LISTA DE ABREVIATURAS

1-MCP	1-metilciclopropeno/1-methylcylclopropene
AA	Amino acid
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
ACD-FC	Atmosfera controlada dinâmica monitorada pela fluorescência de clorofilas
ACD-QR	Atmosfera controlada dinâmica monitorada pelo quociente respiratório
ADH	Álcool desidrogenase
ANA	Ácido naftaleno acético
ANOVA	Análise de Variância
ATP	Adenosina trifosfato
AVG	Aminoetoxivinilglicina/aminoethoxyvinylglycine
BCAT	Branched-chain amino acid aminotransferase
CA	Controlled atmosphere
C_2H_4	Fórmula molecular do Etileno
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
FID	Flame ionization detector
FIRM	Fluorescence Interactive Response Monitor
GC-FID	Cromatógrafo a gás com detector por ionização em chama
GC-MS	Cromatógrafo a gás acoplado à espectrômetro de massa
HS-SPME	Head-space Solid phase microextraction
IEC	Internal Ethylene Concentration
ILOs	Initila Low Oxygen Stress
kg	Quilograma

kPa	Kilopascal
LRI	Linear Retention index
LMO/LOL	Limite mínimo de oxigênio/Lower oxygen limit
LOX	Lipoxigenase
µL L^{-1}	Microlitro por litro
µg	Micrograma
mL	Mililitro
MdACO1	Gene para ACC oxidase em maçã
MdACS1	Gene para ACC sintase em maçã
MdERS1	Genes para receptores de etileno em maçã
MpAAT1	Proteína da AAT1 em *Malus pumila*
mg	Miligrama
mL	Mililitro
mm	Milímetro
N	Newton ou Normal
NAA	Naphathalene acetic acid
N_2	Nitrogênio
NaCl	Cloreto de sódio
NAD^+/ NADH	Nicotinamida adenina dinucleotídeo oxidada/ reduzida
NaOH	Hidróxido de sódio
O_2	Oxigênio
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
RQ	Respiratory quotient
S	Segundo
ULO	Ultralow oxygen (ultrabaixo oxigênio)

SUMÁRIO

1. INTRODUÇÃO E JUSTIFICATIVA

No Brasil, a colheita da maçã é concentrada nos meses de fevereiro a abril, sendo necessário o armazenamento para a comercialização da fruta na entressafra. A produção brasileira de maçã gira em torno de 1 milhão de toneladas anualmente (FAOSTAT, 2016). Deste volume, cerca de 65% é armazenado sob refrigeração convencional e atmosfera controlada (MAIA et al., 2010). As principais cultivares produzidas são a 'Royal Gala', 'Galaxy', 'Imperial Gala' e 'Maxi Gala'. A principal técnica de armazenamento para maçãs no Brasil e no mundo é a atmosfera controlada (BRACKMANN et al., 2010; 2015). Nos últimos anos foram construídas no Brasil dezenas de câmaras de atmosfera controlada dinâmica (ACD), uma nova técnica cuja diferença da AC é que a pressão parcial de O_2 varia ao longo do período de armazenamento (PRANGE et al., 2005), objetivando reduzir ao máximo o metabolismo aeróbico para manter melhor qualidade e reduzir perdas em pós-colheita de maçãs, as quais atingem, em determinados anos, 35% do volume armazenado (CORRENT et al., 2009).

Na ACD, a pressão parcial de O_2 varia de acordo com o limite mínimo de O_2 (LMO), que é a pressão parcial de O_2 mínima tolerado pelo fruto durante o armazenamento. Existem diferentes formas de detectar o LMO, como a detecção do nível de etanol no suco da fruta ou no ar da câmara, pela florescência de clorofilas ou pelo quociente respiratório. O método menos usado é a quantificação do etanol, pois esse composto pode ser metabolizado no fruto e produzir ésteres (BRACKMANN et al., 1993), o que não indica precisamente o metabolismo fermentativo da maçã. Em escala comercial, a técnica mais utilizada é o método que mede a emissão da fluorescência de clorofilas (ACD-FC). No entanto, resultados práticos e de pesquisa no Brasil, demonstram que a pressão parcial de O_2 é variável somente no início do armazenamento, quando ocorre o pico de fluorescência das clorofilas em função da baixa pressão parcial de O_2 (PRANGE et al., 2005; THEWES et al., 2015). Após o pico, a pressão parcial de O_2 é elevada e mantida no mínimo em 0,4 kPa. Outra forma de determinação do LMO é por meio do quociente respiratório (QR) (GASSER et al., 2008; WEBER, 2013), o qual é a razão entre a produção de CO_2 e a absorção de O_2 pelo fruto. Quando ocorre respiração aeróbica, a razão é próxima de 1. Na medida em que o O_2 é insuficiente para o metabolismo aeróbico, o fruto inicia a respiração anaeróbica e aumenta o QR. Pelo fato de muitos compostos voláteis, especialmente os ésteres, terem os álcoois como precursores, é possível que o QR acima de 1 proporcione menor redução na produção de compostos voláteis e mantenha melhor a qualidade da maçã do que a AC convencional e a ACD-FC (BRACKMANN et al., 1993). O prolongado armazenamento em AC reduz a produção de compostos voláteis em maçãs (BRACKMANN et al., 1993; SAQUET et al., 2003; MATTHEIS

et al., 2005). Além disso, baixa concentração (40 pmol mL^{-1}) de etanol no ar, produto da fermentação, reduziu a produção de etileno em brócolis (ASODA et al., 2009), e desta forma, compostos químicos, como 1-metilciclopropeno (1-MCP) e aminoetoxivinilglicina (AVG), provavelmente não sejam necessários. Isso contribuiu para uma redução de produtos químicos utilizados na produção de alimentos.

Associados ao armazenamento em AC e ACD-FC, são utilizados fitorreguladores que inibem a síntese de etileno em pré-colheita, como a AVG, e a ação do etileno em pós-colheita, como o 1-MCP, a fim de reduzir o efeito do etileno no amadurecimento da maçã. A AVG diminui a atividade da enzima ACC sintase e o 1-MCP bloqueia a ação do etileno pela ligação irreversível aos receptores de etileno (SALAS et al., 2011). Todas essas técnicas exercem forte redução na síntese de compostos voláteis, os quais são responsáveis pelo aroma em maçãs (BRACKMANN et al., 1993; MATTHEIS et al., 2005; SALAS et al., 2011). Diante disso, é de suma importância avaliar métodos que não reduzam de forma demasiada a produção de compostos voláteis. Para reduzir as perdas pós-colheita aplicam-se fitorreguladores em pré e pós-colheita, mas que podem afetar a síntese de aroma ou causar outros problemas. Entre eles, o aumento da incidência de podridões em alguns casos com aplicação de 1-MCP (JANISIEWICZ et al., 2003; WEBER, 2013), sendo esta a principal causa de perdas de maçãs em pós-colheita. Portanto, são necessárias alternativas para armazenar maçãs mitigando as perdas de qualidade, principalmente evitar a redução de compostos voláteis, a ocorrência de podridões e distúrbios fisiológicos.

1.1. HIPÓTESES

1) A atmosfera controlada dinâmica, monitorada pelo quociente respiratório (ACD-QR), não reduz a produção de compostos voláteis como acontece na ACD-FC, na AC convencional (1,2 kPa O_2 + 2,0 kPa CO_2) e com a aplicação de 1-MCP, além de aumentar a produção de ésteres e ter melhor efeito na conservação da qualidade de maçãs ‘Galaxy’ e ‘Royal Gala’;
2) ACD-QR permite utilizar temperatura de armazenamento mais elevada do que a AC convencional e ACD-FC;
3) Aplicações pré-colheita de AVG mais ANA, para evitar queda pré-colheita de frutos, e aplicação de 1-MCP na pós-colheita, afetam a produção de compostos voláteis e a qualidade físico-química de maçãs armazenadas em ACD-QR e ACD-FC;
4) O estresse por baixa pressão parcial de O_2 durante o período de cálculo do quociente respiratório possui efeito positivo na conservação da qualidade de maçãs.

1.2. OBJETIVOS

1) Avaliar a produção de compostos voláteis, em maçãs ‘Galaxy’ e ‘Royal Gala’ armazenadas em ACD-QR, ACD-FC, AC convencional e com aplicação de 1-MCP;
2) Identificar se a ACD-QR e a ACD-FC permitem utilizar temperatura mais elevada para o armazenamento de maçãs, sem perda de qualidade;
3) Avaliar o efeito da aplicação da AVG isolada ou combinada com o ANA e do 1-MCP na produção de compostos voláteis e qualidades físico-químicas de maçãs armazenadas em ACD;
4) Identificar se o estresse por baixa pressão parcial de O_2 durante o período de cálculo do quociente respiratório possui efeito na conservação da qualidade de maçãs.

2. REVISÃO DE LITERATURA

2.1 ARMAZENAMENTO DE MAÇÃS

O amadurecimento de frutos é um processo geneticamente programado, o qual nos frutos climatéricos ocorre também após o desligamento da planta (CHITARRA; CHITARRA, 2005). Nesta fase fisiológica, o fruto melhora características como sabor, cor, textura e aroma com a finalidade de atrair agentes dispersantes das sementes. A medida que o fruto se aproxima da senescência, os tecidos tornam-se macios, com menos acidez e mais vulnerável ao ataque de patógenos que causam doenças de pós-colheita. Além disso, ocorre a perda da integridade das membranas da célula, aumentando a incidência de distúrbios fisiológicos como o escurecimento da polpa (FRANCK et al., 2007). Neste sentido, o armazenamento de maçãs tem como objetivo atrasar o amadurecimento, mantendo a qualidade, reduzindo distúrbios fisiológicos e a incidência de podridões. Apesar de existirem modernas técnicas de armazenamento, ainda ocorrem perdas que podem atingir, em determinados anos, 35% do volume, tendo como principais causas a ocorrência de podridões, desordens fisiológicas e amadurecimento avançado (CURRENT et al., 2009; ANTONIOLLI et al., 2011).

No Brasil, a maior parte do volume produzido é armazenado para comercialização na entressafra. Algumas técnicas são utilizadas para prolongar a vida pós-colheita de maçãs, como a baixa temperatura durante o armazenamento refrigerado, a atmosfera controlada (AC), a atmosfera controlada dinâmica (ACD), aplicação de inibidores da síntese ou ação do etileno, aumento e controle da umidade relativa na câmara, entre outras (BRACKMANN et al., 2009a e 2009b; WRIGHT et al., 2012, WEBER, 2013). O armazenamento em AC reduz o metabolismo e atrasa o amadurecimento do frutos. Entretanto, causa uma significativa redução na concentração de compostos voláteis no fruto, os quais são responsáveis pelo aroma (BRACKMANN et al., 1993; MATTHEIS et al., 2005).

2.2 ATMOSFERA CONTROLADA

Uma das técnicas mais primitivas de armazenamento de produtos perecíveis era a conservação dos alimentos em porões, onde a temperatura mantinha-se mais baixa do que o ambiente externo (THOMPSON, 2010). Posteriormente, com a criação da refrigeração, o armazenamento refrigerado tornou-se uma forma mais eficaz de conservar frutas (THOMPSON,

2010). Na década de 40, pesquisadores descobriram que a redução da pressão parcial de oxigênio do ambiente da câmara reduz a respiração dos frutos (KIDD; WEST, 1945). A partir desse conhecimento, surgiu o armazenamento em atmosfera controlada (AC), na qual, além do manejo da temperatura e umidade relativa, se reduz a pressão parcial de O_2 e aumenta a de CO_2 (BRACKMANN et al., 1993; BRACKMANN & SAQUET, 1995).

O armazenamento em AC reduz a produção de etileno (GORNY; KADER, 1997) e a taxa respiratória (BRACKMANN et al., 2009a), o que prolonga a vida pós-colheita dos frutos. Para síntese do etileno é necessário a participação das enzimas ACC sintase, que converte o S-adenosilmetionina a ácido 1-carboxílico-1-amino ciclopropano (ACC), e da ACC oxidase, que faz a conversão do ACC a etileno, sendo necessário O_2 para esta etapa (YANG; HOFFMAN, 1984). O mecanismo pelo qual o baixo O_2 reduz a respiração é devido à inibição da enzima citocromo *c* oxidase, na cadeia de transporte de elétrons, que utiliza O_2 como aceptor final de elétrons (WRIGHT et al., 2015).

As pressões parciais de gases recomendadas para o armazenamento em AC para a maçã 'Gala' e mutantes é de 1 a 1,2 kPa de O_2 e 2 a 2,5 kPa de CO_2 (BRACKMANN et al., 2009a; 2010). A pressão parcial de O_2 em torno de 1 a 1,2 kPa é utilizada durante todo o período de armazenamento (8 a 9 meses). No entanto, estas pressões parciais de O_2 podem serem reduzidas, pois a maçã tolera pressões mais baixas desde que seja utilizado um método de detecção do nível mínimo de O_2. A vantagem do armazenamento em pressões parciais mais baixas de O_2 é uma maior redução no metabolismo e melhor conservação da qualidade. O limite mínimo de O_2 (LMO) que o fruto tolera geralmente é uma pressão parcial de O_2 abaixo do ponto de compensação anaeróbico (PCA), que é o nível de O_2 em que a produção de CO_2 é mínima (BOERSIG et al., 1988). O PCA pode variar entre cultivares, temperatura de armazenamento e fatores pré-colheita. Gasser et al. (2010) encontraram um PCA de 0,2 a 0,3 kPa O_2 para as cultivares Idared, Maigold e Elstar e de 0,4 kPa para 'Braeburn'. Com o aumento da temperatura de 0 °C para 1 °C foi necessário aumentar o nível de O_2 para manter melhor qualidade da maçã 'Royal Gala', o que é explicado pela menor solubilidade do O_2 com aumento da temperatura (WEBER et al., 2013a). Desta forma, a utilização de temperatura mais elevada no armazenamento, provavelmente seja viável somente em ACD, que utiliza níveis muito baixos de O_2 com um mecanismo de detecção do limite mínimo de O_2.

2.3 METABOLISMO FERMENTATIVO

Durante o armazenamento em AC ou ACD, quando o O_2 é limitante para a respiração aeróbica, ocorre uma limitação na cadeia transportadora de elétrons da respiração, reduzindo a produção de energia na forma de adenosina trifosfato (ATP) (TAIZ; ZEIGER, 2013). Em condições normais de O_2, ocorre a produção de 38 ATP por molécula de glicose, sendo que em condições de anoxia (ausência de O_2) ocorre a produção de somente 2 ATP por molécula de glicose (TAIZ; ZEIGER, 2013). A concentração de ATP em maçã 'Jonagold' e pera 'Conference' armazenadas em AC foi menor do que a concentração encontrada nos frutos armazenados sob refrigeração (SAQUET; STREIF; BANGERTH, 2000). Essa menor quantidade de energia disponível pode causar distúrbios fisiológicos como a degenerescência da polpa, caracterizada como escurecimento dos tecidos (SAQUET; STREIF; BANGERTH, 2000; FRANCK et al., 2007).

A respiração aeróbica é dividida em três fases: glicólise, ciclo dos ácidos tricarboxílicos e cadeia de transporte de elétrons. Nesta última fase, com baixa pressão parcial de O_2, a enzima citocromo *c* oxidase, que utiliza o O_2 como aceptor final de elétrons, tem sua atividade reduzida. Desta forma, reduz o fluxo de elétrons na cadeia de transporte de elétrons e diminui entrada de H^+ para o espaço intermembranas na mitocôndria, o que causa menor gradiente eletroquímico e menor produção de ATP pela enzima ATP sintase (TAIZ; ZEIGER, 2013). A indução do metabolismo fermentativo é um mecanismo que a célula vegetal possui para produzir ATP pela via glicolítica e oxidar o NADH, produzindo acetaldeído e etanol no final da rota. Neste caso, o ATP é sintetizado na conversão do 1,3-bifosfoglicerato para 3-fosfoglicerato e na conversão do fosfoenolpiruvato em piruvato (TAIZ; ZEIGER, 2013). Em altas concentrações, o acetaldeído e o etanol podem causar danos às membranas e, consequentemente, o escurecimento dos tecidos da polpa (LEE; MATTHEIS; RUDELL, 2012).

A oxidação do NADH é necessária para que o rota glicolítica continue ocorrendo, o que ocorre com a participação de enzimas como a álcool desidrogenase (ADH) e, em menor grau, pela lactato desidrogenase (SAQUET; STREIF, 2008). Em condições de baixo O_2, menor quantidade de piruvato entra no ciclo dos ácidos tricarboxílicos, sendo, portanto, devido ao fato de que este é utilizado pela piruvato descarboxilase (PDC) para produzir acetaldeído, que é desidrogenado pela ADH para produzir etanol. Saquet; Streif (2008) reportam que em maçã 'Jonagold' a atividade da PDC e da ADH aumentou com o correspondente acúmulo de acetaldeído e etanol em frutos armazenados com alto CO_2 e baixo O_2 (0,5 kPa O_2 + 6,0 kPa CO_2).

Os produtos do metabolismo fermentativo, quando em alta concentração são relacionados ao *off-flavor* (ZANELLA et al., 2005; RAFFO et al., 2009). Entretanto, quando em baixos níveis são importantes para a síntese de aroma (WRIGHT et al., 2015). Liu et al. (2012) aplicaram etanol

em melões, o que resultou em melhoria na composição volátil dos frutos. Além disso, o etanol reduziu a síntese de etileno e manteve a qualidade de frutos (LIU et al., 2012; WEBER et al., 2016). Asoda et al. (2009) reportam que a atividade da ACC sintase, da ACC oxidase e a expressão dos genes *BO-ACO1*, *BO-ACO2* e *BO-ACS1* foram reduzidas em brócolis com a aplicação de etanol. Em maçãs 'Royal Gala', a aplicação mensal de 0,3 mL de etanol por quilograma de maçã melhorou a conservação da qualidade dos frutos (WEBER et al., 2016).

2.4 ATMOSFERA CONTROLADA DINÂMICA

Para a utilização de níveis de O_2 próximos ao limite mínimo de O_2 (LMO), surgiu uma nova forma de armazenamento: a atmosfera controlada dinâmica (ACD). Nesta técnica a concentração de O_2 varia durante o armazenamento em função do LMO, o que reduz a ocorrência de distúrbios fisiológicos e mantém melhor qualidade dos frutos que a AC convencional, onde o nível de O_2 permanece constante acima do LMO (PRANGE et al., 2003; ZANELLA et al., 2005; WRIGHT et al., 2012; WEBER et al., 2015; THEWES et al., 2017a). O LMO pode ser definido como a pressão parcial de O_2 que a fruta pode ser armazenada com segurança, sem a ocorrência de fermentação excessiva, danos na epiderme ou nos tecidos internos do fruto (WRIGHT et al., 2015). Os métodos para determinar o LMO são: a quantificação do nível de etanol no fruto (VELTMAN et al., 2003), o monitoramento da fluorescência de clorofilas (ACD-FC) (PRANGE et al., 2007) e a determinação através do quociente respiratório (ACD-QR) (GASSER et al., 2008; WEBER et al., 2015). O método da ACD-FC já é amplamente utilizado na Europa e em menor escala na América Latina. Já o uso comercial do método da quantificação do etanol encontra algumas dificuldades, pois o etanol pode ser metabolizado no fruto e produzir ésteres, além de, maçãs e peras produzirem etanol inclusive em condições de aerobiose podendo se constituir em um falso indicador do metabolismo fermentativo (BRACKMANN et al., 1993; PRANGE et al., 2015; WRIGHT et al., 2015). Esta forma de DCA é comercializada como o nome de DCS™ pela empresa Storex®, da Holanda. O ACD-QR é o método mais recente que ainda se encontra em evolução e avaliação científica, mas com resultados mais promissores.

2.4.1 Atmosfera controlada dinâmica monitorada pela fluorescência de clorofilas

No início do século XXI, um grupo de pesquisa liderado por Robert Prange, no Canadá, propuseram uma nova forma de monitoramento e variação do O_2, a ACD baseada na fluorescência de clorofilas (ACD-FC) (PRANGE et al., 2003; PRANGE et al., 2005). Quando o fruto está sob

estresse por baixo O_2 ocorre a emissão da fluorescência pelas clorofilas quando estas recebem energia luminosa. Foram publicados alguns artigos, sendo que em 2007 a tecnologia foi patenteada com nome de HarwestWatch^{TM} (PRANGE et al., 2007) e comercializada pela empresa italiana Isolcell. Posteriormente, trabalhos desenvolvidos por outros grupos, ajudaram a comprovar a eficiência da tecnologia HarwestWatch^{TM} em relação à AC convencional, na manutenção da qualidade de maçãs (ZANELLA; CAZZANELLI; ROGGI, 2008; KITTEMANN; MCCORMICK; NEUWALD, 2015). A primeira utilização comercial da ACD-FC foi em 2003/04 em Washington, nos Estados Unidos e no Tirol Sul, na Itália. No Brasil, a empresa Rasip, de Vacaria, RS, foi a primeira a utilizar a ACD-FC no ano de 2010. Posteriormente, outras empresas brasileiras adotaram a técnica, visto que há vantagens na conservação da qualidade comparado à AC convencional. Entretanto, recentemente observações práticas e trabalhos científicos têm demonstrado que a ACD-FC pode não apresentar resultado superior ao armazenamento em AC com ultrabaixo O_2 (ULO) (THEWES et al., 2015). O ULO ($\leq$ 1 kPa O_2) não exige aquisição de equipamentos e software como a ACD-FC, para monitoramento da fluorescência de clorofilas. Thewes et al. (2015) reportam que maçãs mutantes da 'Gala' em ULO apresentaram conservação da qualidade similar a ACD-FC durante o armazenamento por 9 meses. Alguns pontos críticos da ACD-FC são a pouca representatividade dos 6 a 8 frutos por sensor (é recomendado 6 sensores por câmara) e também a detecção do estresse ocorre somente nas primeiras camadas de células da epiderme e não no fruto inteiro (WRIGHT et al., 2015).

Na ACD-FC os sensores FIRM (*Fluorescence Interactive Response Monitor*) são colocados na câmara de armazenamento para monitorar a emissão de fluorescência pela clorofila da epiderme de uma amostra de seis frutos. Quando o nível de O_2 torna-se insuficiente para a respiração aeróbica ocorre o estresse nas clorofilas, o qual é detectado pelos sensores, e, com auxílio de um software, é gerado um gráfico. Quando o nível de O_2 atinge o limite mínimo, ocorre um aumento na emissão da fluorescência, momento no qual deve se elevar a pressão parcial de O_2 em 0,2 kPa na câmara, no entanto, o O_2 deve permanecer sempre acima de 0,4 kPa (PRANGE et al., 2005; WRIGHT et al., 2012). Uma das maneiras da clorofila dissipar o excesso de energia é por meio da emissão de fluorescência, que é um comprimento de onda na região vermelha do espectro. Não se tem clareza do mecanismo fisiológico que faz a clorofila emitir fluorescência quando está sob pressão parcial de O_2 insuficiente para respiração aeróbica, entretanto, existem algumas hipóteses. Uma delas é que, quando há falta de oxigênio ocorre um acúmulo de compostos reduzidos (NADH) no citoplasma, o qual é transportado para o cloroplasto onde é usado para reduzir a plastoquinona (PQ). A PQ reduzida é responsável pelo aumento do Fα, que é uma estimativa da fluorescência mínima (Fo) da fluorescência de clorofilas (WRIGHT et al., 2011;

2015). A acidificação do citoplasma é uma outra justificativa para o aumento na emissão de fluorescência (PRANGE et al., 2005). Estes autores reportam que a anoxia reduz o pH citoplasmático e esta redução é concomitante com a hidrólise do ATP e formação de ácido fosfórico. Além disso, pelo aumento na fermentação ocorre formação de ácido lático, que reduz o pH citosólico. Também pode ocorrer menor entrada de H^+ para o vacúolo, pois com a menor respiração aeróbica ocorre menor disponibilidade de ATP para a H^+-ATPase bombear H^+ para o vacúolo, causando a acúmulo de H^+ no citoplasma (PRANGE et al., 2005). Uma outra explicação é que a acidificação do estroma reduz a interconversão da xantofila em anteroxantina e, posteriormente em zeaxantina pela inibição da enzima zeaxantina epoxidase (TAIZ; ZEIGER, 2013). A conversão da xantofila em zeaxantina e importante para dissipar o excesso de energia na forma de calor, quando há excesso de compostos reduzidos. Quando este processo é inibido, ocorre a emissão de fluorescência de clorofila para dissipar o excesso de energia.

2.4.2 Atmosfera controlada dinâmica monitorada pelo quociente respiratório

No final da primeira década do século XXI, surgiu a ideia de monitorar o LMO por meio do quociente respiratório (QR) na ténica chamada atmosfera controlada dinâmica monitorada pelo QR (ACD-QR), a qual iniciou a ser estudada por alguns grupos de pesquisa ao redor do mundo. O QR é a razão entre a produção de CO_2 e o consumo de O_2 na respiração, cujo objetivo é detectar a pressão parcial de O_2 no qual ocorre um aumento no QR, que é em função do aumento na produção de CO_2 oriundo da respiração anaeróbica (BOERSIG et al., 1988). Se a respiração anaeróbica for excessiva, a pressão parcial de O_2 deve ser aumentada a fim de manter o nível do QR previamente estabelecido, mantendo um mínimo de fermentação, sem causar danos aos frutos. A presença de produtos da fermentação como acetaldeído, etanol e acetato de etila em baixos níveis podem melhorar o aroma da maçã (WRIGHT et al., 2015). O conceito do QR relacionado à respiração anaeróbica e ao ponto de compensação anaeróbico em frutos havia sido reportado na década de 80 por Boersig et al. (1988), entretanto, sua aplicação como forma de monitorar o nível de O_2 durante o armazenamento foi implementada mais de 20 anos depois.

Os primeiros testes com a ACD-QR em nível experimental no Brasil começou em 2010, cujo principal resultado foi que a ACD-QR possui vantagens em termos de conservação da qualidade dos frutos, em alguns casos foi mais eficiente do que a ACD-FC para maçãs ‘Royal Gala’ e ‘Fuji Suprema’ (WEBER et al., 2015; WEBER et al., 2017). As avaliações em nível comercial iniciaram em 2012, na empresa Agropecuária Schio, de Vacaria, RS, com bons resultados práticos. No ano de 2013, pesquisadores da Bélgica patentearam uma técnica de

mensurar o QR em câmara comercial, a qual utiliza modelagem matemática para alterar a concentração de O_2 (EP2547213 B1) (DELELE et al. 2013). No ano seguinte, outro equipamento para mensurar o QR em câmara comercial foi protegido, o qual consiste na colocação de uma minicâmara, dentro da câmara frigorífica, onde é colocado uma amostra de maçãs para calcular o QR (US 2014/0242225A1) (SCHAEFER; BISHOP, 2014). A medição do QR é realizada nesta minicâmara, por meio da conexão com um analisador de gases automatizado. Em 2014, a empresa Isolcell da Itália patenteou outro invento (US2015/0257401A1) para mensuração do QR em câmara comercial, que utiliza duas minicâmaras, uma com e outra sem frutos, invento este desenvolvido pelo grupo do Núcleo de Pesquisa em Pós-colheita da UFSM (BRACKMANN, 2015). A minicâmara sem frutos fornece os níveis de gases de referência no momento da determinação do QR, o que aumenta a precisão do método da medição do QR. Atualmente, a empresa holandesa Van Amerogon CA Techonology, a italiana Isolcell e a americana Storage Control System Inc. comercializam técnica de armazenamento ACD-QR.

Os resultados de pesquisa e a utilização em nível comercial da ACD-QR ainda são incipientes. Weber et al. (2015) encontraram que o armazenamento em ACD-QR2,0 manteve a qualidade de maçãs 'Royal Gala' com a mesma eficiência do que a ACD-FC e melhor do que a AC. Na Holanda, em maçãs 'Granny Smith', Bessemans et al. (2016) reportam uma redução na incidência de escaldadura superficial com a utilização da ACD-QR. Em outro trabalho com QR, foi identificado que pressões parciais de CO_2 de 1,2 a 1,6 kPa são as mais adequadas para manter a qualidade de maçã 'Galaxy' após longos períodos de armazenamento (BRACKMANN et al., 2015). Esta cultivar quando armazenada em ACD-QR1.3 apresentou menor produção de etileno, menor respiração, menor incidência de polpa farinácea e maior firmeza da polpa comparado com a AC, entretanto, não apresentou diferença dos frutos em AC com aplicação de 1-MCP (THEWES et al., 2017a). Estes autores reportam que a ACD-QR1.3 é eficiente em manter a qualidade de maçã nos diferentes estádios de maturação. Em relação aos compostos voláteis, Thewes et al. (2017b) reportam que a ACD-QR1.5 resultou em alta concentração de ésteres no suco extraído da maçã, como acetato de butila, acetato de 2-metil butila, acetato de hexila, 2-metil butanoato de etila e acetato de 2-metil propila. Em maçã 'Royal Gala', Both et al. (2017) reportam que a ACD-QR2.0 e ACD-QR1.5 resultaram em alta concentração de compostos voláteis importantes para o aroma da maçã 'Royal Gala', como acetato de butila e acetato de 2-metil butila. Apesar dos trabalhos já realizados, não foram encontradas informações sobre o efeito da ACD-QR associado à aplicação de 1-MCP, a utilização de temperatura mais elevada em ACD-QR, ACD-FC e também sobre o efeito da aplicação de fitorreguladores e o posterior armazenamento em ACD na conservação da qualidade e perfil volátil dos frutos.

2.3 TEMPERATURA DE ARMAZENAMENTO

O principal fator responsável pela redução do metabolismo dos frutos é a utilização da baixa temperatura (EKMANN; GOLDING; MCGLASSON, 2005). A recomendação de temperatura para o armazenamento em AC de maçãs mutantes da 'Gala' ficava na faixa de 0 a 0,5 °C (BRACKMANN et al., 2009c) até que outros resultados indicaram que a temperatura de 1 °C a 1,5 °C apresentaram melhores resultados em maçã 'Royal Gala' e 'Maxi Gala' quando comparado à temperatura de 0 e 0,5 °C (WEBER et al., 2013a; WEBER et al., 2013b; BOTH, 2015). Desta forma, possivelmente temperatura maior do que 1,5 °C pode ser mais eficiente na manutenção da qualidade do que temperatura mais baixa, desde que se reduza a respiração através da atmosfera da câmara. Algumas cultivares de maçãs produzidas na Europa e EUA apresentaram danos por frio, sendo necessário armazenar entre 2 a 4 °C (JUNG; WATKINS, 2011). Cada cultivar possui uma temperatura mais adequada para conservação da qualidade (KÖPCKE, 2015).

Temperatura mais elevada possui também a vantagem de redução do consumo de energia elétrica pelo sistema de refrigeração da câmara. McCormick; Neuwald; Streif (2010) reportam que houve 35% de redução no consumo de energia quando foi utilizada a temperatura de 4 °C associada à aplicação de 1-metilciclopropeno (1-MCP) em relação a 1,5 °C. Além disso, os frutos na temperatura mais elevada apresentaram maior firmeza de polpa e melhor aceitação pelo consumidor. O 1-MCP é um composto químico sintético que inibe a ação do etileno, atrasando o amadurecimento dos frutos climatéricos (SISLER; SEREK, 1997). Em outro trabalho, Mazzurana et al. (2016) encontraram redução na incidência de degenerescência de polpa e alta firmeza da polpa em maçã do grupo 'Gala' com o aumento da temperatura de 0,7 a 0,8 °C para 1,9 a 2,0 °C com aplicação de 1-MCP. Outra vantagem foi a redução no consumo de energia elétrica em 21% na ventilação e 50% no resfriamento.

Outros trabalhos reportam que a alta temperatura promove a dissipação do pingo-de-mel em maçã 'Gloster', resultando em menor incidência de escurecimento interno (KÖPCKE, 2015). Dierend (2012) reporta que o aumento na temperatura de armazenamento para 4 °C sob condições de AC ou ULO com aplicação de 1-MCP não causou efeito negativo na qualidade dos frutos em comparação a condição em baixa temperatura (1 °C) sem aplicação de 1-MCP. Em maçã 'Fuji', o armazenamento em 2 °C reduziu a incidência de degenerescência da polpa em relação ao armazenamento em 0 °C (KWEON et al., 2013). Asif et al. (2012) reportaram que condições com 1,5 kPa de O_2 mais a aplicação de 1-MCP permitiram o armazenamento da maçã 'Granny Smith' na temperatura de 6,5 °C por 198 dias.

O LMO pode variar com a cultivar e a temperatura de armazenamento (GASSER et al., 2010). Wright et al. (2010) reportam que o LMO aumentou com a temperatura para maçã 'Honeycrisp', onde nas temperaturas de 0; 3,5; 10 e 20 °C apresentaram LMO de 0,08; 0,22; 0,33 e 0,72 kPa, respectivamente. Além disso, reportam que o LMO das cultivares Honeycrisp e Delicious após a colheita foi de 0,8 kPa, e após três meses de armazenamento foi de 0,5 kPa, demonstrando que o LMO altera durante o período de conservação. Desta forma, para utilização de temperatura mais elevada é importante avaliar técnicas de determinação do LMO, como a ACD-FC e a ACD-QR sem aplicação de 1-MCP.

O aumento na temperatura em combinação com a aplicação de 1-MCP é uma ferramenta interessante para reduzir o gasto financeiro com energia elétrica pelas empresas, sem a ocorrência de efeitos negativos para conservação da qualidade dos frutos (ASIF et al., 2012; KITTEMANN; MCCORMICK; NEUWALD, 2015). Entretanto, o 1-MCP é um composto químico, não liberado para produção orgânica, o qual possui elevado custo financeiro para o armazenador. Desta forma, é necessário a investigação de técnicas alternativas ao uso deste composto, como a ACD, para armazenar maçã em temperatura mais elevada.

2.4 COMPOSTOS VOLÁTEIS

O sabor e o aroma da maçã são cruciais para a qualidade e aceitação do consumidor. Os compostos voláteis são os constituintes do aroma da maçã, que, em maçãs, são principalmente ésteres, álcoois, aldeídos, ácidos, cetonas e terpenos (LURIE et al., 2002; FAN; MATTHEIS, 1999; SALAZAR; OROZCO, 2011), sendo os ésteres os principais (FELLMAN et al., 1993). Compostos voláteis têm sua concentração significativamente reduzida durante o armazenamento de maçãs (MATTHEIS et al., 2005). A percepção do aroma pelo consumidor depende da combinação dos compostos voláteis, da concentração e do nível de detecção individual de cada composto (DEFILIPPI; KADER; DANDEKAR, 2005). Em maçãs 'Royal Gala' os principais compostos voláteis são o acetato de 2-metil butila, acetato de butila, acetato de hexila e butanol (YOUNG et al., 1996).

Quanto aos ésteres, a última etapa na síntese desses compostos é mediada pela enzima ácool acetiltransferase (AAT), a qual catalisa a transferência de um acetil do acetil-CoA e de um acil do acil-CoA para um álcool formando um éster (LUCCHETTA et al., 2007). A atividade da AAT e sua transcrição são controladas pelo etileno (FAN; MATTHEIS, 1999; DEFILIPPI; KADER; DANDEKAR, 2005). A enzima álcool desidrogenase (ADH) também participa na

formação de ésteres, por liberar substrato (álcoois) para a formação do composto volátil (DEFILIPPI et al., 2005).

Apesar do impacto positivo na conservação da qualidade da maçã e seu reflexo na economia do país (BRCKMANN & SAQUET, 1995), o prolongado armazenamento em AC, de maneira geral, reduz a produção de compostos voláteis nos frutos (BRACKMANN et al., 1993; MATTHEIS et al., 2005). Brackmann et al. (1993) reportam que o baixo O_2 reduz a síntese de compostos voláteis de cadeia linear pela influência deste gás no metabolismo e/ou síntese de lipídios. Estes autores encontraram redução nas concentrações dos ácidos graxos, principalmente o ácido linoleico, em AC com baixo O_2 e alto CO_2. A supressão na síntese de compostos voláteis foi altamente correlaciona com a concentração do ácido graxo linoleico livre (SAQUET et al., 2003). Frutos armazenados em AC têm reduzida produção de ésteres, álcoois e 1-metoxi-4-(2-propenil)benzeno (MATTHEIS et al., 2005). Saquet et al. (2003) reportam que a supressão na síntese de voláteis foi mais acentuado com o aumento da duração do armazenamento. O armazenamento de maçã 'Royal Gala' em O_2 extremamente baixo (0,5 kPa), durante oito meses, reduziu a produção de alguns ésteres, ao passo que outros compostos voláteis não foram afetados (BOTH et al., 2014). Fellman et al. (1993) encontraram redução na atividade da enzima acetil-Coa álcool transferase, que catalisa a formação do acetato de butila a partir do butanol, após o armazenamento de maçã 'Rome' por nove meses com 0,5 e 1,0 kPa de O_2. No entanto, após nove dias de vida de prateleira a 25 °C a atividade da enzima foi superior nos frutos com 0,5 kPa de O_2 comparado aos armazenados sob refrigeração.

Maçãs sintetizam álcoois de cadeia linear ou ácidos a partir da β-oxidação de ácidos graxos de cadeia longa (BARTLEY, 1985) e álcoois de cadeia ramificada ou ácidos a partir da degradação de aminoácidos (HANSEN; POLL, 1993) que servem de substratos para síntese de ésteres, bem como para a atividade de enzimas que sintetizam ésteres (MATTHEIS et al., 2005). A beta-oxidação e a lipoxigenase, que estão envolvidas na produção de precursores para síntese de ésteres, são dependentes do O_2, assim, o prolongado armazenamento em baixo O_2 pode reduzir a oxidação de lipídios e a concentração dos precursores para ésteres, como o acetil Co-A (BRACKMANN et al., 1993; ECHEVERRÍA et al., 2004). Dependendo do período de armazenamento e das condições de AC pode não haver redução na produção de acetato de 2-metil butila. Maçã 'Fuji' armazenada com 1,0 kPa de O_2 durante o armazenamento por 3 meses não reduziu a produção deste composto (ECHEVERRÍA et al., 2004). Both et al. (2014) armazenando maçã 'Royal Gala' com 0,7 kPa de O_2 encontraram maior produção de acetato de 2-metil butila do que quando utilizaram 1 kPa de O_2.

A disponibilidade do precursor é mais importante do que a atividade de enzimas para o desenvolvimento do aroma durante a maturação de maçã 'Fuji' (ECHEVERRÍA et al., 2004, LARA et al., 2006) e pêra 'Doyenne du Comice' (LARA et al., 2003). Desta forma, o etanol e outros compostos, proveniente da respiração anaeróbica proporcionada pela ACD-QR, talvez possibilite aumento da produção de álcoois e ácidos, precursores de compostos voláteis, predominantemente de ésteres, contribuindo para manter uma melhor qualidade do fruto que a AC convencional e ACD-FC, mesmo com a aplicação de 1-MCP. Esse composto reduz a produção de compostos voláteis em maçãs armazenadas em AC (FAN; MATTHEIS, 1999).

2.5 FITORREGULADORES

2.5.1 Metilciclopropeno

O 1-metilciclopropeno (1-MCP) é um composto químico que se liga de forma irreversível aos receptores de etileno na membrana do retículo endoplasmático da célula, bloqueando a ligação deste fitohormônio ao seu respectivo receptor (SISLER; SEREK, 1997). Desta forma, o 1-MCP evita a sequência de eventos desencadeada pelo etileno, que culmina no amadurecimento e senescência do fruto (SISLER; SEREK, 1997). Este composto atrasa a redução da firmeza da polpa e degradação de ácidos (REBEAUD; GASSER, 2015). Uma vez que o etileno está envolvido na síntese de compostos voláteis (MATTHEIS et al., 2005) o bloqueio de sua ação reduz a produção destes. O 1-MCP é amplamente utilizado por empresas armazenadoras de maçãs em nível mundial, para prolongar a vida pós-colheita de frutos climatéricos (BRACKMANN et al., 2009c).

A redução da produção de ésteres pelo 1-MCP tem impacto negativo no desenvolvimento do aroma (FAN; MATTHEIS, 1999). Mattheis et al. (2005) encontraram menor produção de ésteres em maçãs tratadas com 1-MCP e armazenada durante sete meses, sendo maior a redução quando o 1-MCP foi associado ao armazenamento em AC com 1,0 kPa O_2 mais 2,0 kPa CO_2. Estes autores reportam também que a exposição dos frutos ao etileno, após o tratamento com 1-MCP, aumentou a síntese de ésteres. Ortiz et al. (2010) reportam que algumas enzimas envolvidas na produção de ésteres a partir de ácidos graxos são parcialmente inibidas pelo 1-MCP. Além disso, o 1-MCP aumenta a suscetibilidade à ocorrência de distúrbios fisiológicos, como a degenerescência, principalmente em temperatura mais elevada (2-4 °C) (JUNG; WATKINS, 2011), possivelmente pela menor produção de energia e consequente menor reparação de membranas em função do 1-MCP reduzir indiretamente a respiração.

Em função da ACD-QR permitir um mínimo de fermentação dos frutos, esta poderia manter a qualidade melhor ou igual ao 1-MCP e não reduzir de forma demasiada a produção de compostos voláteis no fruto, uma vez que os produtos da fermentação são precursores de compostos voláteis. O 1-MCP em alguns casos aumenta a incidência de podridões, possivelmente pela redução do mecanismo de defesa do fruto contra o ataque de patógenos (JANISIEWICZ et al., 2003), uma vez que o mecanismo de defesa do fruto necessita de etileno para sua ativação. KÖPCKE (2015) encontrou que alta temperatura promoveu o aumento da dissipação do pingo de mel em maçã 'Gloster', resultando menor escurecimento interno. Por outro lado, o 1-MCP causou redução na dissipação do escurecimento interno, enquanto a ACD-FC não teve efeito no pingo de mel.

2.5.2 Ácido naftaleno acético

Para reduzir a queda pré-colheita e/ou atrasar a colheita de maçãs utilizam-se fitorreguladores. A aminoetoxivinilglicina (AVG) controla a queda pré-colheita e atrasa a maturação, enquanto que o ácido naftaleno acético (ANA), uma auxina sintética, somente reduz a abscisão pré-colheita, entretanto, acelera a maturação dos frutos, pelo aumento na expressão de genes da ACC sintase, e a consequente produção de etileno, reduzindo o potencial de armazenamento (YUAN; CARBAUGH, 2007; LI; YUAN, 2008; UNRATH et al., 2009; BRACKMANN et al., 2014; BRACKMANN et al., 2015). Em maçãs 'Gala', como a semente reduz a produção de auxinas antes da completa maturação do fruto, ocorre redução na relação de auxinas e etileno. Assim, o etileno atua na zona de abscisão do pedúnculo causando a queda do fruto, que pode ser reduzida pelo aumento nos níveis de auxina exógena (UNRATH et al., 2009).

Alguns trabalhos foram realizados com aplicação de ANA em maçã, com posterior armazenamento em AC. Brackmann et al. (2014) reportam que o ANA acelerou o amadurecimento e reduziu a difusão de gases em maçãs 'Brookfield' armazenadas em AC. O ANA aumentou a expressão de genes da biossíntese (*MdACS1* e *MdACO1*), da percepção (*MdERS1*) do etileno, e de enzimas da degradação da parede celular (*MdPG1*) (LI; YUAN, 2008; YUAN; LI, 2008). Shin et al. (2016) reportam que a auxina pode ser crítica na determinação da ativação de genes da biossíntese de etileno (*MdACS3* e *MdACS1*) e na ativação da maturação de maçãs. A hipótese é que ocorre aumento na concentração de compostos voláteis em maçãs com ANA, uma vez que este fitorregulador acelera o amadurecimento em pós-colheita (BRACKMANN et al., 2014), e que a ACD atrasa o amadurecimento dos frutos durante o armazenamento, pois a ACD já apresentou bom resultado independente do ponto de maturação dos frutos (THEWES et al., 2017a).

2.5.3 Aminoetoxivinilglicina

A aminoetoxivinilglicina (AVG) reduz a síntese de etileno pela supressão da expressão de genes da enzima ACC sintase, *MdACS5A* e *MdACS5B* e da ACC oxidase, *MdACO1* (LI; YUAN, 2008; UNRATH et al., 2009). Além disso, reduz indiretamente a expressão de genes de enzimas que degradam a parede celular, na camada de abscisão, como a poligalacturonase, *MdPG2*, e celulase, *MdEG1* (LI; YUAN, 2008). Sua aplicação é realizada cerca de quatro semanas antes da data prevista da colheita. Unrath et al. (2009) avaliaram maçã 'Scarletspur Delicious' durante 10 anos e encontraram que a AVG é mais eficiente em reduzir a queda pré-colheita que o ANA. No entanto, a AVG reduz a síntese de etileno, afetando negativamente a síntese de compostos voláteis. Maçãs 'Golden Delicious' tiveram a síntese de compostos do aroma reduzidos com aplicação de AVG (BANGERTH; STREIF, 1987). Salas et al. (2011) reportam que a AVG reduziu a concentração de álcoois e ésteres de maçã 'Golden Delicious'.

Yuan; Carbaugh (2007) reportam que a aplicação de AVG mais ANA associado ou não com 1-MCP apresentou maior redução na queda pré-colheita de frutos do que qualquer um desses fitorreguladores aplicados isolados. O mecanismo de como a AVG e o 1-MCP inibem o efeito negativo do ANA é pela redução da expressão de genes para enzimas envolvidas na síntese do etileno, receptores de etileno e enzimas que degradam a parede celular, como as celulases e poligalacturonases (LI; YUAN, 2008). O ANA, por aumentar a síntese de etileno, provavelmente aumenta também a produção de compostos voláteis. Neste sentido, não foi encontrado estudo que avalie a síntese de compostos voláteis e outros parâmetros de qualidade após a colheita e o armazenamento em AC ou ACD de maçãs com aplicação de AVG mais ANA na pré-colheita.

3. ARTIGO 1

3.1. Fruit quality and volatile compounds profile of 'Galaxy' apple influenced by dynamic controlled atmosphere storage and 1-methylcyclopropene treatment[1]

Abstract

The aim of this work was to evaluate the effect of dynamic controlled atmosphere monitored by respiratory quotient (DCA-RQ) as compared with DCA monitored by chlorophyll fluorescence (DCA-CF), controlled atmosphere (CA), ultralow oxygen (ULO) and 1-methylcyclopropene (1-MCP) treatment on the quality and volatile profile of 'Galaxy' apple after 9 months of storage plus 7 days shelf life. The treatments were: [1] CA (1.2 kPa O_2 + 2.0 kPa CO_2); [2] ULO (0.4 kPa O_2 + 1.2 kPa CO_2); [3] DCA-CF with 1.2 kPa CO_2; [4] DCA-RQ1.3 + 1.2 kPa CO_2 and [5] DCA-RQ1.5 + 1.2 kPa CO_2. All storage conditions were performed with or without 0.625 µL L^{-1} of 1-MCP application. 'Galaxy' apple stored in a DCA-RQ1.5 maintained a better quality and volatile profile as compared to CA, ULO and DCA-CF. 1-MCP, despite of maintaining some quality attributes, reduced volatile compounds production even under DCA-RQ1.5 stored apples. DCF-CF has similar effect on maintaining apple quality than ULO (0.4kPa). Apples stored under DCA-RQ1.5 increased ester production after 9 months of storage. The recommendation to 'Galaxy' apple storage is DCA-RQ1.3 or DCA-RQ1.5.

Keywords: conservation, *Malus domestica*, oxygen, physiological disorders

3.1.1 Introduction

The apple industry needs to store the fruits to sell them throughout the year. The main form used to maintain the apple quality during long term storage is the controlled atmosphere (CA),

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

which employs low temperature, high relative humidity, high CO_2 and low O_2 partial pressure (pO_2) (Brackmann et al., 1993). In CA storage rooms, the O_2 partial pressure usually is about 1.2 kPa, and, if the O_2 partial pressure is below 1.0 kPa, it will be named ultralow oxygen (ULO) (Raffo et al., 2009). However, low pO_2 reduce ethylene biosynthesis and, consequently enzymes activity that produce volatile compounds, such as lipoxygenase (LOX), alcohol dehydrogenase (ADH) and alcohol acyltransferase (AAT). Several authors found reduction of volatile compounds during CA storage as compared to cold storage (Brackmann et al., 1993; López et al., 2007; Thewes et al., 2017).

The main groups of volatile compounds in apples are aldehydes, alcohols and esters (Young et al., 1996). In 'Gala' mutants, as well as for 'Galaxy', the main compounds are hexyl acetate, butyl acetate, butanol and 2-methylbutyl acetate (Young et al, 1996; Salas et al., 2011; Both et al., 2014). Esters are produced by esterification of alcohol and acyl-CoAs, catalyzed by AAT. Fatty acids, degraded by beta-oxidation or LOX, are precursors of straight-chain volatile, as hexyl acetate (Defilippi et al., 2005; Contreras et al., 2013; Contreras et al., 2016) and amino acid form branched-chain volatile as 2-methylbutyl acetate and 2-methylpropyl acetate (Yang et al., 2016). Enzymes involved in volatile biosynthesis as LOX, ADH and AAT are dependent of ethylene (Harb et al., 2011; Schiller et al., 2015). On the other hand, branched-chain amino acid transaminases (BCAT), which degraded amino acids into volatile compounds, showed high activity without ethylene action (Yang et al., 2016).

Throughout the last few years, new methodologies to monitor the pO_2 during CA storage were developed, named dynamic controlled atmosphere (DCA) storage, based on measurements of a biological response of the stored fruit to low pO_2 (Zanella, 2003). In this system, pO_2 changes during the storage period according to the lowest oxygen limit (LOL). For apple produced in Brazil, Weber et al. (2015) used pO_2 below of anaerobic compensation point (ACP) and found good quality maintenance of 'Royal Gala' apple. ACP is the pO_2 where O_2 uptake and CO_2

production is minimal (Boersig et al., 1988), which is the pO_2 above of the LOL tolerated by the apple. Below of ACP the fermentative metabolism is predominant in relation aerobic metabolism. To monitor the LOL, there are three methods available to apple storers based on ethanol measurement in the air of the storage room (DCS – dynamic control system) or in the fruit (ILOS-Plus) (Veltman et al., 2003), based on chlorophyll fluorescence (DCA-CF) (Prange et al., 2007), and, recently, based on respiratory quotient (DCA-RQ) (Brackmann, 2015).

Veltman et al. (2003) studied DCS on 'Elstar' apples, who found that this system maintained higher flesh firmness and color as compared to conventional CA storage with 1.2 kPa O_2 and 2.5 kPa CO_2. However, the measurement of ethanol to monitor LOL is not precise because of ethanol may be metabolized and did not show precise correlation with anaerobic respiration (Brackmann et al., 1993). DCA-CF is the main widespread DCA system used around the world to store apples, which monitors fruits with chlorophyll fluorescence as a response to low O_2 (Prange et al., 2007; Wright et al., 2012; Wright et al., 2015). Another form to monitoring LOL is the respiratory quotient (RQ), which is defined as the ratio of the CO_2 production to the O_2 consumption of the stored fruit (Boersig et al., 1988; Weber et al., 2015). On DCA-RQ, the RQ value proposed by some studies is above 1, which induces anaerobic respiration with a little ethanol production (Weber et al., 2015; Brackmann et al., 2015; Bessemans et al., 2016).

When compared with the conventioanl CA storage, the DCA-CF maintained higher the apple flesh firmness, the titratable acidity and reduced the occurrence of physiological disorders such as flesh breakdown and mealiness (Zanella; Rossi, 2015; Weber et al. 2015; Thewes et al., 2015a; Köpcke, 2015). There are few studies focused on DCA-CF on volatile biosynthesis. Raffo et al. (2009) found that the DCA-CF can preserve the aroma compounds better than ULO plus 0.625 μLL^{-1} of 1-MCP during long-term storage, but did not compare with conventional CA and DCA-CF plus 1-MCP. 1-MCP is an ethylene action inhibitor, which reduced volatile biosynthesis during CA and ULO storage (Raffo et al., 2009; Ortiz et al., 2010; Thewes et al., 2015b). Few reports

were found about DCA-RQ, one of them is Bessemans et al. (2016), which reported that DCA-RQ maintains ‘Granny Smith’ apple quality better as compared to CA and CA plus 1-MCP. Weber et al. (2015) found that DCA-RQ2.0 maintained ‘Royal Gala’ apple quality compared to DCA-CF. However, the effect of DCA-RQ with and without 1-MCP on quality and volatile profile needs to be investigated. The DCA-RQ induced a little of anaerobic respiration, where are produced aldehydes and alcohols (Gasser et al., 2010). Thewes et al. (2017) found about 1,000 mg L^{-1} of ethanol concentration after DCA-RQ storage. So, it is possible that esters production will be increased by substrates (ethanol and acetyl-CoA) available in fruits under DCA-RQ. Moreover, maybe the reduction on volatile compounds caused by 1-MCP can be minimized with DCA-RQ storage.

Therefore, the aims of this work was to evaluate the effect of DCA-RQ as compared with DCA-CF, CA, ULO with or without 1-MCP applications on the quality and volatile profile of ‘Galaxy’ apple after 9 months of storage plus shelf life.

3.1.2 Materials and methods

3.1.2.1 Plant material, orchard location, harvest maturity and sample preparation

The apple cultivar Galaxy, a ‘Gala’ strain, were harvested in a commercial orchard located at Vacaria, RS, Southern Brazil. ‘Galaxy’ apple fruit were grafted on M9 rootstocks and a density of 3,575 plants ha^{-1} was used in the orchard. During the 2013 growing season, the following fertilization was applied: 80 kg N ha^{-1} and 120 kg K ha^{-1}.

Immediately after harvest, fruit were transported to the Postharvest Research Center of the Federal University of Santa Maria, RS, Brazil. At the laboratory, fruit were selected, aiming to eliminate the those with physical damage, defects or irregular size. Thereafter, samples of 25 fruit each were performed with 4 samples per treatment.

At harvest, initial analysis was determined in 3 replicates of 20 fruit each. At this analysis, fruit showed iodine-starch index of 6.4, soluble solids of 13.4%, titratable acidity of 0.34 mg malic acid $100g^{-1}$, respiration rate of 14.4 µg CO_2 kg^{-1} h^{-1}, ethylene production of 1.72 ng C_2H_4 kg^{-1} h^{-1} and flesh firmness of 77 N.

3.1.2.2 Temperature, relative humidity, CA, ULO, DCA-CF and DCA-RQ conditions

After the sample preparation, fruit were stored in a 0.23 m^3 experimental CA rooms and after that, the following conditions were established: [1] controlled atmosphere (CA) (1.2 kPa O_2 + 2.0 kPa CO_2); [2] Ultra-low oxygen (0.4 kPa O_2 + 1.2 kPa CO_2); [3] Dynamic controlled atmosphere monitored by chlorophyll florescence (DCA-CF) with 1.2 kPa CO_2; [4] Dynamic controlled atmosphere monitored by respiratory quotient 1.3 (DCA-RQ1.3) + 1.2 kPa CO_2 and [5] DCA-RQ1.5 + 1.2 kPa CO_2. All treatments were performed with or without 0.625 µL L^{-1} of 1-MCP application, accounting 10 treatments.

The storage temperature was seat at 1.5 ± 0.1 °C and monitored daily during the 9 months of storage period with mercury thermometers inserted inside the fruit flesh to control the pulp temperature. Inside the storage room, where the CA rooms were, the relative humidity was manually monitored with psycrometers and adjusted with calcium chloride, which absorbed the excess of humidity maintaining an average in the range of 94 ± 2%. Was used 0.15 kg of calcium chloride per treatment.

3.1.2.3 1-MCP treatment

In order to perform the 1-MCP application fruit were put into a 230-L chamber, which was placed inside a cold room (1.5 ± 0.1 C). Afterward the 1-MCP was applied, a final concentration of 0.625 µL L^{-1} was obtained in the chamber (SmartFresh, 0.14% of active ingredient) .The chamber was hermetically closed for 24 h. During this time, the air contained inside the chamber

was circulated 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 the above reported condition.

3.1.2.4 CA, ULO and DCA setup and maintenance

The CA storage rooms were sealed and the storage conditions within 24 h established. At the first storage day, the temperature was reduced to 5 °C and thereafter, lowered to 1.5 °C in 5 d. When the storage temperature reached 1.5 °C, the CA, DCA-CF and DCA-RQ were established, to obtain the desired atmospheric condition. For this procedure, the desied low oxygen partial pressure was got flushing nitrogen to reach 1.2 kPa for the conventional CA, to 0.4 kPa O_2 in the ULO condition, to 0.5 kPa O2 for DCA-CF and DCA-RQ conditions. For this oxygen pull down were need 5 d. The carbon dioxide partial pressure was established by fruit respiration. Thus, during the first 5 d storage, only the temperature was reduced immediately to 1.5 °C, and from the 5th d up to the 10th d the CA, ULO, DCA-CF and DCA-RQ conditions were installed. This procedure was adopted to simulate the commercial storage condition.

Throughout the storage period, the oxygen partial pressure was changing according to the fruit metabolism in DCA and maintained static during CA and ULO storage. To control pO_2 at DCA-RQ conditions, the respiratory quotient (RQ) was measured two times within a week, according to the methodology proposed by Weber et al. (2015). Thus, the RQ was seat at 1.3 and 1.5, and the oxygen partial pressure changed accordingly to maintain these RQ values (Figure 1A and 1B). The RQ was calculated considering an interval of 13 h between the CA room sealing and the second gas measurement. Therefore, the RQ values were calculated by the ratio between the CO_2 released and the O_2 uptake. In relation to the CA and ULO conditions, they were maintained according to the methodology proposed by Thewes et al. (2015a).

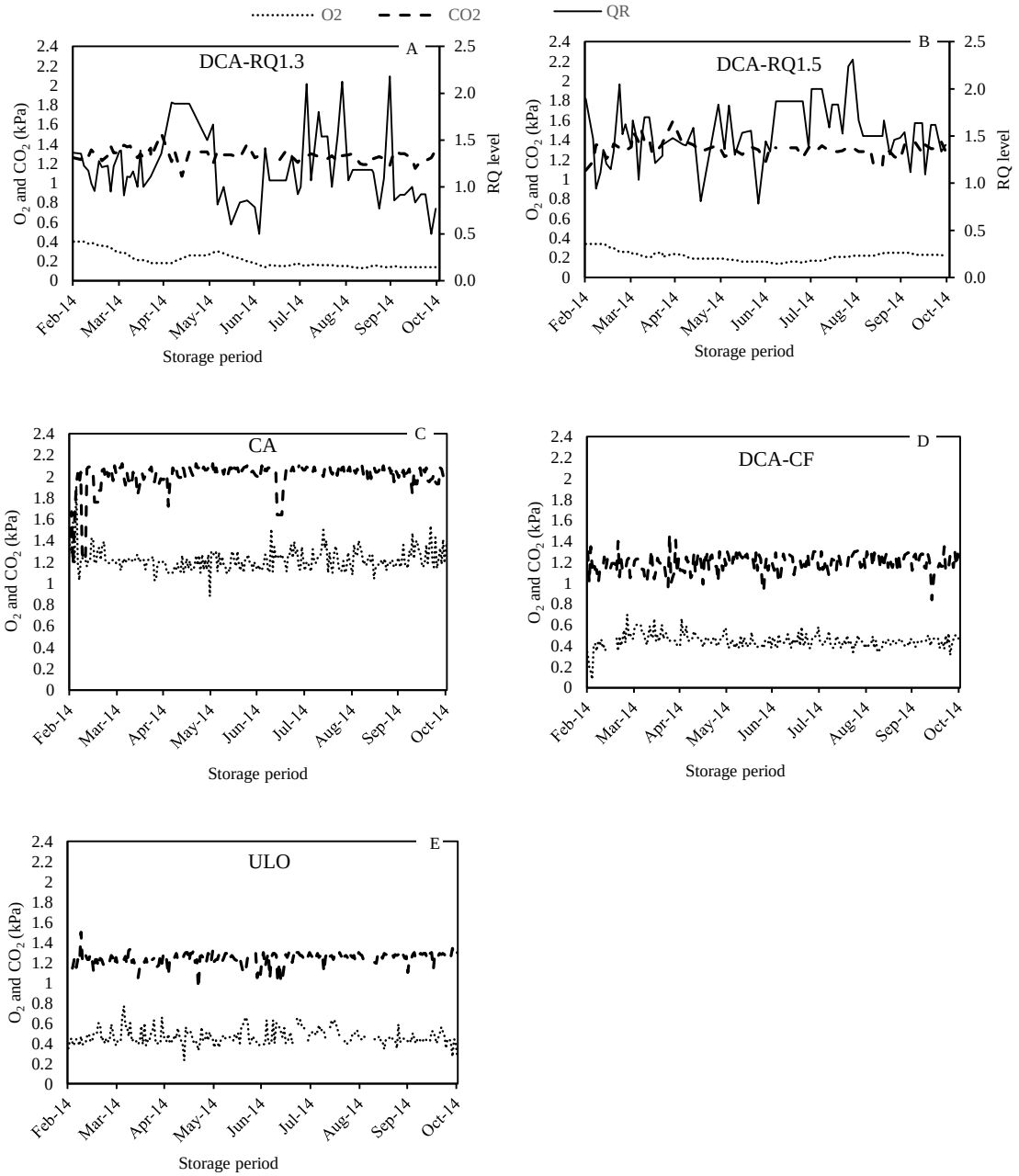


Fig. 1. Changes in oxygen and carbon dioxide partial pressures, and respiratory quotient (RQ) during DCA-RQ1.3 (A) and DCA-RQ1.5 (B), CA (C), DCA-CF (D) and ULO (E) during 9 months of storage of 'Galaxy' apple fruit. Santa Maria, Brazil, 2016. DCA-RQ1.3: Maximum O_2: 0.40 kPa; Minimum O_2: 0.14 kPa; Average O_2: 0.23 kPa; DCA-RQ1.5: Maximum O_2: 0.34 kPa; Minimum O_2: 0.14 kPa; Average O_2: 0.21 kPa.

The DCA-CF was monitored according to Prange et al. (2007). The chlorophyll fluorescence was monitored in six pples during exposure to low O_2. Thus, apples cooled to 1.5 °C were placed in a perforated plastic container (18 cm width, 27 cm length, 25 cm height) with the

fluorescence sensors installed on the top of the container. The container was placed inside an experimental CA room; the CA room was sealed, and covered with a black plastic to protect from the light. The fluorescence monitoring system was activated and then the pO_2 was reduced to 0.5 kPa by N_2 flushing. Afterwards, the respiration process reduced the pO_2 until a change in fluorescence was detected. The lowest O_2 set point was determined by identifying the O_2 partial pressure where an inflection in the fluorescence signal was detected, and then by increasing O_2 by 0.2 kPa as a safety factor (Figure 1 D). Chlorophyll fluorescence was monitored every hour for the entire storage period during the experiment.

3.1.2.4 *Metabolism and volatile compounds analyses*

These analyses were carried out at harvest and after 9 months of storage followed by 7 d shelf life at 20 (± 2 °C) and a relative humidity of 80 (± 2%).

3.1.2.4.1 Internal ethylene concentration (IEC): Determined according to Mannapperuma et al. (1991). The internal air of the carpellar cavity of the fruit was withdraw and two samples (1 mL) were 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, the injector and the detector were 90, 140 and 200 °C respectively. The results were expressed in ng C_2H_4 L^{-1}. Were evaluated 4 repetition per treatment. .

3.1.2.4.2 Ethylene production and respiration rate: To determine the ethylene production and respiration rate, fruit were stored inside a 5-L sealed glass jar during 1 h. After that, 2 samples of 1 mL were removed from the headspace and injected in the same gas chromatograph used to determine the IEC. Considering the glass volume, the fruit weight, the ethylene concentration inside the glass jars and the time of incubation, 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 glass jars was circulated through an electronic gas analyzer (Schele®, model KB7, Germany),

which determined the CO_2 concentration inside the glass. The respiration rate was expressed as µg CO_2 kg^{-1} s^{-1}. Were evaluated 3 repetition per treatment.

3.1.2.4.3 ACC oxidase: This enzyme was evaluated according to the methodology developed by Bufler (1986). The results were expressed in pg C_2 H_4 kg^{-1} s^{-1}. Were evaluated 3 repetition per treatment.

3.1.2.4.4 Total soluble solids: Slices of the equatorial region of the 25 fruit of each repetition were taken off and a juice was made with a juicer (Philips Walita®). From this juice was measured the soluble solids with a manual refractometer. The results were expressed in %.

3.1.2.4.5 Acidity: From the same sample of juice to determine soluble solids, it was taken an aliquot of 10 mL and diluted in 100 mL of distillated water. This solution was titrated with a 0.1 N of NaOH solution up to pH 8.1. The results were expressed in mg malic acid $100g^{-1}$.

3.1.2.4.6 Flesh firmness: Determined in two opposite sides of the equatorial region of the fruit flesh, where previously the skin was removed, with the aid of 11 mm tip penetrometer. The results expressed in Newton (N).

3.1.2.4.7 Decay: Evaluated by counting the fruit showing typical fungal lesions larger than 5 mm in diameter. The results were expressed in percentage.

3.1.2.4.8 Mealiness: Evaluated by slicing the fruits in the equatorial region and visualization of any symptom of mealy pulp. The results were expressed as a percentage of the total fruit.

3.1.2.4.9 Flesh breakdown: Evaluated by slicing the fruit on the equatorial region and visualization of any symptom of flesh browning. The results were expressed as a percentage of the total fruit.

3.1.2.4.10 Healthy fruit: This was quantified taken into account the total number of fruit per replicate (25 fruit) minus fruit with any symptom of decay, pulp cracking, mealiness and flesh breakdown incidence. The results were expressed as a percentage of healthy fruit.

3.1.2.4.11 Volatile compounds analysis:

3.1.2.4.11.1 Sample preparation: In order to prepare the samples for volatile compounds analysis, 10 – 15 fruit was cooled down to 0 °C. Immediately after the pulp cooling, horizontal slices (2-3 cm) of the equatorial region of the fruit were taken, discharged the seeds, and centrifuged under low temperature, to avoid the chemical and enzymatic oxidation of the samples (the maximum juice temperature during sample preparation was 5 °C). The juice was placed inside of 100 mL amber flash and immediately frozen to -30 °C up to the volatile compounds analysis.

3.1.2.4.11.2 Volatile compound quantification: the juice 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 until the juice was liquid (temperature 2 °C). An aliquot of 10 mL of this juice was taken, mixed with 3 g NaCl and 10 µL of 3-octanol standard solution (10 µg mL^{-1}) inside a 20 mL vial sealed hermetically with a PTFE-coated silicone lid. From this solution, the volatile compounds were extracted via head space solid phase microextraction (HS-SPME). A Divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (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 and 35 °C.

The volatile compounds were measured with a Dani® gas chromatograph (Dani Instruments Spa., Viale Brianza, Cologno Monzese, Italy) 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 to separate the volatile compounds. Nitrogen was used as carrier gas with a constant flow of 1 mL min^{-1}. The temperature of the ramp used during the analysis was: the initial temperature was 35 °C, and kept during 3 min, then a ramp with a temperature of 2 °C min^{-1} up to 80 °C, thereafter, another ramp of 5 °C min^{-1} till 230 °C and kept at 230 °C during 5 min. The temperature of the FID detector was 230 °C. To calculate the linear retention index it was analyzed

a serie of n-alkanes in the same chromatographic conditions used to analyze the volatile compounds. The analytes concentration was determined according to the methodology proposed by Both et al. (2014a).

3.1.2.4.11.3 Volatile compounds identification: The volatile compounds were identified with 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 at a flow rate of 1.4 mL min^{-1}. The detector was operated in the electron impact ionization, with an 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 the 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. In the table 1 it is showed a linear retention index and analysis at the harvest for volatile compounds.

Table 1 – Linear retention index and analysis at harvest of volatile compounds of 'Galaxy' apple.

Volatile Compounds	LRI_{exp}	OTH[a]	At Harvest (µg L^{-1})
		Esters	
Methyl acetate	839	8300[a]	0.09
Ethyl acetate	897	13,500[a]	1.00
Ethyl propanoate	962	40[f]	0.01
Ethyl 2-Methylpropanoate	968	NF	0.02
Propyl acetate	983	2000[a]	1.73
Methyl 2-Methylbutanoate	1015	8[f]	0.36
2-Methylpropyl acetate	1018	66[c]	3.08
Ethyl butanoate	1042	1[a]	0.02
Ethyl 2-methylbutanoate	1057	0.06[a]	388.2
Butyl acetate	1083	66[a]	19.7
2 Methylbutyl acetate	1128	11[a]	159.4
Butyl propanoate	1137	25[a]	0.08
3-Methylbutyl acetate	1168	2[d]	39.2
4-Pentenyl acetate	1192	NF	0.06
Butyl butanoate	1202	100[a]	2.40
Z-2-penten-1-yl-acetate	1241	NF	1.16
Hexyl acetate	1262	2[a]	420.9
Z-2-Hexenyl acetate	1286	NF	0.44
Z-3-Hexenyl acetate	1290	8[c]	3.42

E-3-Hexen-1-yl acetate	1294	NF	0.17
5-Hexen-1-yl, acetate	1316	NF	5.23
E-2-hexenyl acetate	1321	7[c]	9.43
Heptyl acetate	1364	NF	0.09
Butyl hexanoate	1394	700[c]	0.07
Benzyl acetate	1726	364[c]	0.24
		Aldehydes	
Acetaldehyde	644	120[d]	0.08
Butanal	890	37[d]	0.22
Pentanal	920	NF	0.02
Hexanal	1099	5[a]	0.08
Z-3-Hexenal	1148	NF	3.08
Z-2-Hexenal	1205	NF	*nd*
E-2-Hexenal	1222	17[b]	75.1
		Alcohols	
Ethanol	945	100,000[d]	0.32
1-Butanol	1162	500[a]	33.7
4-Methyl-2-pentanol	1176	NF	0.31
3-Hexanol	1201	NF	0.06
2-Methyl-1-butanol	1211	250[a]	7.16
1-Pentanol	1249	4,000[a]	2.03
2-Methyl-2-buten-1-ol	1297	3[c]	*nd*
1-Hexanol	1352	500[a]	93.1
E-3-Hexen-1-ol	1361	NF	0.46
Z-3-Hexen-1-ol	1380	70[b]	0.17
E-2-Hexen-1-ol	1399	400[d]	9.26
E-5-Hexen-1-ol	1407	NF	1.57
E-1-Octen-3-ol	1445	NF	0.21
1-Heptanol	1454	3[d]	1.42
6-Methyl-5-hepten-2-ol	1465	2,000[d]	0.21
2-Ethyl 1-hexanol	1485	270,000[d]	0.47
		Ketones	
2-Propanone	831	500,000[d]	0.20
6-methyl-5-heptene-2-one	1334	50[d]	0.01

nd: not detectated. LRI_{exp}: Experimental Linear Retention Index; NF: not found
Odor threshold. References: [a] López et al. (2007); [b] Mehinagic et al. (2006); [c] Pino and Quijano (2012); [d] Leffingwell and Leffingwell (1991); [e] Takeoka et al. (1990); [f] Komthong et al. (2006); [g] Mass spectrum and retention time comparable to standard (Positively identified) * Concentration were calculated relative to an internal standard (3-octanol).

3.1.2.3 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 autoscaled 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. The data that showed significant difference

by ANOVA was subjected to the Tukey's test at 5% error probability. The experiment was conducted in a completely randomized scheme with a factorial arrangement (5-storage conditions × with or without 1-MCP application).

3.1.3 Results

3.1.3.1 Volatile compounds profile

The volatile compounds have high importance in the odor of the apple. Odor is a quality attribute that is reduced during controlled atmosphere storage. A total of 51 volatile compounds was identified in 'Galaxy' apple after storage: 25 esters, 17 alcohols, 7 aldehydes and 2 ketones (Figures 4, 5 and 6). Fruit without 1-MCP application stored in DCA-RQ1.5 showed high total esters concentration after storage (Figure 4Z). The ULO, DCA-CF and DCA-RQ1.3 storage condition showed the lowest esters. The main esters were high in fruit stored under DCA-RQ1.5, as methyl acetate (Figure 4A), ethyl acetate (Figure 4B), ethyl propanoate (Figure 4C) and ethyl 2-methylpropanoate (Figure 4D), as compared to other storage conditions.

In order to better visualize the effects of the storage conditions on the volatile compounds and quality parameters, we performed PCA as an exploratory multivariate analysis. The principal components I and II (PC I and PC II) explained the 33.2% and 25.5% of the overall variance, respectively (Figure 2A). These analyses allowed a separation according to storage atmosphere condition and 1-MCP application, where, along PC I, the treatments with and without 1-MCP application were located in the opposite side, except DCA-RQ1.3 which is located together with 1-MCP treatment. The main volatile compounds, like butyl acetate, hexyl acetate, 2-methylbutyl acetate, 2-methylpropyl acetate were located at the right-side of PC I, which are treatments without 1-MCP (Figure 2B). The flesh firmness is more correlated with ULO + 1-MCP and DCA-CF + 1-MCP. According to PC I and PC III (it explains 15.50% of the overall variance), we can separate treatments with and without 1-MCP again (Figure 3A). Along to PC III there is a clear separation

of CA and DCA-RQ1.5. Although the CA is related to the main volatile compounds, it is related with ethylene, internal ethylene concentration, ACC oxidase activity, flesh breakdown and mealiness. On the other hand, fruit stored under DCA-RQ1.5 are related with the main apple esters, like ethyl esters, hexyl acetate, 3-methylbutyl acetate and 2-methylpropyl acetate (Figure 3B).

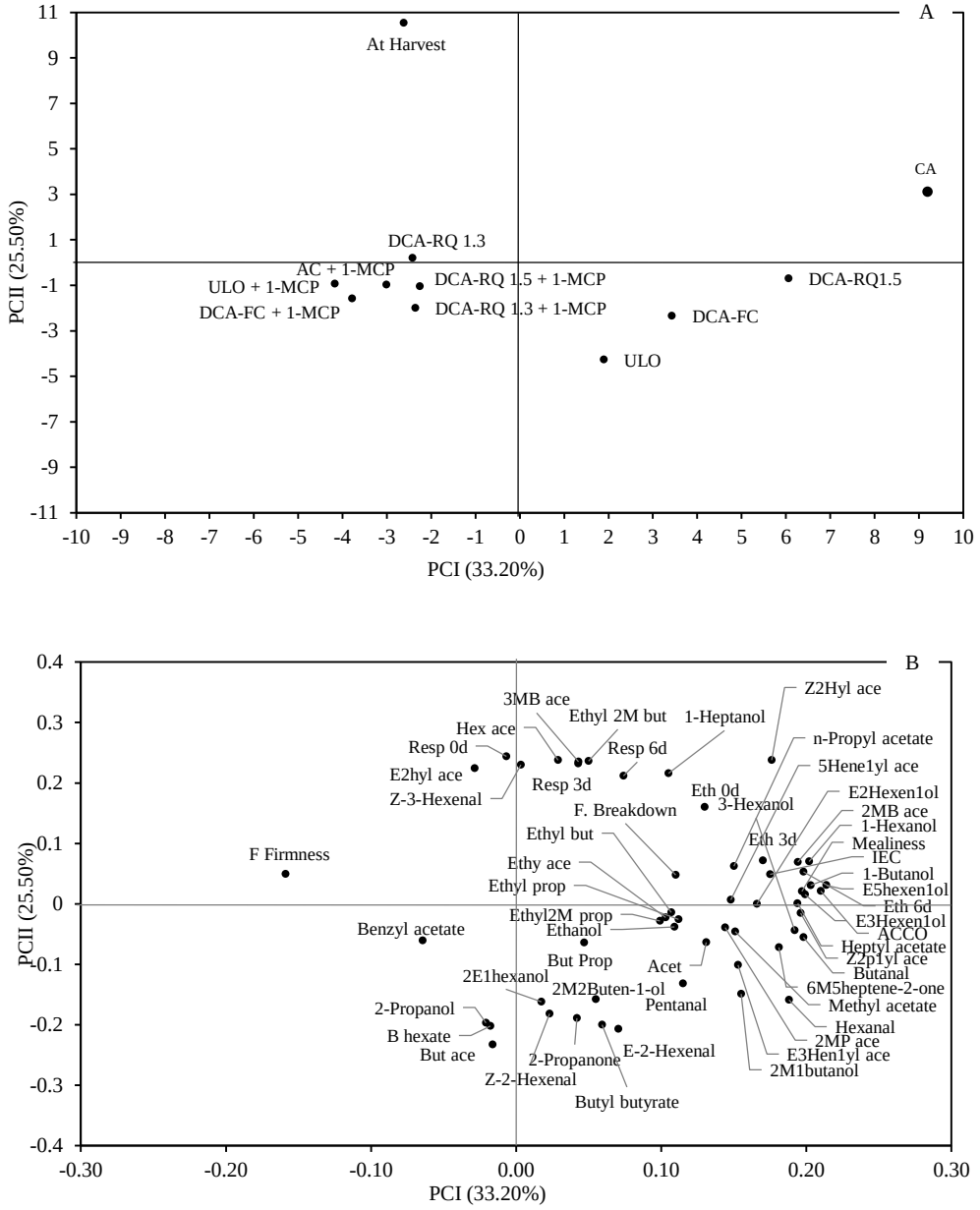


Fig. 2. (A) Scores (treatments) and (B) loadings (variables) plots showing the PCI and PCII components of 'Galaxy' apples stored under controlled atmosphere, dynamic controlled atmosphere (DCA) and ultralow oxygen levels with or without 1-MCP, during 9 months storage plus 7 days of shelf life. **F. Breakdown**: Flesh breakdown; **IEC**: internal ethylene concentration; **Eth 0d**: Ethylene production at 0 days at 20 °C; **Resp 0d**: respiration at 0 days at 20 °C; **F Firmness**: Flesh Firmness; **ACCO**: ACC oxidase activity; **Acet**: Acetaldehyde; **Ethy ace**: Ethyl acetate; **Ethyl prop**: Ethyl propanoate; **Ethyl2M prop**: Ethyl 2-Methylpropanoate; **2MP ace**: 2-Methylpropyl acetate; **Ethyl but**: Ethyl butanoate; **Ethyl 2M but**: Ethyl 2-methyl butanoate; **But ace**: Butyl acetate; **2MB ace**: 2 Methyl butyl acetate; **But Prop**: Butyl Propanoate; **3MB ace**: 3-methylbutyl acetate; **2M1butanol:** 2-Methyl-1-butanol; **Z2p1yl**

ace: Z-2-penten-1-yl-acetate; **Hex ace**: Hexyl acetate; **Z2Hyl ace**: Z-2-Hexenyl acetate; **E3Hen1yl ace**: E - 3-Hexen-1-yl-acetate; **5Hene1yl ace**: 5-Hexene-1-yl acetate; **2M2Buten-1-ol**: 2-Methyl 2-buten-1-ol; **E2hyl ace**: E-2-hexenyl acetate; **6M5heptene-2-one:** 6-methyl-5-heptene-2-one; **E3Hexen1ol:** E-3-Hexen-1-ol; **B hexate**: Butyl hexanoate; **E2Hexen1ol:** E-2-Hexen-1-ol; **E5hexen1ol:** E-5-hexen-1-ol; **2E1hexanol**: 2-Ethyl 1-hexanol.

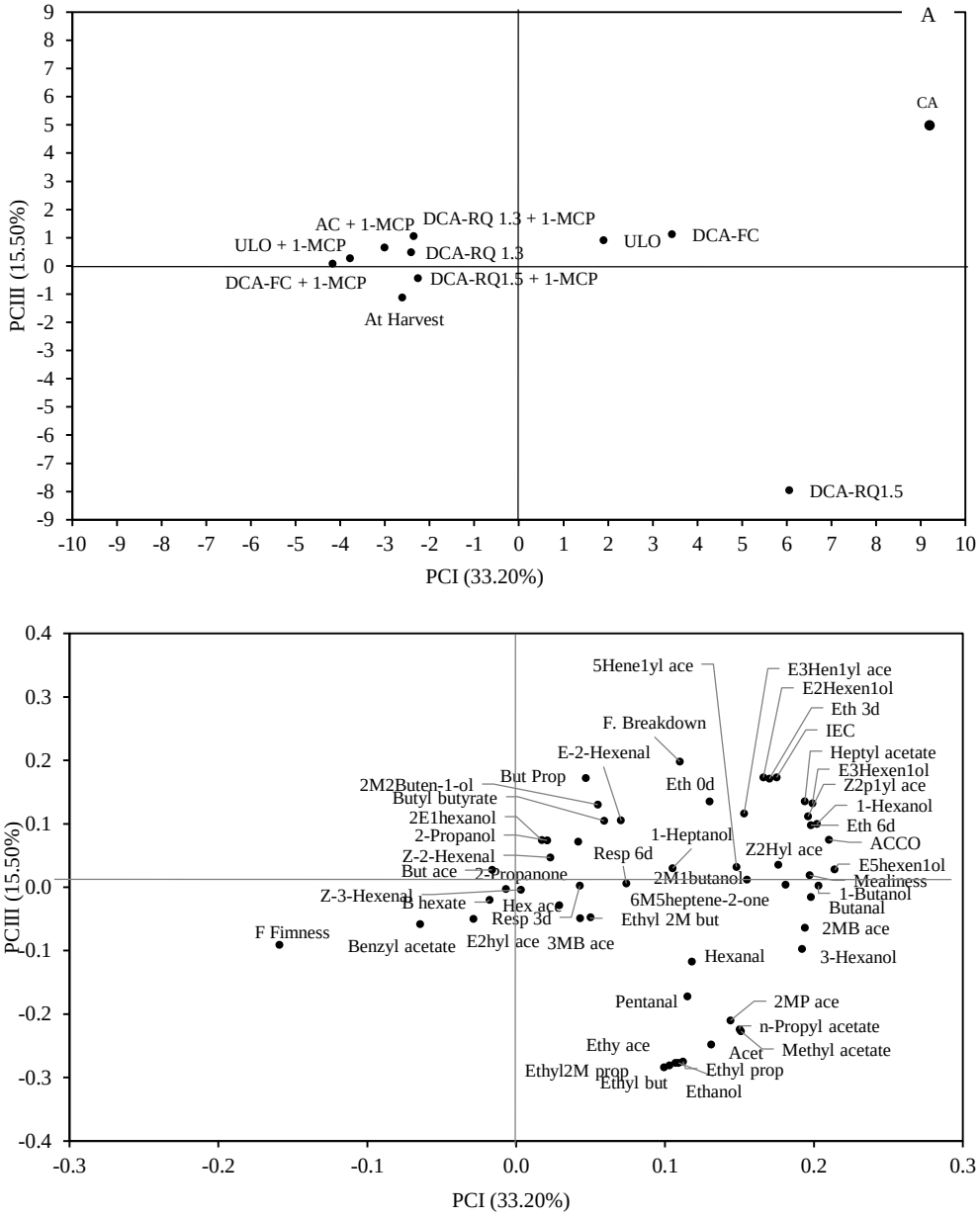
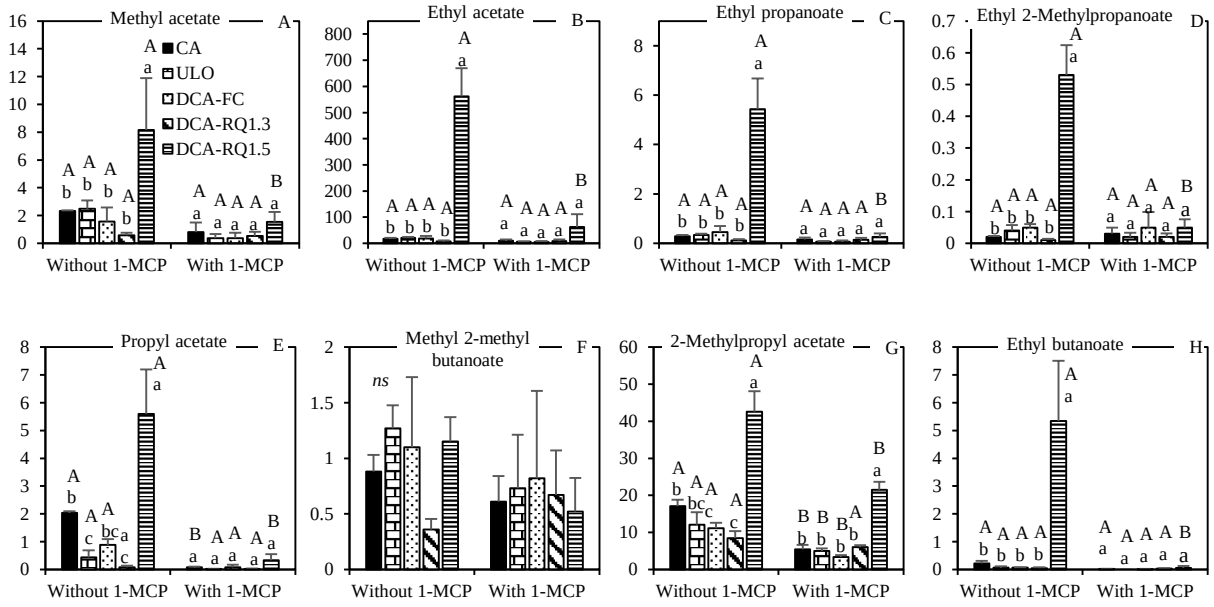


Fig. 3. (A) Scores (treatments) and (B) loadings (variables) plots showing the PCI and PCIII components of 'Galaxy' apples stored under controlled atmosphere, dynamic controlled atmosphere (DCA) and ultralow oxygen levels with or without 1-MCP, during 9 months storage plus 7 days of shelf life. **F. Breakdown**: Flesh breakdown; **IEC**: internal ethylene concentration; **Eth 0d**: Ethylene production at 0 days at 20 °C; **Resp 0d**: respiration at 0 days at 20 °C; **F Firmness**: Flesh Firmness; **ACCO**: ACC oxidase activity; **Acet**: Acetaldehyde; **Ethy ace**: Ethyl acetate; **Ethyl prop**: Ethyl propanoate; **Ethyl2M prop**: Ethyl 2-Methylpropanoate; **2MP ace**: 2-Methylpropyl acetate; **Ethyl but**: Ethyl butanoate; **Ethyl 2M but**: Ethyl 2-methyl butanoate; **But ace**: Butyl acetate; **2MB ace**: 2 Methyl butyl acetate; **But Prop**: Butyl Propanoate; **3MB ace**: 3-methylbutyl acetate; **2M1butanol:** 2-Methyl-1-butanol; **Z2p1yl ace**: Z-2-penten-1-yl-acetate; **Hex ace**: Hexyl acetate; **Z2Hyl ace**: Z-2-Hexenyl acetate; **E3Hen1yl ace**: E - 3-Hexen-1-yl-acetate; **5Hene1yl ace**: 5-Hexene-1-yl acetate; **2M2Buten-1-ol**: 2-Methyl 2-buten-1-ol; **E2hyl ace**: E-2-hexenyl

acetate; **6M5heptene-2-one:** 6-methyl-5-heptene-2-one; **E3Hexen1ol:** E-3-Hexen-1-ol; **B hexate**: Butyl hexanoate; **E2Hexen1ol:** E-2-Hexen-1-ol; **E5hexen1ol:** E-5-hexen-1-ol; **2E1hexanol**: 2-Ethyl 1-hexanol.

The main esters of 'Galaxy' apple were butyl acetate, 2-methylbutyl acetate, hexyl acetate, 2-methylpropyl acetate, ethyl 2-methyl butanoate. The apples stored under ULO without 1-MCP treatment, showed high butyl acetate contents (Figure 4J). 1-MCP did not reduce the butyl acetate concentration during CA, DCA-RQ1.5, DCA-CF and ULO storage. DCA stored apples did not differ from the ones stored in CA. On the other hand, DCA-RQ1.5 and CA showed high 2-methylbutyl acetate (Figure 4K) and 3-methylbutyl acetate (Figure 4M) concentration without 1-MCP. This compound was lower with 1-MCP in all the storage conditions, except under DCA-RQ1.3. Another important ester to 'Gala' apple mutants is hexyl acetate (Yang et al., 1996), which was high in fruits under CA, followed by DCA-RQ1.5, in fruits without 1-MCP (Figure 4Q).



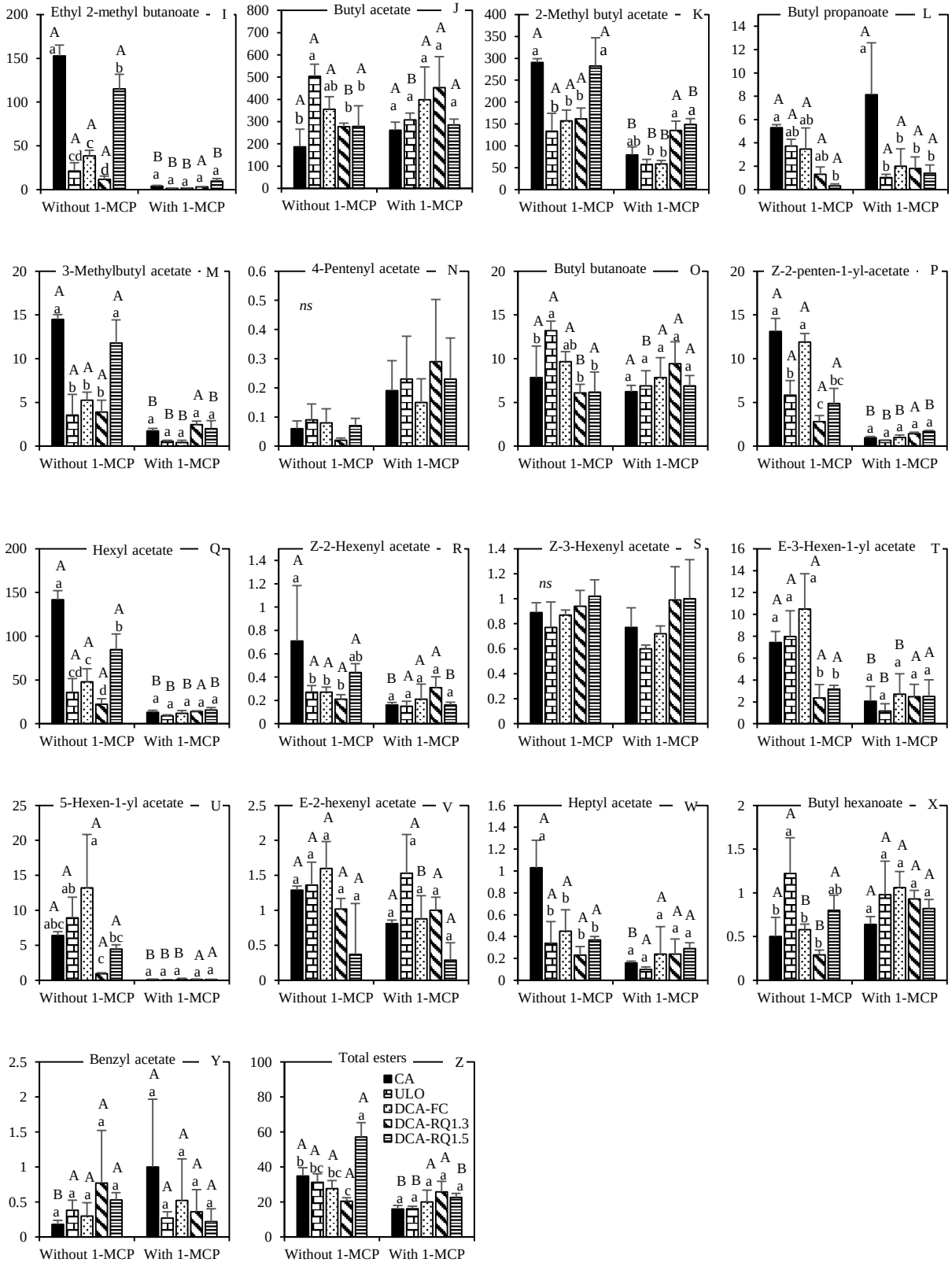


Figure 4 – Esters (µg L^{-1}) of 'Galaxy' apple under controlled atmosphere, dynamic controlled atmosphere and 1-MCP application, after 9 months of storage at 1.5 °C plus 7 days at 20 °C. Bars with the same lower case letter in the same 1-MCP application (with or without), and each bar with the same upper

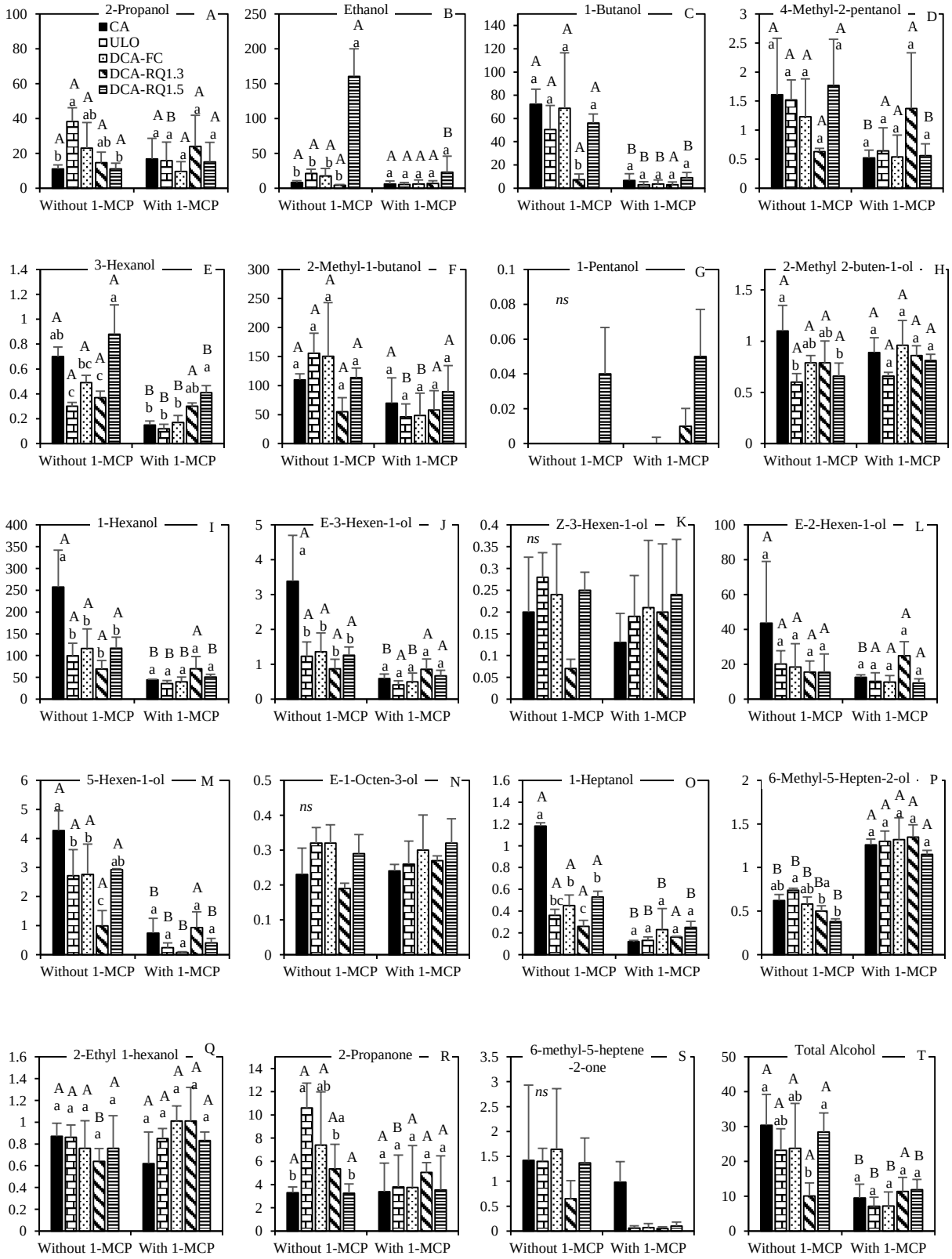
case letter in different 1-MCP application are not significantly different by Tukey's test, at 5% probability; ns: no significant; Error bars mean standard deviation.

DCA-RQ1.5 provided high 2-methylpropyl acetate concentration, both with 1-MCP or without this regulator (Figure 4G). Fatty acids like linolenic and linoleic acid, produce C6 volatile compounds. Esters from fatty acids like E-3-hexen-1-yl-acetate, 5-Hexen-1-yl-acetate, Z-2-penten-1-yl-acetate showed low concentration in 'Galaxy' apple under DCA-RQ1.3 and DCA-RQ1.5 as compared to CA without 1-MCP (Figure 4T, 4U and 4P). The Z-3-hexenal, a product of linolenic acid degradation, was higher in apples under CA and ULO without 1-MCP (Figure 6E). Fruits with 1-MCP showed a reduction of Z-3-hexenal in CA, ULO and DCA-CF. E-2-hexenal was higher in ULO and DCA-CF without 1-MCP. On the other hand, E-2-hexenal did not differ among atmosphere conditions on apples with 1-MCP application.

Ethyl 2-methyl butanoate was high in fruits stored in CA without 1-MCP (Figure 4I). DCA-RQ1.5 showed lesser ethyl 2-methyl butanoate as compared to CA, but much higher to ULO, DCA-CF and DCA-RQ1.3. DCA-RQ1.5 provided higher amounts of propyl acetate (Figure 4E), ethyl butanoate (Figure 4H), ethyl 2-methylpropanoate (Figure 4D) and ethyl propanoate (Figure 4C) over other storage conditions without 1-MCP, which have fruit odor. Fruit with 1-MCP application did not differ among storage conditions for these esters. DCA-RQ1.5 did not increase ethyl esters in apple fruits with 1-MCP.

Alcohols were linked with acyl moieties to produce esters compounds (Beekwilder et al., 2004). The total of alcohol was reduced by 1-MCP application, except under DCA-RQ1.3 (Figure 5T). This result is related with the total esters, which were lesser with 1-MCP application. 1-hexanol is a precursor of hexyl acetate, which was reduced by 1-MCP application, except under DCA-RQ1.3 (Figure 5I). The 'Galaxy' apple under CA with 1-MCP showed high 1-hexanol concentration. Another important alcohol is 2-methyl-1-butanol, which is precursor of 2-methylbutyl acetate. 2-Methyl-1-butanol did not differ among storage atmosphere, but 1-MCP

reduced this alcohol in fruits under ULO and DCA-CF (Figure 5F). Volatile derivative from linoleic acid as E-2-hexen-1-ol was reduced by 1-MCP application in CA (Figure 5L).



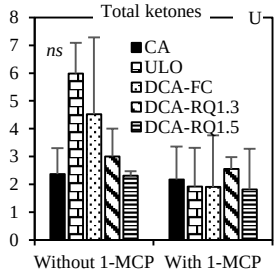


Figure 5 – Alcohols and ketones (µg L^{-1}) of 'Galaxy' apple under controlled atmosphere, dynamic controlled atmosphere and 1-MCP application, after 9 months of storage at 1.5 °C plus 7 days at 20 °C. Bars with the same lower case letter in the same 1-MCP application (with or without), and each bar with the same upper case letter in different 1-MCP application are not significantly different by Tukey's test, at 5% probability; ns: no significant; Error bars mean standard deviation.

The total of aldehydes was lower in fruit storage in DCA-RQ1.3 and DCA-RQ1.5 without 1-MCP (Figure 6H), although it does not differ from CA and DCA-CF. Interestingly, 1-MCP reduces the total of aldehydes only in ULO. E-2-hexenal, produced by LOX from linolenic acid was higher in fruit under ULO and DCA-CF without 1-MCP (Figure 6G). Acetaldehyde was higher in DCA-RQ1.5 without 1-MCP. ULO, DCA-CF and DCA-RQ1.5 with 1-MCP application showed lower acetaldehyde in relation to the fruit without 1-MCP (Figure 6A).

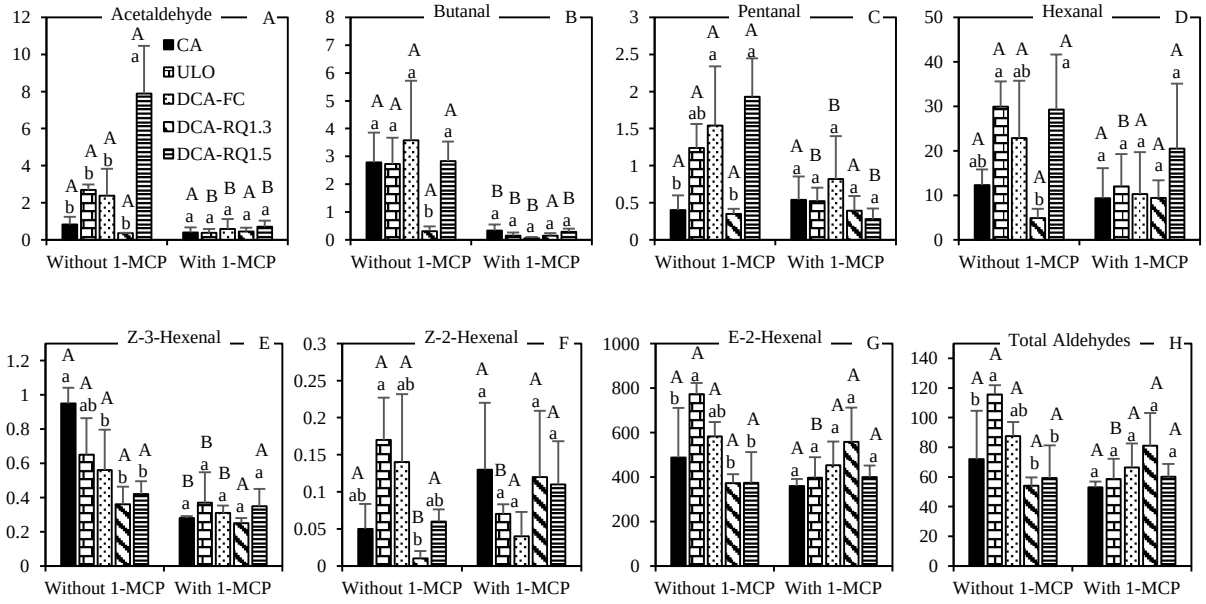


Figure 6 – Aldehydes (µg L^{-1}) of 'Galaxy' apple under controlled atmosphere, dynamic controlled atmosphere and 1-MCP application, after 9 months of storage at 1.5 °C plus 7 days at 20 °C. Bars with the

same lower case letter in the same 1-MCP application (with or without), and each bar with the same upper case letter in different 1-MCP application are not significantly different by Tukey's test, at 5% probability; ns: no significant; Error bars mean standard deviation.

3.1.3.2 ACC oxidase activity

ACC oxidase activity was reduced by 1-MCP application in all treatments (Figure 7A). CA without 1-MCP showed higher ACC oxidase activity, this resulted in high IEC and ethylene production (Figure 7B, C, D and E). Without 1-MCP, this enzyme showed lower activity under DCA-RQ1.3 and ULO. Another important result is that 1-MCP reduces the IEC and ethylene production only with higher pO_2 (CA and DCA-CF). Treatment with 1-MCP did not differ among storage atmosphere for IEC and ethylene production.

3.1.3.3 Respiration rates

CA showed high respiration during shelf life compared with other storage condition (Figure 7F, G and H). Fruit with 1-MCP stored under DCA-CF showed lower respiration at open chamber and after 3 days at 20 °C. At the end of shelf life, apples with 1-MCP stored in ULO and DCA-CF showed lower respiration.

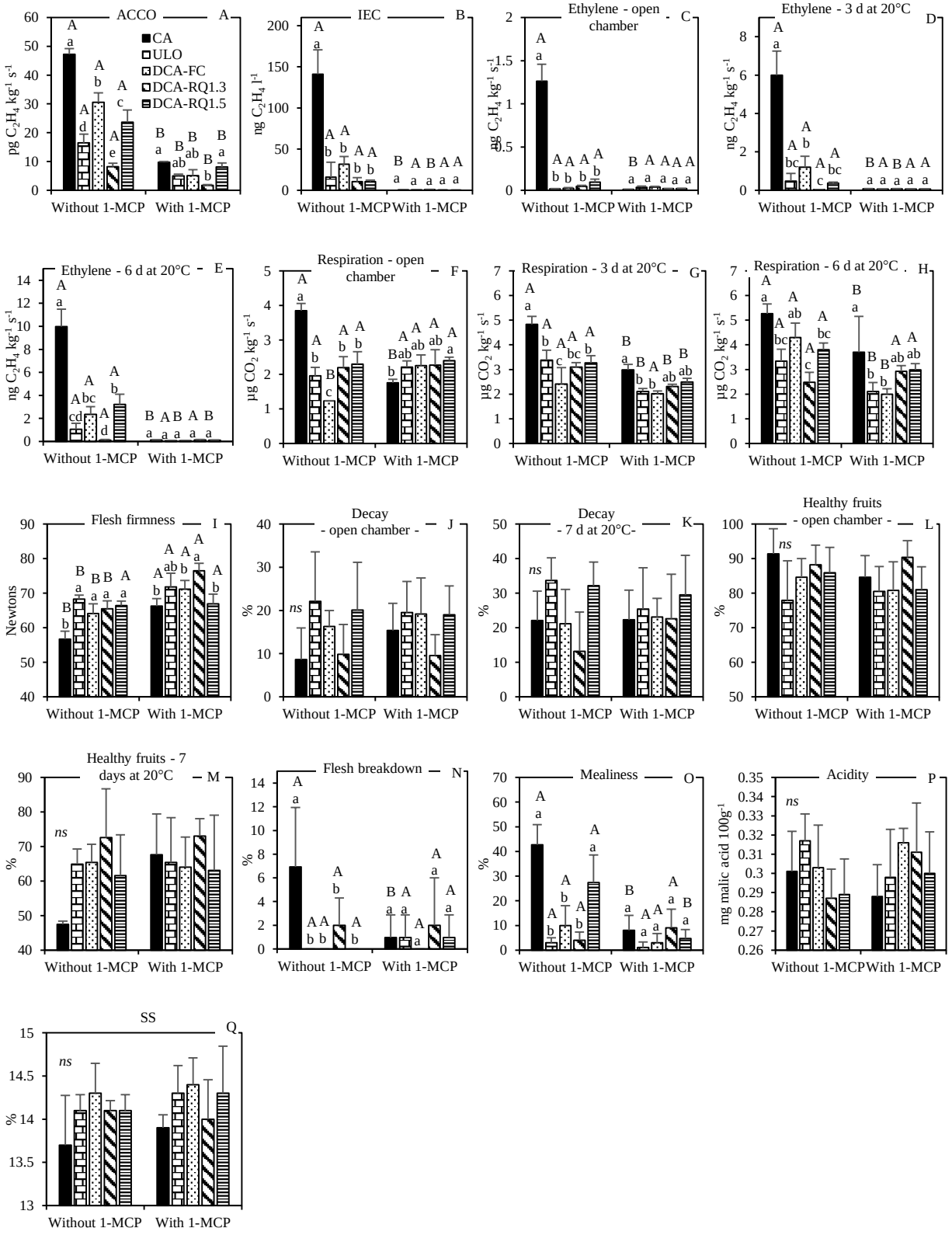


Figure 7 – ACC oxidase activity (ACCO), internal ethylene concentration (IEC), ethylene production, respiration, flesh firmness, decay, healthy fruits, flesh breakdown, mealiness, acidity and soluble solids (SS) of 'Galaxy' apple under controlled atmosphere, dynamic controlled atmosphere and 1-MCP application, after 9 months of storage at 1.5 °C plus 7 days at 20 °C. Bars with the same lower case letter in the same 1-MCP application (with or without), and each bar with the same upper case letter in different

1-MCP application are not significantly different by Tukey's test, at 5% probability; ns: no significant; Error bars mean standard deviation.

3.1.3.4 Flesh firmness

Flesh firmness is one of the most important characteristic to stores and consumers. Treatment with 1-MCP maintained higher flesh firmness in apple stored in CA, ULO, DCA-CF and DCA-RQ1.3, but did not show effect in DCA-RQ1.5 (Figure 7I). Fruit without 1-MCP stored under CA showed lower flesh firmness than other treatments. With 1-MCP application, DCA-RQ1.3 and DCA-CF showed higher flesh firmness.

3.1.3.5 Decay incidence and healthy fruit

In relation to the decay incidence and healthy fruits, no difference was observed among the storage condition (Figure 7J, K, L and M). Flesh breakdown was reduced by 1-MCP application only in CA (Figure 7N). DCA and ULO reduced flesh breakdown in relation to CA in fruits without 1-MCP, whereas 1-MCP did not differ among storage condition. Mealiness is another important apple disorder, which was low in ULO, DCA-CF and DCA-RQ1.3 in apple without 1-MCP (Figure 7O). In relation to 1-MCP, this regulator reduced mealiness only in CA and DCA-RQ1.5. Acidity and soluble solids did not differ among treatments.

3.1.4 Discussion

3.1.4.1 Volatile compound profile

Volatile compounds reduction during storage under CA + 1-MCP application were reported by several authors (Fan; Mattheis, 1999; Mattheis et al., 2005; Ortiz et al., 2010; Thewes et al., 2015b). However, the effect of the controlled dynamic atmosphere (DCA) with respiratory quotient (DCA-RQ), with or without 1-MCP application on volatile compounds is still unclear.

Thus, we did this work to give information to apple storers about innovative storage technologies to maintain the 'Galaxy' apple volatile profile and fruit general quality.

Esters were the main volatile compounds of the apple. The total esters were higher in fruits under DCA-RQ1.5 without 1-MCP, which is showed by the PCA analysis, where DCA-RQ1.5 showed correlation with several volatile compounds (Figure 3A, 3B, 4A and 4B). Esters are produced by esterification of alcohol and acyl-CoAs, catalyzed by alcohol acyltransferase (AAT). Ethylene has effect on gene expression of the AAT. Harb et al (2011) found 1-MCP application reduced gene expression of this enzyme, which reduced esters in apple stored under CA (Thewes et al., 2015b). The DCA-RQ1.5 without 1-MCP showed lower ethylene production, about 3 ng kg^{-1} s^{-1} at 6 days at 20 °C (Figure 7E). It is possible that this low ethylene concentration is enough to AAT production and activation, once Ban et al. (2010) found incomplete inhibition of *pMdAAT* by 1-MCP, and that AAT gene expression may occur in low ethylene levels. Moreover, low O_2 partial pressure on DCA-RQ1.5 (Figure 1D) increases fermentation and may increase acetyl-CoA availability, once tricarboxylic acid cycle and oxidative phosphorylation is inhibited by low O_2 availability. Probably, the high acetyl-CoA concentration associated with high anaerobic respiration products, like alcohols, contributed to more esters production.

By the principal components analysis, one important conclusion is that 1-MCP application in 'Galaxy' apple stored in either DCA-RQ, DCA-CF or ULO reduced volatile compounds. Even in DCA-RQ1.5, condition that increase ester production, 1-MCP application reduced volatile compounds. There are several reports that 1-MCP reduced volatile in apples stored under cold storage and CA (Fan; Mattheis, 1999; Mattheis et al., 2005; Ortiz et al., 2010; Thewes et al., 2015b), but it is the first report in the literature evaluating DCA-RQ associated with 1-MCP. 1-MCP block ethylene receptor and therefore physiological response (Sisler; Serek, 2003). Ethylene is required to activate several key enzymes on volatile biosynthesis, like LOX, pyruvate decarboxylase (PDC), ADH and AAT activity (Ortiz et al., 2010; Schiller et al., 2015).

The main volatile compounds to ‘Gala’ mutants are butanol, hexyl acetate, butyl acetate, 2-methylpropyl acetate and 2-methylbutyl acetate (Young et al, 1996; Salas et al., 2011; Both et al., 2014a). Butyl acetate was higher in apple stored under ULO without 1-MCP, which is synthetized from 1-butanol. The hexyl acetate is synthetized from hexanol. The enzyme lipoxygenase (13-LOX) oxygenates carbon 13 of linoleic acid to form precursors of C6 volatile compounds, such as hexanal and hexanol (Schiller et al., 2015). Both et al. (2014) showed lower emission of straight-chain esters under extremely low O_2 (0.5 kPa), while branched-chain esters were not significantly affected in this condition. Hexyl acetate was higher in fruits under CA, followed by DCA-RQ1.5 in fruits without 1-MCP. O_2 partial pressure on CA storage was 1.2 kPa, which results on high ethylene production (Figure 4Q), this may have maintained high AAT activity and hexyl acetate production. On the other hand, apples under DCA-RQ1.5, with lower O_2 partial pressure (0.21 kPa in average), even with lower ethylene production, probably showed high acetyl-CoA that was used by AAT to esters production. DCA-RQ1.5 showed low ethylene, but enough to AAT activity (Ban et al., 2010). It is important to highlight that DCA-RQ1.5 increases the total of esters without 1-MCP, however DCA-RQ1.5 with 1-MCP application did not increase the total of esters production. The reduction in ethylene production in fruit maintained in DCA-RQ1.5 + 1-MCP, mainly in 6 days at 20 °C (Figure 7E), probably inhibited the PDC, which decarboxylate pyruvate to produce aldehydes and inhibited the ADH which produces alcohols from aldehydes. Ortiz et al. (2010) found reduction on PDC, ADH and AAT activity with 1-MCP application. 1-Hexanol was higher in fruits stored under CA, 1-MCP application reduced this compounds in all storage condition, except DCA-RQ1.3 (Figure 5I).

2-Methylbutyl acetate is the main volatile compound of ‘Gala’ apple mutants (Yang et al., 1996). DCA-RQ1.5 and CA showed high 2-methylbutyl acetate and 3-methylbutyl acetate concentration without 1-MCP. These compounds were synthetized from amino acids (AA) isoleucine and leucine, respectively (Hadi et al., 2013; Yang et al., 2016). Amino acids are

degraded firstly by branched-chain amino acids aminotransferase (BCAT) (Gonda et al., 2010; Yang et al., 2016) to produce precursors of esters. Volatile compounds from amino acids are not reduced by low O_2 partial pressure (Brackmann et al., 1993). It is proved by the high 2-methylbutyl acetate and 3-methylbutyl acetate in DCA-RQ1.5 with extremely low pO_2, where it probably had high acetyl-CoA availability to esterification. CA showed higher concentration of 2-methylbutyl acetate and 3-methylbutyl acetate by higher ethylene production (Figure 7C, D and E). It is likely that pyruvate and acetyl-CoA was not high, but had higher AAT activity in relation to the other treatment, which resulted in higher 3-methylbutyl acetate. Fruits with 1-MCP application showed high 2-methylbutyl acetate under DCA-RQ1.3 and DCA-RQ1.5 in relation to DCA-CF and ULO, this strengthens the hypothesis that low O_2 partial pressure provides acetyl-CoA to ester synthesis, by higher glycolytic pathway activity. In this case, acetyl-CoA combined with alcohols from amino acids degradation form esters. The highest glycolytic activity occurs to produce energy at the substrate level (Pesis, 2005; Pesis et al., 2010) for the cell survive at low O_2. As the O_2 partial pressure in DCA-CF is not as low as DCA-RQ, it is likely that the fruit stored in this condition did not have high availability acetyl-CoA to esterify with alcohols.

2-Methylbutyl acetate is synthetized from esterification of 2-methyl-1-butanol with acetyl-CoA (Brackmann et al., 1993; Gonda et al., 2010; Yang et al., 2016). 2-Methyl-1-butanol did not differ among storage atmosphere without 1-MCP application. This result confirms that the high amount of 2-methylbutyl acetate in fruits under CA is due to the higher amount of ethylene, which probably increases the activity of AAT in relation to other treatments. Likely, the reason for the high amount of 2-methylbutyl acetate in fruits under DCA-RQ1.5 is the high availability of acetyl-CoA, and not just the availability of the alcohol precursor.

Fruits with and without 1-MCP stored under DCA-RQ1.5 showed high 2-methylpropyl acetate (Figure 4G), which is synthetized from the amino acid valine (Bekele et al., 2015; António et al., 2016). These authors found that hypoxia conditions increased some amino acids

concentration, like valine, alanine, leucine, proline, phenylalanine and serine. It is possible that fruits with low O_2 partial pressure, as used to obtain RQ 1.5, increased the accumulation of valine and 2-methylpropyl acetate. On the other hand, pO_2 slightly above of the one used in DCA-RQ 1.5 reduced the accumulation of 2-methylpropyl acetate in treatments such as CA, DCA-CF, ULO and DCA-RQ1.3.

Several ethyl esters were higher in fruits under DCA-RQ1.5 as ethyl acetate, ethyl propanoate, ethyl 2-methylpropanoate, ethyl butanoate and ethyl 2-methyl butanoate (Figure 4B, C, D, H and I). In strawberries ethyl esters are synthetized from alanine (Perez et al., 1992; Beekwilder et al., 2004). In the same way of valine, it is likely that the low pO_2 induced alanine accumulation (Bekele et al., 2015; 2016; António et al., 2016), which was the precursor of ethyl esters. Alanine is produced from pyruvate by alanine aminotransferase (António et al., 2016). The enzyme branched-chain amino acid transaminases (BCAT) degraded the amino acids into volatile compounds, which showed high activity without ethylene action (Kochevenko et al., 2012; Yang et al., 2016). The first compound produced from AA degradation is an α-ketoacid, which is decarboxylated to an aldehyde (Gonda et al., 2010). AA can also be the precursors of Acyl-CoAs, which are used in alcohol esterification reactions catalyzed by AAT (Hadi et al., 2013). Our results highlight that DCA-RQ1.5 is a promising storage condition for maintaining apple quality and volatile profile.

Fatty acids, degraded via beta-oxidation or LOX, are precursors of straight-chain volatile as C6 volatile compounds (Contreras et al., 2016). Esters from fatty acids like E-3-Hexen-1-ol-acetate, 5-Hexen-1-ol-acetate, Z-2-penten-1-ol-acetate were lesser in 'Galaxy' apple under DCA-RQ1.3 and DCA-RQ1.5 as compared to CA without 1-MCP. The Z-3-hexenal, a product of linolenic acid degradation, was higher in apples under CA and ULO without 1-MCP. In CA, probably LOX showed high activity because of the high ethylene production (Figure 7C, D and E). Fruits with 1-MCP showed reduction of Z-3-hexenal in CA, ULO and DCA-CF, as a result of

the ethylene action inhibition by 1-MCP. The isomerization of Z-3-hexenal forms E-2-hexenal, that was higher in ULO and DCA-CF without 1-MCP, which probably occurs by higher isomerization under O_2 partial pressure used in DCA-CF and ULO (about 0.4 kPa) (Figure 6G).

The total of alcohol was reduced by 1-MCP application, except under DCA-RQ1.3 (Figure 5T). This result is related with the total of esters, which were lesser with 1-MCP application. This is because 1-MCP reduced PDC, ADH and AAT activity and reduced the gene expression (Harb et al., 2010; 2011; Ortiz et al., 2010). 1-Hexanol and 1-butanol were reduced by 1-MCP application, except under DCA-RQ1.3, which is a result of the inhibition of ADH by 1-MCP, because the ethylene is needed to produce and activate this enzyme. These alcohols are precursors of important esters, like hexyl acetate from 1-hexanol, and butyl acetate from 1-butanol. Interestingly, hexyl acetate was reduced by 1-MCP, while butyl acetate was less affected by 1-MCP application, but both the precursor of 1-hexanol and 1-butanol, respectively, were reduced by 1-MCP application (Figure 5C). It is likely that AAT genes, which are required to esterify 1-butanol and produce butyl acetate, were less affected by 1-MCP, or the affinity of AAT for 1-butanol was higher than 1-hexanol at the concentration of these alcohols. Souleyre et al. (2005) found that many factors contribute to the ability of a fruit to synthetize its aroma, including substrate availability, number of AATs, their regulation and kinetic characteristic under different substrate concentration. DCA-RQ1.5 without 1-MCP showed higher ethanol production, which contributed to higher ethyl acetate. The anaerobic metabolism is induced in low O_2 partial pressure, as under DCA-RQ1.5, to maintain glycolytic pathway and produce low amount of ATP.

3.1.4.2 Ethylene, ACC oxidase activity and internal ethylene concentration

The effect of 1-MCP on the reduction of ethylene production, ACC oxidase activity and IEC under CA is already known (Brackmann et al., 2014; Both et al., 2014b). The important fact is that with extremely low O_2 used in DCA, especially in DCA-RQ1.3 and DCA-RQ1.5, 1-MCP had no

effect to reduce IEC and ethylene. This is important to apple storers, because 1-MCP application is not needed when apples are stored under DCA-RQ. There are some advantages, such as reduced financial cost with 1-MCP application and better volatile profile by storage under DCA-RQ without 1-MCP. The low O_2 may have two effects on ethylene biosynthesis reduction, by direct effect on ACC oxidase and indirect by inducing anaerobic metabolism, in which their products reduced the genes expression to ethylene biosynthesis, as *BO-ACO1*, *BO-ACO2* e *BO-ACS1* (Asoda et al., 2009). Ethylene has an effect on the activation of enzymes that degrade the cell wall and reduce the flesh firmness, as poligalacturonase, pectinamethylesterase and ß-galactosidade (Prasanna et al., 2007).

3.1.4.3 Flesf firmness

For this reason, 1-MCP maintains higher flesh firmness in CA, ULO, DCA-CF and DCA-RQ1.3, but did not have effect in DCA-RQ1.5. The ethylene biosynthesis is reduced by low O_2 partial pressure (Yang and Hoffman, 1984). This strengthens the evidence that apples can be stored under DCA-RQ1.5 without 1-MCP to maintain better quality. DCA-CF maintains the flesh firmness better than CA without 1-MCP application, however it did not differ from the other treatments. It is important to notice that that DCA-CF has the same effect on the quality of the 'Galaxy' apple as ULO (0.4kPa), which is confirmed by flesh firmness, ethylene production, internal ethylene concentration and ACC oxidase. Another work already obtained similar conclusions between these conditions (Thewes et al., 2015a). It is likely that O_2 partial pressure (0.4kPa) used on DCA-CF during all period of storage, except at the initial storage period, has the same effect on apple quality than O_2 partial pressure used in ULO (0.4kPa) (Figure 1).

3.1.4.4 Physiological disorders

1-MCP reduced the flesh breakdown only in CA stored apple. This disorder is associated with ethylene, which is caused by oxidation and browning of tissue. Franck et al. (2007) found that browning disorder is a direct consequence of membrane disintegration, which facilitated polyphenol oxidase enzyme acting. The CA showed high ethylene, which was crucial to increase the flesh breakdown. On the other hand, 'Galaxy' apples under DCA and ULO, with low ethylene production showed low flesh breakdown incidence. The important fact is that DCA has the same effect as CA + 1-MCP in the flesh breakdown reduction. Thus, low pO_2 decrease strongly the ethylene production, which can eliminate 1-MCP application in apples stored under DCA-RQ1.5. Other studies found low or none 1-MCP effect on apples stored under ULO (Brackmann et al., 2012; Both et al., 2014b).

Mealiness is another important physiological disorder, which was lower in ULO, DCA-CF and DCA-RQ1.3 in apple without 1-MCP (Figure 7O). Mealiness is characterized by abnormal softness and lack of free juice in the fruit. This occurs by the degradation of the pectin in the middle lamella and causes the rupture of contact from cell to cell (Huang; Lu, 2010). This disorder is accelerated by ethylene, which increases pectin degradation enzymes (Prasanna et al., 2007). In treatments with low ethylene production occurred low incidence of mealiness, except by DCA-RQ1.5 without 1-MCP.

3.1.5 Conclusion

The 'Galaxy' apple stored in a controlled dynamic atmosphere based on respiratory quotient 1.5 (DCA-RQ1.5) maintained a better volatile profile as compared to controlled atmosphere, ultra-low oxygen (ULO) and controlled dynamic atmosphere based on chlorophyll fluorescencece (DCA-CF).

1-Methylcyclopropene, despite of maintaining some quality attributes, reduced volatile compounds production even under DCA-RQ1.5 stored apples.

Apples sotred under DCA-RQ1.3 with 1-MCP application maintained higher flesh firmness. DCF-CF has similar effect on maintaining apple quality than ULO (0.4kPa).

Apples stored under DCA-RQ1.5 increased ester production after 9 months of storage.

3.1.6. Acknowledgements

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3.1.7. References

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4. ARTIGO 2

4.1 Dynamic controlled atmosphere, stress by low oxygen, temperature and 1-methylcyclopropene on quality of the 'Galaxy' apple during long term of storage [2]

Abstracts

The aim of this study was to evaluate the effect of dynamic controlled atmosphere storage monitored with chlorophyll fluorescence (DCA-CF) and monitored with respiratory quotient 1.3 (DCA-RQ1.3) methods using higher temperatures (2.0 and 2.5 °C), with and without 1-MCP application (0.625 μL L^{-1}) on 'Galaxy' apples; and also to evaluate if the stress caused by extremely low O_2 partial pressure (pO_2) employed during DCA-RQ calculation has additional effects in preserving apple quality. Two experiments were performed. On the experiment 1 the treatments evaluated were: [1] controlled atmosphere (CA) (1.2 kPa O_2 + 2.0 kPa CO_2), [2] dynamic controlled atmosphere with chlorophyll fluorescence (DCA-CF) with 1.2 kPa CO_2 and [3] dynamic controlled atmosphere monitored by respiratory quotient (DCA-RQ) 1.3 with 1.2 kPa CO_2, both stored under temperatures of 1.5, 2.0 and 2.5 °C (±0.1 °C). All storage conditions were combined with or without 1-MCP application, totaling 18 treatments. In experiment 2, the following treatments were evaluated: [1] CA, [2] DCA-CF, [3] DCA-RQ1.3 with 1.2 kPa CO_2 with RQ calculation and [4] DCA-RQ1.3 with 1.2 kPa CO_2 without RQ calculation, only maintaining the O_2 set point the same as determined in treatment 3 of the experiment 2. The temperature in experiment 2 was kept under 1.5 °C. DCA-RQ1.3 and DCA-CF allow the storage of 'Galaxy' apples under higher temperatures (2.0 and 2.5 °C) than typically recommended (1.5 °C). Application of 1-MCP did not bring additional effects when 'Galaxy' apples were stored under DCA-RQ1.3 and DCA-CF under higher temperatures. Without 1-MCP application, DCA-RQ1.3 at a low temperature (1.5 °C) is better than DCA-CF because it helps maintain higher flesh

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

firmness and lower mealiness. Under DCA-RQ1.3, the stress caused by low O_2 partial pressure during RQ calculation has a beneficial effect on the quality of 'Galaxy' apples after storage, maintaining higher flesh firmness, lower mealiness and lower decay incidence when compared with fruit stored without stress by low O_2 partial pressure.

Keywords: chlorophyll fluorescence, conservation, *Malus domestica*, physiological disorders, respiratory quotient

4.1.1 Introduction

New technologies and improvements related to apple storage are crucial in reducing quality losses. Apples postharvest losses account 35% of the stored volume (Corrent et al., 2009). About 75% of Brazil's apple production is stored in controlled atmosphere (CA) (Brackmann et al., 2008; Agapomi, 2016). Recently, new storage methods such as dynamic controlled atmosphere (DCA) are being studied and used by apple storehouses (Zanella, 2003; Prange et al., 2007; Wright et al., 2012; Brackmann, 2015; Weber et al., 2015; Both et al., 2017; Thewes et al., 2017). However, more research is still necessary to properly investigate the effects of different storage temperatures and the ideal temperature in relation to other variables. Using DCA storage, O_2 partial pressure (pO_2) is modified according to the physiological response of the fruit (Zanella, 2003), which allows the storage of the fruit near the lowest oxygen limit (LOL) tolerated by apples. To monitor the LOL, three methods are available to apple storers: a) based on the release of ethanol measured in the headspace of the storage room (DCS – dynamic control system) or in the fruit juice (ILOS-Plus) (Veltman et al., 2003); b) based on chlorophyll fluorescence emission (DCA-CF) (Prange et al., 2007) and; c) based on respiratory quotient (DCA-RQ) (Gasser et al., 2008; Weber et al., 2015; Brackmann, 2015). The least used methods is DCS, because ethanol does not show a precise correlation with anaerobic respiration, since this alcohol may be metabolized within the fruit

producing esters, or ethanol may be produced in any other condition not necessarily under hypoxic environment (Brackmann et al., 1993; Prange et al., 2015; Wright et al., 2015). The DCA-CF method detects the fluorescence emitted by chlorophyll when the fruit is submitted to the stress of low O_2 partial pressure (Prange et al., 2007; Wright et al., 2012; Wright et al., 2015). In the last few years, the DCA-RQ method was developed in Brazil and other countries (Gasser et al., 2008; Weber et al., 2015; Bessemanns et al., 2016; Both et al., 2017; Thewes et al., 2017), and is based on the ratio between the production of CO_2 and the consumption of O_2 by the stored fruit (Boersig et al., 1988; Weber et al., 2015). For RQ calculation, the storage chamber is closed for 13 h, O_2 and CO_2 being measured before and after this period (Thewes et al., 2017). During this period, O_2 is reduced to near anoxia, which may helps maintain apple quality during DCA-RQ storage.

Associated with the change in pO_2, storage also employs low temperature, which has a direct effect on the speed of chemical and biochemical reactions (Ekman et al., 2005). Under CA storage, the recommended temperature for 'Gala' apple mutants is between 0.5 to 1.5 °C (Brackmann et al., 2008; 2009; 2010; Weber et al., 2013). There are some studies with the application of 1-methylcyclopropene (1-MCP), which allows the use of higher temperatures (around 2.0 or 3.0 °C) than the ones recommend (McCormick et al., 2012; Kittermann et al., 2015; Mazzurana et al., 2016). Köpcke (2015) reported that DCA, monitored by chlorophyll florescence (DCA-CF) plus 1-MCP application kept 'Elstar', 'Jonagold' and 'Gloster' apples' quality under temperatures of 2.0 and 3.5 °C. The main advantage of setting a higher temperature is the reduction of energy consumption, compared with lower temperatures. By applying 1-MCP and increasing the storage temperature from 0.7 °C to 2.0 °C, Mazzurana et al. (2016) found a 21% reduction in energy usage of fans plus 50% reduction of the air cooling system.

At the end of the last century the 1-MCP molecule was studied for its ability of blocking the action of ethylene (Sisler; Serek, 1997; Blankenship and Dole, 2003). 1-MCP blocks the ethylene receptor, inhibiting the ethylene's binding to receptors and therefore reducing its

physiological effect on ripening (Sisler; Serek, 1997; Lacey and Binder, 2014). This growth regulator has the effect of delaying fruit ripening under CS and CA, reducing the biosynthesis of ethylene and maintaining flesh firmness and fruit acidity (Corrent et al., 2004; Watkins, 2006). However, besides being a chemical product, 1-MCP reduces volatile biosynthesis, as found in several works (Raffo et al., 2009; Ortiz et al., 2010; Thewes et al., 2015a), and may increase decay incidence (Janisiewicz et al., 2003; Both, 2015) and flesh breakdown in apples (Jung and Watkins, 2011; Köpcke, 2015). Therefore, developing an alternative technology to 1-MCP is very important to reduce the use of chemical products in the apple industry, without quality loss during postharvest life.

Thus, the aim of this study was to evaluate the effect of using higher temperatures (2.0 and 2.5 °C) on the 'Galaxy' apples storage under DCA-CF and DCA-RQ1.3 methods, with and without 1-MCP application; and also to evaluate if the stress caused by extremely low pO_2 employed during DCA-RQ calculation has additional effects in preserving apple quality.

4.1.2 Materials and methods

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

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

Immediately after harvest, the fruit was transported to the Postharvest Research Center of the Federal University of Santa Maria, RS, Brazil. At the Postharvest Research Center the fruit was submitted to a selection process, aiming to eliminate the damaged ones and homogenize fruit size in the samples. Thereafter, samples were selected containing 25 fruit each, a total of 4 samples per treatment. At harvest, fruit showed iodine-starch index of 7.2, soluble solids of 11.2%,

titratable acidity of 0.36 mg malic acid $100g^{-1}$, respiration rate of 7.76 µg CO_2 kg^{-1} h^{-1}, ethylene production of 0.11 ng C_2H_4 kg^{-1} h^{-1} and flesh firmness of 75.0 N.

4.1.2.2 CA, DCA-CF, DCA-RQ, temperature and relative humidity conditions

After the sample preparation, fruit was stowed into 0.233 m^3 experimental chambers and the following treatments applied for experiment 1: [1] Controlled atmosphere (CA) (1.2 kPa O_2 + 2.0 kPa CO_2) under temperatures of 1.5, 2.0 and 2.5 °C (±0.1); [2] Dynamic controlled atmosphere with chlorophyll fluorescence (DCA-CF) with 1.2 kPa CO_2 under temperatures of 1.5, 2.0 and 2.5 °C; [3] Dynamic controlled atmosphere monitored by respiratory quotient (DCA-RQ) 1.3 with 1.2 kPa CO_2, stored under temperatures of 1.5, 2.0 and 2.5 °C. All storage conditions were combined with or without 1-MCP application.

In experiment 2, the following treatments were evaluated: [1] Controlled atmosphere (CA) (1.2 kPa O_2 + 2.0 kPa CO_2); [2] Dynamic controlled atmosphere with chlorophyll fluorescence (DCA-CF); [3] Dynamic controlled atmosphere with respiratory quotient (DCA-RQ) of 1.3 with 1.2 kPa CO_2 with RQ calculation; [4] Dynamic controlled atmosphere with respiratory quotient (DCA-RQ) of 1.3 with 1.2 kPa CO_2 without RQ calculation, only maintaining the O_2 set point as determined in treatment 3. The temperature in all treatments of this experiment was 1.5 °C.

The storage temperature was set at 1.5, 2.0 or 2.5 °C ± 0.1 °C (according to each experiment) and monitored daily during the 9 months of storage with the aid of mercury thermometers inserted inside the fruit flesh to determine pulp temperature. Inside the storage chamber, the relative humidity was monitored manually with psychrometers and controlled by the allocation of calcium chloride, which allowed absorb the excess of humidity inside of the chamber, maintaining an average relative humidity of 94 % ± 2%.

4.1.2.3 CA, DCA-CF and DCA-RQ setup and maintenance

The experimental chambers were hermetically closed and the storage conditions installed. In the first day of storage, the temperature was reduced down to 5.0 °C and thereafter gradually down to 1.5, 2.0 and 2.5 °C in about 5 days. The day that the temperature reached the pre-established level, the CA and DCA were setup to obtain the desired atmospheric condition, with the chambers being flushed with nitrogen until the desired oxygen level for CA (1.2 kPa) was reached; and reduced down to 0.5 kPa for DCA conditions. This process was also carried out in 5 days. The carbon dioxide partial pressure was obtained by its accumulation in the storage chamber from fruit respiration. Thus, during the first 4-5 days of storage only the temperature was reduced, and from the fifth day up to the tenth day the CA and DCA conditions were installed. This procedure was carried out to simulate the conditions of commercial storage.

Throughout the storage period, the oxygen partial pressure was changed according to fruit metabolism in DCA and kept constant in CA. To measure the LOL during storage, the respiratory quotient (RQ) was measured two times a week, according to the methodology proposed by Weber et al. (2015). Thus, the RQ was set at 1.3, and the oxygen partial pressure changed accordingly to maintain these RQ levels (Figure 1). The RQ was calculated with a chamber closure of 13 hours between the first and second reading. The RQ was calculated by the ratio between CO_2 production and O_2 uptake.

In experiment 2, treatment 4, the O_2 set point was adjusted according to treatment 3, where RQ was calculated. Because of this, treatment 4 did not inflict stress from extremely low O_2 during the RQ calculation period (Figures 3A and 3B). In relation to the CA conditions, they were maintained according to the methodology proposed by Thewes et al. (2015b), where fruit was kept at 1.2 kPa O_2 plus 2.0 kPa CO_2.

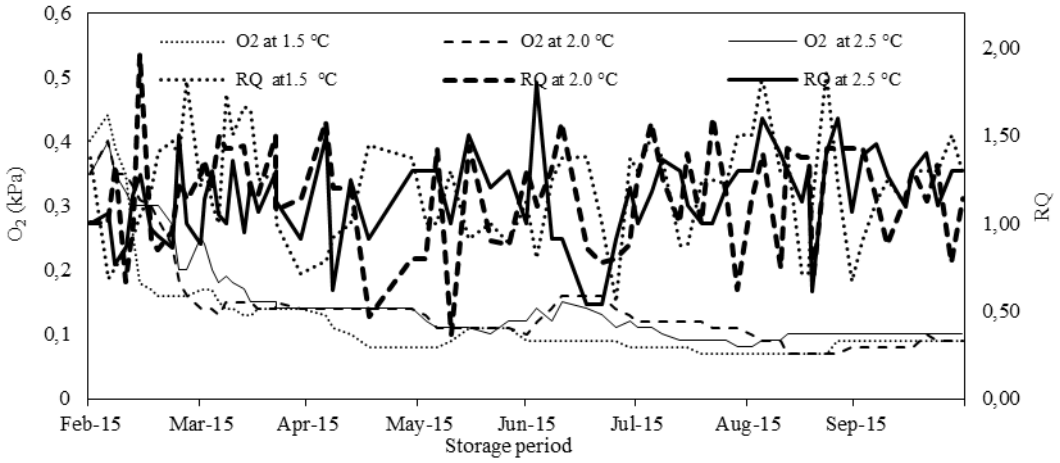

Fig. 1. Oxygen and respiratory quotient (RQ) level variation in DCA-RQ1.3 of 'Galaxy' apple under 1.5. 2.0 and 2.5 °C during 9 months of storage. CO_2 partial pressure was 1.2 kPa in DCA-RQ.

The DCA-CF was monitored according to Prange et al. (2007). The chlorophyll fluorescence was monitored from six apples during fruit exposure to low O_2. Thus, apples cooled to each treatment temperature were placed in a perforated plastic container (18 cm width, 27 cm length, 25 cm height) with the fluorescence sensors attached to the inside of the container's top. The container was placed inside an experimental chamber; the chamber was sealed, and covered with black plastic to exclude light. The fluorescence monitoring system was activated and then O_2 was subsequently reduced to 0.5 kPa by N_2 injection. Afterwards, the respiration process reduced the O_2 partial pressure until a change in fluorescence was detected. The lowest O_2 set point was determined by identifying the O_2 partial pressure where an inflection in the fluorescence signal was detected, and then increasing O_2 by 0.2 kPa as a safety factor. After the fluorescence signal the O_2 was kept at 0.4kPa (Prange et al., 2007). Chlorophyll fluorescence was monitored every hour for the entire storage period during the experiment.

4.1.2.4 1-MCP treatment

The fruit of the treatment with 1-MCP was stowed inside an experimental chamber of 0.233 m^3 and thereafter submitted to 1-MCP treatment. A solution containing 0.625 µL L^{-1} 1-MCP was

prepared (SmartFresh®, 0.14% of active ingredient) and allocated into petri dishes inside the chamber. Immediately after, the chamber was hermetically closed during 24 hours. During the 24 hours, the air inside the chamber was stirred with a fan. This process was carried out at the storage temperature for each treatment. After the 1-MCP treatment period the fruit was stored according to the conditions above described.

4.1.2.5 Metabolism and overall quality analyses

The following analyses were carried out after 9 months of storage and after 7 days of shelf life (at 20 ± 2 °C and a relative humidity of 80 % ± 2 %): Internal ethylene concentration, ethylene production, respiration rate, ACC oxidase, soluble solids, acidity, flesh firmness, decay, mealiness and flesh breakdown, according to described in article 1. Electrolyte leakage was evaluated for each replicate, where 10 discs (5 mm thickness and diameter) were taken from different fruit. These discs were stowed into 20 mL 0.4 M mannitol solution during 1 hour (20± 1 °C) before measuring the conductivity of the suspension. The suspension was then placed 120 °C for 30 minutes 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.

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). Additionally, a variance analysis (ANOVA) at a 5% probability of error was carried out. Data that showed significant difference by ANOVA were subjected to the Tukey's test at a 5% probability of error. Experiment 1 was conducted in a completely randomized scheme with a trifactorial arrangement (3 storage

conditions x 3 temperatures × with or without 1-MCP application). Experiment 2 was conducted in a completely randomized scheme with an unifactorial arrangement.

4.1.3 Results

4.1.3.1 Experiment 1

The last step of ethylene biosynthesis is the catalysed by 1-aminocyclopropane-1-carboxylic acid oxidase (ACC oxidase) (Yang & Hoffman, 1984). Apple under CA, with or without 1-MCP had an increase in ACC oxidase activity under higher temperatures (Table 1). When 'Galaxy' apples were kept under DCA-CF and DCA-RQ1.3, fruit did not show an increase on ACC oxidase activity with the increase of temperature. The 1-MCP, an inhibitor of ethylene action, reduced this enzyme in apple stored under CA at the three temperature, and under DCA-CF at 1.5 °C. Under the DCA-RQ1.3 conditions 1-MCP did not have effect. Storage conditions with lower O_2 partial pressure (DCA-CF and DCA-RQ1.3), with or without 1-MCP, presented a reduced ACC oxidase activity under higher temperatures.

Table 1 – ACC oxidase enzyme activity of 'Galaxy' apple stored in controlled atmosphere and dynamic controlled atmosphere at three different temperatures, with and without 1-MCP for 9 months and 7 days at 20 °C.

		ACC oxidase (ng C_2H_4 kg^{-1} s^{-1}) Temperature (°C)		
	1-MCP	1.5	2.0	2.5
CA (1.2+2.0)*	Without	13.6 Ca**	21.7 Ba	26.0 Aa
	With	4.41 Bb	6.65 ABb	8.52 Ab
DCA-CF	Without	7.23 Aa	3.74 Aa	4.75 Aa
	With	2.14 Ab	3.18 Aa	3.14 Aa
DCA-RQ1.3	Without	4.81 Aa	3.70 Aa	3.07 Aa
	With	3.01 Aa	2.33 Aa	2.46 Aa
Without 1-MCP	CA	13.6 a	21.7 a	26.0 a
	DCA-CF	7.23 b	3.74 b	4.75 b
	DCA-RQ1.3	4.81 b	3.70 b	3.07 b
With 1-MCP	CA	4.41 a	6.65 a	8.52 a
	DCA-CF	2.14 a	3.18 ab	3.14 b

	DCA-RQ1.3	3.01 a	2.33 b	2.46 b
CV(%)			27.4	

* CA: 1.2 kPa O_2 + 2.0 kPa CO_2; DCA-CF: dynamic controlled atmosphere by chlorophyll fluorescence with 1.2 kPa of CO_2; DCA-RQ1.3: dynamic controlled atmosphere by respiratory quotient 1.3 with 1.2 kPa of CO_2.
** Means followed by equal letters in upper case in the line and lower case in the columns, do not differ by Tukey's test at 5% of error probability.

'Galaxy' apples stored in CA without 1-MCP had a higher ethylene production and internal ethylene concentration under high temperatures, as compared to 1.5 °C at the opening of chambers and after 7 days of shelf life (Table 2, 3 and 4). On the other hand, fruit treated with 1-MCP, under DCA-CF and DCA-RQ1.3 did not present and increase in ethylene production and internal ethylene concentration with higher temperatures. An interesting result is that the application of 1-MCP only reduced ethylene production and internal ethylene concentration in fruit stored under CA at the three temperatures. 1-MCP did not bring additional effect on ethylene production and IEC of 'Galaxy' apples stored under DCA-CF and DCA-RQ1.3 in neither of the two periods of evaluation. DCA storage presented reduced ethylene production and IEC only on apples without 1-MCP. Those treated with 1-MCP did not show any effects in DCA.

Table 2 – Ethylene production of 'Galaxy' apple stored in controlled atmosphere and dynamic controlled atmosphere at three different temperatures, with and without 1-MCP, for 9 months at the open chamber.

		Ethylene – open chamber (ng C_2H_4 kg^{-1} s^{-1}) Temperature (°C)		
	1-MCP	1.5	2.0	2.5
CA (1.2+2.0)*	Without	0.46 Ba**	1.37 Aa	1.23 Aa
	With	0.05 Ab	0.04 Ab	0.05 Ab
DCA-CF	Without	0.21 Aa	0.17 Aa	0.06 Aa
	With	0.03 Aa	0.04 Aa	0.03 Aa
DCA-RQ1.3	Without	0.06 Aa	0.05 Aa	0.04 Aa
	With	0.03 Aa	0.03 Aa	0.03 Aa
Without 1-MCP	CA	0.46 a	1.37 a	1.23 a
	DCA-CF	0.21 ab	0.17 b	0.06 b
	DCA-RQ1.3	0.06 b	0.05 b	0.04 b
With 1-MCP	CA	0.05 a	0.04 a	0.05 a

	DCA-CF	0.03 a	0.04 a	0.03 a
	DCA-RQ1.3	0.03 a	0.03 a	0.03 a
CV(%)			57.0	

* CA: 1.2 kPa O_2 + 2.0 kPa CO_2; DCA-CF: dynamic controlled atmosphere by chlorophyll fluorescence with 1.2 kPa of CO_2; DCA-RQ1.3: dynamic controlled atmosphere by respiratory quotient 1.3 with 1.2 kPa of CO_2. ** Means followed by equal letters in upper case in the line and lower case in the columns, do not differ by Tukey's test at 5% of error probability.

Table 3 – Ethylene production of 'Galaxy' apple stored in controlled atmosphere and dynamic controlled atmosphere at three different temperatures, with and without 1-MCP, for 9 months and 7 days at 20 °C.

		Ethylene – 7 days at 20 °C (ng C_2H_4 kg^{-1} s^{-1}) Temperature (°C)		
	1-MCP	1.5	2.0	2.5
CA (1.2+2.0)*	Without	0.67 Ca**	6.63 Aa	4.93 Ba
	With	0.02 Ab	0.04 Ab	0.04 Ab
DCA-CF	Without	0.31 Aa	0.25 Aa	0.08 Aa
	With	0.02 Aa	0.05 Aa	0.04 Aa
DCA-RQ1.3	Without	0.09 Aa	0.07 Aa	0.02 Aa
	With	0.02 Aa	0.04 Aa	0.08 Aa
Without 1-MCP	CA	0.67 a	6.63 a	4.93 a
	DCA-CF	0.31 ab	0.25 b	0.08 b
	DCA-RQ1.3	0.09 b	0.07 b	0.02 b
With 1-MCP	CA	0.02 a	0.04 a	0.04 a
	DCA-CF	0.02 a	0.05 a	0.04 a
	DCA-RQ1.3	0.02 a	0.04 a	0.08 a
CV(%)			35.0	

* CA: 1.2 kPa O_2 + 2.0 kPa CO_2; DCA-CF: dynamic controlled atmosphere by chlorophyll fluorescence with 1.2 kPa of CO_2; DCA-RQ1.3: dynamic controlled atmosphere by respiratory quotient 1.3 with 1.2 kPa of CO_2. ** Means followed by equal letters in upper case in the line and lower case in the columns, do not differ by Tukey's test at 5% of error probability.

Table 4 – Internal Ethylene Concentration of 'Galaxy' apple stored in controlled atmosphere and dynamic controlled atmosphere at three different temperatures, with and without, 1-MCP for 9 months and 7 days at 20 °C.

		Internal ethylene concentration (µg C_2H_2 L^{-1}) Temperature (°C)		
	1-MCP	1.5	2.0	2.5
CA (1.2+2.0)*	Without	6.39 Ca**	12.98 Ba	46.16 Aa
	With	0.36 Ab	0.54 Ab	0.11 Ab
DCA-CF	Without	2.40 Aa	1.63 Aa	1.57 Aa
	With	0.29 Ab	0.25 Aa	0.11 Ab
DCA-RQ1.3	Without	0.62 Aa	0.33 Aa	0.23 Aa
	With	0.12 Aa	0.87 Aa	0.37 Aa
Without 1-MCP	CA	6.39 a	13.0 a	46.2 a
	DCA-CF	2.40 ab	1.63 b	1.57 b
	DCA-RQ1.3	0.62 b	0.33 b	0.23 b
With 1-MCP	CA	0.36 a	0.54 a	0.11 a
	DCA-CF	0.29 a	0.25 a	0.11 a
	DCA-RQ1.3	0.12 a	0.87 a	0.37 a
CV(%)			50.5	

* CA: 1.2 kPa O_2 + 2.0 kPa CO_2; DCA-CF: dynamic controlled atmosphere by chlorophyll fluorescence with 1.2 kPa of CO_2; DCA-RQ1.3: dynamic controlled atmosphere by respiratory quotient 1.3 with 1.2 kPa of CO_2.
** Means followed by equal letters in upper case in the line and lower case in the columns, do not differ by Tukey's test at 5% of error probability.

At the opening of chambers, respiration rate increased with higher temperature only under DCA-RQ1.3 without 1-MCP (Table 5). After 7 days at 20 °C, fruit stored under CA without 1-MCP and DCA-RQ1.3 with 1-MCP had higher respiration with increase of temperature. Respiration was reduced by 1-MCP, except under DCA-CF at 2.5 °C at the opening of chambers and after 7 days at 20 °C, and under DCA-RQ1.3 at 2.0 °C. Generally, respiration rate on apples without 1-MCP was lower in fruit under DCA-CF and DCA-RQ1.3 at the opening of chambers, and after 7 days of shelf life.

Table 5 – Respiration rate of 'Galaxy' apple stored in controlled atmosphere and dynamic controlled atmosphere at three different temperatures, with and without 1-MCP, for 9 months at the open chamber.

		Respiration – open chamber (µg CO_2 kg^{-1} s^{-1}) Temperature (°C)		
	1-MCP	1.5	2.0	2.5
CA (1.2+2.0)*	Without	8.06 Aa**	7.42 Aa	7.79 Aa
	With	4.91 Ab	5.04 Ab	4.46 Ab
DCA-CF	Without	6.49 Aa	6.24 Aa	4.74 Ba
	With	4.20 Ab	4.15 Ab	4.17 Aa
DCA-RQ1.3	Without	5.17 Ba	4.63 Ba	6.44 Aa
	With	4.26 Ab	3.98 Aa	3.69 Ab
Without 1-MCP	CA	8.06 a	7.42 a	7.79 a
	DCA-CF	6.49 b	6.24 b	4.74 c
	DCA-RQ1.3	5.17 b	4.63 b	6.44 b
With 1-MCP	CA	4.91 a	5.04 a	4.46 a
	DCA-CF	4.20 a	4.15 a	4.17 a
	DCA-RQ1.3	4.26 a	3.98 a	3.69 a
CV(%)			10.3	

* CA: 1.2 kPa O_2 + 2.0 kPa CO_2; DCA-CF: dynamic controlled atmosphere by chlorophyll fluorescence with 1.2 kPa of CO_2; DCA-RQ1.: dynamic controlled atmosphere by respiratory quotient 1.3 with 1.2 kPa of CO_2. ** Means followed by equal letters in upper case in the line and lower case in the columns, do not differ by Tukey's test at 5% of error probability.

Table 6 – Respiration rate of 'Galaxy' apple stored in controlled atmosphere and dynamic controlled atmosphere at three different temperatures, with and without 1-MCP, for 9 months and 7 days at 20 °C.

		Respiration – 7 days at 20 °C (μg CO_2 kg^{-1} s^{-1}) Temperature (°C)		
	1-MCP	1.5	2.0	2.5
CA (1.2+2.0)*	Without	2.97 Ba**	3.58 Aa	3.74 Aa
	With	1.82 Ab	1.96 Ab	1.81 Ab
DCA-CF	Without	2.44 Aa	2.35 Aa	2.18 Aa
	With	1.71 Ab	1.88 Ab	1.83 Aa
DCA-RQ1.3	Without	2.32 Aa	2.26 Aa	1.87 Ab
	With	1.71 Bb	1.55 Bb	2.47 Aa
Without 1-MCP	CA	2.97 a	3.58 a	3.74 a
	DCA-CF	2.44 ab	2.35 b	2.18 b
	DCA-RQ1.3	2.32 b	2.26 b	1.87 b
With 1-MCP	CA	1.82 a	1.96 a	1.81 b
	DCA-CF	1.71 a	1.88 a	1.83 b
	DCA-RQ1.3	1.71 a	1.55 a	2.47 a
CV(%)			12.3	

* CA: 1.2 kPa O_2 + 2.0 kPa CO_2; DCA-CF: dynamic controlled atmosphere by chlorophyll fluorescence with 1.2 kPa of CO_2; DCA-RQ1.3: dynamic controlled atmosphere by respiratory quotient 1.3 with 1.2 kPa of CO_2. ** Means followed by equal letters in upper case in the line and lower case in the columns, do not differ by Tukey's test at 5% of error probability.

One of the main parameters used by apple consumers to evaluate fruit quality is flesh firmness (Harker et al., 2008). Flesh firmness was reduced with the increase of temperatures in apples stored under CA, independent of the inhibition of ethylene action (Table 7). The important result is that fruit storage under DCA-CF and DCA-RQ1.3 did not reduce flesh firmness under higher temperatures. In addition, DCA-CF without the application of 1-MCP showed higher flesh firmness under 2.0 and 2.5 °C then under 1.5 °C. Similar to ethylene and IEC, 1-MCP had effects in maintaining flesh firmness only in apples under CA at the three temperatures and under DCA-CF at 1.5 °C. For the other storage conditions, 1-MCP was not efective to maintain higher flesh firmness. DCA-CF and DCA-RQ1.3 without 1-MCP maintained higher flesh firmness in fruit stored at 2.0 and 2.5 °C, compared with CA. At 1.5 °C, only DCA-RQ resulted in fruit with higher

flesh firmness, being better than DCA-CF. With the application of 1-MCP, the two DCA methods maintained higher flesh firmness in fruit stored at 2.0 °C.

Table 7 – Flesh firmness of 'Galaxy' apple stored in controlled atmosphere and dynamic controlled atmosphere at three different temperatures, with and without 1-MCP, for 9 months and 7 days at 20 ° C.

		Flesh firmness (N) Temperature (°C)		
	1-MCP	1.5	2.0	2.5
CA (1.2+2.0)*	Without	59.9 Ab**	53.1 Bb	54.1 Bb
	With	63.7 Aa	63.5 Aa	59.0 Ba
DCA-CF	Without	57.3 Bb	61.3 Aa	61.8 Aa
	With	62.2 Aa	62.4 Aa	63.1 Aa
DCA-RQ1.3	Without	63.3 Aa	61.3 Aa	62.9 Aa
	With	64.9 Aa	62.9 Aa	63.6 Aa
Without 1-MCP	CA	59.9 b	53.1 b	54.1 b
	DCA-CF	57.3 b	61.3 a	61.8 a
	DCA-RQ1.3	63.3 a	61.3 a	62.9 a
With 1-MCP	CA	63.7 a	63.5 a	59.0 b
	DCA-CF	62.2 a	62.4 a	63.1 a
	DCA-RQ1.3	64.9 a	62.9 a	63.6 a
CV(%)			2.93	

* CA: 1.2 kPa O_2 + 2.0 kPa CO_2; DCA-CF: dynamic controlled atmosphere by chlorophyll fluorescence with 1.2 kPa of CO_2; DCA-RQ1.3: dynamic controlled atmosphere by respiratory quotient 1.3 with 1.2 kPa of CO_2. ** Means followed by equal letters in upper case in the line and lower case in the columns, do not differ by Tukey's test at 5% of error probability.

Physiological disorders are a key cause of apple losses around the world, being mealiness one of them. Temperatures higher than 1.5 °C reduced mealiness in fruit stored under DCA-CF without 1-MCP (Table 8). 1-MCP reduced mealiness only in apples under CA at 2.0 °C and DCA-CF at 1.5 °C. 1-MCP did not have any effect on 'Galaxy' apples stored in DCA-RQ1.3 at any of the three temperatures used in tests. Fruit without 1-MCP had lower mealiness under DCA-RQ1.3 in all temperatures, while DCA-CF reduced this disorder in apples at 2.0 and 2.5 °C. When fruit received 1-MCP application, DCA-CF and DCA-RQ1.3 reduced mealiness in fruit at 1.5 °C and 2.5 °C.

Table 8 – Mealiness of 'Galaxy' apple stored in controlled atmosphere and dynamic controlled atmosphere at three different temperatures, with and without 1-MCP, for 9 months and 7 days at 20 ° C.

		Mealiness (%) Temperature (°C)		
	1-MCP	1.5	2.0	2.5
CA (1.2+2.0)*	Without	8.4 Aa**	16.2 Aa	13.5 Aa
	With	6.0 ABa	3.6 Bb	12.5 Aa
DCA-CF	Without	11.8 Aa	4.0 ABa	1.9 Ba
	With	0.0 Ab	2.8 Aa	2.0 Aa
DCA-RQ1.3	Without	1.7 Aa	2.8 Aa	0.0 Aa
	With	0.0 Aa	0.0 Aa	2.0 Aa
Without 1-MCP	CA	8.4 ab	16.2 a	13.5 a
	DCA-CF	11.8 a	4.0 b	1.9 b
	DCA-RQ1.3	1.7 b	2.8 b	0.0 b
With 1-MCP	CA	6.0 a	3.6 a	12.5 a
	DCA-CF	0.0 b	2.8 a	2.0 b
	DCA-RQ1.3	0.0 b	0.0 a	2.0 b
CV(%)			76.9	

* CA: 1.2 kPa O_2 + 2.0 kPa CO_2; DCA-CF: dynamic controlled atmosphere by chlorophyll fluorescence with 1.2 kPa of CO_2; DCA-RQ1.3: dynamic controlled atmosphere by respiratory quotient 1.3 with 1.2 kPa of CO_2. ** Means followed by equal letters in upper case in the line and lower case in the columns, do not differ by Tukey's test at 5% of error probability.

A temperature of 2.5 °C reduced soluble solids under DCA-RQ1.3, however, there was no difference from CA. Fruits with 1-MCP had higher soluble solids at 2.0 °C (Table 9). Acidity was higher in apples without 1-MCP under DCA-CF and DCA-RQ1.3 (Table 10). 1-MCP maintained acidity only on apples under CA. Under DCA, this growth regulator did not have effects on acidity. Decay incidence, the main cause of apple losses (Li et al., 2017), was reduced by 1-MCP application under DCA-RQ1.3. Under the other conditions, there were no differences (Table 10).

Table 9 – Soluble solids of 'Galaxy' apple stored in controlled atmosphere and dynamic controlled atmosphere at three different temperatures, with and without 1-MCP, for 9 months and 7 days at 20 °C.

	SS (%) Temperature (°C)		
	1.5	2.0	2.5
CA (1.2+2.0)*	11.7 Aa**	11.8 Aa	11.9 Aab
DCA-CF	11.8 Aa	11.8 Aa	12.0 Aa
DCA-RQ1.3	11.9 ABa	12.0 Aa	11.6 Bb
Without 1-MCP	11.8 Aa	11.7 Ab	11.8 Aa
With 1-MCP	11.8 Aa	12.0 Aa	11.9 Aa
CV(%)		2.28	

* CA: 1.2 kPa O_2 + 2.0 kPa CO_2; DCA-CF: dynamic controlled atmosphere by chlorophyll fluorescence with 1.2 kPa of CO_2; DCA-RQ1.3: dynamic controlled atmosphere by respiratory quotient 1.3 with 1.2 kPa of CO_2. ** Means followed by equal letters in upper case in the line and lower case in the columns, do not differ by Tukey's test at 5% of error probability.

Table 10 – Acidity and decay of 'Galaxy' apple stored in controlled atmosphere and dynamic controlled atmosphere at three different temperatures, with and without 1-MCP, for 9 months and 7 days at 20 ° C.

	Acidity (mg malic acid $100g^{-1}$) Atmosphere condition		
	CA (1.2+2.0)*	DCA-CF	DCA-RQ1.3
Without 1-MCP	0.22 Bb**	0.28 Aa	0.27 Aa
With 1-MCP	0.27 Aa	0.28 Aa	0.27 Aa
CV(%)		9.08	
		Decay – (%)	
Without 1-MCP	21.1 Aa**	23.3 Aa	28.7 Aa
With 1-MCP	19.9 Aa	25.2 Aa	19.9 Ab
CV(%)		20.6	

* CA: 1.2 kPa O_2 + 2.0 kPa CO_2; DCA-CF: dynamic controlled atmosphere by chlorophyll fluorescence with 1.2 kPa of CO_2; DCA-RQ1.3: dynamic controlled atmosphere by respiratory quotient 1.3 with 1.2 kPa of CO_2. ** Means followed by equal letters in upper case in the line and lower case in the columns, do not differ by Tukey's test at 5% of error probability.

4.1.3.2 Experiment 2

This experiment was performed to evaluate the effect of stress by low O_2 caused during RQ calculation. One treatment was monitored by DCA-RQ1.3 and O_2 partial pressure change

according RQ calculation (Figure 2A). Another treatment was conducted maintaining the same O_2 set point of DCA-RQ1.3, however, without the low O_2 stress during RQ calculation (Figure 2B). These treatments were compared to standard condition (CA) and DCA-CF. During the period of RQ calculation (13 hours) in the storage chamber, O_2 partial pressure decreased strongly (Figure 1A). In another chamber, without RQ calculation, the O_2 partial pressure remained near the set point (hysteresis = 0.02 kPa O_2), without low O_2 stress to the apple (Figure 1B).

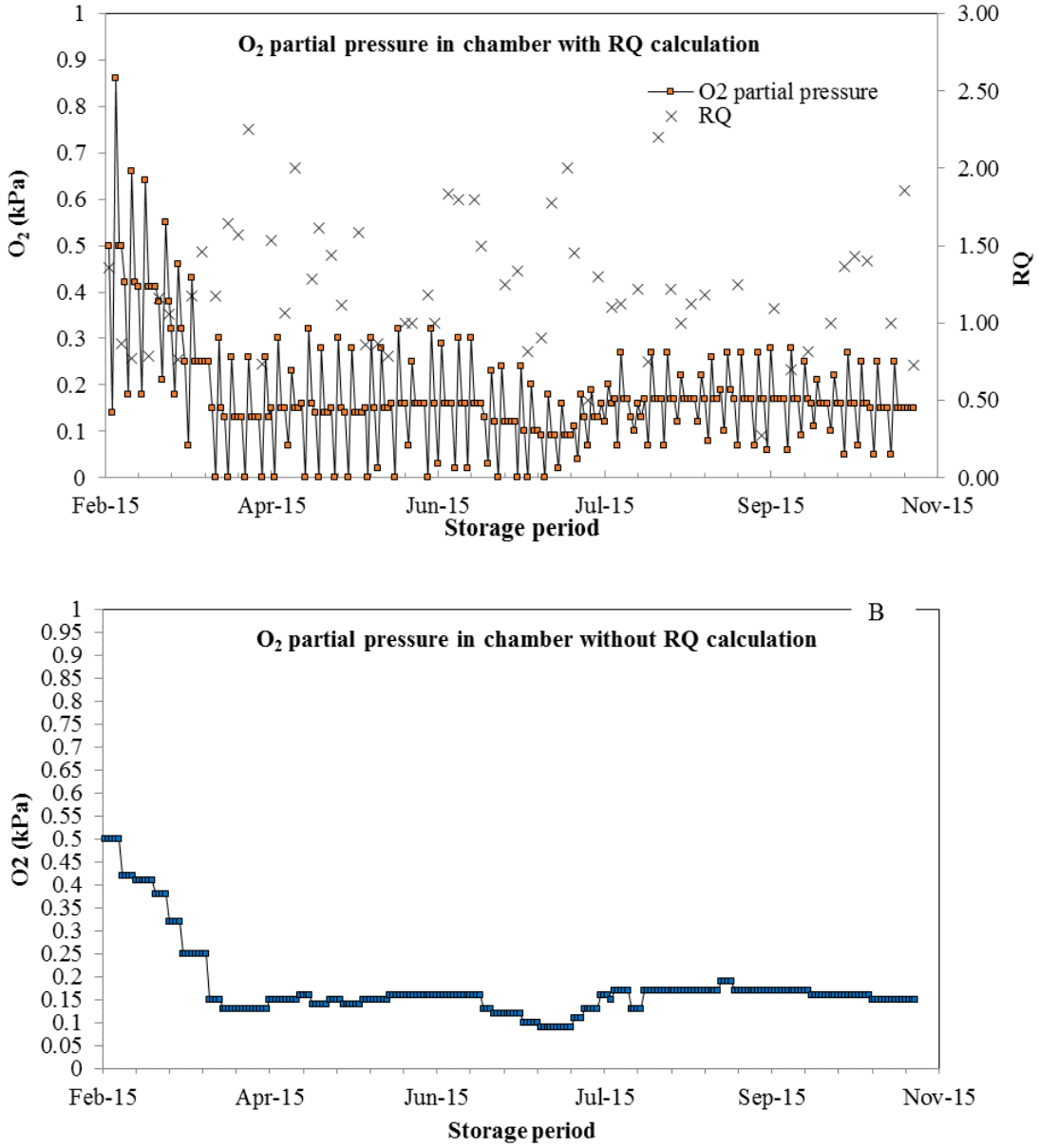

Figure 2 – Oxygen partial pressure with RQ calculation (A) and without RQ calculation (B) in 'Galaxy' apple stored by 9 months at 1.5 °C. CO_2 partial pressure was 1.2 kPa to DCA-RQ. Hysteresis was 0.02 kPa of O_2.

Ethylene production and internal ethylene concentration were higher in CA (Figure 4A and 4D), which is demonstrated by PCA, where by PCI, CA is more related with ethylene production and IEC (Figure 3). DCA-CF and DCA-RQ1.3 with RQ calculation and DCA-RQ1.3 without RQ calculation (without stress) showed lower ethylene production in relation to CA. The last enzyme in ethylene pathway biosynthesis is ACC oxidase, which had its activity reduced by DCA (Figure 4C). DCA-RQ1.3 with RQ calculation caused a higher suppression of ACC oxidase activity than DCA-CF. There was a tendency of fruit under DCA-RQ1.3 without RQ calculation presenting less reduction of this enzyme's activity.

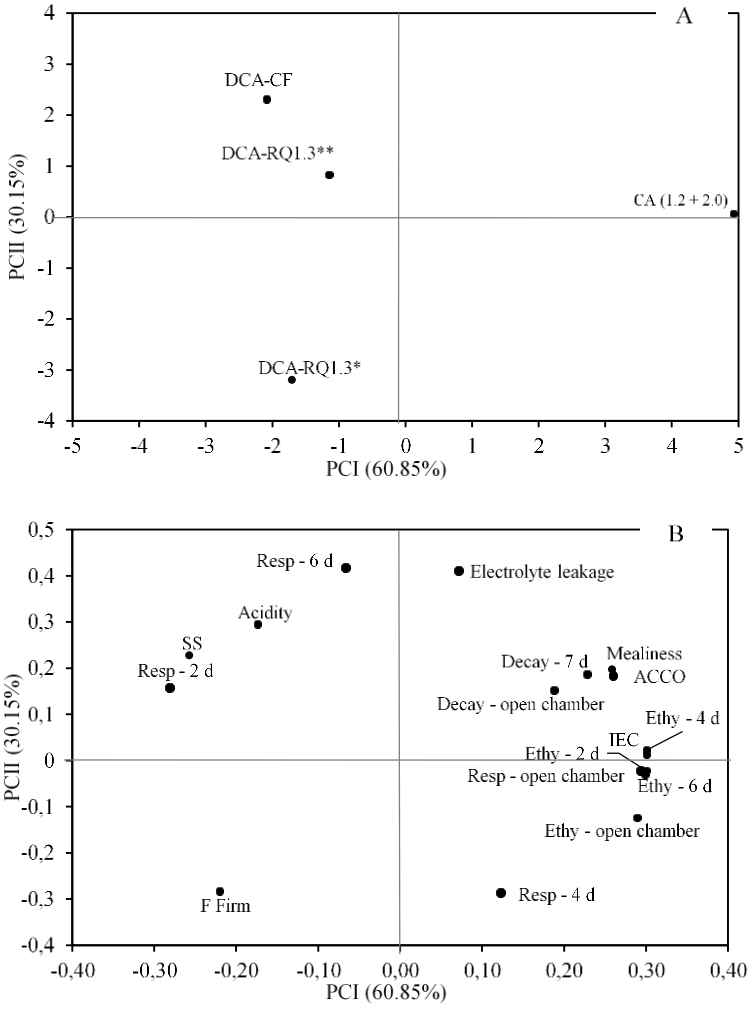

Fig. 3. (A) Scores (treatments) and (B) loadings (variables) plots showing the two major principal components of 'Galaxy' apples stored under controlled atmosphere, dynamic controlled atmosphere (DCA), during 9 months storage plus 7 days of shelf life. CA: controlled atmosphere; DCA-RQ1.3*: DCA-

RQ1.3 with RQ calculation (stress by low O_2); DCA-RQ1.3**: DCA-RQ1.3 without RQ calculation (without stress); DCA-CF: dynamic controlled atmosphere by chlorophyll florescence; IEC: internal ethylene concentration; SS: soluble solids; Ethy: ethylene; F firm: flesh firmness; Resp: respiration rate; ACCO: ACC oxidase activity.

Flesh firmness was higher in fruits under DCA-RQ1.3 with RQ calculation in relation to DCA-RQ1.3 without RQ calculation (Table 4E). DCA-RQ1.3 with RQ calculation resulted in apples with higher flesh firmness than DCA-CF and CA. DCA-RQ1.3 without RQ calculation maintained higher flesh firmness than CA, and did not differ from DCA-CF.

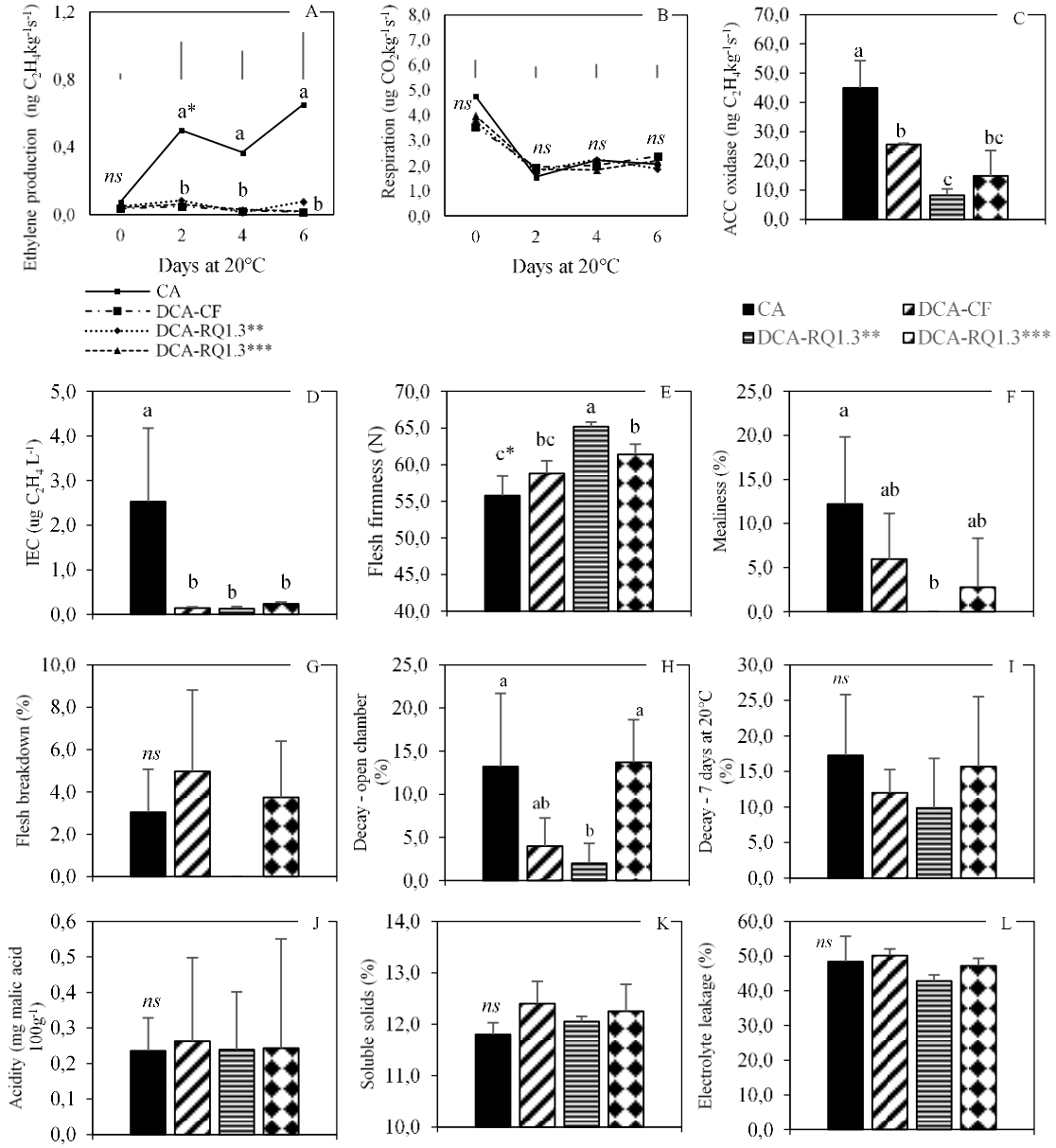

Figure 4 – Metabolism and quality of ‘Galaxy’ apple stored under controlled atmosphere, dynamic controlled atmosphere (DCA), during 9 months storage plus 7 days of shelf life. *Means followed by equal letters do not differ by Tukey’s test at 5% of error probability. Error bars mean standard deviation; *ns*: no significant; **DCA-RQ1.3: DCA-RQ1.3 with RQ calculation (stress by low O_2); ***DCA-RQ1.3: DCA-RQ1.3 without RQ calculation (without stress).

Fruit losses are caused mainly by decay and physiological disorders. Mealiness is a common physiological disorder found in apples, which was lower in fruit under DCA-RQ1.3 with RQ calculation, although only different from CA (Figure 4F). In general, there were no noticeable differences in regards to flesh breakdown. However, apples under DCA-RQ1.3 with RQ calculation had no fruit with this disorder (Figure 4G). At the opening of the chambers, ‘Galaxy’ apples under DCA-RQ1.3 with RQ calculation had lower decay incidence than DCA-RQ1.3 without RQ calculation and CA (Table 4H). After 7 days of shelf life there were differences, although there was a tendency of less decay under DCA-RQ1.3 with RQ calculation. Acidity, SS and membrane permeability did not differ among treatments (Figure 4J, 4K, and 4L).

4.1.4 Discussion

4.1.4.1 Experiment 1

Principal component analysis I (PCI) allowed separate CA and DCA storage (Figure 5). In CA storage temperatures show higher correlation with ethylene production, respiration rate, mealiness, internal ethylene concentration (IEC) and ACC oxidase activity. PCI represents more than 67% of the all variation between treatments, PCII represents 10.36% and PCIII represents 9.00%. Except DCA-CF at 1.5 °C, through PCI, the other treatments did not show a clear separation, which is in according with the results obtained through the Tukey test presented on Tables 1 to 11.

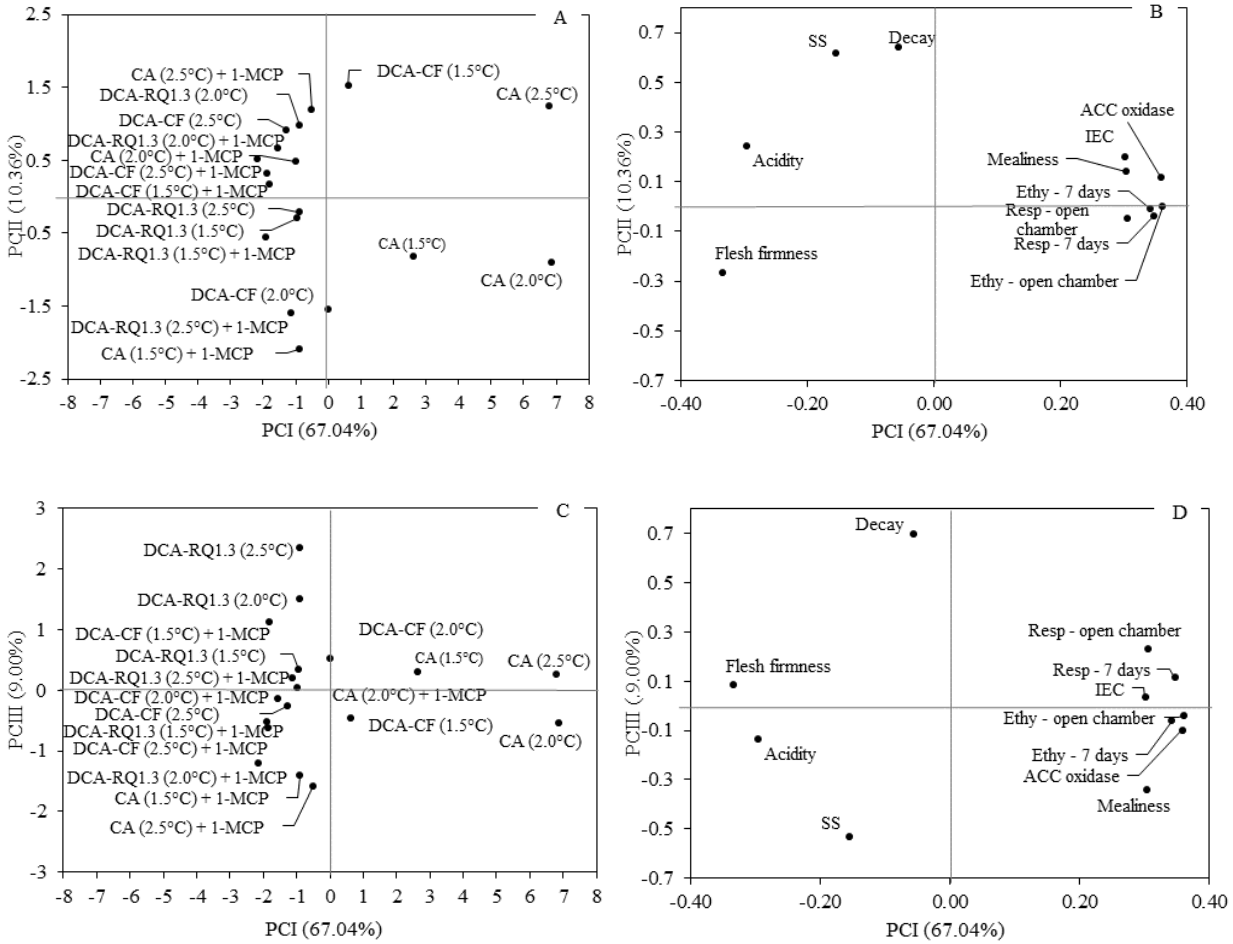

Fig. 5. (A and C) Scores (treatments) and (B and D) loadings (variables) plots showing the two major principal components of 'Galaxy' apples stored under controlled atmosphere, dynamic controlled atmosphere (DCA), during 9 months plus 7 days of shelf life. CA: controlled atmosphere; DCA-RQ1.3*: DCA-RQ1.3 with RQ calculation (stress by low O_2); DCA-RQ1.3**: DCA-RQ1.3 without RQ calculation (without stress); DCA-CF: dynamic controlled atmosphere by chlorophyll florescence; IEC: internal ethylene concentration; SS: soluble solids; Ethy: ethylene; F firm: flesh firmness; Resp: respiration rate; ACCO: ACC oxidase activity; M permeability: membrane permeability.

Temperature and oxygen partial pressure are the most important postharvest factors that affect fruit ripening (Yang & Hoffman, 1984; Ekman et al., 2005). 'Galaxy' apples stored without 1-MCP under CA showed higher ACC oxidase activity, IEC, ethylene production and respiration rate than apples stored under DCA conditions. Fruit temperature has a direct effect on the speed of chemical and biochemical reactions (Ekman et al., 2005). O_2 is required in the last step of ethylene biosynthesis, catalyzed by ACC oxidase (Yang & Hoffman, 1984). It is possible to

observe that DCA-RQ1.3 and DCA-CF, which employs low O_2 partial pressure during storage, results in lower ACC oxidase activity. This low enzyme activity results in lower IEC and ethylene production in fruit. In broccoli, Asoda et al. (2009) found that anaerobic respiration products produced by low O_2 partial pressure reduced ethylene biosynthesis by inhibiting gene expression (*BOACO1*, *BO-ACO2* and *BO-ACS1*) in the ethylene biosynthesis pathway.

1-Methylcyclopropene has a strong effect on ethylene action inhibition and, consequently, reduced ACC oxidase activity, IEC and ethylene production (Ferenczi et al., 2006; Brackmann et al., 2010) in apples stored under CA. On the other hand, 1-MCP did not have additional effect on ACC oxidase, IEC and ethylene production in 'Galaxy' apples stored under DCA-RQ at the three temperatures and DCA-CF at 2 °C. This demonstrated that 1-MCP has no additional effect when apples are stored under extremelly low pO_2. In relation to high temperatures, ACC oxidase and ethylene production increase with higher temperatures (Table 1, 2 and 3) under CA. However, fruit under DCA-RQ and DCA-CF did not show an increase in ethylene production and ACC oxidase activity, neither with or without the application of 1-MCP, which confirms previous results showed in article 2 of this thesis.

Flesh firmness was better maintained in fruit under CA with 1-MCP, for all the three temperatures. This is explained by the fact that 1-MCP blocked ethylene action and, as a consequence, enzymes that degrade cell walls such as polygalacturonase, β-galactosidase and pectin methylesterase (Prasanna et al., 2007) has its activity decreased. Kittermann et al. (2015) found that the increase of storage temperature in combination with 1-MCP treatment can be an useful tool to reduce energy usage during apple storage without a negative influence on fruit quality. Interestingly, when 1-MCP was applied and then apples were stored under DCA-RQ1.3, at three tested temperatures, as well as under DCA-CF at 2.0 and 2.5 °C, there was not effect of 1-MCP in the maintenance of flesh firmness. This information is very important to apple storers because it demonstrates that it is possible to keep 'Galaxy' apples under higher temperatures (2.0

and 2.5 °C) without 1-MCP when stored under DCA. Other works report low or none 1-MCP effect on apples stored under ultra low oxygen (Brackmann et al., 2012; Both et al., 2014). For apples without 1-MCP at 1.5 °C, only DCA-RQ had fruit with higher flesh firmness, presenting better results than DCA-CF. DCA-RQ1.3 at 1.5 °C without 1-MCP showed low ethylene production and IEC, which explains this result. With these results in hand, we generated a new question: could stress caused by extremely low pO_2 employed during DCA-RQ calculation (Figure 3) have any additional effect in maintaining apple quality? To answer this, we performed experiment 2.

Acidity is another characteristic that 1-MCP helped maintain under CA (Jung and Watkins, 2011). However, when 'Galaxy' apples were kept under DCA-CF and DCA-RQ1.3, 1-MCP did not have any additional effects on acidity maintenance. Low O_2 partial pressure employed under DCA storage have the same effect on the acidity degradation than 1-MCP. Probably, this fact occurs because of low ethylene production and respiration rate in apples under DCA when compared with CA, which reduced acidity degradation. Soluble solids (SS) showed an elevated percentage under DCA-RQ1.3 at 2.0 °C. At 2.5 °C, DCA-CF showed higher SS when compared with DCA-RQ1.3. The SS has an effect on fruit acceptance, and this is dependent on cultivar and flesh firmness (Harker et al., 2008). These authors found that with 'Gala' apples, SS and acidity were important for fruit acceptance only in fruit with higher flesh firmness. On the other hand, in regards to fruits with low flesh firmness, there was no effect of SS and acidity on consumer acceptance.

Fruit with mealiness have soft flesh and lack of juiciness during consumption (Nobile et al., 2011; Arefi et al., 2015). Temperatures of 2.0 and 2.5 °C did not increase this disorder. DCA-CF without 1-MCP showed a reduction in mealiness at 2.0 and 2.5 °C when compared to 1.5 °C. This can be explained by the reduction in ethylene production and IEC under higher temperatures, despite not being statistically different. 1-MCP reduced the incidence of mealiness on fruit stored

under CA at 2.0 °C and under DCA-CF at 1.5 °C. This condition is related with low ethylene production. Storage under low O_2 partial pressure (DCA-RQ and DCA-CF) reduced mealiness under a higher temperature (2.5 °C), regardless of the application of 1-MCP, which is resulted of delay of ripening in these conditions. Nobile et al. (2011) demonstrated the association of a novel α-L-arabinofuranosidase (α-AFase), a hydrolase acting on the pectic component of the cell walls, with mealiness of apple fruit. These authors found that the *MdAF3*, gene to α-AFase, is differentially regulated in distinct genomic contexts. They also showed that α-AFase activity, as well as *MdAF3* transcriptional levels, are not solely responsible for the complex mealy phenotype and its effect is modulated by the genomic context.

In Brazil, it is unanimous among storers that the main cause of fruit losses is decay. In regards to fruit decay, there was no difference in results from the tested storage conditions. The 1-MCP was effective in reducing decay in fruit stored under DCA-RQ1.3. The extremely low O_2 partial pressure associated with blocking ethylene action probably reduced fungi and decay development. On the other hand, some works report that 1-MCP may actually increase decay incidence (Janisiewicz et al., 2003; Both, 2015). Li et al. (2017) found that 5 uL L^{-1} of 1-MCP reduced decay incidence, lesion expansion, mycelial growth and spore germination of the *Penicillium expansum*. They have identified that 1-MCP enhanced the level of reactive oxygen species and increased damage to fungi's cell membrane. The difference in 1-MCP effects on decay incidence may be dependend ofcultivar and 1-MCP concentration.

In summary, between the two DCA conditions tested, DCA-RQ and DCA-CF, we can conclude that both allow the employment of higher temperatures (2.0 and 2.5 °C) for 'Galaxy' apples without application of 1-MCP.

4.1.4.2 Experiment 2

During the RQ calculation period (13 hours) for the storage chambers, O_2 partial pressure lowered drastically (Figure 1A). We hypostatized that this fact has contributed to the maintenance of apple quality, which was confirmed by the results shown above. Through PCA, we can see a clear separation between treatments (Figure 3A). PCI and PCII together explain more than 90% of the all variation. Along with PCI, CA treatment was separated from DCA storage conditions. Apple under CA has higher relation with mealiness, decay, ACC oxidase, ethylene production and respiration rate at the opening of the chambers and after 4 days shelf life (Figure 3A and 3B). Through PCII, there was a separarion between DCA-RQ1.3 with RQ calculation from DCA-RQ1.3 without RQ calculation and DCA-CF. DCA-RQ1.3 with RQ calculation has higher relation with flesh firmness. This indicates that the stress caused by low O_2 (Figure 2A) from RQ calculation process (13 hours two times a week) can be beneficial to apple quality conservation. It is probable that low O_2 stress produces anaerobic respiration products, such as ethanol and acetaldehyde (Saquet; Streif, 2008). Some works found that a short anaerobic period was beneficial to reduce physiological disorders and reduce the ethylene production in apples (Pesis, 2005; 2007; 2010; Asoda et al., 2009). Ethanol application at the 0.3 mL kg^{-1} apple $month^{-1}$ dose was effective to prevent fruit softening and to decrease ACC oxidase activity and ethylene production in 'Royal Gala' apples during CA storage (Weber et al., 2016).

All DCA storage conditions reduced ethylene production, but CA showed higher production of this vegetal hormone. This is explained by the higher O_2 partial pressure employed under CA, which allowed more ACC oxidase activity (Figure 4C). O_2 is required by ACC oxidase to catalyze the conversion of ACC into ethylene (Yang & Hoffman, 1984). Interestingly fact is that ACC oxidase under DCA-CF was lower than under CA, probably because of the lower O_2 partial pressure (0.4 kPa) employed under DCA-CF when compared to CA (1.2kPa). Fruit kept under DCA-RQ1.3 with RQ calculation showed lower ACC oxidase activity than DCA-CF. There was a tendency of apples kept under DCA-RQ1.3 with RQ calculation to show a lower ACC

oxidase activity than DCA-RQ1.3 without RQ calculation, which indicated that stress employed during RQ calculation is beneficial to maintain low fruit metabolism.

Flesh firmness is the primary edible quality factor (of apples) that contributes to consumer acceptance and preference (Harker et al., 2008). According to articles 1 and 2 of this thesis, DCA storage conditions were the most effective in maintaining flesh firmness during storage period and shelf life. In this experiment, fruit stored under DCA-RQ1.3 with RQ calculation showed the highest flesh firmness (Figure 4E). On the other hand, fruit stored under DCA-RQ1.3 without RQ calculation showed lower flesh firmness than fruit under DCA-RQ1.3 with RQ calculation. Even though the O_2 set point during storage period stayed at the same level under the two conditions, apples with RQ calculation suffered a reduction of O_2 during the closed period for the RQ measurement, which at some point has reached anoxia for some hours. In some cultivars, initial stress by low O_2 is beneficial to apple quality maintenance, such as to reduce superficial scald, maintain flesh firmness and green color (Pesis et al., 2010; Sabban-Amin et al., 2011). Regarding apple varieties produced in Brazil, several works were performed to evaluate initial O_2 stress (0.2 at 0.4kPa of O_2 on the first week), however, none show positive results (Brackmann et al., 2012; 2013; Both et al., 2014). In this experiment, the difference from the previous works is that O_2 stress was employed during all storage period, which can be a promising storage technique, along with RQ, to maintain apple quality with low fruit losses. However, more research is still needed. This is the first work evaluating stress by low O_2 during RQ calculation on apple quality maintenance.

Mealiness is a physiological disorder caused by pectin degradation in middle lamella which causes cell to cell contact rupture (Huang and Lu, 2010). This disorder is accelerated by ethylene, which increases pectin degradation enzymes (Prasanna et al., 2007). Fruit with low ethylene production and IEC had lower incidence of mealiness (Figure 4A, 4D and 4F). Apples kept under DCA-RQ1.3 with RQ calculation did not present this disorder, differing only from CA. On the other hand, fruit under DCA-RQ1.3 without RQ calculation had more than 2% of mealiness, which

indicates a tendency of lower mealiness in fruit submitted to stress by low O_2 during RQ calculation. Although the processes did not differ statistically, is important to highlight that 'Galaxy' apples under DCA-RQ1.3 with RQ calculation did not show fruit with flesh breakdown. In relation to decay incidence, fruit under DCA-RQ1.3 with RQ calculation showed lower percentage of decay than DCA-RQ1.3 without RQ calculation at the opening of the chambers. This demonstrated that apples submitted to stress by low O_2 partial pressure during RQ calculation had reduced fungi development and reduced fruit losses from decay.

It seems that stress caused by low O_2 partial pressure from RQ calculation, for any storage period is beneficial for apple quality maintenance. It is probable that it is associated with the induction of anaerobic respiration by extremely low O_2 partial pressure during RQ calculation (Figure 2A). After this induction, the O_2 increase reduces the anaerobic respiration metabolism. Thus, beneficial effect of low ethanol concentration is reached and its toxic effect is not reached, because O_2 is immediately increased above the set point, reducing anaerobic respiration. This is supported by some works, such as Asoda et al. (2010), that found effects of ethanol on ethylene biosynthesis reduction; and Weber et al. (2016), which found that ethanol application in low doses can be beneficial to apple quality conservation. In this case, it is possible that ethanol production was induced by extremely low O_2 weekly exposure during all storage period, resulting in a positive effect. It was proved that low O_2 at the beginning of storage period is no adequate to apple quality conservation (Brackmann et al., 2012; 2013; Both et al., 2014).

4.1.5 Conclusion

DCA-RQ1.3 and DCA-CF allowed the storage of 'Galaxy' apples under higher temperatures (2.0 and 2.5 °C) than typically recommended (1.5 °C).

Application of 1-MCP did not bring additional effects when 'Galaxy' apples were stored under DCA-RQ1.3 and DCA-CF at higher temperatures.

Without 1-MCP application, DCA-RQ1.3 at a low temperature (1.5 °C) is better than DCA-CF because it helps maintain higher flesh firmness and lower mealiness.

Under DCA-RQ1.3, the stress caused by low O_2 partial pressure during RQ calculation has a beneficial effect on the quality of 'Galaxy' apples after storage, maintaining higher flesh firmness, lower mealiness and lower decay incidence when compared to fruit stored without stress by low O_2.

4.1.6 Acknowledgements

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5. ARTIGO 3

5.1 Higher storage temperature in dynamic controlled atmosphere based on respiratory quotient influence volatile profile of 'Galaxy' apples[3]

Abstract

The aim of this study was to evaluate the effect of higher storage temperature (2.0 and 2.5 °C) on the production of volatile compounds of 'Galaxy' apple stored under dynamic controlled atmosphere monitored by respiratory quotient. The following conditions evaluated were: [1] controlled atmosphere (CA) (1.2 kPa O_2 + 2.0 kPa CO_2); [2] dynamic controlled atmosphere monitored by respiratory quotient 1.3 (DCA-RQ1.3) and [3] DCA-RQ1.5, both under 1.2 kPa CO_2 all treatments under temperature of 1.5, 2.0 and 2.5 °C (±0.1 °C). Higher temperatures (2.0 and 2.5 °C) in comparison with low temperature (1.5 °C) can be used under DCA-RQ1.3 without loss of important volatile compounds of 'Galaxy' apple, such as 2-methylpropyl acetate, butyl acetate, hexyl acetate, 2-methylbutyl acetate and 1-butanol. In DCA-RQ1.5 higher temperatures reduced total of esters and total of alcohols compounds in relation to lower temperature (1.5 °C). DCA-RQ1.3 and DCA-RQ1.5 at higher temperatures increase butyl acetate concentration in relation to CA condition. Higher temperature (2.0 and 2.5 °C) reduced ethylene production and respiration in 'Galaxy' apple under DCA-RQ1.5, this lower ethylene production reduced mealiness in those conditions.

Keywords: *Malus domestica*, aroma, anaerobic metabolism, ethylene, conservation, quality.

5.1.1 Introduction

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

The apple production in Brazil is based on few cultivars, being ‘Galaxy’ apple, a mutant of ‘Royal Gala’, the main apple produced in this country. Apples are generally stored in cold storage and controlled atmosphere (CA). The main factor that reduce fruit metabolism is the low temperature, which has direct effects on the velocity of chemical and biochemical reactions (Ekman et al., 2005). Together with temperature, low O_2 and high CO_2 partial pressure have strength effect on metabolism reduction. This alteration on gaseous composition in storage room reduces ethylene biosynthesis and respiration, consequently, maintain flesh firmness, acidity and reduce physiological disorders (Brackmann et al., 2015; Weber et al., 2015; Both et al., 2017).

There are several studies for apple stored under higher temperature during CA storage, however, in higher temperature the 1-methylcyclopropene (1-MCP) application is needed. 1-MCP is a chemical compound that inhibits ethylene action (Sisler; Serek, 1997). This compound reduced volatile production such showed in article 1 and by other authors (Raffo et al., 2009; Ortiz et al., 2010; Thewes et al., 2015a; Thewes et al., 2017), and may increase decay (Janisiewicz et al., 2003; Both, 2015) and flesh breakdown incidence in apple (Köpcke, 2015). According to article 2, apples storers can keep ‘Galaxy’ apple under higher temperatures (2.0 and 2.5 °C) if employ dynamic controlled atmosphere (DCA) without 1-MCP application.

DCA-RQ is a recent method to monitor low oxygen limit (LOL) tolerated by apple along to storage (Weber et al., 2015). Respiratory quotient is defined as the ratio of the CO_2 production to the O_2 consumption of the stored fruit (Boersig et al., 1988; Weber et al., 2015). Another method to monitor LOL is based on the ethanol production by fruit (Veltman et al., 2003) and through chlorophyll fluorescence (Prange et al., 2007; Wright et al., 2012). The RQ value proposed by some studies for DCA storage is above 1.0, which induces anaerobic respiration with a little ethanol production (Weber et al 2015; Brackmann et al., 2015). The first research employing DCA-RQ was published by Weber et al. (2015) on ‘Royal Gala’, which found that DCA-RQ2.0 maintain fruit quality comparable as those stored in DCA-CF and better than those stored in CA. In an

another work, ‘Galaxy’ apple stored on DCA-RQ1.5 showed lower ethylene biosynthesis, higher flesh firmness, lower mealiness and higher percentage of healthy fruit than DCA-CF and CA (Brackmann et al., 2015). Later, Bessemans et al. (2016) found that DCA-RQ control superficial scald and maintained flesh firmness and color on ‘Granny Smith’ apple. According to Both et al. (2017), ‘Royal Gala’ under DCA-RQ1.5 and DCA-RQ2.0 had higher key volatile compounds, such as butyl acetate and 2-methylbutyl acetate. This method is also efficient to maintain quality of apple at different maturity stages (Thewes et al., 2017).

Volatile compounds are important to fruit aroma, which is an important quality characteristic. The main apple aroma groups are aldehydes, alcohol and esters (Young et al., 1996), which are reduced by the storage under CA in comparison with cold storage (Brackmann et al., 1993; Bangerth at al., 2012). Esters contribute more than 80% of volatile compounds and produce fruity aromas in apple (Yang et al., 2016). In ‘Gala’ apples mutants, the key volatile compounds of aroma are butanol, hexyl acetate, butyl acetate, 2-methylpropyl acetate and 2-methylbutyl acetate (Young et al, 1996; Salas et al., 2011; Both et al., 2014). CA storage reduces ethylene biosynthesis and inhibits normal volatile production by limit ester biosynthesis, lipoxygenase activity and beta-oxidation (Brackmann et al., 1993; Lumpkin et al., 2014). This reduction depends on O_2 partial pressure (pO_2) employed. Both et al. (2014) showed that ‘Royal Gala’ apple under CA with pO_2 of 0.5 kPa (ultra low oxygen) does not have a negative effect on the 2-methylbutyl acetate as compared to the storage under CA (1.0 kPa). In another study, ‘Royal Gala’ stored under 0.7 kPa O_2 at 0.5 °C had a higher production of straight-chain esters, and the reduction of O_2 down to 0.5 kPa did not significantly suppress the production of most branched-chain esters, but reduces ethylene production and respiration rate (Both et al., 2016). In a recent research, Both et al. (2017) reported that apples stored at 1.0 °C under DCA-RQ1.5 and DCA-RQ2.0 showed a higher key volatile compound, such as butyl acetate and 2-methylbutyl acetate than DCA-CF. However, there are no information about effect of DCA-RQ under higher storage temperatures (2.0 and 2.5 °C) on

volatile compounds profile. Higher temperature for apple storage is important because reduce electrical energy cost, as reported by several authors (McCormick et al., 2012; Kittermann et al., 2015; Mazzurana et al., 2016).

Therefore, the aim of this study was to evaluate the effect of higher storage temperature (2.0 and 2.5 °C) on the production of volatile compounds of 'Galaxy' apple stored under dynamic controlled atmosphere monitored by respiratory quotient.

5.1.2. Materials and methods

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

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

Immediately after harvest, the fruits were transported to the Postharvest Research Center of the Federal University of Santa Maria, RS, Brazil. At the Postharvest Research Center, the same fruit were submitted to a selection process, aiming to eliminate fruit with any damage and homogenize the fruit size. Thereafter, samples of 25 fruit each were performed, 3 samples per treatment. At harvest, fruit showed iodine-starch index of 6.4, soluble solids of 13.4%, titratable acidity of 0.34 mg malic acid $100g^{-1}$, respiration rate of 14.4 µg CO_2 kg^{-1} h^{-1}, ethylene production of 1.72 ng C_2H_4 kg^{-1} h^{-1} and flesh firmness of 77 N.

5.1.2.2. CA, DCA-RQ, temperature and relative humidity conditions

After the sample preparation, fruit were put into 0.233 m^3 experimental chambers with the following conditions (treatments): [1] Controlled atmosphere (CA) (1.2 kPa O_2 + 2.0 kPa CO_2); [2] Dynamic controlled atmosphere with respiratory quotient (DCA-RQ) 1.3 and [3] DCARQ1.5 with 1.2 kPa CO_2. The temperatures was 1.5, 2.0 and 2.5 °C for all treatments.

The storage temperatures were monitored daily during the 9 months of storage with mercury thermometers inserted in the fruit to determine the pulp temperature. Inside the storage chamber, the relative humidity was monitored manually with psycrometers and controlled by calcium chloride trap, which allowed to absorb the excess of humidity inside the chamber, maintaining an average relative humidity of 94 ± 2%.

5.1.2.3. CA and DCA setup and maintenance

The experimental chambers were hermetically closed and the storage conditions setup. At the first storage day, the temperature was reduced down to 5.0 °C and thereafter gradually down to 1.5, 2.0 and 2.5 °C in about 4-5 days. At the day that the temperature reached the pre-established level, the CA and DCA-RQ conditions were setup to obtain the desired atmospheric condition, therefore the chambers were flushed with nitrogen until to the oxygen pre-established level 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 4-5 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 storage condition setup.

Throughout the storage period the oxygen partial pressure was changed according to the fruit metabolism in DCA and maintained constant for CA. To measure the LOL in real time during storage, the respiratory quotient (RQ) was measured twice a week, according to the methodology proposed by Brackmann (2015), Brackmann et al. (2015) and Weber et al. (2015). Thus, the RQ was seated at 1.3 and 1.5, and the oxygen partial pressure changed accordingly to maintain this RQ level (Figure 1B and C). The RQ was calculated with a chamber closure of 13 hours between the first and second O_2 and CO_2 measurement. The RQ was calculated by the ratio between CO_2

production and O_2 uptake. In relation to the CA conditions, they were maintained according to the methodology proposed by Thewes et al. (2015b).

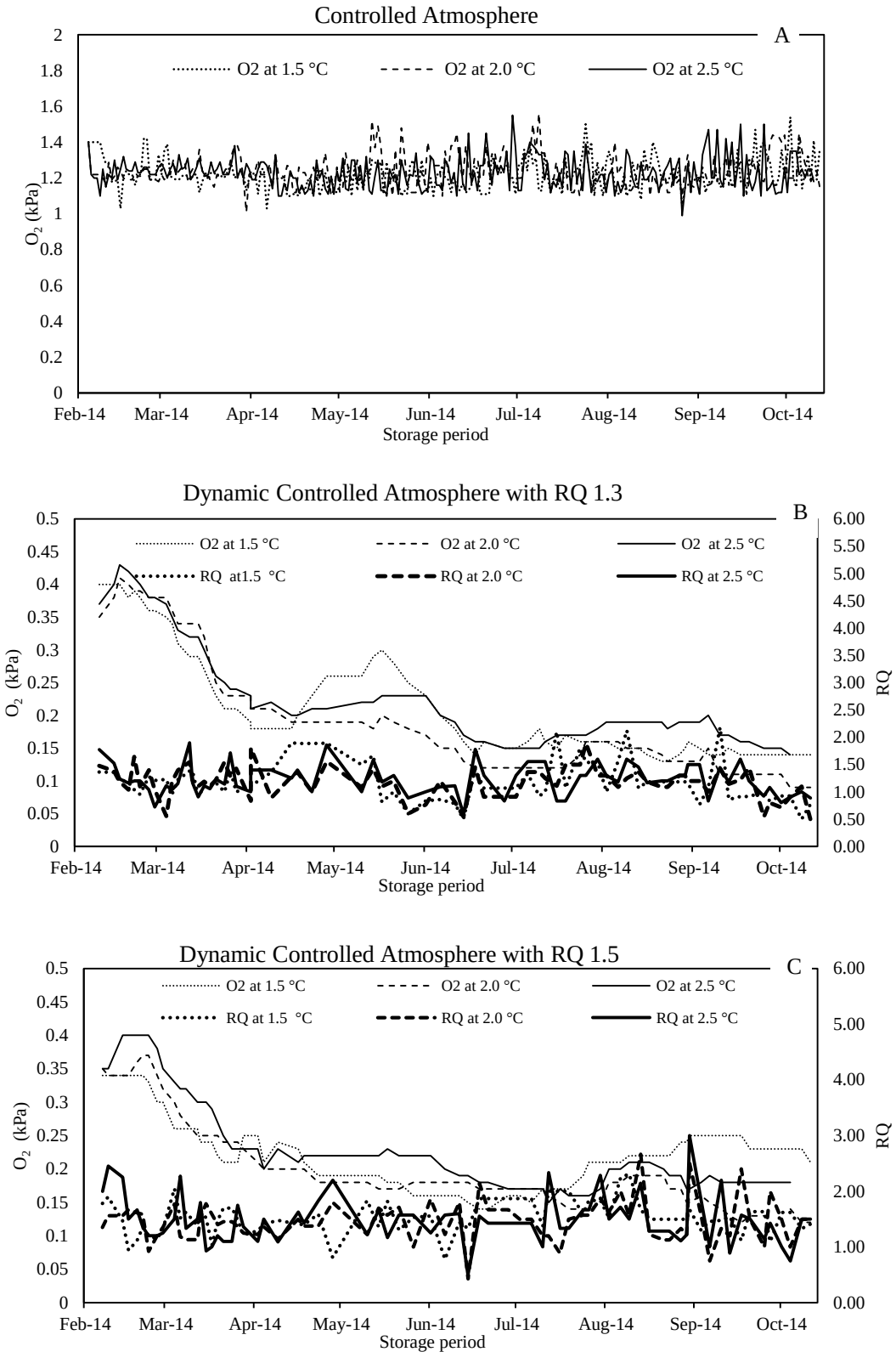

Fig. 1. Oxygen and respiratory quotient (RQ) level variation in CA (A), DCA-RQ1.3 (B) and DCA-RQ 1.5 (C) of 'Galaxy' apple under 1.5, 2.0 and 2.5 °C during 9 months of storage. CO_2 partial pressure was 1.2 kPa to DCA-RQ and 2.0 kPa to CA. Santa Maria. Brazil. 2016.

5.1.2.4. Metabolism and volatile compounds analysis

These following analysis were carried out at harvest and after 9 months of storage under storage conditions plus 7 days of shelf life at 20 ± 2 °C and relative humidity of 80 ± 2%: ethylene production, respiration rate, mealiness, and volatile compounds analysis were evaluated according to described in article 1. In the Table 1 is shown linear retention index and analysis at harvest to volatile compounds and other variables.

Table 1 – Linear retention index and analysis at harvest of volatile compounds of 'Galaxy' apple.

Volatile Compounds	LRI_{exp}	OTH[a]	At Harvest (μg L^{-1})
		Esters	
Methyl acetate	839	8,300[a]	0.09
Ethyl acetate	897	13,500[a]	1.00
Ethyl propanoate	962	40[f]	0.01
Ethyl 2-Methylpropanoate	968	NF	0.02
Propyl acetate	983	2000[a]	1.73
Methyl 2-Methylbutanoate	1015	8[f]	0.36
2-Methylpropyl acetate	1018	66[c]	3.08
Ethyl butanoate	1042	1[a]	0.02
Ethyl 2-methylbutanoate	1057	0.06[a]	388.2
Butyl acetate	1083	66[a]	19.7
2 Methylbutyl acetate	1128	11[a]	159.4
Butyl propanoate	1137	25[a]	0.08
3-Methylbutyl acetate	1168	2[d]	39.2
4-Pentenyl acetate	1192	NF	0.06
Butyl butanoate	1202	100[a]	2.40
Z-2-penten-1-yl-acetate	1241	NF	1.16
Hexyl acetate	1262	2[a]	420.9
Z-2-Hexenyl acetate	1286	NF	0.44
Z-3-Hexenyl acetate	1290	8[c]	3.42
E-3-Hexen-1-yl acetate	1294	NF	0.17
5-Hexen-1-yl acetate	1316	NF	5.23
E-2-hexenyl acetate	1321	7[c]	9.43
Heptyl acetate	1364	NF	0.09
Butyl hexanoate	1394	700[c]	0.07
Benzyl acetate	1726	364[c]	0.24

		Aldehydes	
Acetaldehyde	644	120[d]	0.08
Butanal	890	37[d]	0.22
Pentanal	920	NF	0.02
Hexanal	1099	5[a]	0.08
Z-3-Hexenal	1148	NF	3.08
Z-2-Hexenal	1205	NF	*nd*
E-2-Hexenal	1222	17[b]	75.1
		Alcohols	
Ethanol	945	100,000[d]	0.32
1-Butanol	1162	500[a]	33.7
4-Methyl-2-pentanol	1176	NF	0.31
3-Hexanol	1201	NF	0.06
2-Methyl-1-butanol	1211	250[a]	7.16
1-Pentanol	1249	4,000[a]	2.03
2-Methyl-2-buten-1-ol	1297	3[c]	*nd*
1-Hexanol	1352	500[a]	93.1
E-3-Hexen-1-ol	1361	NF	0.46
Z-3-Hexen-1-ol	1380	70[b]	0.17
E-2-Hexen-1-ol	1399	400[d]	9.26
E-5-Hexen-1-ol	1407	NF	1.57
E-1-Octen-3-ol	1445	NF	0.21
1-Heptanol	1454	3[d]	1.42
6-Methyl-5-hepten-2-ol	1465	2,000[d]	0.21
2-Ethyl 1-hexanol	1485	270,000[d]	0.47
		Ketones	
2-Propanone	831	500,000[d]	0.20
6-methyl-5-heptene-2-one	1334	50[d]	0.01

nd: not detectated. LRI_{exp}: Experimental Linear Retention Index; NF: not found

Odor threshold. References: [a] López et al. (2007); [b] Mehinagic et al. (2006); [c] Pino and Quijano (2012); [d] Leffingwell and Leffingwell (1991); [e] Takeoka et al. (1990); [f] Komthong et al. (2006); [g] Mass spectrum and retention time comparable to standard (Positively identified)

* Concentration were calculated relative to an internal standard (3-octanol).

5.1.2.5. 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). 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 bifactorial arrangement (3 storage conditions x 3 temperatures).

5.1.3. Results

After storage of 'Galaxy' apple under DCA and CA in three temperatures, 51 volatile compounds were identified, being 25 esters, 17 alcohols, 7 aldehydes and 2 ketones (Figures 4, 5 and 6). Analysis of principal component (PC) I, II and III explain together more than 83% of total variation of the treatments. PCI separated CA on three temperatures from the DCA-RQ1.3 in all temperatures and DCA-RQ1.5 at 2.0 and 2.5 °C (Fig. 2A). In PCI, CA is related with important apple volatile compounds like 2-methylbutyl acetate, hexyl acetate and 1-butanol, but is strongly correlated with respiration and ethylene production (Fig. 2B). Except DCA-RQ1.5 at 1.5 °C, other treatments were correlated with butyl acetate. DCA-RQ1.5 at 1.5 °C is not related with other DCA condition, in PCI and PCIII this treatment is correlated with CA (Fig. 3A), which are related with some important volatile compounds such as 2-methylpropyl acetate, 2-methylbutyl acetate, hexyl acetate and 1-butanol (Fig. 3B). Between temperatures, a clear separation is shown in DCA-RQ1.5, where DCA-RQ1.5 at 1.5 °C is correlated with more number of volatile compounds compared to DCA-RQ1.5 at 2.0 and 2.5 °C. However, DCA-RQ1.5 under higher temperature showed

association with butyl acetate. By PCI and PCII there are not separation of temperature under DCA-RQ1.3.

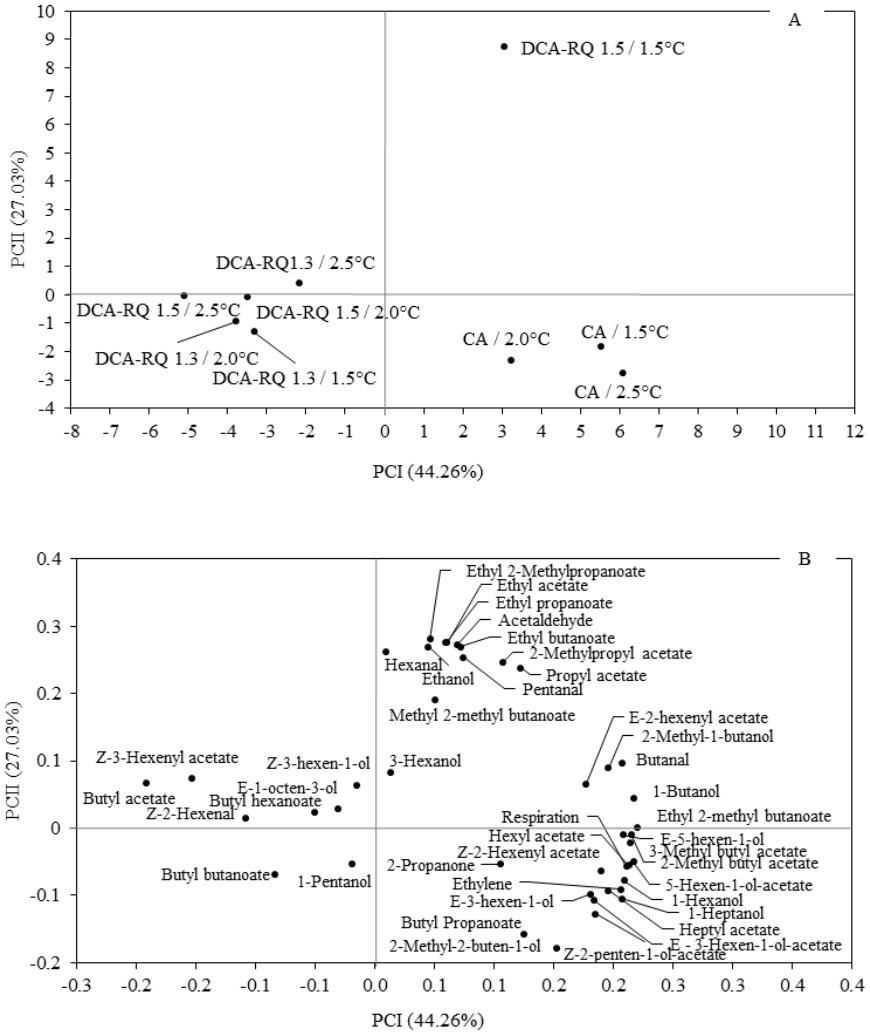

Fig. 2. (A) Scores (treatments) and (B) loadings (variables) plots showing the two major principal components (PCI versus PCII) of ‘Galaxy’ apples stored under controlled atmosphere and dynamic controlled atmosphere (DCA), during 9 months storage plus 7 days of shelf life.

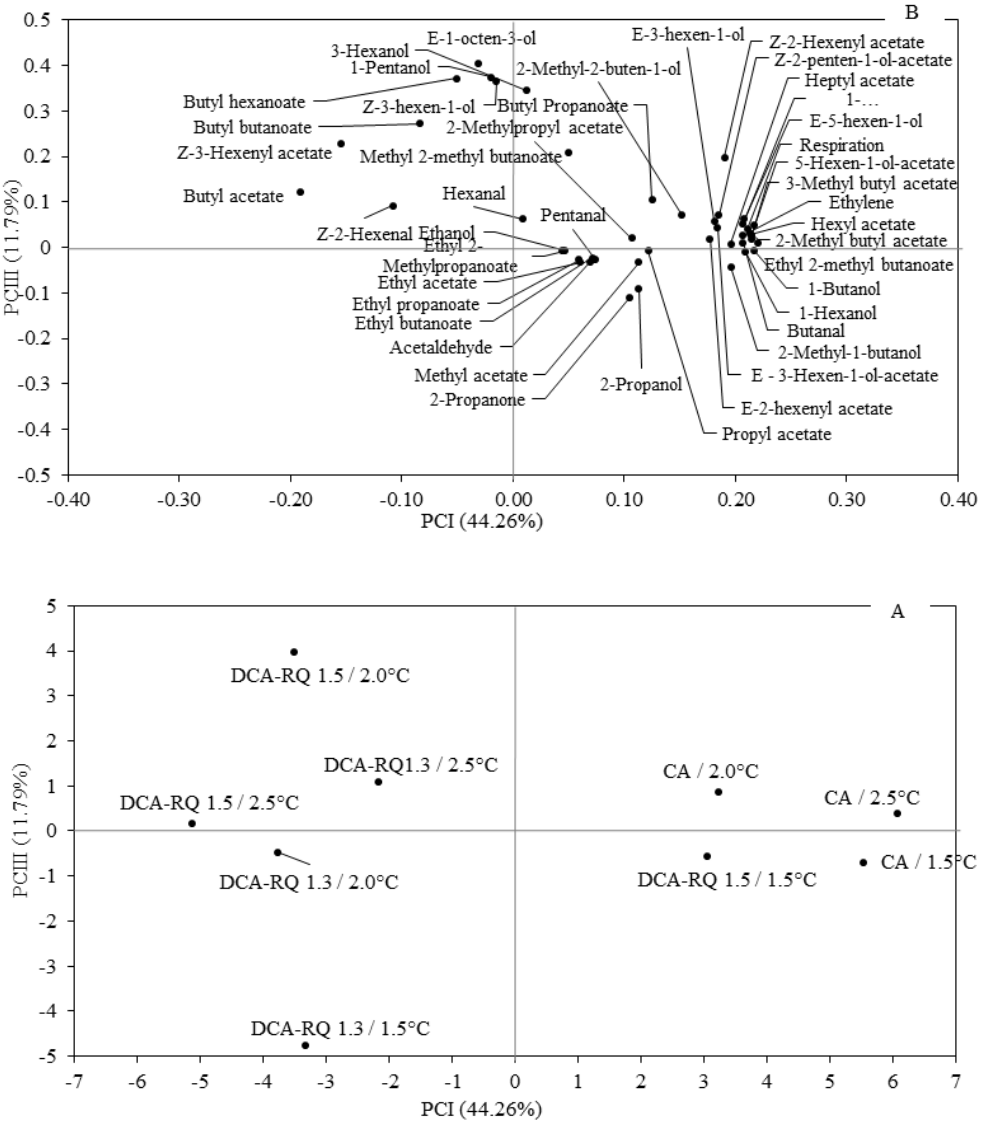

Fig. 3. (A) Scores (treatments) and (B) loadings (variables) plots showing the two major principal components (PCI versus PCIII) of ‘Galaxy’ apples stored under controlled atmosphere and dynamic controlled atmosphere (DCA), during 9 months storage plus 7 days of shelf life.

Higher temperature did not change total esters in ‘Galaxy’ apple under CA and DCA-RQ1.3. On the other hand, fruit in DCA-RQ1.5 stored at 2.0 and 2.5 °C showed reduction in total esters. Main apple odor is given by esters, among them butyl acetate have great participation, which gives apple odor. This compound had an increase under low O_2 partial pressure employed in DCA-RQ1.3 and DCA-RQ1.5 in temperatures of 2.0 and 2.5 (Figure 4K). When compared temperature

in each atmosphere condition, there is no change on butyl acetate concentration under higher temperature.

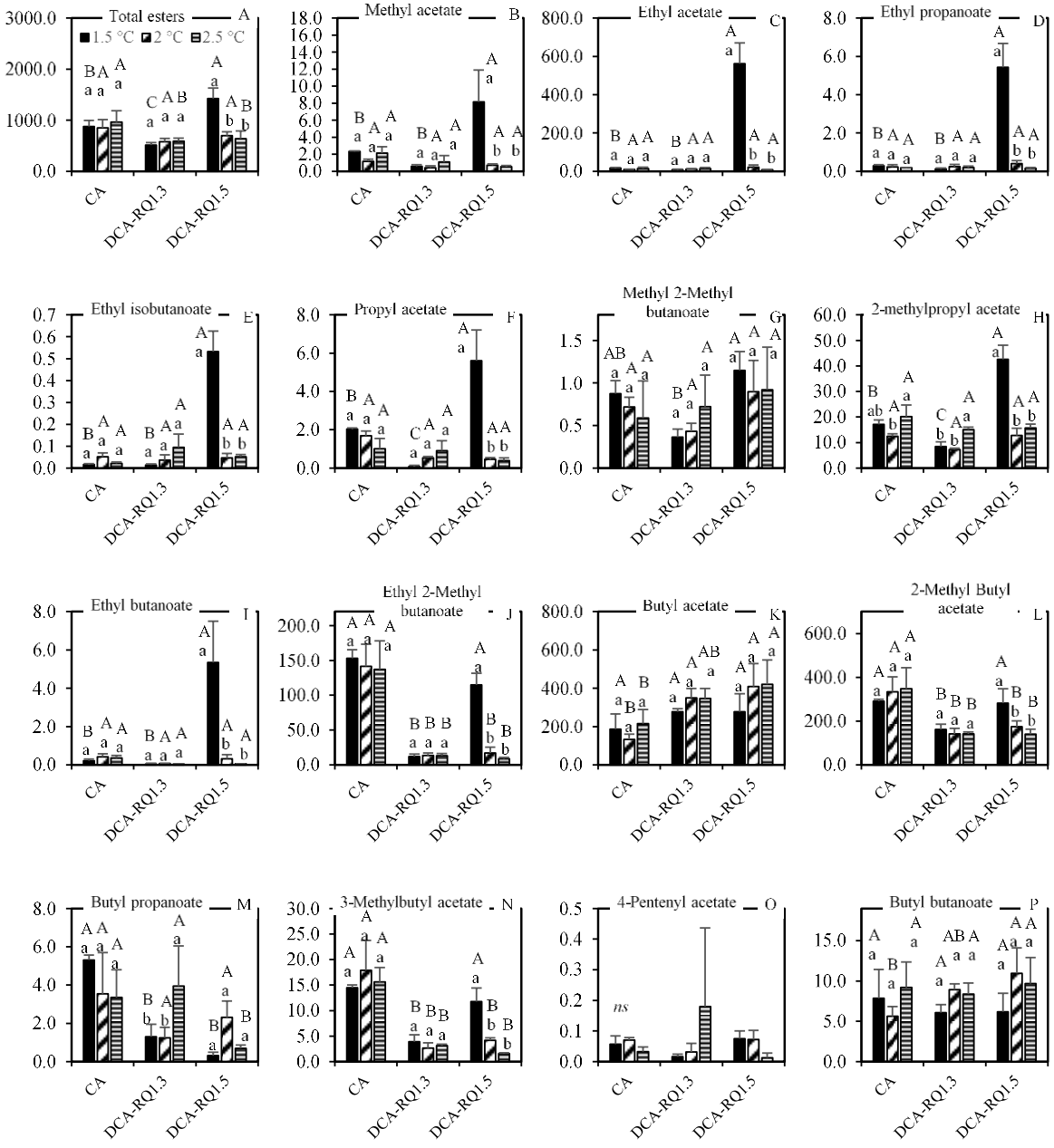

Figure 4 – Esters (µg L^{-1}) of 'Galaxy' apple under dynamic controlled atmosphere based on respiratory quotient (DCA-RQ) at 1.5, 2.0 and 2.5 °C, after 9 months of storage plus 7 days at 20 °C. Bars with the same lower case letter in the same atmosphere storage condition, and each bar with the same upper case letter in different atmosphere storage condition are not significantly different by Tukey's test, at 5% probability; ns: no significant; Error bars mean standard deviation.

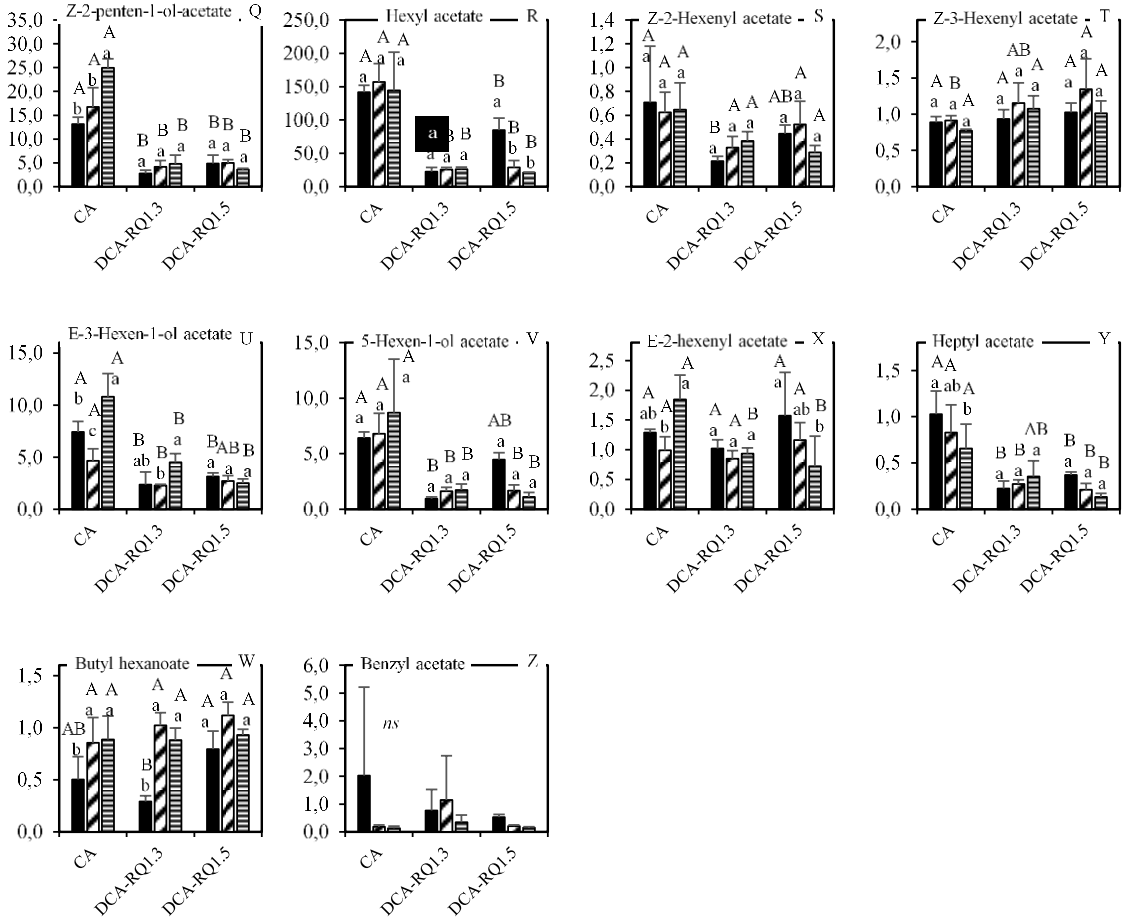

Figure 4 – Continued.

2-Methylbutyl acetate is another important volatile compounds to ‘Gala’ apple group, which showed reduction in apple stored at 2.0 and 2.5 °C under DCA-RQ1.5 in relation to 1.5 °C (Figure 4L). 3-Methylbutyl acetate showed the same results than 2-methylbutyl acetate (Figure 4N). High temperature did not have effect under CA and DCA-RQ1.3 on those esters. With exception of DCA-RQ1.5 at 1.5 °C, all DCA conditions showed lower 2-methylbutyl acetate and 3-methylbutyl acetate concentration, but higher than its odor threshold (Table 1). Another ester with apple and banana odor is 2-methylpropyl acetate. This compound had a decrease at 2.0 and 2.5 °C under DCA-RQ1.5 (Figure 4H), on the other hand, high temperature did not reduce 2-methylpropyl acetate under CA and DCA-RQ1.3.

There are many ethyl esters in fruit. In this work, ethyl acetate, ethyl propanoate, ethyl butanoate ant ethyl 2-methyl butanoate were identified (Figure 4C, 4D, 4E, 4I and 4J). Not all of these compounds changed with increase of temperature when stored in CA and DCA-RQ1.3. The interesting result is that DCA-RQ1.5 stored fruit had high ethyl esters under 1.5 °C, and low ethyl esters with higher temperature. Straight-chain ester as hexyl acetate (Figure 4R) showed similar result than ethyl esters which did not change with higher temperature under CA and DCA-RQ1.3. Hexyl acetate showed reduced concentration at 2.0 and 2.5 °C under DCA-RQ1.5. Moreover, DCA-RQ1.3 and DCA-RQ1.5 showed lower hexyl acetate concentration than CA (Figure 4R).

Many esters are produced from fatty acids, such hexanol, butanol, butyl butanoate, butyl hexanoate, Z-2-penten-1-ol-acetate, among others. Z-2-penten-1-ol-acetate showed higher concentration in CA at 2.5 °C (Figure 5Q). In DCA conditions, there are no differences between temperatures for this compound. DCA-RQ1.3 and DCA-RQ1.5 had lower Z-2-penten-1-ol-acetate, E-3-hexen-1-ol-acetate, 5-hexen-1-ol-acetate and heptyl acetate as compared to CA (Figure 4Q, 4U, 4V and 4Y). High temperatures in all storage conditions did not have effect in Z-2-hexenyl acetate. Linoleic acid is precursor of Z-2-hexenyl acetate and Z-3-hexenyl acetate (Figure 4S and 4T).

Such as total esters, the total alcohols did not change with different temperatures under CA and DCA-RQ1.3, but under DCA-RQ1.5, the temperatures of 2.0 and 2.5 °C showed lower alcohols production. 2-Methyl-1-butanol is a precursor of the 2-methylbutyl acetate. The concentration of this alcohol did not change with the increase of temperature under CA and DCA-RQ1.3 (Figure 5H). Similar with its ester, 2-methyl-1-butanol was reduced at 2.0 and 2.5 °C when stored in DCA-RQ1.5. One important alcohol in apple odor is 1-butanol, which is precursor of the butyl acetate. High temperature (2.0 and 2.5 °C) provides low 1-butanol concentration under CA and DCA-RQ1.5, but under DCA-RQ1.3 temperature did not have effect on this compound. In relation to ethanol, there is a clear interaction between O_2 partial pressure and temperature (Figure

5D). Apples stored at 2.5 °C under DCA-RQ1.3 showed higher ethanol concentration, while when stored at 2.5 °C with DCA-RQ1.5 the apples showed low ethanol concentration. The same tendency is shown in other alcohols such as 2-methyl-1-butanol and total alcohols (Figure 5A, 5E and 5H). Another important alcohol is 1-hexanol, which showed low concentration at 2.0 as 2.5 °C under CA, but under DCA did not show any difference between temperatures. Temperature did not change 2-propanone and total of ketones concentration under DCA-RQ1.3 and DCA-RQ1.5 (Figure 5B and 5T). However, apple stored at 2.5 °C under CA had higher 2-propanone and total of ketones.

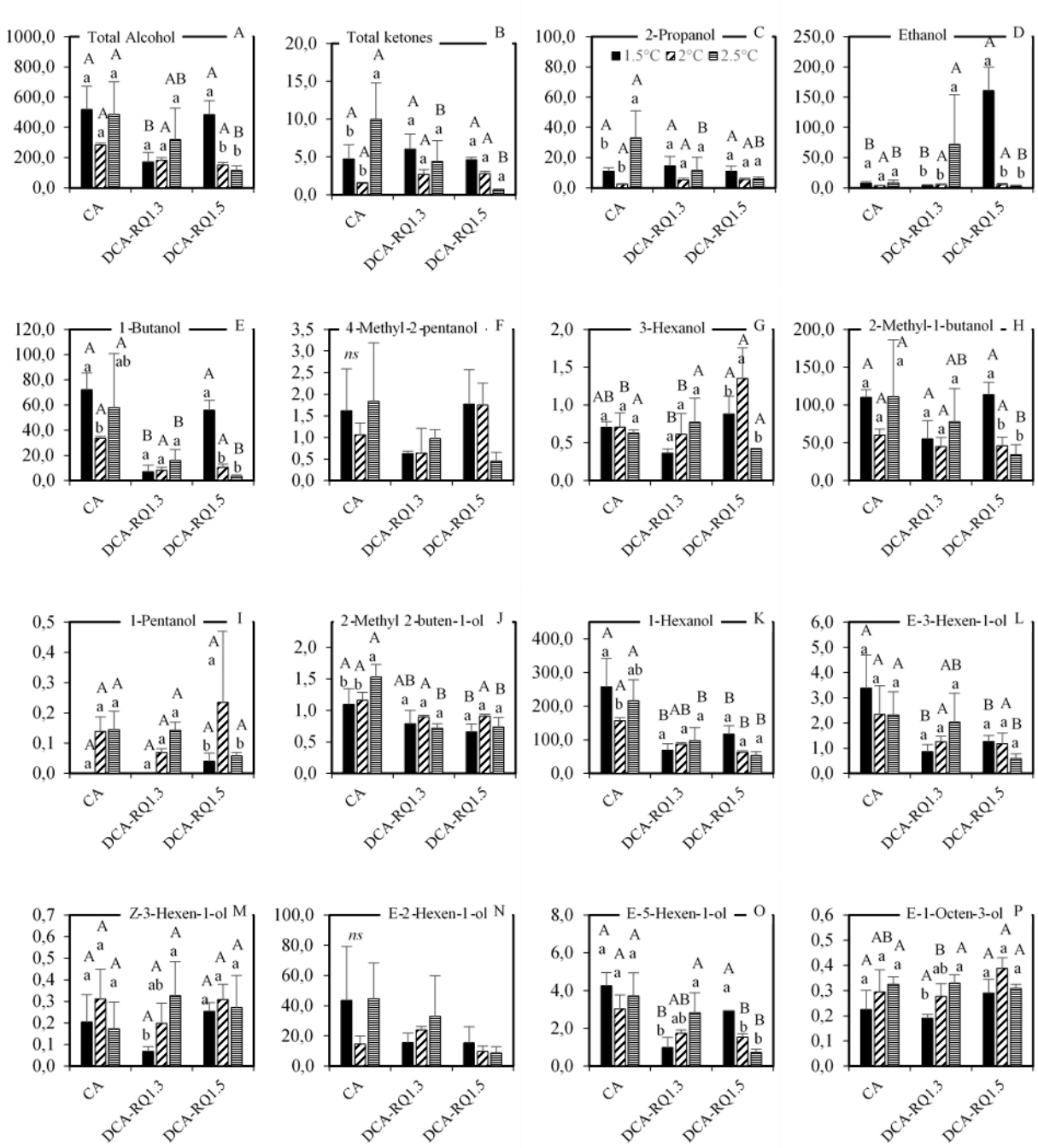

Figure 5 – Alcohol and ketones (μg L^{-1}) of 'Galaxy' apple under dynamic controlled atmosphere based on respiratory quotient (DCA-RQ) at 1.5, 2.0 and 2.5 °C, after 9 months of storage plus 7 days at 20 °C. Bars with the same lower case letter in the same atmosphere storage condition, and each bar with the same upper case letter in different atmosphere storage condition are not significantly different by Tukey's test, at 5% probability; ns: no significant; Error bars mean standard deviation.

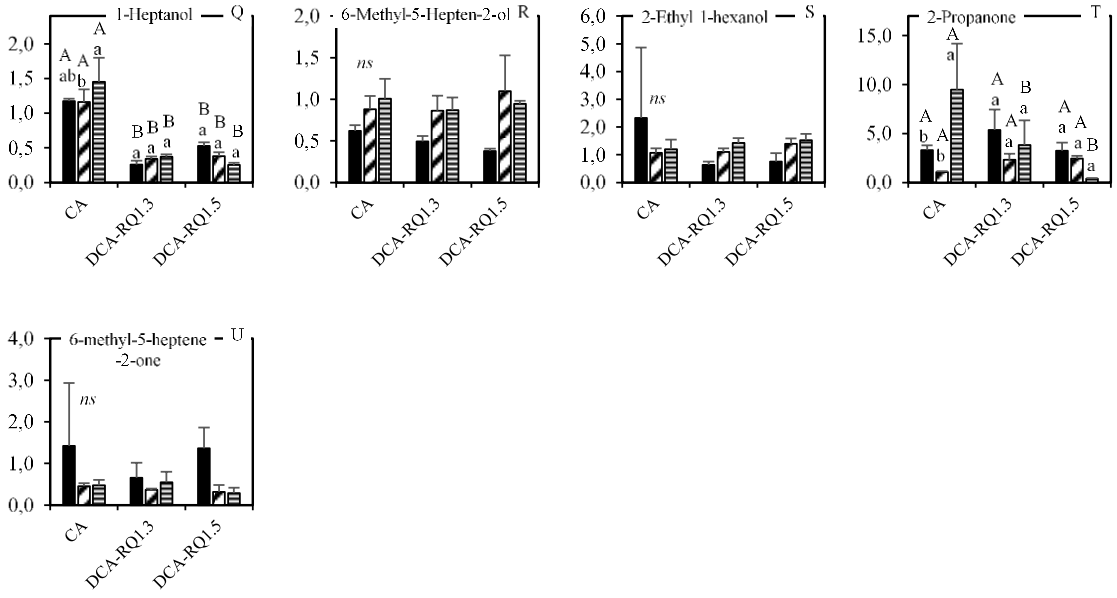

Figure 5 – Continued.

There are no any difference in total aldehydes by temperature and atmosphere conditions. However, important aldehydes such as acetaldehyde, butanal, hexanal and Z-2-hexanal were affected by storage condition (Figure 6A, 6B, 6C, 6E and 6G). Temperature did not change acetaldehyde and butanal under CA and DCA-RQ1.3 (Figure 6B and 6C), at the same time, apple under DCA-RQ1.5 showed higher acetaldehyde and butanal at 1.5 °C. Similar results were obtained to pentanal and hexanal (Figure 6D and 6E). 2-Hexenal was identified with cis and trans isomers (Figure 6G and 6H). These compounds are synthetized from linolenic fatty acid (Contreras et al., 2016). E-2-hexenal did not show difference between treatments, though Z-2-hexenal had higher concentration at 2.5 °C under DCA-RQ1.5.

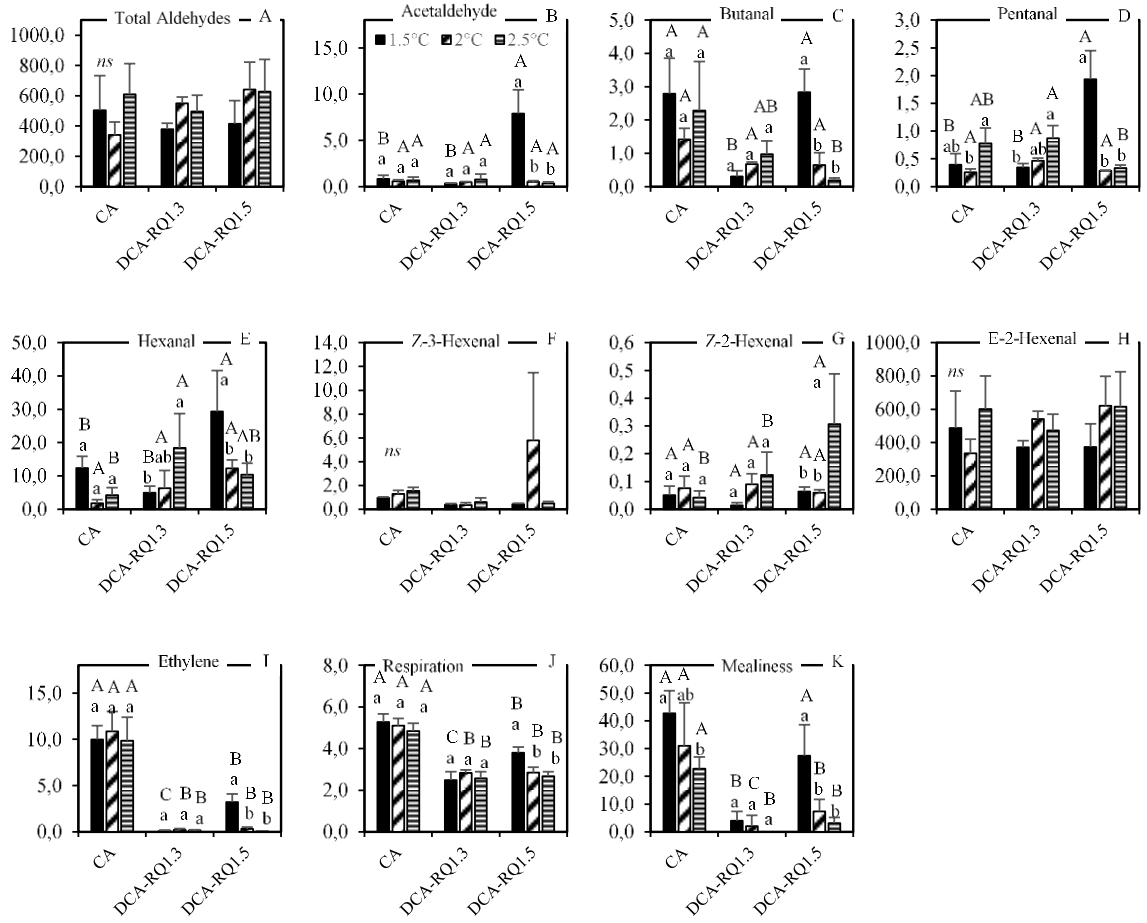

Figure 6 – Aldehydes (µg L^{-1}), ethylene, respiration and mealiness of 'Galaxy' apple under dynamic controlled atmosphere based on respiratory quotient (DCA-RQ) at 1.5, 2.0 and 2.5 °C, after 9 months of storage plus 7 days at 20 °C. Bars with the same lower case letter in the same atmosphere storage condition, and each bar with the same upper case letter in different atmosphere storage condition are not significantly different by Tukey's test, at 5% probability; ns: no significant; Error bars mean standard deviation.

Ethylene production and respiration were strongly reduced under DCA-RQ1.3 and DCA-RQ1.5 as compared to CA (Figure 6I and 6J). In relation to temperature, only under DCA-RQ1.5 there are differences. Higher temperature (2.0 and 2.5 °C) reduced ethylene production and respiration in 'Galaxy' apple under DCA-RQ1.5, this lower ethylene production reduced mealiness in those conditions.

5.1.4. Discussion

According to article 2, it is possible to store ‘Galaxy’ apple under DCA-RQ in higher temperature (2.0 and 2.5 °C) because it maintains high fruit quality without 1-MCP application. It is known that 1-MCP reduce volatile compounds (Raffo et al., 2009; Ortiz et al., 2010; Thewes et al., 2015a). According to article 2, when apples are stored under DCA-RQ or DCA-CF, 1-MCP did not have additional effect. For this reason, in this paper we evaluated the influence of the high temperatures (2.0 and 2.5 °C) on volatile biosynthesis of ‘Galaxy’ apple stored under DCA-RQ. The general information is that a higher temperature did not reduce the volatile compounds in this apple when stored under CA and DCA-RQ1.3.

Higher temperature did not change the total of esters in ‘Galaxy’ apple under CA and DCA-RQ1.3. On the other hand, fruit in DCA-RQ1.5 stored at 1.5 °C increased the total esters compared to 2.0 and 2.5 °C. This occurs because majoritarian esters such as ethyl acetate, 2-methylbutyl acetate and hexyl acetate had low concentration under higher temperatures in DCA-RQ1.5. This DCA condition at 1.5 °C resulted in higher anaerobic metabolism than at 2.0 and 2.5 °C, which is demonstrated by higher acetaldehyde and ethanol in fruit under DCA-RQ1.5 at 1.5 °C (Figure 5D and 6B). This fact results in high availability of esters precursors, such as alcohol and probably more acetyl-CoA to esters formation. Esters are synthetized by link an acetyl moiety from acetyl CoA with the appropriate alcohol (Defilippi et al., 2005). Despite the low concentration of some volatile such as 2-methylbutyl acetate and hexyl acetate in fruit stored in DCA-RQ1.3 and DCA-RQ1.5 at 2.0 and 2.5 °C their concentration are higher than the odor threshold (Table 1). It is important to highlight that ethyl acetate is related to off-flavors, however, in this work, the concentration was lower than the odor threshold in all storage conditions (Table 1).

Butyl acetate is a volatile compound with great importance in ‘Galaxy’ apple odor, which did not show change with high temperature, but showed higher concentration when stored under low O_2 partial pressure used in DCA-RQ1.3 and DCA-RQ1.5 (Figure 4K). 1-Butanol is a precursor of butyl acetate (Gonda et al., 2010; Yang et al., 2016). Under higher temperature, this alcohol

showed lower concentration under DCA-RQ1.3 and DCA-RQ1.5 than CA. This result is associated with the production of butyl acetate, which showed higher concentration in apple under DCA-RQ1.3 and DCA-RQ1.5 at 2.0 and 2.5 °C than CA. Probably, 1-butanol was used on esterification in apple under DCA-RQ1.3 and DCA-RQ1.5 at higher temperature. Low O_2 partial pressure and higher temperature may be offer more precursors, such as alcohol and acetyl-CoA to ester synthesis. Both et al. (2017) found an increase of butyl acetate in 'Royal Gala' apple stored under extremely low O_2 partial pressure (0.13 kPa) used during DCA-RQ2.0. This result is because branched-chain amino acid transaminases (BCAT) enzymes, which degraded amino acid into intermediaries to volatile compounds synthesis such as butyl acetate, showed high activity without ethylene action (Yang et al., 2016). Shin; Liao (2008) showed in bacteria that 1-butanol and 1-propranol, which produce butyl acetate and propyl acetate, are synthetized from threonine. Another pathway to synthesis butyl acetate is from glucose (Figure 7). Shen; Liao (2008), Lee et al. (2008) and Shen et al. (2011) found that in bacteria, glucose could be produce 1-butanol, which is esterified to butyl acetate production. In apple under extremely low O_2 partial pressure (DCA-RQ1.5) it is probable that butyl acetate is synthetized from glucose, because this pathway makes available more NAD^+ to use in glycolytic pathway to produce ATP at substrate level (Figure 7 – Pathway B).

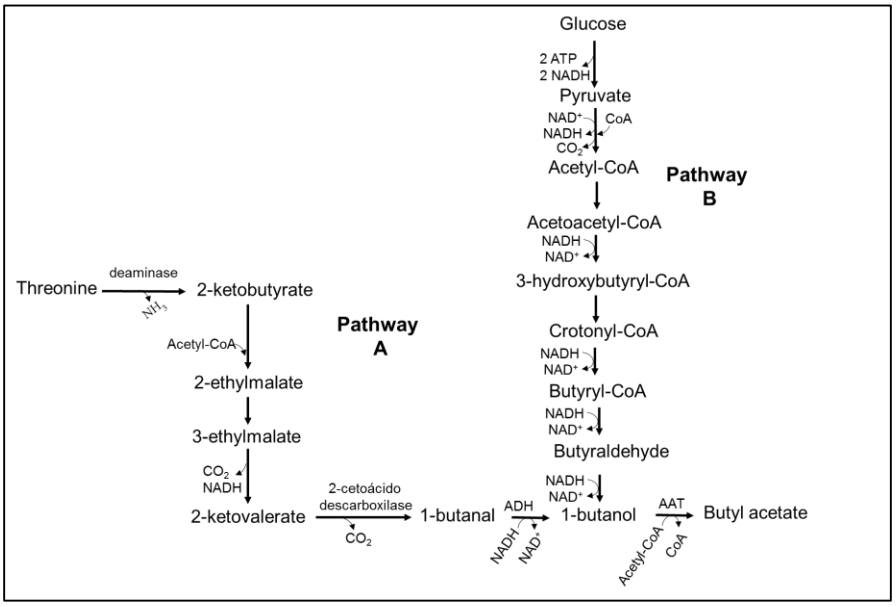

Figure 7 – 1-Butanol and butyl acetate biosynthesis from amino acid threonine (Pathway A) and glucose (Pathway B). Adapted from: Shen; Liao, 2008; Lee et al., 2008; Shen et al 2011.

Generally, ethyl esters did not show difference between atmosphere condition at higher temperature (2.0 and 2.5 °C) (Figure 4C, 4D, 4E and 4I). In the other hand, 'Galaxy' apple stored at 1.5 °C under DCA-RQ1.5 showed higher ethyl esters. These compounds are synthetized from amino acid alanine (Perez et al., 1992). Low O_2 partial pressure on 'Jonagold' apple (Bekele et al., 2015) and on soybean roots (António et al., 2016) resulted in alanine, valine and leucine accumulation. It is possible that low O_2 used in DCA-RQ1.5 at 1.5 °C increases glycolytic pathway rate in apple and accumulate pyruvate. This compound might be decarboxylase by pyruvate decarboxylase (PDC) or used to alanine synthesis. The higher alanine availability might be used to produce ethyl esters.

According to Young et al. (1996), 2-methylbutyl acetate is a key volatile compound in 'Gala' strain. In all treatments this compound showed a higher concentration than the odor threshold (Table 1). Higher temperature in apple kept under CA and DCA-RQ1.3 did not have influence on 2-methylbutyl acetate, which is related to 2-methyl-1-butanol (Figure 5J), the precursor of this ester. Isoleucine, a branched-chain amino acids, is precursor of intermediary compounds such as

α-keto-3-methylvalerate by action of branched-chain aminotransferase enzyme. The α-keto acid is decarboxylated to produce 2-methyl-1-butanal and, after, this aldehyde is dehydrogenated to produce corresponding alcohol, 2-methyl-1-butanol (Sugimoto et al., 2011; Kochevenko et al., 2012). It is probable that higher temperatures (2.0 and 2.5 °C) under CA and DC-RQ1.3, which did not use extremely low O_2 during all storage period (Figure 1), did not change the enzyme expression or activity for the branched-chain aminotransferase, keto-acid decarboxylase, alcohol dehydrogenase and alcohol acyltransferase. On the other hand, 'Galaxy' apple stored DCA-RQ1.5 at higher temperature showed reduced 2-methylbutyl acetate and 2-methyl-1-butanol. Most likely higher temperature associated with extremely low O_2 used in DCA-RQ1.5 caused reduction in one or more enzyme expressions or activities of the 2-methyl-1-butanol biosynthesis. Likely, the same behavior occurs with 3-methylbutyl acetate, which showed lower concentration in higher temperature when kept in DCA-RQ1.5. The 3-methylbutyl acetate is synthetized from α-ketoisocaproate, a keto-acid produced from amino acid leucine by branched-chain aminotransferase (Kochevenko et al., 2012). Another possible explanation to low concentration of these compounds is related to precursor availability (Brackmann et al., 1993; Ferenczi et al., 2006). Maybe apples under higher temperature in DCA-RQ1.5 showed lower glucose degradation into pyruvate, which reduced ethanol concentration (Figure 5D) and can be reduced precursor of methyl esters, such as keto-acid. The keto-acids are produced from pyruvate at the amino acid in the volatile pathway synthesis (Sugimoto et al., 2011).

Linoleic and linolenic fatty acids are precursor of important C6 volatile compounds such hexyl esters (Contreras et al., 2016). Linoleic is precursor of straight-chain ester such as hexanol and hexyl acetate. These compounds showed lower concentration in apple stored under DCA-RQ1.3 and DCA-RQ1.5 (Figure 4R, 5K). Low O_2 partial pressure inhibits enzyme such as AAT and ADH because low O_2 reduced ethylene biosynthesis (Figure 6I). Harb et al. (2011) found reduction on gene expression of AAT and ADH when applied 1-MCP, an ethylene action inhibitor.

Although, ester biosynthesis is more limited by reduced substrate synthesis than enzymes activity (Brackmann et al., 1993; Ferenczi et al., 2006). Inhibition of ethylene action caused lower apple volatile concentration (Thewes et al., 2015a). In relation to temperature, DCA-RQ1.5 at 2.0 and 2.5 °C showed lower concentration of hexyl acetate than DCA-RQ1.5 at 1.5 °C. In 'Royal Gala' apple stored under ultralow oxygen partial pressure (0.5 kPa) a significantly reduction on straight-chain ester was observed, but branched-chain esters were not reduced by oxygen lowering (Both et al., 2014).

Fatty acids, degraded by beta-oxidation or lipoxygenase (LOX), are precursors of straight-chain volatile (Defilippi et al., 2005; Contreras et al., 2013; Contreras et al., 2016). Linolenic acid is a precursor of esters such as Z-3-hexenyl acetate and E-2-hexenyl acetate (Contreras et al., 2016). Z-3-hexenyl acetate did not change concentration in different temperatures (Figure 4T) in all storage conditions. Temperature do not have effect on other C6 compounds such as Z-2-hexenyl acetate and 5-hexen-1-ol acetate. 5-Hexen-1-ol acetate showed lower concentration in apple under DCA-RQ1.3 and DCA-RQ1.5, it is in accord with Both et al (2017) that found lower concentration of these esters with DCA-CF, DCA-RQ1.5 and DCA-RQ2.0 in 'Royal Gala' apple.

The total of the alcohols did not change between temperatures in apple under CA and DCA-RQ1.5 (Figure 5A). DCA-RQ1.5 under low temperature (1.5 °C) provided higher alcohol concentration and aldehydes (Figure 6A). This occurs because DCA-RQ 1.5 at 1.5 °C caused higher mealiness in 'Galaxy' apple, which might reduce volatile compounds diffusion during storage and shelf life. The mealiness may be a chilling injury expressed under low temperature associated with extremely low O_2 partial pressure. Brackmann et al. (2014) in 'Brookfield' apples and Anese et al. (2016) 'Royal Gala' and 'Galaxy' apples found inverse correlation between mealiness incidence and gas diffusion rate in the fruit flesh. Alcohols such as 1-butanol and 2-methyl-1-butanol showed lower concentration under DCA-RQ1.5 at 2.5 °C than CA. This might occur because fruit under CA showed advanced maturity stage, with more ethylene production and

precursor for esters synthesis. In all temperatures, 1-heptanol and it ester heptyl acetate showed lower concentration under DCA-RQ1.3 and DCA-RQ1.5.

Total ketones and 2-propanone showed higher concentration under 2.5 °C under CA. Higher temperature did not change these compounds under DCA storage. At 2.5 °C, 2-propanone and total ketones showed lower concentration under DCA-RQ1.3 and DCA-RQ1.5. Both et al. (2014) found reduction in 2-propanone concentration when used low O_2 partial pressure in 'Royal Gala' apple. Aldehydes are precursors of alcohols and consequently of esters, which confers green flavor to apple. Temperature and atmosphere conditions did not change the total of aldehydes (Figure 6A). Z-3-hexenal and E-2-hexenal are two aldehydes from linolenic acid (Schwab et al., 2008), which did not change with temperature and atmosphere conditions. 13-LOX convert linolenic acid into hydroperoxides, after hydroperoxide lyase produce aldehydes. Aldehydes such as acetaldehyde showed higher concentration in apple under DCA-RQ1.5 at 1.5 °C. Both et al. (2017) associated higher acetaldehyde with physiological disorders in 'Royal Gala' apples. Acetaldehyde produces ethanol, which also showed higher concentration under DCA-RQ1.5 at 1.5 °C. It may be occur chilling injury in apple stored under low temperature (1.5 °C) with low O_2 partial pressure and induces anaerobic respiration, with acetaldehyde and ethanol production. Treatment under DCA-RQ1.5 at 1.5 ºC were kept under low O_2 partial pressure, mainly at the beginning of storage period (Figure 1C). The acetaldehyde and ethanol compounds, when in to high concentration can increase the physiological disorders (Pesis, 2005).

Mealiness incidence was related with ethylene production and respiration rate (Figure 5I, J and K). Apples under DCA-RQ1.3 or DAC-RQ1.5 showed lower ethylene beucause lower pO_2 was employed in these conditions, which resulted in lower respiration and mealiness. Mealiness is characterized by abnormal softness and lack of free juice in the fruit. This occurs by the degradation of the pectin in the middle lamella and causes the rupture of contact from cell to cell

(Huang; Lu, 2010). This disorder is accelerated by ethylene, which increases pectin degradation enzymes (Prasanna et al., 2007).

5.1.5. Conclusion

Higher temperatures (2.0 and 2.5 °C) in comparison with low temperature (1.5 °C) could be used under DCA-RQ1.3 without loss of important volatile compounds of 'Galaxy' apple, such as 2-methylpropyl acetate, butyl acetate, hexyl acetate, 2-methylbutyl acetate and 1-butanol. In DCA-RQ1.5 higher temperatures reduced these volatile compounds in relation to lower temperature (1.5 °C).

DCA-RQ1.3 and DCA-RQ1.5 at higher temperatures increase butyl acetate concentration in relation to CA condition.

DCA-RQ1.5 at higher temperatures reduces ethylene production, respiration and mealiness incidence of the 'Galaxy' apples.

5.1.6. Acknowledgements

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6. ARTIGO 4

6.1. Effect of growth regulators on quality traits and volatile compounds profile of 'Royal Gala' apple at harvest and after dynamic controlled atmosphere storage[4]

Abstract

The aim of this study was to evaluate the effects of naphthalene acetic acid (NAA) and aminoethoxyvinylglycine (AVG), isolated and combined, on the quality and volatile compounds profile of 'Royal Gala' apples at harvest and after 9 months of storage under controlled atmosphere (CA), dynamic controlled atmosphere with chlorophyll florescence (DCA-CF) and dynamic controlled atmosphere with respiratory quotient 1.3 (DCA-RQ1.3) conditions. Pre-harvest treatments were: [1] Control: water only; [2] NAA (40g ha^{-1} of NAA - Fruitone^{TM}) applied 7 days before harvest (BH); [3] AVG (0.83 kg $ha^{-1}$of Retain at 15% a.i.) 30 days BH; [4] AVG + NAA (30 + 7 days BH, respectively). Each pre-harvest treatment was stored under the following conditions: [1] CA (1.2 kPa O_2 + 2.0 kPa CO_2); [2] DCA-CF with 1.2 kPa CO_2; [4] DCA-RQ1.3 + 1.2 kPa CO_2. AVG+NAA applications in preharvest caused a higher increase in some important volatile compounds such as 2-methylbutl acetate, hexyl acetate and butyl acetate in 'Royal Gala' apples after long term storage under CA, when compared to DCA-CF and DCA-RQ1.3 storage. AVG+NAA application in preharvest, associated with storage under DCA-CF or DCA-RQ1.3, is a promising tool to maintain higher flesh firmness, reduce physiological disorders and maintain higher percentage of healthy fruit of 'Royal Gala' apples. However, NAA and AVG did not increase the concentration of volatile compounds in 'Royal Gala' apples stored under DCA-CF and DCA-RQ1.3.

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

Keywords: aminoethoxyvinylglycine; naphthalene acetic acid; quality; physiological disorders

6.1.2. Introduction

'Gala' apples and mutants show fruit drop before fruit has developed an optimum red color, size and maturity, which causes expressive economic losses (Yuan; Carbaugh, 2007; Unrath et al., 2009). Thus, apple growers apply growth regulators to reduce fruit drop, such as naphthaleneacetic acid (NAA) and aminoethoxyvinylglycine (AVG) (Steffens et al., 2006; Brackmann et al., 2014; 2015; Arseneault; Cline, 2016). There is a lack of information about the effects of these growth regulators on quality and volatile profile of the apples stored under controlled atmosphere (CA) and dynamic controlled atmosphere (DCA).

Different from the CA storage process, which employs static O_2 partial pressure (pO_2), DCA changes pO_2 according to the physiological response of the fruit (Zanella, 2003; Weber et al., 2015), which allows store the fruit near the lowest oxygen limit (LOL) tolerated by apples. To keep apples near LOL, it is necessary to monitor stress by low pO_2 to avoid damages caused by excessive anaerobic respiration. For this, there are some methods available, based on the measurement of ethanol in the air inside the storage room (DCS – dynamic control system) or in fruit (ILOS-Plus) (Veltman et al., 2003); based on chlorophyll fluorescence (DCA-CF) (Prange et al., 2007); and based on respiratory quotient (DCA-RQ) (Weber et al., 2015; Brackmann, 2015). The least used method is DCS, because ethanol does not show a precise correlation with anaerobic respiration, since this alcohol may be metabolized in the fruit into synthetized esters (Brackmann et al., 1993; Wright et al., 2015). Another method is DCA-CF, which measures the fluorescence emitted by chlorophyll when fruit is submitted to stress by low pO_2 (Prange et al., 2007; Wright et al., 2012; Wright et al., 2015). In recent years, the DCA-RQ method was developed and studied in Brazil and other countries (Weber et al., 2015; Bessemanns et al., 2016; Both et al., 2017). RQ is defined as the ratio between CO_2 production and O_2 consumption by the stored fruit (Boersig et

al., 1988; Weber et al., 2015). Both et al. (2017) and Thewes et al. (2017a) have found that DCA-RQ1.5 and DCA-RQ2.0 showed higher levels of key volatile compounds in ‘Royal Gala’ and ‘Galaxy’, respectively, such as butyl acetate, 2-methylbutyl acetate and hexyl acetate, than CA. Volatile compounds are synthetized from amino acids and fatty acids by the action of enzymes such as lipoxygenase (LOX), alcohol acyltransferase (AAT), which are affected by ethylene concentration (Defilippi et al., 2005; Contreras et al., 2013; Contreras et al., 2016; Yang et al., 2016).

The NAA is a synthetic auxin that reduces apple abscission, even though it accelerates postharvest fruit ripening by increase of ACC (1- aminocyclopropane-1-carboxylate) synthase, consequently increasing ethylene production and fruit softening, reducing storage potential (Yuan; Carbaugh, 2007; Li; Yuan, 2008; Unrath et al., 2009; Brackmann et al., 2014). Moreover, NAA increases gene expression of the ethylene biosynthesis (*MdACS1* and *MdACO1*), of the ethylene perception (*MdERS1*) and of apple cell wall degradation (*MdPG1*) (Li; Yuan, 2008; Yuan; Li, 2008). Shin et al. (2016) found that auxin could be critical in determining the time of activation of ethylene biosynthesis genes (*MdACS3* and then *MdACS1*) in maturing apple fruit. Brackmann et al. (2014) found that NAA applied in the field, increases the ripening rate and reduces gas diffusion of ‘Brookfield’ apple after storage under CA. We hypothesize that NAA can increase the concentration of volatile compounds in ‘Royal Gala’ apples stored under DCA-CF and DCA-RQ.

Another tool used by apple growers is AVG, which can reduce fruit drop, delay fruit ripening during storage and increase fruit size. AVG suppresses the gene expression of ACC synthase (*MdACS5A* and *MdACS5B*), ACC oxidase (*MdACO1*), and indirectly reduces gene expression of enzymes such as polygalacturonase (*MdPG2*) and cellulase (*MdEG1*) (Li; Yuan, 2008). Salas et al. (2011) found that the application of AVG suppresses volatile concentration, including alcohols and esters, in ‘Golden Delicious’ after 35 days at 8 °C. It is possible that apples treated with AVG during pre-harvest and stored under DCA will not show lower volatile compounds concentration

since DCA-RQ can enhance the concentration of volatile compounds (Both et al., 2017; Thewes et al., 2017a).

The aim of this study was to evaluate the effects of NAA and AVG, isolated and combined, on the quality and volatile profile of 'Royal Gala' apples at harvest and after 9 months of storage under CA, DCA-CF and DCA-RQ1.3 conditions.

6.1.3. Materials and methods

6.1.3.1. Plant material, orchard location, harvest maturity, sample preparation and treatments applied

Apples of the cultivar Royal Gala, a 'Gala' strain, were harvested in a commercial orchard located in Vacaria, RS, Brazil. The 'Royal Gala' apples were grafted on M9 rootstocks. A density of 3,575 plants ha^{-1} was used in the orchard. During the growing season, the following fertilization was carried out: 80 kg ha^{-1} of nitrogen and 120 kg ha^{-1} of potassium. Pre-harvest treatments were: [1] Control: water only; [2] NAA (40g ha^{-1} of NAA - Fruitone^{TM}) applied 7 days before harvest (BH); [3] AVG (0.83 kg $ha^{-1}$of Retain at 15% a.i.) 30 days BH; [4] AVG + NAA (30 + 7 days BH, respectively). An output of 1,000 L ha^{-1} of water was used in the treatments carried out in the field, according to Steffens et al. (2006). Fruit of treatment [1] control, [2] NAA and [4] AVG+NAA were harvested in 02-02-2015. Treatment [3] AVG was harvested one week later, 02-12-2015, a practice similar to the one used by apple growers.

Immediately after harvest, the fruit was transported to the Postharvest Research Center of the Federal University of Santa Maria, RS, Brazil. At the Postharvest Research Center the fruit was submitted to a selection process, aiming to eliminate fruit with any damage and homogenize fruit size. Thereafter, samples of 25 fruit each were picked, 4 samples per treatment. Each pre-harvest treatment was stored under the following conditions: [1] controlled atmosphere (CA) (1.2 kPa O_2 + 2.0 kPa CO_2); [2] Dynamic controlled atmosphere with chlorophyll florescence (DCA-

CF) with 1.2 kPa CO_2; [4] Dynamic controlled atmosphere with respiratory quotient 1.3 (DCA-RQ1.3) + 1.2 kPa CO_2, totalizing 12 treatments.

6.1.3.2. CA, DCA-RQ, DCA-CF, temperature and relative humidity conditions

After the sample preparation, fruit was put into 0.233 m^3 experimental chambers to set up each storage condition described above. The storage temperature was set 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 psychrometers and controlled with a calcium chloride trap, which allowed the absorption of excess of humidity inside the chamber, maintaining an average relative humidity of 94% ± 2%.

6.1.3.3. CA and DCA setup and maintenance

The experimental chambers were hermetically closed and the storage conditions installed. In the first storage day, the temperature was reduced down to 5.0 °C and then gradually down to 1.5 °C over 5 days. At the day that the temperature reached the pre-established level (1.5 °C), the CA, DCA-CF and DCA-RQ1.3 were setup, to obtain the desired atmospheric condition. Then, the chambers were flushed with nitrogen until the pre-established oxygen level for CA (1.2 kPa) was achieved, and reduced down to 0.5 kPa for DCA-CF and DCA-RQ1.3 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, and from the fifth day up to the tenth day the CA, DCA-CF and DCA-RQ1.3 conditions were installed. This procedure was carried out to simulate commercial storage conditions.

Throughout the storage period, the oxygen partial pressure (pO_2) was changed according to fruit metabolism in DCA and maintained constant for CA. To measure the LOL during storage, the respiratory quotient (RQ) was measured two times a week, according to the methodology

proposed by Weber et al. (2015). Thus, the RQ was set at 1.3, and the pO_2 changed accordingly in order to maintain this RQ level. The average pO_2 set point of the chamber to obtain DCA-RQ1.3 was 0.13kPa (minimum 0.07kPa and maximum 0.50kPa). The RQ was calculated with a chamber closure of 13 hours between the first and second measurement. The RQ was calculated by the ratio between CO_2 production and O_2 uptake. In relation to the CA conditions, they were maintained according to the methodology proposed by Thewes et al. (2017b), where pO_2 was 1.2kPa (±0.2kPa) and pCO_2 was 2.0 (±0.2kPa).

DCA-CF was monitored according to the instructions of Prange et al. (2007). The chlorophyll fluorescence was monitored from six apples during fruit exposure to low O_2. Thus, apples cooled to 1.5 °C were placed in a perforated plastic container (18 cm width, 27 cm length, 25 cm height) with the fluorescence sensors attached to the inside of the container top. The container was placed inside an experimental chamber; the chamber was sealed, and covered with black plastic to exclude light. The fluorescence monitoring system was activated and then O_2 was subsequently reduced to 0.5 kPa through N_2 injection. Then, the respiration process reduced the O_2 partial pressure until a change in fluorescence was detected. The lowest pO_2 was determined by identifying the pO_2 point where an inflection in the fluorescence signal was detected, and then by increasing O_2 up until 0.4 kPa as a safety factor. Chlorophyll fluorescence was monitored every hour for the entire storage period during the experiment. The average pO_2 set point of the chamber in DCA-CF was 0.4 kPa (minimum 0.00 kPa, during stress and fluorescence chlorophyll emission, and maximum 0.50kPa).

6.1.3.4. Metabolism and volatile compounds analyses

The metabolism and volatile compounds were analysed at harvest and after 9 months of storage under different storage conditions, plus 7 days of shelf life at 20 ± 2 °C and a relative humidity of 80% ± 2%.

6.1.3.4.1. Red skin color index: assessed subjectively through the identification of the skin area with red color, on a scale of 0 – 3, where 0 = < 25 % of the fruit skin red; 1 = ≥ 25 % up to 50 % of the fruit skin red; 2 = ≥ 50 % up to 75 % of the fruit skin red; 3 = ≥ 75 % to 100% of the fruit skin red, according to Brackmann et al. (2015). The average was obtained by multiplying the total number of fruit by their respective red skin level, the results was then divided by the total number of fruit in the sample.

6.1.3.4.2. Starch index: evaluated using the methodology proposed by Streif (1984), with an index of 1 (all fruit with starch) to 10 (without starch).

6.1.3.4.3. Skin color (*h*°): evaluated with a Minolta colorimeter, model CR 310. Results were expressed in hue angle (0° red; 90° yellow; 180°green and 270° blue);

6.1.3.4.4. Ethylene production, respiration rate, internal ethylene concentration (IEC), ACC oxidase, soluble solids, acidity, flesh firmness, decay, mealiness, flesh breakdown, healthy fruit and volatile compounds analysis were evaluated according to described in article 1. On table 1, the linear retention index and results of the analysis at harvest are shown.

Table 1 – Volatile concentration of 'Royal Gala' apple at harvest submitted to growth regulator treatments in orchard.

	LRI_{exp}	OT[1] (μg kg^{-1})	**Control**	**NAA**	**AVG**	**AVG+NAA**
			Esters			
Total esters[*]			13,063.2(±2,173)b[**]	62,673.8(±9,955)ab	53,930.1(±22,782)ab	123,616.1(±70,443)a
Ethyl acetate[g]	897	13,500[a]	2.76(±0.52)b	11.0(±2.04)ab	38.6(±20.9)a	18.6(±12.1)ab
2-Methylpropyl acetate	1,018	66[c]	10.5(±3.52)b	39.0(±10.9)ab	125.5(±73.3)ab	244.7(±146.6)a
Ethyl butanoate	1,042	1[a]	1.38(±0.47)[ns]	4.82(±2.03)	7.91(±6.21)	3.06(±2.15)
Butyl acetate	1,083	66[a]	6,374.4(±1,120)b	32,447.5(±5,869)ab	27,134.6(±14,733)ab	62,853.5(±37,461)a
2-Methylbutyl acetate	1,128	11[a]	1,495.3(±296)b	8,069.4(±1,523)ab	6,888.9(±3,495)ab	11,799.9(±6,743)a
Pentyl acetate			Nd	nd	91.4(±38) [ns]	247.6(±139)
Z-2-Penten-1-ol acetate	1,255	NF	64.4(±7.4) [ns]	295.2(±109)	Nd	nd
Hexyl acetate	1,262	2[a]	4,916.1(±741)b	21,458.6(±2,753)ab	19,124.2(±5,836)ab	47,836.3(±25,651)a
Z-3-Hexenyl acetate	1,290	8[c]	71.9(±15.4)a	39.1(±13.6)ab	41.5(±18.1)ab	19.2(±12.2)b
5-Hexeneyl acetate	1,316	NF	89.1(±16.8)b	249.7(±58.4)ab	176.9(±33.0)ab	342.0(±169)a
E-2-Hexenyl acetate	1,321	7[c]	Nd	nd	81.6(±15.6)[ns]	104.1(±48.3)
Heptyl acetate	1,364	NF	16.8(±3.16)[ns]	40.3(±14.9)	38.9(±18.4)	123.9(±85.6)
Benzyl acetate	1,726	364[c]	20.6(±5.16)[ns]	19.2(±10.7)	18.7(±6.01)	23.3(±21.0)
			Alcohols			
Total alcohols			4,005.3(±617)[ns]	12,365.0(±2,842)	11,514.5(±6,471)	25,228.3(±18,200)
2-Propanol	954		nd	nd	nd	nd
Ethanol[g]	945	100,000[d]	18.7(±8.70)[ns]	121.9(±25.1)	353.3(±238.6)	226.0(±148.0)
3-Methyl-Buten-2-ol			0.25(±0.11)[ns]	0.28(±0.16)	nd	nd
3-Methyl-2-butanol			78.6(±57.0)b	3,510.5(±718)a	71.3(±49.7)b	95.1(±55.6)b

1-Butanol[g]	1,162	500[a]	1,236.8(±229)[ns]	2,631.6(±542)	3,645.7(1,993)	7,266.2(±5,400)
1-Pentanol	1,249	4000[a]	44.8(±2.43)[ns]	134.3(±25.7)	158.0(±106)	121.2(±9.6)
2-Methyl-1-butanol	1,211	250[a]	275.2(±96.1)[ns]	380.5(±66.2)	774.5(±483)	1,362.9(±994)
Hexanol	1,362	500a	2,079.0(±203.4)[ns]	5,046.4(±1,332)	5,574.3(±2,950)	15,109.5(±10,835)
E-3-Hexen-1-ol	1,361	NF	59.1(±9.08)[ns]	164.6(±44.2)	100.1(±86.4)	245.9(±196)
Z-3-Hexen-1-ol	1,380	70[b]	17.9(±1.14)[ns]	23.1(±12.8)	51.5(±38.5)	47.7(±31.7)
E-2-Hexen-1-ol	1,399	400[d]	83.0(±11.0)[ns]	199.8(±51.7)	467.7(±445)	374.8(±284)
E-3-Hepten-1-ol			15.9(±2.02)[ns]	34.4(±10.2)	96.6(±80.5)	67.4(±63.1)
2-Methyl-1-pentanol			77.6(±10.3)[ns]	55.6(±19.7)	167.3(±117)	191.2(±129)
6-Methyl-5-Hepten 2 ol	1,465	2000[d]	8.68(±2.71)b	39.4(±10.2)a	9.99(±5.46)b	18.3(±16.0)ab
1-octanol	1,565	130d	1.82(±0.60)b	7.85(±2.90)b	22.7(±7.01)ab	48.3(±27.3)a
Menthol			0.67(±0.34)[ns]	4.46(±1.83)	4.00(±3.89)	5.45(±2.08)
2-undecanol			6.27(±1.79)[ns]	5.04(±0.75)	12.8(±6.37)	29.9(±31.6)
Phenethyl alcohol	1,854	750d	0.88(±0.31) [ns]	5.41(±1.49)	4.75(±3.41)	18.6(±30.1)
			Aldehydes			
Total aldehydes			8,554.3(±2,777)[ns]	22,918.8(±7,115)	10,628.2(±4,057)	44,991.2(±27,877)
Acetaldehyde[g]	644	120[d]	3.94(±1.86)[ns]	7.16(±0.82)	4.43(±3.23)	13.5(±9.07)
2-Methyl Butanal	<700		20.3(±5.93)[ns]	23.3(±4.35)	47.0(±34.9)	50.1(±30.5)
Hexanal	1099	5[a]	1,312.2(±359)b	4,792.7(±991)ab	3,039.6(±1,554)ab	9,549.3(±5,688)a
E-2-Hexenal	1,222	17[b]	7,217.3(±2,425)[ns]	18,095.6(±6,119)	7,524.0(±2,478)	35,373.0(±22,203)
E-2-nonenal	1,165	0.08[d]	0.69(±0.58)[ns]	nd	13.1(±14.0)	5.33(±3.22)
			Acids			
Total acids			366.3(±12.1)[ns]	546.7(±217)	418.7(±143)	700.1(±353)
Ethanoic acid			345.7(±13.9)[ns]	521.0(±214)	352.8(±100)	645.7(±319)
Hexanoic acid	1,782	3000[d]	9.46(±4.70)[ns]	13.7(±3.09)	35.8(±23.9)	12.7(±8.45)
Octanoic acid	1,977	3000[d]	6.93(±3.25)[ns]	9.68(±1.21)	23.7(±18.8)	38.8(±41.7)
Nonanoic acid	<2,000	3000[d]	nd	1.52(±0.42)[ns]	3.85(±3.03)	0.39(±0.10)
2-Ethylhexanoic acid	1,952	NF	4.15(±3.58)[ns]	0.86(±0.32)	2.50(±1.06)	2.45(±0.51)
			Ketones			
6-Methyl-5 Hepten-2-one	1,348	50[b]	nd	nd	162.4(±96.5)	180.5(±150.5)

[*]Means followed by equal letters do not differ by the Tukey test, at 5% probability; *ns*: no significant. nd: not detected NAA: naphthalene acetic acid; AVG: aminoethoxyvinylglycine. LRI_{exp}: Experimental Linear Retention Index; NF: not found Odor threshold. References: [a] López et al. (2007); [b] Mehinagic et al. (2006); [c] Pino and Quijano (2012); [d] Leffingwell and Leffingwell (1991); [e] Takeoka et al. (1990); [f] Komthong et al. (2006); [g] Mass spectrum and retention time comparable to standard (Positively identified) [**]Concentration was calculated relative to an internal standard (3-octanol).

6.1.3.5. Statistical analysis

All data was 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). Additionally, a variance analysis (ANOVA) with a 5% probability of error was carried out. Data showing a significant difference by ANOVA was subjected to Tukey's test at 5% probability of error. The experiment was conducted in a completely randomized scheme with a bifactorial arrangement (4 growth regulators x 3 storage conditions).

6.1.4. Results and discussion

6.1.4.1. Effect of growth regulator after harvest

Fruit previously treated with NAA, AVG and NAA+AVG were evaluated after harvest for quality parameters and volatile compounds concentration.

6.1.4.1.2. Metabolism and quality parameters

At harvest, there was no stimulation of maturation with the application of NAA. Fruit with NAA did not show differences from control in regards to ethylene production, internal ethylene concentration (IEC), ACC oxidase, respiration and starch index (Table 2). Other researchers found that naphthaleneacetic acid (NAA) accelerated apple postharvest ripening (Li; Yuan, 2008; Yuan; Li, 2008, Brackmann et al., 2014).

The fruit with AVG application, harvested one week later than control, did not show delay in ripening through the analysis of starch index, ACC oxidase activity, IEC and ethylene production (Table 2). Delay of harvest allowed fruit to synthesize ethylene and ripe normally. Brackmann et al. (2015) found a lower ethylene production at harvest on 'Brookfield' apple treated with AVG, however, harvest was performed in the same date of untreated fruit. On the other hand, apples with AVG+NAA application, harvested together with control, were less ripe than control. This occurred because AVG reduced ACC synthase activity, and reduced ethylene syntheses and their dependent processes. AVG suppressed the gene expression of ACC synthase (*MdACS5A* and *MdACS5B*), ACC oxidase (*MdACO1*), and indirectly reduced gene expression of enzymes such as polygalacturonase (*MdPG2*) and cellulase (*MdEG1*) (Li; Yuan, 2008).

Table 2 – Quality of 'Royal Gala' apple at harvest submitted to growth regulator treatments in orchard.

	Red skin color index (1-4)*	IEC (µg L^{-1})	Ethylene production (ngC_2H_4 $kg^{-1}s^{-1}$)	Respiration (µg CO_2 $kg^{-1}s^{-1}$)	Starch index (1-10)**	ACC oxidase (ng C_2H_4 $kg^{-1}s^{-1}$)
Control	2.73a***	1.19*ns*	0.23ab	6.11a	5.89ab	10.1ab
NAA	2.68a	1.40	0.41a	6.44a	5.40ab	5.13b
AVG	2.92a	0.64	0.25ab	4.86b	6.45a	17.8a
AVG+NAA	1.88b	0.58	0.16b	5.86a	4.69b	4.98b
cv (%)	4.40	36.7	28.3	4.77	9.20	43.1

	SS (%)	Acidity (mg malic acid $100g^{-1}$)	Flesh firmness (N)	Skin color (°*h*)
Control	11.0*ns*	0.37*ns*	84.1*ns*	50.0ab
NAA	11.5	0.40	84.9	41.2b
AVG	11.6	0.39	81.1	42.8b
AVG+NAA	11.2	0.41	80.6	57.5a
cv (%)	3.42	6.40	3.54	10.8

* index 1: 0 to 25% of the red skin color; 2: ≥25 to 50%; 3: ≥50 to 75% and 4: ≥75 to 100%;
** index 1: 100% of the starch; index 10: without starch;
*** Means followed by equal letters do not differ by the Tukey test, at 5% probability; *ns*: no significant.
NAA: naphthalene acetic acid; AVG: aminoethoxyvinylglycine

In some works, the application of AVG was associated with the reduction of red skin color in apples (Steffens et al., 2006). We did not find negative effects of AVG in red skin color, evaluateing both by index and colorimetric, in relation to control (Table 2). Red skin color is one of the main apple attributes considered by consumers when purchasing. This result can be explained by the harvest of fruit with AVG happening a week later than control, which allowed fruit metabolism to reestablish ethylene biosynthesis and to synthesize pigments normally, resulting in red skin color. AVG is a growth regulator widely used by apple growers to delay the start of harvest, and consequently optimize labor. Thewes et al. (2017b) report that due to the short harvest window, apples are either harvested before or after the correct maturity stage. When AVG+NAA was applied, fruit had a reduction in red skin color (index), despite not presenting differences from control in the evaluation performed through colorimeter. The fruit was harvested together with control, which can explain this result. Moreover, it showed low ethylene production

and starch index, indicating they were less ripe. Growth regulators did not influence IEC, soluble solids, acidity and flesh firmness at harvest.

6.1.4.1.3. Volatile compounds profile

There were changes in the concentration of volatile compounds with the application of growth regulators. After harvest, 41 volatile compounds were identified in 'Royal Gala' apples, being 13 esters, 17 alcohols, 5 aldehydes, 5 acids and 1 ketone (Table 1). Expressive change occurred in esters compounds, being total esters higher in fruit with AVG+NAA application, although differing only from control. This is an interesting fact because fruit with AVG+NAA had lower ethylene production, but had higher concentration of esters when compared to control. Esters are produced by esterification of alcohol and acyl-CoAs, catalyzed by alcohol acyltransferase (AAT). Fatty acids, degraded by beta-oxidation or lipoxygenase (LOX), are precursors of straight-chain volatiles, as hexyl acetate (Defilippi et al., 2005a; Contreras et al., 2013; Contreras et al., 2016), and amino acid form branched-chain volatiles as 2-methylbutyl acetate and 2-methylpropyl acetate (Yang et al., 2016). The main esters of the 'Gala' apple group, such as butyl acetate, hexyl acetate, 2-methylbutyl acetate and 2-methylpropyl acetate (Young et al., 1996), had higher concentration in appled with AVG+NAA application (Table 1), but did not differ from NAA and AVG applications, isolated. Therefore, 'Royal Gala' apples with NAA, AVG+NAA or AVG harvested later, can probably have better acceptance by consumer at harvest than fruit without a growth regulator, because of the higher amounts of volatile compounds.

Salas et al. (2011) found that AVG application suppresses volatile concentration, including alcohols and esters, in 'Golden Delicious' stored for 35 days at 8 °C. However, apples with AVG application were harvested in the same date of those without AVG application. We harvested apples treated with AVG one week later, similar to the procedures used by apple growers, who use AVG to delay harvest and reduce fruit drop. In our work, 'Royal Gala' apples did not present

differences between AVG and control in regards to esters, alcohols, aldehydes and acids concentrations (Table 1). There was a tendency of apples with AVG to have higher esters and alcohols. This is a result of the delay in harvesting, that allowed the fruit to start ripening, with higher production of ethylene. Enzymes involved in volatile biosynthesis as LOX, alcohol dehydrogenase (ADH) and AAT are dependent of ethylene (Harb et al., 2011; Schiller et al., 2015). The intriguing fact is that apples with AVG+NAA, although showing a lower ethylene concentration, had higher concentration of esters. Probably that a small concentration of ethylene is sufficient for AAT activation and esterification. Ban et al. (2010) found incomplete inhibition of *pMdAAT* by 1-methylcyclopropene (an ethylene action blocker), and that AAT gene expression may occur in low ethylene levels.

6.1.4.1.4. Effect of growth regulator after storage

By using principal component analysis, it is possible to separate along with PC I the fruit stored under CA from those under DCA-RQ1.3 and DCA-CF, regardless of growth regulator (Figure 1A and B). Fruit under CA showed higher correlation with important volatile compounds, such as butyl acetate, 2-methylbutyl acetate, hexyl acetate and 2-methylpropyl acetate. However, they also showed correlation with mealiness, flesh breakdown, decay, ACC oxidase, respiration rate and ethylene production, which are not good for apple quality maintenance. This occurred because CA employed higher O_2 partial pressure than DCA storage condition, which accelerated ripening through higher ACC oxidase activity and ethylene production. Ethylene production depends of pO_2 (Yang and Hoffmann, 1984). Along to PCII, there was a separation of the NAA/CA and control/CA from those fruit treated with AVG and AVG+NAA stored under CA (Figure 1A). This is explained by the fact that fruit under CA with AVG and AVG+NAA had higher ACC oxidase activity, ethylene production and IEC after storage, but fruit with AVG+NAA under CA also had higher number of healthy fruit, better flesh firmness and acidity (Table 4 and 5). In PCI,

PCII and PCIII there was no separation between DCA-RQ1.3 and DCA-CF when we observed fruit with the same growth regulator (Figure 1A and C). Both DCA types are related to conditions with less volatile concentration, although have higher relation with flesh firmness, acidity and healthy fruit, which are extremely important characteristics to in apple industry.

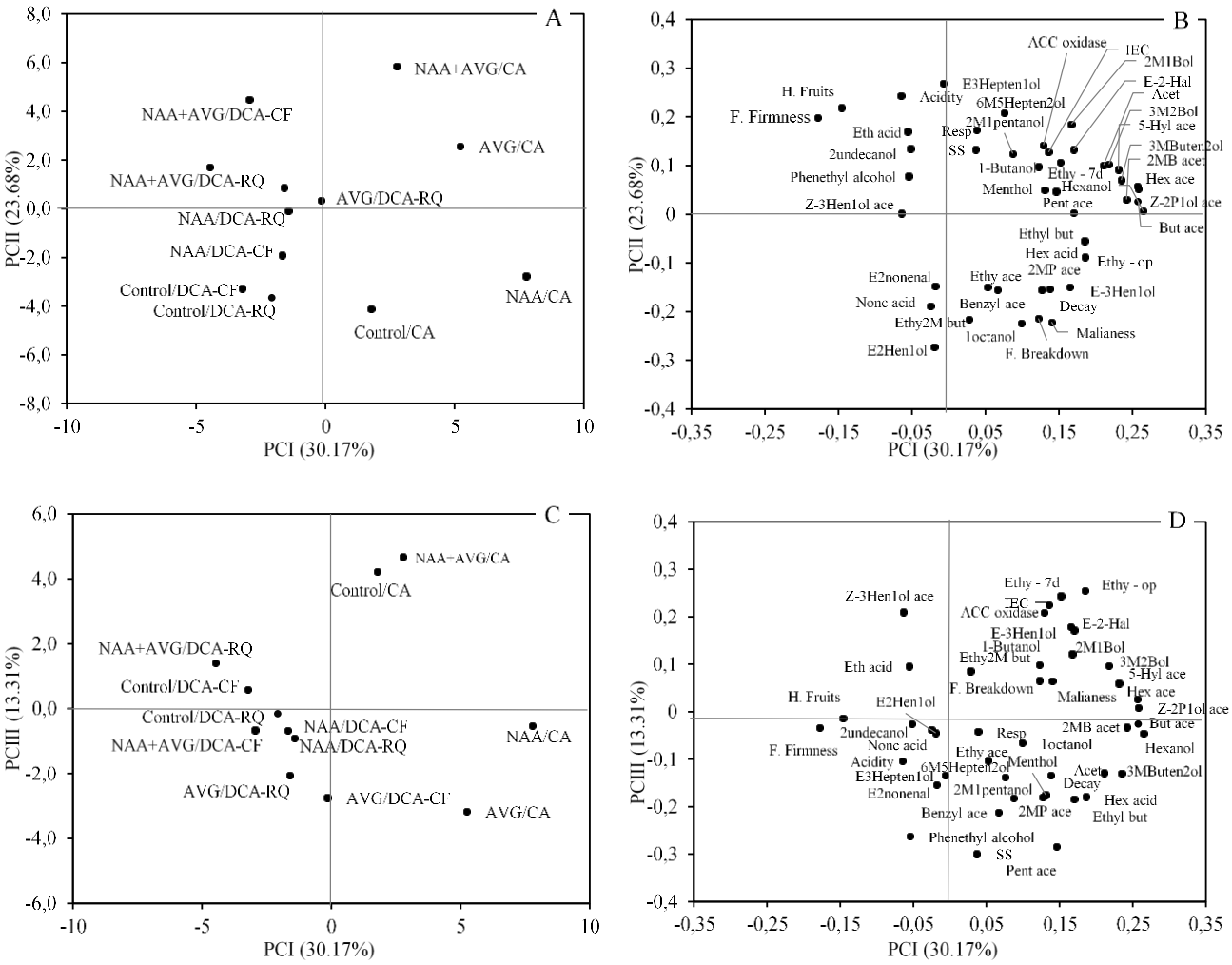

Figure 1 – (A and C) Scores (treatments) and (B and D) loadings (variables) plots showing the two major principal components of the 'Royal Gala' apple submitted to preharvest growth regulator application and stored under controlled atmosphere and dynamic controlled atmosphere during 9 months plus 7 days at 20 °C. Abreviation**:** NAA: naphthalene acetic acid; AVG: aminoethoxyvinylglycine; Control: without growth regulator application; CA: Controlled atmosphere; DCA-CF: dynamic controlled atmosphere with chlorophyll fluorescence; DCA-RQ1.3: dynamic controlled atmosphere with respiratory quotient 1.3; **Acet:** Acetaldehyde; **Ethy ace:** Ethyl acetate; **2MP ace:** 2-Methylpropyl acetate; **Ethyl but:** Ethyl butanoate; **3MButen2ol:** 3-Methyl-Buten-2-ol; **Ethy2M but**: Ethyl-2-methyl butanoate; **But ace**: Butyl acetate; **2MB acet**: 2-Methylbutyl acetate; **3M2Bol**: 3-Methyl-2-butanol; **Pent ace**: Pentyl acetate; **2M1Bol:** 2-Methyl-1-butanol; **E-2-Hal**: E-2-Hexenal; **Z-2P1ol ace**: Z-2-Penten-1-ol acetate; **Hex ace**: Hexyl acetate; **Z-3Hen1ol ace:** Z-3-Hexenyl acetate; **5-Hyl ace**: 5-Hexenyl acetate; **E-3Hen1ol:** E-3-Hexen-1-ol; **E2Hen1ol:** E-2-Hexen-1-ol; **E3Hepten1ol:** E-3-Hepten-1-ol; **Eth acid**: Ethanoic acid;

2M1pentanol: 2-Methyl 1 pentanol; **6M5Hepten2ol:** 6-Methyl-5-Hepten 2 ol; **Benzyl ace**: Benzyl acetate; **Hex acid**: Hexanoic acid; **Nonc acid**: Nonanoic acid; **F. Breakdown**: Flesh breakdown; **H. Fruits**: Healthy fruits; **F. Firmness**: Flesh firmness; **IEC**: internal ethylene concentration; **SS**: soluble solids; **ACC oxidase**: 1-Aminocyclopropane-1-Carboxylate oxidase; **Ethy – op**: Ethylene production at the open chamber; **Ethy - 7d**: Ethylene production after 7 days at 20 °C; **Resp:** respiration rate.

6.1.4.1.5. Metabolism and quality parameters

Ripening of the climacteric fruit is a process that depends on ethylene. In the ethylene biosynthesis pathway, there are enzymes, such as ACC oxidase, which convert ACC into ethylene employing O_2 to its activity (Yang; Hoffman, 1984). In this work, apples kept under DCA-CF or DCA-RQ1.3 had lower ACC oxidase activity, ethylene production and IEC than CA after storage, independent of the application of a growth regulator (Table 3). DCA employed lower pO_2 than CA, which reduces ethylene production more effectively. By the fact that DCA-RQ1.3 employs lower pO_2 than DCA-CF, there was a tendency of DCA-RQ1.3 to show lower ethylene production than DCA-CF, although without statistical difference.

Table 3 – Physical and chemical characteristic of 'Royal Gala' apple with preharvest treatments with growth regulators and stored under controlled atmosphere and dynamic controlled atmosphere during 9 months plus 7 days at 20 °C.

	Ethylene – opening of chambers ($ngC_2H_4\ kg^{-1}s^{-1}$)			Ethylene – 7 days at 20 °C ($ngC_2H_4\ kg^{-1}s^{-1}$)			
	CA*	DCA-CF	DCA-RQ1.3	CA	DCA-CF	DCA-RQ1.3	
Control	1.09Aab**	0.12Ba	0.05Ba	1.28Ac	0.14Aa	0.09Aa	
NAA	1.18Aa	0.13Ba	0.04Ba	4.24Ab	0.07Ba	0.08Ba	
AVG	0.95Aab	0.08Ba	0.03Ba	6.79Aa	0.82Ba	0.08Ba	
AVG+NAA	0.86Ab	0.13Ba	0.02Ba	8.08Aa	0.22Ba	0.04Ba	
cv (%)	28.7			34.1			
	ACC oxidase (ng $C_2H_4\ kg^{-1}s^{-1}$)			Respiration (µg $CO_2\ kg^{-1}s^{-1}$)			
Control	34.0Ac	14.9Bb	18.7ABab	4.42	2.88	3.01	
NAA	37.5Ac	16.4Bb	6.15Bb	5.28	2.20	3.22	
AVG	57.3Ab	40.4Aa	25.9Ba	5.63	3.34	2.76	
AVG+NAA	107.8Aa	20.5Bb	8.69Bab	5.00	3.27	2.73	
				5.08A	2.92B	2.93B	
cv (%)	26.6			21.4			
	IEC (µgL L^{-1})			Flesh firmness (N)			
Control	2.07Ac	0.51Aa	0.31Aa	52.7B	62.1Ab	65.5Ab	
NAA	19.3Ab	3.03Ba	0.20Ba	51.1Bb	60.7Ab	64.1Ab	
AVG	23.7Ab	2.13Ba	0.20Ba	53.2Bb	60.7Ab	63.5Ab	
AVG+NAA	42.9Aa	2.05Ba	0.26Ba	65.9Ba	71.7Aa	71.7Aa	
cv (%)	60.3			4.19			
	SS (%)			Acidity (mg malic acid $100g^{-1}$)			
Control	11.5Bc	12.1Ab	12.0Ac	0.27	0.30	0.29	0.29b
NAA	12.1Ab	12.4Ab	12.4Ab	0.29	0.29	0.31	0.30ab
AVG	12.7Aa	12.9Aa	13.0Aa	0.29	0.30	0.31	0.30ab
AVG+NAA	12.3Aab	12.1Ab	12.0Abc	0.31	0.32	0.31	0.32a
				0.29^{ns}	0.30	0.31	
cv (%)	2.01			7.63			

* Controlled atmosphere with 1.0 kPa of O_2 + 1.2 kPa CO_2. DCA dynamic controlled atmosphere with chlorophyll fluorescence (DCA-CF) or respiratory quotient (DCA-RQ1.3).

**Means followed by equal letters, lowercase in the columns and uppercase in the lines, do not differ by the Tukey test, at 5% probability; ns: no significant. RH: 94%; Temperature: 1.5 °C; CO_2 partial pressure to DCA = 1.2 kPa.

NAA: naphthalene acetic acid; AVG: aminoethoxyvinylglycine

Apples with NAA and AVG (applied isolated or combined), when stored under DCA-CF and DCA-RQ1.3 did not present a difference in ethylene production from the control (Table 3).

On the other hand, fruit kept under CA had higher ethylene production with NAA application at the opening of the chamber and with AVG and NAA+AVG after 7 days at 20 °C. IEC also did not present any difference when fruit was kept under DCA-RQ1.3 and DCA-CF. These results demonstrated that lower pO_2 used under DCA methods reduced ethylene production independent of the application of a growth regulator on preharvest. After storage, preharvest NAA application increased ethylene production after shelf life when fruit were kept under CA. On the other hand, fruit with NAA application and stored under DCA-CF and DCA-RQ1.3 did not increase ethylene production, due to the low pO_2 used on these conditions. After storage under CA, apples with AVG+NAA had higher IEC, ACC oxidase activity and ethylene production after 7 days at 20 °C. This is explained by less ripening of this fruit in the beginning of storage (Table 1). For these results, it is probable that climacteric of this fruit is happening, once fruit with AVG+NAA had higher flesh firmness after storage.

IEC reveals how much ethylene is in the intercellular space of the apple. Fruit with growth regulator when stored under DCA-RQ1.3 and DCA-CF had lower IEC (Table 3). Apples without growth regulators did not show a reduction in IEC with DCA storage. Aerobic respiration is physiological event dependent of O_2. This gas acts in the last step of respiration, as the final acceptor of electrons from the electron transport chain that happens in the inner membrane of mitochondria (Millar et al., 2011). Apples under DCA-RQ1.3 and DCA-CF had lower respiration rates than under CA. This occurred because CA had higher pO_2, which allowed higher aerobic respiration.

Flesh firmness is the primary edible quality factor (of apples) that contributes to consumer acceptance and preference (Harker et al., 2008). Brazilian legislation allows a minimum flesh firmness of 9 lb (±40N) (Instrução Normativa nº5, 2006). In this work, all conditions maintained flesh firmness higher than this minimum (Table 3). However, apples under DCA-CF and DCA-RQ1.3 had higher firmness than CA, regardless of the usage of a growth regulator. This can be

attributed to lower ethylene production in fruit under DCA, once ethylene activates cell wall enzyme degradation, such as polygalacturonase, β-galactosidase and pectinmetylesterase (Prasanna et al., 2007). Among the growth regulators, AVG+NAA-treated fruit had higher flesh firmness than other treatments in all storage conditions (Table 3). AVG inhibits ACC synthase activity, resulting in lower ACC accumulation and reduced ethylene biosynthesis (Li; Yuan, 2008; Yuan; Li, 2008), and with lower ethylene production, there was lower cell wall degradation and higher flesh firmness. Brackmann et al. (2015) found higher flesh firmness after CA storage in 'Brookfield' apples treated with AVG+NAA preharvest. We found that 'Royal Gala' apples with AVG+NAA application stored under DCA-CF or DCA-RQ1.3 maintains higher flesh firmness than fruits with AVG+NAA stored under CA after 9 months plus 7 days of shelf life. With this result, apple growers have an important tool to maintain fruit quality during storage. Li; Yuan (2008) found that AVG inhibits the negative effects of NAA through the reduction of gene expression of the ethylene synthesis, ethylene receptor and of enzymes of the cell wall degradation.

Fruit without growth regulators had higher soluble solids (SS) under DCA-CF and DCA-RQ1.3 than CA (Table 3). Maybe, apples with AVG had a reduced or delayed sugar consumption. When AVG or NAA were applied, isolated or together, there were no differences among storage conditions. Acidity did not reflect an interaction between storage condition and growth regulation application (Table 3). Under average atmospheric conditions, fruit with AVG+NAA application had higher acidity, although differing only from control.

Decay incidence is an important cause of fruit losses around the world. Fruit with AVG or AVG+NAA had lower incidence of decay (Table 4), but did not differ from control. Apples with these growth regulators have a delayed ripening, which can reduce fungi development and decay occurrence. Another important result is that AVG+NAA application had fruit with lower incidence of mealiness in all storage atmospheres (Table 4). Is probable that lower ethylene levels in the beginning of storage period contributes to reduce mealiness, since this disorder occurs by

degradation of middle lamella by ethylene dependent enzymes (Storch et al., 2015). Among storage atmospheres, ‘Royal Gala’ apples under DCA-CF and DCA-RQ1.3 had lower mealiness than CA fruit, with AVG and NAA applications. Apples with AVG+NAA did not present differences among storage atmosphere conditions; moreover, apples with AVG+NAA stored under DCA-RQ1.3 presented no fruit with mealiness.

Table 4 – Decay, physiological disorders and healthy fruits of the 'Royal Gala' apple submitted to preharvest growth regulators application and stored under controlled atmosphere and dynamic controlled atmosphere during 9 months plus 7 days at 20 °C.

	Decay (%) – opening of chambers			Decay (%) – 7 days at 20 °C			
	CA	DCA-CF	DCA-RQ1.3	CA	DCA-CF	DCA-RQ1.3	
Control	0.0^{ns}	0.0	1.0	4.0	5.0	6.8	5.3ab**
NAA	1.0	3.1	1.0	11.8	6.8	10.0	9.5a
AVG	0.0	0.0	2.0	1.0	4.0	6.0	3.7b
AVG+NAA	2.0	1.8	0.0	3.0	0.9	0.0	1.3b
				4.9^{ns}	4.2	5.7	
cv (%)	20.1			89.9			
	Mealiness (%)			Flesh Breakdown (%)			
Control	27.0Aa**	10.0Ba	6.9Bab	10.0	4.0	4.8	6.3a
NAA	24.2Aa	16.4ABa	10.3Ba	5.9	5.1	2.0	4.3ab
AVG	23.3Aa	9.4Bab	7.1Ba	11.8	3.1	5.0	6.6a
AVG+NAA	3.0Ab	1.0Ab	0.0Ab	2.0	0.0	1.0	1.0b
				7.4^{ns}	3.1	3.2	
cv (%)	50.7			84.5			
	Healthy fruits (%) – opening of chambers			Healthy fruits (%) – 7 days at 20 °C			
Control	100.0^{ns}	100.0	99.0	70.0	85.0	91.1	82.0b**
NAA	99.0	96.9	97.0	68.7	77.5	86.8	77.7b
AVG	99.0	100.0	97.0	77.9	86.4	87.9	84.1b
AVG+NAA	98.0	98.2	100.0	96.0	97.1	99.0	97.4a
		6.49		78.1C	86.5B	91.2A	
cv (%)					10.0		

*Controlled atmosphere with 1.0 kPa of O_2 + 1.2 kPa CO_2. DCA dynamic controlled atmosphere with chlorophyll fluorescence (DCA-CF) or respiratory quotient (DCA-RQ1.3).

**Means followed by equal letters, lowercase in the columns and uppercase in the lines, do not differ by the Tukey test, at 5% probability; ns: no significant. RH: 94%; Temperature: 1.5 °C; CO_2 partial pressure to DCA = 1.2 kPa.

NAA: naphthalene acetic acid; AVG: aminoethoxyvinylglycine

Flesh breakdown is also associated with ripening, when advanced ripening causes loss of membrane integrity and oxidation of phenolic compounds (Franck et al., 2007). Like mealiness, fruit with AVG+NAA had lower incidence of flesh breakdown (Table 4), and these results, together, contribute to a higher percentage of healthy fruit treated with these growth regulators. However, is important to highlight that fruit kept under DCA-RQ1.3 had higher percentage of healthy fruit than DCA-CF and CA.

6.1.4.1.6. Volatile compounds profile

After storage, 43 volatile compounds were identified, being 14 esters, 18 alcohols, 5 aldehydes, 1 ketone and 5 acids. Esters are usually the most important volatile compounds of the 'Royal Gala' apple aroma, such as hexyl acetate, butyl acetate and 2-methylbutyl acetate. These were higher in apples treated with AVG and NAA on preharvest and stored under CA (Fig. 2A, K, F and G). The PC I demonstrated that AVG and NAA application with storage under CA were more related with those important volatile compounds of 'Royal Gala' apples (Fig. 1). However, those treatments were also related with decay, mealiness and flesh breakdown, which is a negative factor for apple storers. The apples stored under DCA-CF and DCA-RQ1.3 had lower concentrations of butyl acetate, hexyl acetate and total esters than CA, regardless of growth regulator application. As found in articles 1 and 3, and by other researchers, DCA-RQ1.5 and DCA-RQ2.0 enhance volatile compounds on 'Galaxy' and 'Royal Gala' apples (Both et al., 2017; Thewes et al., 2017a). However, DCA-RQ1.3 employed in this work may not increase anaerobic respiration such as reported by Both et al. (2017) and Thewes et al. (2017a), who used extremely low pO_2 (average of 0.13kPa), which could increase the concentration of precursors and esters. This was confirmed by low acetaldehyde, ethanol and ethyl acetate in fruit stored under DCA-RQ1.3 (Fig. 2B, 3Q and 4F).

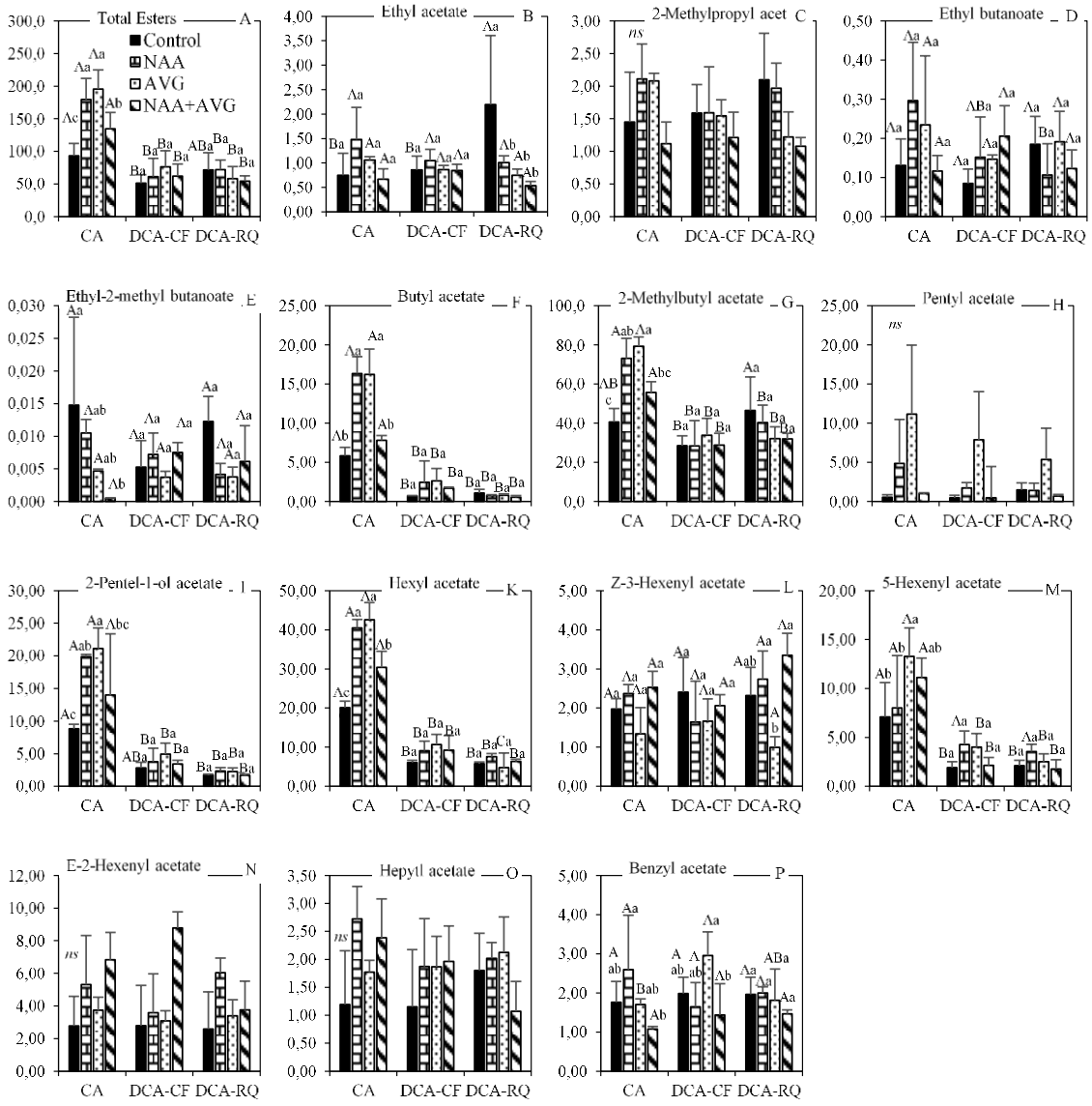

Figure 2 – Esters (µg L^{-1}) of 'Royal Gala' apples submitted to preharvest growth regulators application and stored under controlled atmosphere and dynamic controlled atmosphere (DCA-CF and DCA-RQ1.3) during 9 months plus 7 days at 20 °C. Bars with the same lowercase letter in the same storage atmosphere condition, and each bar with the same uppercase letter in different storage atmosphere condition are not significantly different by Tukey's test, at 5% probability; ns: no significant; Error bars mean standard deviation.

In relation to ethyl butanoate, compound that confers aroma apples, is synthetized from ethanol with butanoic acid (Souleyre et al., 2005). This compound was not affected by the application of growth regulators under all storage conditions (Table 2D). Apple with NAA application and stored under CA had higher ethyl 2-methylpropanoate concentration than in DCA-RQ1.3. Maybe a higher ethylene production in apples with NAA and stored under CA resulted in

higher AAT activity and synthesis of esters. The suppression of ethylene production on DCA-CF and DCA-RQ1.3 reduced enzymatic activity and/or precursor synthesis, reducing ethyl butanoate concentration in fruit.

Among straight-chain esters, butyl acetate has a greater impact on the aroma of the apple. It is synthetized from 1-butanol, by the action of AAT (Holland et al., 2005). Apples with NAA and AVG application stored under CA had higher butyl acetate than control and AVG+NAA treated fruit. Hexyl acetate and 2-methylbultyl acetate showed the same behavior (Fig. 2F and 2K). Apple with AVG application had higher ethylene production than control, which could stimulate AAT activity and/or contribute to a higher availability of precursors for esterification.

Apples with a growth regulator and stored under DCA-CF and DCA-RQ1.3 had lower 2-methylbutyl acetate concentrations, compared to CA (Fig. 2G). On the other hand, apples without growth regulator, stored under DCA-RQ1.3 had higher 2-methylbutyl acetate concentration than DCA-CF stored fruit, even though not different from CA. 2-Methylbutyl acetate is synthetized from amino acid isoleucine (Hadi et al., 2013; Yang et al., 2016). This amino acid is first degraded by branched-chain amino acid aminotransferase (BCAT) to produce precursors of esters (Gonda et al., 2010; Yang et al., 2016). Volatile compounds from amino acids are not reduced by low O_2 partial pressure (Brackmann et al., 1993). Isoleucine can be synthetized from threonine, which is produced from oxaloacetate (Bekele et al., 2015). Oxaloacetate, a compound of tricarboxylic cycle acid, was often used for amino acid biosynthesis under low pO_2 as the employed in DCA-RQ1.3. These amino acids were used to volatile biosynthesis, which can oxide NADH. This NAD^+ can be used in the glycolytic pathway to produce adenosine triphosphate (ATP). Analyzing atmosphere conditions, growth regulators did not change 2-methylbultyl acetate concentration under DCA-CF and DCA-QR 1.3. On the other hand, when fruit were stored under CA, apples with AVG application had higher 2-methylbutyl acetate. Maybe, higher pO_2 under CA and higher ethylene production after shelf life were crucial to elevate the concentration of that compound.

‘Royal Gala’ apples stored under DCA-CF and DCA-RQ1.3 had lower hexyl acetate concentration than in CA, regardless of the application of growth regulators (Fig. 2K). Like 2-methylbutyl acetate, apples with AVG and NAA had higher hexyl acetate concentration under CA. The hexyl acetate is synthesized from linoleic acid (LA). Lipoxygenase (13-LOX) enzyme oxygenates carbon 13 of LA to form precursors of C6 volatile compounds, like hexanal and hexanol (Schiller et al., 2015). LOX activity responds to ethylene (Defilippi et al., 2005b; Schiller et al., 2015) which can explain higher hexyl acetate under condition with higher ethylene production at the chamber opening or after shelf life. Both et al. (2014) showed lower emissions of straight-chain esters under extremely low pO_2 (0.5 kPa), while branched-chain esters were not significantly affected in such condition.

In relation to total alcohols, there was no difference among treatments (Fig. 3A). Individual alcohols such as hexanol, precursor of hexyl acetate (Holland et al., 2005), showed changes with treatments. Apples with growth regulators and stored under DCA-CF and DCA-RQ1.3 had lower hexanol concentration than fruit kept under CA (Fig. 3K). This low hexanol concentration in fruit under DCA leads to a lower concentration of hexyl acetate in these conditions, because hexanol is a precursor of hexyl acetate. Substrate availability is more relevant to the synthesis of volatile compounds after storage than AAT activity (Brackmann et al., 1993; Lara et al., 2006). 2-Methyl-1-butanol, precursor of 2-methylbutyl acetate did not show difference among treatments (Fig. 3N). ‘Royal Gala’ apples with AVG+NAA had reduced 1-butanol concentration when kept under DCA-RQ1.3 (Fig. 3O). The alcohol 1-butanol is synthetized by alcohol dehydrogenase (ADH) enzyme. Some early papers have reported that 1-MCP application decreases the ADH expression and activity in apples (Harb et al., 2010; Harb et al., 2011) and peaches (Ortiz et al., 2010). This demonstrated that ethylene is needed by ADH. Likely, AVG+NAA applications reduced ethylene production and ADH activity and/or expression, resulting in lower 1-butanol concentration.

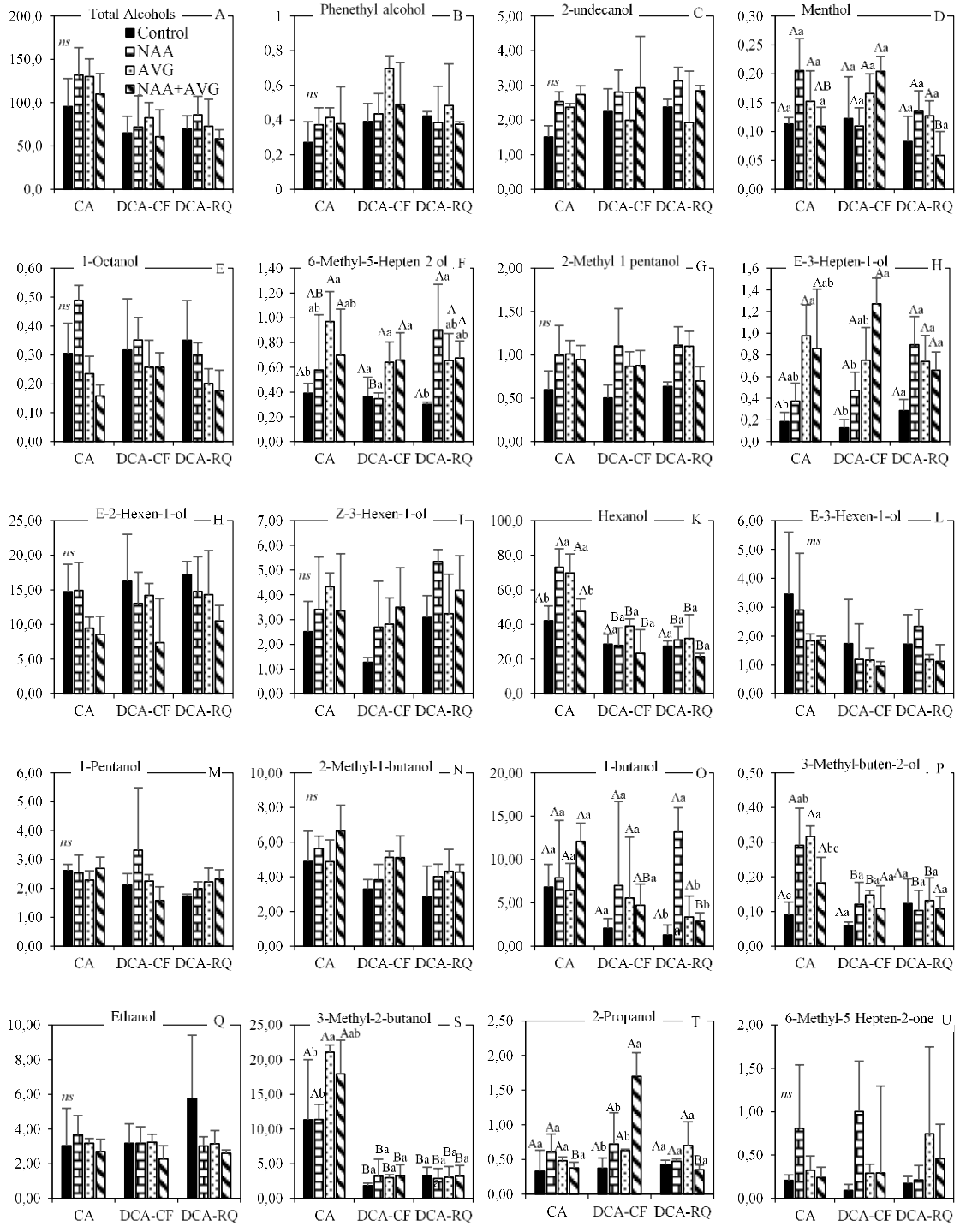

Figure 3 – Alcohol and ketone (µg L^{-1}) of 'Royal Gala' apples submitted to preharvest growth regulators application and stored under controlled atmosphere and dynamic controlled atmosphere (DCA-CF and DCA-RQ1.3) during 9 months plus 7 days at 20 °C. Means followed by equal letters, lowercase and uppercase, do not differ by the Tukey test, at 5% probability; ns: no significant; Error bars mean standard deviation.

For total of aldehydes, there was no difference among growth regulator and atmosphere conditions (Fig. 4). Some acids identified in 'Royal Gala' apples changed depending on treatments, such as nonanoic acid, hexanoic acid and ethanoic acid (Fig. 4D, E and F). Hexanoic acid had lower concentration under DCA-CF in apples with NAA application (Fig. 4E). Under DCA-RQ1.3, apples showed a higher concentration of nonanoic acid with growth regulators (Fig. 4D). In relation to ethanoic acid, apples under DCA-RQ1.3 with NAA had higher concentration of this acid (Fig. 4F).

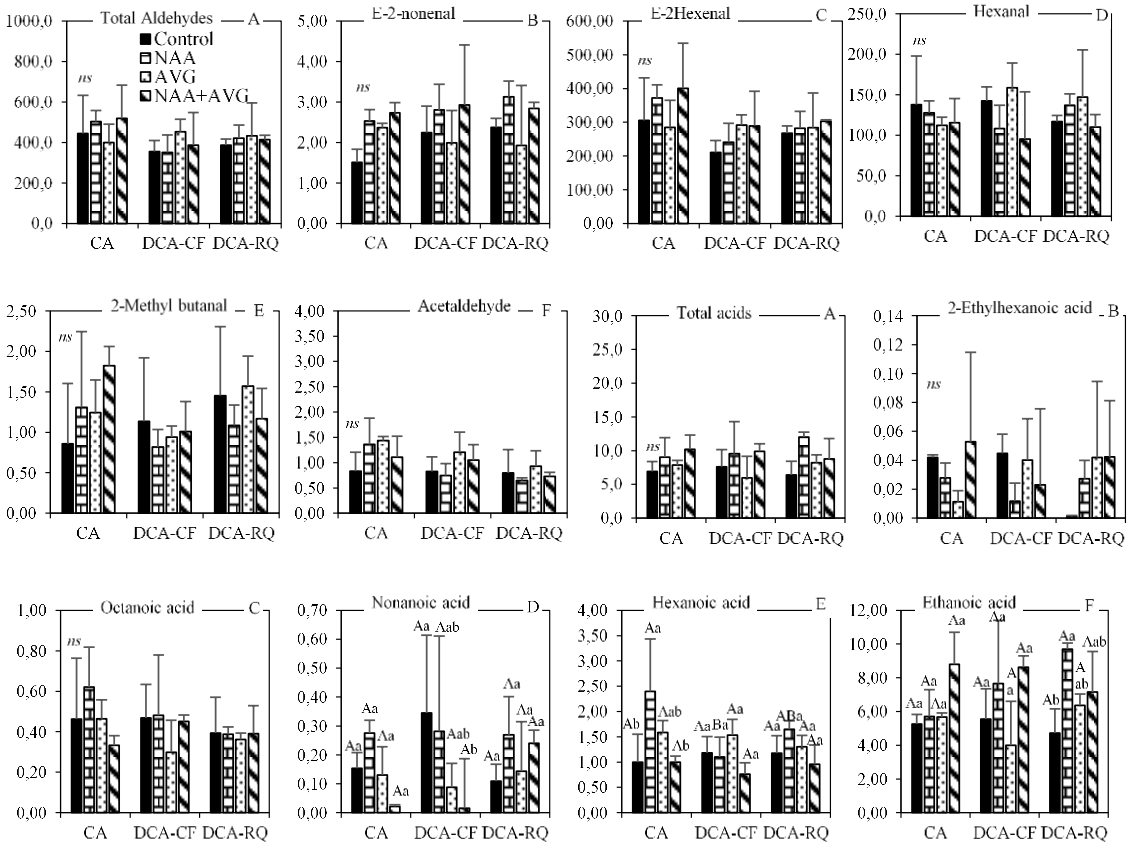

Figure 4 – Aldehydes and acids (μg L^{-1}) of 'Royal Gala' apples submitted to preharvest growth regulators application and stored under controlled atmosphere and dynamic controlled atmosphere (DCA-CF and DCA-RQ1.3) during 9 months plus 7 days at 20 °C. Bars with the same lowercase letter in the same storage atmosphere condition, and each bar with the same uppercase letter in different storage atmosphere condition are not significantly different by Tukey's test, at 5% probability; ns: no significant; Error bars mean standard deviation.

6.1.5. Conclusion

AVG+NAA applications in preharvest caused a higher increase in some important volatile compounds such as 2-methylbutl acetate, hexyl acetate and butyl acetate in ‘Royal Gala’ apples after long term storage under CA, when compared to DCA-CF and DCA-RQ1.3 storage.

AVG+NAA application in preharvest, associated with storage under DCA-CF or DCA-RQ1.3, is a promising tool to maintain higher flesh firmness, reduce physiological disorders and maintain higher percentage of healthy fruit of ‘Royal Gala’ apples.

NAA and AVG did not increase the concentration of volatile compounds in ‘Royal Gala’ apples stored under DCA-CF and DCA-RQ1.3. Maybe a more elevated RQ level on DCA-RQ storage can increase some volatile compounds.

6.1.6. Acknowledgements

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6.1.7. References

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7. DISCUSSÃO GERAL

Em função da busca de melhorias para o setor de armazenamento de maçãs, buscamos preencher algumas lacunas no conhecimento para auxiliar os armazenadores na obtenção de frutas de melhor qualidade, com redução de perdas em pós-colheita. No primeiro artigo buscamos avaliar o efeito de diferentes formas de armazenamento de maçãs (atmosfera controlada -AC, ultrabaixo O_2 - ULO, atmosfera controlada dinâmica com fluorescência de clorofilas - ACD-FC e ACD com quociente respiratório - ACD-QR), com e sem a aplicação de 1-metilciclopropeno (1-MCP), sob a concentração de compostos voláteis e outros parâmetros de qualidade de maçãs 'Galaxy'. A ACD-QR1,5 manteve melhor qualidade e concentração de compostos voláteis que a AC, ULO e a ACD-FC, o que certamente foi devido à menor pressão parcial de O_2 usada na ACD-QR1,5, que induziu o metabolismo fermentativo. Produtos da fermentação podem ter efeito na redução da síntese de etileno (Asoda et al., 2009), além disso, a fermentação aumentou a disponibilidade de precursores para síntese de compostos voláteis. Na ACD-QR1,5, mesmo com baixa produção de etileno houve alta produção de ésteres, o que parece contraditório considerando que a atividade da enzima álcool acetiltransferase, que participa na rota de síntese de ésteres, é dependente do etileno. Entretanto, Ban et al. (2010) observaram incompleta inibição de alguns genes da AAT pela inibição da ação do etileno, o que ajuda a explicar que mesmo com baixo nível de etileno ocorra alta produção de ésteres em ACD-QR1,5.

A partir dos resultados do primeiro artigo, e sabendo que o 1-MCP possui menor efeito quando as maçãs são armazenadas em níveis de O_2 extremamente baixos (BRACKMANN et al., 2012; BOTH et al., 2014), avaliamos o efeito da ACD quando os frutos são armazenados em temperatura mais elevada. A temperatura mais elevada possui grande importância na redução de custos com o armazenamento, pois quanto menor a temperatura da câmara, maior é o consumo de energia elétrica pelo sistema de refrigeração. O que se conhecia até então era que é possível armazenar maçãs em AC em temperaturas mais elevadas (2,0 a 2,5 °C) do que a atualmente recomendada desde que se aplique 1-MCP (MCCORMICK et al., 2012; KITTEMANN et al., 2015; MAZZURANA et al., 2016). Entretanto, o 1-MCP é um produto químico sintético, não interessante no sentido de buscarmos uma agricultura mais sustentável. Além disso, a sua aplicação possui elevado custo financeiro para o armazenador. Nesse trabalho, observamos que, quando maçãs 'Galaxy' são armazenadas em ACD-QR1,3 e em ACD-FC em temperaturas mais elevadas (2,0 e 2,5 °C), não é necessário aplicar 1-MCP, pois não possui efeito adicional. O potencial da ACD em reduzir a produção de etileno se equivale ao armazenamento em AC com a aplicação de 1-MCP, em temperatura mais elevada.

Para calcular o QR no armazenamento em ACD-QR, a pressão parcial de O_2 atinge valores extremamente baixos, próximo da anoxia. Foi constatado que o estresse por baixo O_2, devido a variação do nível de O_2 durante a determinação do QR duas vezes por semana durante o período de armazenamento, possui efeito benéfico na qualidade dos frutos, não sendo somente a variação do nível de O_2 (*set point*) no armazenamento em ACD-QR o responsável pela manutenção da qualidade dos frutos. É possível que curtos períodos de estresse (12 a 14h) durante o armazenamento em ACD induza um nível do metabolismo fermentativo que possui forte efeito na redução do metabolismo sem causar efeitos deletérios na qualidade do fruto. Os produtos da fermentação, quando em níveis elevados causam danos às membranas da célula e perda de compartimentalização, resultando em distúrbios fisiológicos (SAQUET; STREIF, 2008).

Para avaliar o efeito da temperatura mais elevada, no terceiro artigo foi avaliado a concentração de compostos voláteis em maçãs submetidas a ACD-QR1,3 e ACD-QR1,5, sem a aplicação de 1-MCP devido ao fato de este reduzir a concentração de compostos voláteis mesmo em ACD-QR1,5, conforme constatado no primeiro artigo. Uma maior temperatura (2,0 e 2,5 °C) pode ser usada em ACD-QR1,3 sem redução de compostos voláteis importantes como acetato de 2-metil propila, acetato de butila, acetato de hexila, acetato de 2-metil butila e 1-butanol. Entretanto, quando os frutos foram armazenados em ACD-QR1,5, a alta temperatura reduziu a concentração destes compostos comparado a 1,5 °C. Por outro lado, a ACD-QR1,5 em alta temperatura reduziu a respiração, produção de etileno e a incidência de polpa farinácea, o que também possui grande importância prática para o armazenador e consumidor.

Em virtude do efeito benéfico da ACD na conservação da qualidade de maçãs e pelo aumento na concentração de compostos voláteis, foi avaliado o efeito da ACD em maçãs 'Royal Gala' tratadas em pré-colheita com aminoetoxivinilglicina (AVG) e ácido naftaleno acético (ANA). Em trabalho prévio de Salas et al. (2011) foi observado que o AVG reduziu a concentração de compostos voláteis em maçã 'Golden Delicious'. Com relação a aplicação de ANA, Brackmann et al. (2014) reportam aceleração do amadurecimento durante o armazenamento em AC. Entretanto, quando os frutos foram armazenados em ACD-QR apresentaram baixa incidência de polpa farinácea, atividade da ACC oxidase, produção de etileno e alta firmza da polpa. Desta forma, a ACD-QR conseguiu suprimir o metabolismo mesmo de maçãs tratadas em pré-colheita com ANA. O ANA aumenta a expressão de genes da síntese de etileno (*MdACS1* e *MdACO1*), da percepção do etileno (*MdERS1*) e da degradação da parede celular (*MdPG1*) (LI; YUAN, 2008; YUAN; LI, 2008). Quando os frutos tratados com AVG foram armazenados em ACD-QR1,3 e ACD-CF, não apresentaram aumento na concentração de compostos voláteis. Talvez com níveis de QR mais elevado poderia haver um aumento na concentração de compostos voláteis, pois

ocorreria maior indução do metabolismo fermentativo conforme observado em outros trabalhos (BOTH et al., 2017; THEWES et al., 2017b). A aplicação de AVG (30 dias antes da data prevista de colheita) associada à aplicação de ANA (7 dias antes da colheita) apresentou aumento na concentração de compostos voláteis, como acetato de 2-metil butila, acetato de hexila e acetato de butila, quando os frutos foram armazenado em AC. Por outro lado, a aplicação associada de AVG mais ANA e o posterior armazenamento em ACD-QR1,3 ou ACD-FC manteve melhor firmeza da polpa, reduziu a incidência de distúrbios fisiológicos e manteve alto percentual de frutos sadios.

8. CONSIDERAÇÕES FINAIS

Após a realização desses trabalhos, pode-se destacar que a maçã ‘Galaxy’ quando armazenada em atmosfera controlada dinâmica com quociente respiratório 1,5 (ACD-QR1,5) mantem melhor qualidade do que o armazenamento em atmosfera controlada (AC), ultra baixo O_2 (ULO) e a ACD com fluorescência de clorofilas (ACD-FC), além de aumentar a concentração de ésteres. Além disso, o 1-metilciclopropeno (1-MCP) reduz a concentração de compostos voláteis, mesmo em frutos armazenados em ACD-QR1,5. Também, a ACD-FC possui efeito similar ao armazenamento em ULO com 0,4 kPa de O_2.

A ACD-QR1,3 e a ACD-FC permitem armazenar maçã ‘Galaxy’ em temperatura mais elevada (2,0 e 2,5 °C) do que a atualmente recomendada (1,5 °C). Também, a aplicação de 1-MCP não possui feito adicional em condições de temperatura mais elevada quando os frutos são armazenados em ACD. Alta temperatura pode ser usada em ACD-QR1,3 sem perda de compostos voláteis importantes.

No armazenamento em ACD-QR, o estresse por baixo O_2 que os frutos são submetidos durante o cálculo do QR possui efeito benéfico, pois mantém alta firmeza da polpa, baixa incidência de polpa farinácea e podridões comparado aos frutos que não sofreram estresse por baixo O_2.

O armazenamento de maçãs ‘Royal Gala’ em ACD-QR1,3 e ACD-FC tratadas em pré-colheita com aminoetoxivinilglicina (AVG) e ácido naftaleno acético (ANA) não aumentaram a concentração de compostos voláteis. Entretanto, a aplicação associada de AVG mais ANA e o armazenamento em ACD-QR1,3 e ACD-FC é uma excelente alternativa, pois mantém a firmeza da polpa, reduz distúrbios fisiológicos mantendo alta percentagem de frutos sadios.

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