UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS RURAIS
PROGRAMA DE PÓS-GRADUAÇÃO EM AGRONOMIA

Eraní Eliseu Schultz

QUALIDADE DE MAÇÃS ARMAZENADAS EM ATMOSFERA CONTROLADA DINÂMICA COM DIFERENTES NÍVEIS DE CO_2 E EM NÍVEIS EXTREMAMENTE BAIXOS DE OXIGÊNIO COM ESTRESSES PERIÓDICOS POR HIPOXIA ATRAVÉS DE DIFERENTES HISTERESES

Santa Maria, RS
2021

Erani Eliseu Schultz

QUALIDADE DE MAÇÃS ARMAZENADAS EM ATMOSFERA CONTROLADA DINÂMICA COM DIFERENTES NÍVEIS DE CO_2 E EM NÍVEIS EXTREMAMENTE BAIXOS DE OXIGÊNIO COM ESTRESSES PERIÓDICOS POR HIPOXIA ATRAVÉS DE DIFERENTES HISTERESES

Tese de Doutorado apresentado 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

Santa Maria, RS
2021

This study was financied in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001

SCHULTZ, ERANI ELISEU
QUALIDADE DE MAÇÃS ARMAZENADAS EM ATMOSFERA CONTROLADA DINÂMICA COM DIFERENTES NÍVEIS DE CO2 E EM NÍVEIS EXTREMAMENTE BAIXOS DE OXIGÊNIO COM ESTRESSES PERIÓDICOS POR HIPOXIA ATRAVÉS DE DIFERENTES HISTERESES / ERANI ELISEU SCHULTZ.- 2021.
142 p.; 30 cm

Orientador: AURI BRACKMANN
Tese (doutorado) - Universidade Federal de Santa Maria, Centro de Ciências Rurais, Programa de Pós Graduação em Agronomia, RS, 2021

1. Compostos voláteis 2. Desordens fisiológicas 3. Vida de prateleira 4. Malus domestica 5. Frutos sadios I. BRACKMANN, AURI II. Título.

Sistema de geração automática de ficha catalográfica da UFSM. Dados fornecidos pelo autor(a). Sob supervisão da Direção da Divisão de Processos Técnicos da Biblioteca Central. Bibliotecária responsável Paula Schoenfeldt Patta CRB 10/1728.

Declaro, ERANI ELISEU SCHULTZ, para os devidos fins e sob as penas da lei, que a pesquisa constante neste trabalho de conclusão de curso (Tese) foi por mim elaborada e que as informações necessárias objeto de consulta em literatura e outras fontes estão devidamente referenciadas. Declaro, ainda, que este trabalho ou parte dele não foi apresentado anteriormente para obtenção de qualquer outro grau acadêmico, estando ciente de que a inveracidade da presente declaração poderá resultar na anulação da titulação pela Universidade, entre outras consequências legais.

Eraní Eliseu Schultz

QUALIDADE DE MAÇÃS ARMAZENADAS EM ATMOSFERA CONTROLADA DINÂMICA COM DIFERENTES NÍVEIS DE CO_2 E EM NÍVEIS EXTREMAMENTE BAIXOS DE OXIGÊNIO COM ESTRESSES PERIÓDICOS POR HIPOXIA ATRAVÉS DE DIFERENTES HISTERESES

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.**

Aprovado em 06 de agosto de 2021:

_____
Auri Brackmann, **Prof. Dr.** (UFSM – por parecer)
(Presidente/Orientador)

_____
Adriano Arriel Saquet, Prof. Dr. (IFFAR – por parecer)

_____
Anderson Weber, Prof. Dr. (UNIPAMPA – por parecer)

_____
Andréia Hansen Oster, Dra. (EMBRAPA – por parecer)

_____
Vanderlei Both, Prof. Dr. (UFSM – por parecer)

Santa Maria, 06 de agosto de 2021

DEDICATÓRIA

Dedico essa dissertação à minha família,
em especial aos meus pais Claudio (in memoriam) e Renate Schultz pela educação, apoio e exemplo de vida, bem como, aos meus irmãos Enéias, Elis e Emerson pelo companheirismo durante esse período.

DEDICO.

AGRADECIMENTOS

À Deus, por me guiar durante a Graduação e Pós-Graduação;

A Capes, pela bolsa de Doutorado concedida;

À Universidade Federal de Santa Maria e ao Programa de Pós-Graduação em Agronomia, pela oportunidade de realizar este estudo;

Ao professor Dr. Auri Brackmann, pela orientação e ensinamentos durante a minha Graduação e Pós-Graduação;

Aos colegas de Pós-Graduação pelas contribuições ao tema estudado;

A todos os colegas de iniciação científica do Núcleo de Pesquisa em Pós-colheita, pela ajuda na condução dos experimentos;

Ao Professor Dr. Vanderlei Both, pelas valiosas contribuições ao trabalho.

A todos os familiares e amigos que, de alguma forma, contribuíram para realização deste trabalho.

MUITO OBRIGADO.

RESUMO

QUALIDADE DE MAÇÃS ARMAZENADAS EM ATMOSFERA CONTROLADA DINÂMICA COM DIFERENTES NÍVEIS DE CO_2 E EM NÍVEIS EXTREMAMENTE BAIXOS DE OXIGÊNIO COM ESTRESSES PERIÓDICOS POR HIPOXIA ATRAVÉS DE DIFERENTES HISTERESES

AUTOR: Eraní Eliseu Schultz
ORIENTADOR: Auri Brackmann

O armazenamento de maçãs em atmosfera controlada dinâmica (ACD), onde é monitorado o limite mínimo de oxigênio (LMO), permite utilizar níveis extremamente baixos de oxigênio. Dentre as técnicas mais estudadas estão fluorescência de clorofila (FC) e o quociente respiratório (QR). O monitoramento do LMO é importante para evitar a produção excessiva de compostos do metabolismo anaeróbico, causados pela redução excessiva do O_2. O estudo do efeito de diferentes pressões parciais de CO_2 em níveis extremamente baixos de O_2 em ACD é importante a fim de propiciar melhorias na conservação dos frutos e redução do custo com absorção de CO_2 nas câmaras frigoríficas. Além disso, a adoção de níveis estáticos de O_2 extremamente baixos e a histerese da variação destes níveis ainda carecem de mais estudos. Nesse contexto, no presente trabalho foram desenvolvidos três artigos científicos com o objetivo de: [1] avaliar diferentes pressões parciais de CO_2 em ACD-QR e seu efeito na qualidade geral de maçãs 'Maxi Gala' após nove meses de armazenamento seguido de sete dias de vida de prateleira, além de comparar com as técnicas de atmosfera controlada (AC) com aplicação de 1-MCP e ACD-FC; [2] avaliar o efeito de níveis de CO_2 em ACD-QR na qualidade geral e perfil volátil de maçãs após nove meses de armazenamento mais 21 dias de vida de prateleira e compara com a AC- 1-MCP e ACD-FC e [3] estudar o efeito do armazenamento em níveis extremamente baixos de oxigênio (*ELO -Extreme low oxygen*) com indução de estresses por baixo O_2 (histereses) na qualidade geral e emissão de compostos voláteis após nove meses de armazenamento mais 7, 14 e 21 dias de vida de prateleira, e compará-las com AC + 1-MCP (0,650 µL L^{-1}), ACD-FC e ACD-QR. O armazenamento de maçãs 'Maxi Gala' durante nove meses em ACD-QR 1,3 com 1,6 e 2,0 kPa de CO_2 mais sete dias de vida de prateleira, mantém a qualidade dos frutos similar a ACD-QR 1,3 com 1,2 kPa de CO_2. ACD-QR 1,3 com 1,2 e 1,6 kPa CO_2 mantém alta qualidade dos frutos após nove meses de armazenamento mais 21 dias de vida de prateleira a 20 °C. A adoção de baixo CO_2 (0,4 kPa), em ACD-QR 1,3, resulta em menor firmeza de polpa durante 21 dias de vida de prateleira. A ACD-QR 1,3 com 2,0 kPa de CO_2 mantém a qualidade dos frutos similar a ACD-QR 1,3 com 1,2 e 1,6 kPa de CO_2 até o 14º dia de vida de prateleira. A ACD-QR 1,3 com 1,2, 1,6 e 2,0 kPa de CO_2 resultam em maior emissão de voláteis (principalmente ésteres), aos 21 dias de vida de prateleira sem diferir da AC. O armazenamento em *ELO*, com ou sem histereses, mantém a qualidade dos frutos similar à ACD-FC, AC+1-MCP, e superior a AC após sete dias de vida de prateleira. Após 21 de vida de prateleira, *ELO* com 0,4 O_2 + 0,4 e 0,6 de histerese mantém maior porcentagem de frutos sadios em relação a ACD-FC, AC +1-MCP, AC e similar a ACD-QR, mas com menor firmeza de polpa que a ACD-QR e AC + 1-MCP. *ELO* com ou sem histereses reduzem a emissão dos principais ésteres, como acetato de 2-metilbutila e o acetato de etila (com exceção do acetato de butila) aos sete e 14 dias, mas com incremento aos 21 dias. Maçãs armazenadas em ACD-FC e AC + 1-MCP apresentam baixa emissão de compostos voláteis durante os 21 dias de vida de prateleira, enquanto que aquelas em AC mantém alta produção durante toda a vida de prateleira. Os resultados dos três trabalhos evidenciam que o armazenamento de maçãs 'Maxi Gala' em oxigênio extremamente baixo (0,4 kPa) com histereses de 0,4 a 0,6 permitem uma conservação da qualidade superior a ACD-FC e que em condições de *ELO* é possível elevar o nível de CO_2 a 1,6 kPa, o que reduz custos com energia elétrica no processo de adsorção da câmara de AC.

Palavras-chave: Compostos voláteis. Desordens fisiológicas. Firmeza de polpa. Frutos sadios. *Malus domestica*. Vida de prateleira.

ABSTRACT

QUALITY OF APPLES STORED IN DYNAMIC CONTROLLED ATMOSPHERE WITH DIFFERENT CO_2 LEVELS AND EXTREME LOW OXYGEN LEVELS WITH PERIODIC HYPOXIA STRESS THROUGH DIFFERENT HYSTERESIS

AUTHOR: Eraní Eliseu Schultz
ADVISOR: Auri Brackmann

The storage of apples in a dynamic controlled atmosphere (DCA), where the low oxygen limit (LOL) is monitored, allows the use of extremely low levels of oxygen. Among the most studied techniques are chlorophyll fluorescence (CF) and respiratory quotient (RQ). Monitoring LOL is important to avoid excessive induction of anaerobic metabolism compounds caused by excessive O_2 reduction. The study of the effect of different partial pressures of CO_2 on extremely low levels of O_2 in DCA is important in order to provide improvements in fruit conservation and a reduction in the cost of CO_2 absorption in the chambers. Furthermore, the adoption of extremely low static O_2 levels and the hysteresis of the variation in these levels still need further studies. In this context, in the present work, three papers were developed with the aim of: [1] evaluating different pCO_2 in DCA-RQ and its effect on the overall quality 'Maxi Gala' apples after 9 months of storage plus 7 days of shelf life, in addition, compare with controlled atmosphere with application of 1-methylcyclopropene (1-MCP) and DCA-CF; [2] evaluate the effect of CO_2 levels in DCA-RQ on the overall quality and volatile profile of apples after 9 months of storage plus 21 days of shelf life and compare with CA +1-MCP and DCA-CF and [3] study the effect of storage at extreme low oxygen levels (ELO) with induction of stresses by low O_2 (hysteresis) on the overall quality and emission of volatile compounds after 9 months of storage plus 7, 14 and 21 days of shelf life, and compare them with CA + 1-MCP, DCA-CF and DCA-RQ. The storage of 'Maxi Gala' apples during 9 months in DCA-RQ 1.3 with 1.6 and 2.0 kPa of CO_2 plus 7 days of shelf life maintains fruit quality similar to DCA-RQ 1.3 with 1.2 kPa of CO_2. DCA-RQ 1.3 with 1.2 and 1.6 kPa CO_2 maintains high fruit quality after 9 months of storage plus 21 days of shelf life at 20 °C. Storage in DCA-RQ 1.3 with 0.4 kPa CO_2 maintains a high percentage of healthy fruit, but reduce flesh firmness during the entire shelf life (21 days). DCA-RQ 1.3 with 2.0 kPa CO_2 maintains similar fruit quality to DCA-RQ 1.3 with 1.2 and 1.6 kPa CO_2 up to 14 days of shelf life. DCA-QR 1.3 with 1.2, 1.6 and 2.0 kPa of CO_2 result in higher emission of volatiles (mainly esters), at 21 days of shelf life, with no difference for CA. Storage at ELO levels with or without hysteresis maintains fruit quality similar to DCA-CF, CA+1-MCP, and superior to CA after 7 days of shelf life. After 21 days of shelf life, ELO with 0.4 O_2 + 0.4 and 0.6 hysteresis maintain a higher percentage of healthy fruit compared to DCA-CF, CA +1-MCP, CA and similar to DCA-RQ , but with lower flesh firmness than DCA-RQ and CA + 1-MCP. Conditions with ELO with or without hysteresis reduce the emission of the main esters, such as 2-methylbutyl acetate and ethyl acetate (with the exception of butyl acetate) at 7 and 14 days, but with an increase at 21 days. DCA-CF and CA + 1-MCP have low emission of volatile compounds during 21 days of shelf life. Apples stored in CA maintain high volatile compounds production throughout the shelf life. The results of the three works show that the storage of 'Maxi Gala' apple in extreme low oxygen (0.4 kPa) with hysteresis of 0.4 to 0.6 allow a better quality conservation in comparison to DCA-CF and that it is possible to increase the CO_2 level to 1.6 kPa in ELO conditions , which reduce cost of electrical energy for CO_2 adsorption from the CA storage room.

Keywords: Flesh firmness. Healthy fruit. *Malus domestica*. Physiological disorders. Shelf life. Volatile compounds.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS

%	Porcentagem
µg	Micrograma
µL L^{-1}	Microlitro por litro
1-MCP	1-metilciclopropeno/1-methylcylclopropene
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 – EtOH	Atmosfera controlada dinâmica monitorada pela emissão de etanol
ACD-FC	Atmosfera controlada dinâmica monitorada pela emissão de fluorescência de clorofilas
ACD-QR	Atmosfera controlada dinâmica monitorada pelas variações no quociente respiratório
Ace	Acetaldehyde
ACP	Anaerobic compensation point
ADH	Álcool desidrogenase
ANOVA	Análise de variância
AR	Armazenamento refrigerado
ATP	Adenosina trifosfato
AVG	Aminoethoxyvinylglycine
CA	Controlled atmosphere
CO_2	Dióxido de carbono (gás carbônico)
DCA	Dynamic controlled atmosphere
DCA-CF	Dynamic controlled atmosphere – chlorophyll fluorescence
DCA-RQ	Dynamic controlled atmosphere – respiratory quotient
EBO	Extremamente Baixo Oxigênio
ELO	Extreme Low Oxygen
FID	Flame ionization detector
GC-FID	Cromatógrafo a gás com detector por ionização em chama
GC-MS	Cromatógrafo a gás acoplado à espectrômetro de massa
HS-SPME	Solid phase microextraction
HY	Hyterese
IEC	Internal Ethylene Concentration
ILOs	Initila Low Oxygen Stress
kg	Quilograma
kPa	Kilopascal
L	Litro
LMO/LOL	Limite mínimo de oxigênio/Low oxygen limit
LRI	Linear retention index

MdAAT1/2	Gene para enzima Álcool Acetil Transferase em maçã
MdACO1	Gene para ACC oxidase em maçã
MdACS1	Gene para ACC sintase em maçã
MdERS1/MdERS2	Genes para receptores de etileno em maçã
MdLOX1	Gene para enzima lipoxigenase em maçã
mg	Miligrama
mL	Mililitro
mm	Milímetro
N	Newton ou Normal
N_2	Nitrogênio
Na	Sódio
NaCl	Cloreto de sódio
NaOH	Hidróxido de sódio
Ng	Nano grama
NIST	National Institute of Standards and Technology
NPP-UFSM	Núcleo de Pesquisa em Pós-colheita da Universidade Federal de Santa Maria
O_2	Oxigênio
°C	Temperatura em graus Celsius
PC	Principal Component
PCA	Ponto de Compensação anaeróbico e Principal Component Analysis
pCO_2	Pressão parcial de gás carbônico
pH	Potencial hidrogeniônico
pO_2	Pressão parcial de oxigênio
QR	Quociente respiratório
RH	Relative humidity
RQ	Respiratory quotient
s	Segundo
SPI	Starch pattern index

SUMÁRIO

1 INTRODUÇÃO E JUSTIFICATIVA

A maçã (*Malus x domestica* Borkh.) é uma fruta de colheita sazonal, por isso a maior parte da sua produção necessita ser armazenada para que possa ser ofertada durante todo o ano. Atualmente, a principal forma de armazenamento empregada é a atmosfera controlada (AC), que tem como características o uso de baixas pressões parciais e O_2 (1,0 a 1,2 kPa) e altas de CO_2 (2,0 kPa para Gala e mutantes e < 0,5 kPa para Fuji e mutantes), além de baixa temperatura (-0,5 a 2 °C) e alta umidade relativa do ar (±94 %).

Apesar da AC possibilitar o armazenamento de maçãs por sete a oito meses, há perdas consideráveis principalmente pela ocorrência de podridões, perda de firmeza de polpa e distúrbios fisiológicos (BOTH et al., 2017; THEWES et al., 2017a). Para diminuir essas perdas, atualmente é muito utilizado pelos armazenadores o fitorregulador 1-metilciclopropeno (1-MCP). Esse produto tem como principal vantagem manter maior firmeza de polpa dos frutos (WEBER et al., 2017; WATKINS et al., 2006; ZANELLA et al., 2008), porém sua aplicação tem um custo elevado e pode causar maior incidência de degenerescência de polpa em maçãs (KOPCKE, 2015), além de ser proibido na produção orgânica (REBEAUD; GASSER, 2015). No armazenamento em AC as perdas ocorrem principalmente porque as maçãs são armazenadas em pressões parciais de O_2 acima do limite mínimo O_2 (LMO) tolerado pelos frutos. O LMO é a pressão parcial de O_2 em que o metabolismo dos frutos é mínimo (BESSEMANS et al., 2016). Em AC há uma maior perda de qualidade devido à maior atividade metabólica em comparação aos frutos armazenados em pressões parciais de O_2 próximas ao LMO (BESSEMANS et al., 2016; WEBER et al., 2015) Com a necessidade de reduzir as perdas durante o armazenamento, reduzir custos com a aplicação de produtos químicos na pós-colheita, a pesquisa evoluiu para a chamada atmosfera controlada dinâmica (ACD), técnica que possibilita monitorar o LMO tolerado pelos frutos durante o armazenamento. Essa técnica permite ao armazenador ajustar a concentração do O_2 em função do metabolismo dos frutos e, assim, reduzir as perdas de qualidade e prolongar o armazenamento (THEWES et al., 2020; WEBER et al., 2017).

Existem três técnicas principais de ACD a nível mundial. A primeira técnica está baseada na avaliação da produção de etanol pelos frutos na atmosfera da câmara ou no suco (SCHOUTEN et al., 1997; VELTMANN et al., 2003). Essa técnica apresenta como principal dificuldade, a medição do etanol no interior das câmaras comerciais ou no suco, por isso é pouco empregada. A segunda técnica está baseada na emissão da fluorescência de clorofilas (ACD-FC) (PRANGE et al., 2003), que atualmente é a técnica de ACD mais empregada em

câmaras comerciais. A terceira técnica está baseada na determinação do quociente respiratório (QR), que é determinado pela relação da produção de CO_2 e a absorção de O_2 pelos frutos na câmara (BESSEMANS et al., 2016; BRACKMANN, 2015; GASSER et al., 2005; WEBER et al., 2015). Essa técnica tem apresentado alta preservação da qualidade dos frutos de maçã, com resultados iguais ou superiores a ACD-FC (BOTH et al., 2017; THEWES et al., 2017a; WEBER et al., 2017). A pressão parcial de O_2 durante o armazenamento em ACD-QR é geralmente inferior a 0,3 kPa, causando uma maior redução da respiração dos frutos e, com isso, melhor manutenção da qualidade (THEWES et., 2020).

Atualmente, os melhores níveis/valores de QR para o armazenamento de maçãs em ACD-QR se situam entre 1,1 a 1,5 (ANESE et al., 2020; THEWES et al., 2017a). Nesses níveis de QR a pressão parcial de CO_2 empregada é de 1,2 kPa para a cultivar Gala e suas mutantes. O CO_2 é liberado pela respiração dos frutos e acumula na câmara. Altas pressões parciais de CO_2 ($\geq$2,0 kPa) durante o armazenamento podem reduzir a qualidade dos frutos (BRACKMANN; WEBER; BOTH, 2015). Para reduzir a pressão parcial de CO_2 nas câmaras comerciais, os armazenadores utilizam adsorvedores de carvão ativado movidos à energia elétrica. Outra forma de reduzir a pressão parcial de CO_2 é o uso da cal hidratada no interior das câmaras, que absorve o CO_2. Geralmente são usados apenas adsorvedores de carvão ativado em maçãs da cultivar Gala e mutantes, por serem armazenadas em pressões parciais de CO_2 acima de 1,0 kPa. Acima desse nível de CO_2 a eficiência do adsorvedor é maior. Já para maçãs da cultivar Fuji e mutantes, que precisam ser mantidas em pressões parciais de CO_2 menores que 1,0 kPa devido à maior sensibilidade a ocorrência de distúrbios fisiológicos causados pelo CO_2, é utilizado a cal hidratada.

A energia elétrica é um dos principais custos do armazenamento. Se for possível utilizar pressões parciais de CO_2 maiores que 1,2 kPa (atualmente utilizado em ACD para maçãs ‘Gala’), haverá economia de energia elétrica, porque o número e o tempo de adsorção, em que o equipamento estará ligado, será menor. Outra alternativa para reduzir os custos pode ser a utilização da cal hidratada no interior da câmara, para absorção do CO_2 em maçãs ‘Gala’ e mutantes. Com o uso da cal hidratada a pressão parcial de CO_2 na câmara permanece próximo a zero. Em termos financeiros, a cal é um produto com menor custo se comparado com o custo da energia elétrica e a aquisição do adsorvedor de carvão ativado. Portanto, é necessário avaliar o efeito das pressões parciais de CO_2 em ACD-QR sobre a qualidade de maçãs ‘Maxi Gala’.

Em estudo realizado no Núcleo de Pesquisa em Pós-colheita (NPP) da Universidade Federal de Santa Maria (UFSM), que avaliou a aplicação de estresses por baixo O_2 durante o armazenamento de maçãs ‘Galaxy’ em ACD, verificou-se que essa prática manteve uma melhor

qualidade quando comparado ao tratamento sem a aplicação de estresses. O armazenamento de maçãs em pressões parciais extremamente baixas de O_2 sem monitorar o LMO podem incrementar a emissão de compostos do metabolismo anaeróbico, que podem ocasionar danos nas células dos frutos, reduzindo a qualidade dos frutos (THEWES et al., 2021). Diante disso, é importante avaliar qual o efeito da frequência de estresses (1 e 2 vezes por semana) e a intensidade (histereses de 0,8; 0,6; 0,4 e 0,2 kPa O_2) na manutenção da qualidade de maçãs armazenadas em ultrabaixo O_2 (ELO – *Extreme Low Oxygen* com *setpoint* de 0,5 e 0,4 kPa O_2). A técnica de exposição dos frutos a estresses periódicos por baixo O_2 não necessita de equipamentos adicionais nas câmaras, o que é uma vantagem em relação à ACD-QR e ACD-FC. No entanto, esta técnica necessita ser avaliada quanto à conservação da qualidade das maçãs e comparada com as técnicas de ACD-FC, ACD-QR, AC convencional e AC com aplicação de 1-metilciclopropeno (1-MCP), fitorregulador que inibe a ação do etileno e que, geralmente, é empregado no armazenamento em AC convencional.

1.1 HIPÓTESES

Alta pressão parcial de CO_2 (2,0 kPa) em ACD-QR mantém a mesma qualidade de maçãs ‘Maxi Gala’ que a pressão parcial de CO_2 empregada atualmente (1,2 kPa) após nove meses de armazenamento mais 21 dias de vida de prateleira;

Baixo nível de CO_2 (0,4 kPa) combinado com QR 1,3 reduz a respiração anaeróbica e mantém qualidade similar as maçãs ‘Maxi Gala’ armazenadas na pressão parcial de CO_2 1,2 kPa, possibilitando o uso da cal hidratada nas câmaras.

A aplicação de estresses por hipóxia durante o armazenamento em *ELO* mantém qualidade dos frutos similar à ACD-QR e ACD-FC e superior a AC convencional de maçãs ‘Maxi Gala’, após nove meses de armazenamento mais 21 dias de vida de prateleira;

O emprego da modalidade *ELO* com estresses por baixo O_2 mantém a qualidade de maçãs de forma similar à AC com aplicação de 1-MCP, tornando dispensável a aplicação desse produto.

O armazenamento em QR 1,3 com 0,4, 1,6 e 2,0 e *ELO* com estresses por baixo O_2 aumentam o perfil dos produtos voláteis que compõe o aroma de maças ‘Maxi Gala’.

1.2 OBJETIVOS GERAL E ESPECÍFICOS

1.2.1 Objetivo geral

Aperfeiçoar a técnica de ACD, avaliando o efeito de pressões parciais de CO_2 (0,4; 1,2, 1,6 e 2,0 kPa) combinado com ACD-QR 1.3 e determinar a eficácia dos estresses por hipóxia no armazenamento em condições de oxigênio extremamente baixo, a fim de reduzir custos com o armazenamento e prolongar a oferta de maçãs com maior qualidade.

1.2.2 Objetivos específicos

Avaliar a possibilidade de elevar a pressão parcial de CO_2 de 1,2 para 1,6 ou 2,0 kPa em câmaras de ACD com maçãs 'Maxi Gala' para economizar energia elétrica na absorção do CO_2 e manter a qualidade dos frutos;

Verificar se é possível usar baixa pressão parcial de CO_2 (0,4 kPa) no armazenamento em ACD-QR 1,3, de modo a viabilizar o uso apenas da cal hidratada para absorção de CO_2;

Avaliar a qualidade geral e perfil volátil de maçãs 'Maxi Gala' armazenadas em ACD-QR 1.3 em diferentes pressões parciais de CO_2 (0,4; 1,2; 1,6 e 2.0) após nove meses de armazenamento mais 7, 14 e 21 dias de vida de prateleira a 20 °C;

Identificar a melhor frequência e intensidade de estresses por hipóxia para manutenção da qualidade no armazenamento em *ELO* de maçãs 'Maxi Gala' por meio da variação da histerese do oxigênio;

Avaliar o efeito do armazenamento em *ELO* com estresses por hipóxia na qualidade geral e perfil volátil de maçãs 'Maxi Gala' após 7, 14 e 21 dias de vida de prateleira a 20 °C.

2 REVISÃO DE LITERATURA

2.1 PRODUÇÃO E ARMAZENAMENTO DE MAÇÃS

A maçã (*Malus x domestica* Borkh.) foi a terceira fruta mais produzida no mundo no ano de 2019, com cerca de 87,24 milhões de toneladas (FAOSTAT, 2021). No Brasil ela ocupou a oitava posição, com 1,1 milhão de toneladas na safra 2017/18, sendo que 61,6 % corresponde a cultivar Gala e suas mutantes, 31,9% a cultivar Fuji e mutantes e 6,5 % a outras maçãs (KIST et al., 2019). As cultivares/mutantes com maior produção no país são a Maxi Gala, Galaxy, Gala, Imperial Gala, Fuji, Fuji Suprema e Fuji Mishima (KIST et al., 2016).

A maçã é uma fruta de colheita sazonal que, no Brasil, é realizada a partir de meados de janeiro a início de maio. Em função disso é necessário armazenar cerca de 70% da produção para que haja oferta durante a entressafra. A principal forma de armazenamento empregada no Brasil é a atmosfera controlada (AC), que corresponde a cerca de 70% da capacidade de armazenagem de um total de 922,8 mil toneladas (KIST et al., 2016). A AC é mais utilizada em função da melhor manutenção da qualidade por um período maior que o armazenamento refrigerado (AR). A AC possibilita o armazenamento em até sete ou oito meses, enquanto que o AR apenas três a quatro meses. Isso acorre porque na AC a pressão parcial de O_2 é reduzida e de CO_2 aumentada, o que favorece uma maior redução do metabolismo dos frutos, prolongando o período de armazenamento e com melhor manutenção da qualidade comparado ao AR (BRACKMANN et al., 2008).

Apesar da melhoria na conservação de maçãs armazenadas em AC, ainda ocorrem perdas consideráveis, principalmente quando o período de armazenamento é de oito a nove meses, com excessiva redução na firmeza de polpa e de perdas por podridões, resultando em menor porcentagem de frutos sadios (BOTH et al., 2017; BRACKMANN et al., 2013; THEWES et al., 2015). Com o objetivo de reduzir essas perdas, nas últimas duas décadas foram desenvolvidas técnicas que empregam pressões parciais de O_2 extremamente baixas ($\leq$ 0,4 kPa), técnicas como ACD monitorada pelo produção de etanol (ACD-ET) (SCHOUTEN et al., 1997; VELTMAN et al., 2003), a ACD pela emissão de fluorescência de clorofilas (PRANGE et al., 2003; WRIGHT et al., 2015) e a ACD monitorada pelo quociente respiratório (BESSEMANS et al., 2016; BRACKMANN, 2015; GASSER et al., 2005). Estas técnicas resultam na melhoria da manutenção da qualidade em comparação à AC convencional (BESSEMANS et al., 2016; PRANGE et al., 2003; VELTMANN et al., 2003, WEBER et al., 2015).

2.2 EXTREMELY LOW OXYGEN (*ELO*) E ESTRESSES POR HIPÓXIA NO ARMAZENAMENTO DE MAÇÃS

O armazenamento de maçãs em Extremely Low Oxygen (*ELO*) se caracteriza pela adoção de pressões parciais de O_2 igual ou menores que 0,4 kPa durante o armazenamento e o método de estresses por hipóxia se caracteriza pela redução da pressão parcial de O_2 na câmara abaixo de 0,4 kPa durante um curto período de tempo e várias vezes durante o armazenamento em Extremely Low Oxygen (*ELO*). Essa redução de O_2 induz à respiração anaeróbica nos frutos que pode ser benéfica para manutenção dos frutos. Estudos verificaram que compostos produzidos durante a respiração anaeróbica, como o etanol, têm efeito na redução do metabolismo dos frutos (ASODA et al., 2009; JIN et al., 2013; WEBER et al., 2016). Esses autores verificaram que o etanol atua na inibição da expressão de genes envolvidos na síntese de etileno, hormônio que desencadeia o processo de amadurecimento de frutos climatéricos. Além disso, a adoção de pressões parciais de O_2 extremamente baixas não necessita de aquisição de equipamentos adicionais, não aumentando os custos de armazenamento. Por outro lado, a redução de O_2 a pressões parciais extremamente baixas sem o monitoramento do limite mínimo tolerado pelos frutos, pode ocasionar danos celulares e o surgimento de distúrbios fisiológicos nas maçãs, resultando em perdas para as empresas armazenadoras.

Nos estudos realizados até o momento foram avaliados apenas o efeito do estresse inicial por baixo O_2 no início do armazenamento e após o O_2 era mantido estático (entre 0,4 e 0,8 kPa) (BOTH et al., 2014; BRACKMANN et al., 2013; MATTÉ et al., 2005; THEWES et al., 2015; WANG; DILLEY, 2000). A principal vantagem da realização do estresse inicial está no controle da escaldadura, que os autores atribuem à redução do O_2 próximo ao LMO (MATTÉ et al., 2005; SABBAN-AMIN et al., 2011; WANG; DILLEY, 2000). Porém, outros pesquisadores não verificaram benefícios do estresse inicial por baixo O_2 em maçãs ‘Royal Gala’ e ‘Galaxy, pelo contrário, houve aumento da ocorrência de degenerescência de polpa (BOTH et al., 2014; BRACKMANN et al., 2013; THEWES et al., 2015). O armazenamento de maçãs ‘Royal Gala’ com 0,25 kPa de O_2 durante todo o armazenamento reduziu a firmeza de polpa comparado a pO_2 de 0,4; 0,5 e 0,7 kPa (Magno et al., 2019). Nesse sentido surgiu a ideia de testar variações na pO_2 durante o armazenamento em *ELO* com a indução de estresses.

Estudo realizado em 2016 no NPP/UFSM com níveis e quantidades de estresses em ACD mostrou que a exposição dos frutos a diversos estresses é benéfica na manutenção da qualidade. A partir disso, é importante estudar níveis e quantidades de estresses no armazenamento em oxigênio extremamente baixo (*ELO* - 0,5, 0,4 e kPa), pois é uma técnica

que não necessita de equipamentos adicionais, podendo ser uma alternativa aos diversos métodos de ACD e a aplicação de 1-MCP.

2.3 ATMOSFERA CONTROLADA DINÂMICA (ACD)

A ACD tem como objetivo manter os frutos armazenados em concentrações de oxigênio na qual os frutos apresentam menor metabolismo, resultando em menor perda de qualidade por um longo período de armazenamento. Isto é possível através do monitoramento constante do limite mínimo de oxigênio (LMO) tolerado pelos frutos por meio das técnicas de ACD (PRANGE et al., 2007; VELTMANN et al., 2003; WEBER et al., 2015).

2.3.1 Atmosfera controlada dinâmica monitorada pela fluorescência de clorofilas (ACD-FC)

A ACD-FC é a técnica de ACD mais utilizada no mundo. Esta técnica possibilita monitorar o limite mínimo de O_2 (LMO) tolerado pelos frutos através da fluorescência de clorofilas (PRANGE et al., 2003; SCHOUTEN et al., 1997). A técnica de ACD-FC, consiste na instalação de sensores da fluorescência de clorofila em uma caixa plástica com seis frutos, essa caixa é alocada no interior da câmara. No início do armazenamento o O_2 é reduzido até que ocorra a emissão de um pico de fluorescência, geralmente com $O_2 \leq 0,1$ kPa. Nesse momento o *set point* do O_2 deve ser aumentado em 0,2 a 0,3 kPa, porém este não deve ser menor que 0,4 kPa (PRANGE et al., 2003, PRANGE et al., 2007). A partir desse momento o LMO continua sendo monitorado até o final do armazenamento para verificar a ocorrência de um novo pico de fluorescência. Se isso ocorrer o O_2 deverá ser aumentado novamente, caso não ocorra mais nenhum pico, o O_2 será mantido em 0,4 kPa até o fim do armazenamento.

Frutos armazenados em ACD-FC apresentam melhor conservação comparado à AC convencional, em função das baixas pressões de O_2 empregadas, que favorecem uma maior redução na respiração e produção de etileno do que os frutos armazenados em AC (BOTH et al., 2017; THEWES et al., 2017b; THEWES et al., 2015). A redução do O_2 diminui a atividade de enzimas dependentes de O_2 essenciais na respiração, como a citocromo *c* oxidase e na produção de etileno, como a 1-aminociclopropano-1-carboxílico (ACC) oxidase (TAIZ; ZEIGER, 2013). As principais melhorias na manutenção da qualidade, comparado a AC, estão na redução da incidência de escaldadura superficial e da perda de firmeza de polpa (AUBERT et al., 2015; DeLONG et al., 2004; (LAFER, 2008; WATKINS, 2008; ZANELLA et al., 2005),

menor ocorrência de degenerescência de polpa e polpa farinácea (ANESE et al., 2020; BOTH et al., 2017; THEWES et al., 2017a; THEWES et al., 2017b). Por outro lado, em maçãs ‘Royal Gala’ e ‘Fuji Suprema’ foi verificado menor concentração de compostos voláteis importantes para o aroma dessas maçãs, com adoção da ACD-FC em comparação a AC e ACD-QR (BOTH et al., 2017; THEWES et al., 2017b).

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

O QR é a relação entre a produção de CO_2 e o consumo de O_2. Quando o valor dessa relação for 1,0 ou menor, indica que os frutos estão em condição aeróbica e quando for maior que 1,0 indica uma predominante respiração anaeróbica. O objetivo dessa técnica é manter os frutos sempre com QR um pouco acima de 1,0, com isso haverá pequena produção de etanol. A produção de etanol em baixas concentrações auxilia na manutenção da qualidade dos frutos, em função do etanol inibir a produção de etileno, hormônio que atua no amadurecimento e senescência de frutos (ASODA et al., 2009; JIN et al., 2013; WEBER et al., 2016). O cálculo do QR para monitorar o LMO começou a ser utilizado pelo Núcleo de Pesquisa em Pós-colheita da Universidade Federal de Santa Maria (NPP-UFSM) (BRACKMANN, 2015; WEBER et al., 2015).

Estudos realizados com o armazenamento de maçãs em ACD-QR mostraram que a técnica é eficiente na manutenção da qualidade dos frutos (BESSEMANS et al., 2016; BOTH et al., 2017; GASSER et al., 2008; THEWES et al., 2017a; WEBER et al., 2017). Maçãs ‘Royal Gala’ armazenadas em ACD-QR 1,5 apresentaram menor produção de etileno, menor taxa respiratória e atividade da enzima ACC oxidase (BOTH et al., 2017). O armazenamento de maçãs em ACD-QR 1,5 e 2,0 apresentaram maior produção de compostos voláteis em comparação a ACD-FC (BOTH et al., 2017; THEWES et al., 2017b). Estudos recentes demostraram que os níveis de QR 1,3 e 1,5 são os mais indicados para maçã ‘Gala’ (ANESE et al., 2020; BOTH et al., 2018; BRACKMANN et al., 2015; THEWES et al., 2017c) e maçãs ‘Fuji’ entre QR 1,5 e 2,0 (DONADEL et al., 2019; THEWES et al., 2017).

O nível de CO_2 empregado nos estudos de armazenamento em QR é de 1,2 kPa para maçãs mutantes da Gala (BOTH et al., 2017; THEWES et al., 2017a; WEBER et al., 2015). Desta maneira, é importante verificar o efeito de diferentes níveis de CO_2 em ACD-QR, principalmente CO_2 acima de 1,2 kPa. Um nível maior de CO_2 pode reduzir os custos com a energia elétrica na eliminação deste gás da câmara comercial, em função da maior eficiência

do equipamento de absorção, quando as pressões parciais de CO_2 são mantidas mais altas durante o armazenamento.

2.3.3 Efeito do CO_2 no armazenamento de maçãs

A pressão parcial de CO_2 utilizada durante o armazenamento de maçãs é dependente da cultivar de maçã e também da pressão parcial de O_2 empregada. Maçãs da cultivar Fuji e mutantes devem ser armazenados em pressões parciais menores que 1,0 kPa de CO_2, em função da maior sensibilidade à ocorrência de distúrbios fisiológicos, principalmente a degenerescência de polpa (BRACKMANN et al., 2005; CORRÊA et al., 2010; KWEON et al., 2013). Já as maçãs da cultivar Gala e mutantes toleram maiores pressões parciais de CO_2 (até 2,5 kPa) (BRACKMANN et al., 2008). Outro fator que influencia na escolha da pressão parcial de CO_2 a ser utilizada durante o armazenamento é a pressão parcial de O_2 (BEAUNDRY, 1999). Quando a pressão parcial de O_2 é maior que 1,0 kPa, que é o caso do armazenamento em AC, a pressão parcial de CO_2 pode ser maior (2,0 a 2,5 kPa para Gala). Quando os níveis de O_2 empregados são menores, como as adotadas no armazenamento em *ULO*, *ELO* e ACD, o nível de CO_2 deveria ser menor.

A manutenção de menores níveis de CO_2 em câmaras comerciais em ACD e *ULO* aumenta os custos com energia elétrica, em função do maior tempo que os adsorvedores estarão ligados, pois sua eficácia reduz em baixas concentrações de CO_2. Atualmente, a pressão parcial de CO_2 mais utilizada no armazenamento em ACD é de 1,2 kPa para maçãs da cultivar Gala e mutantes (BOTH et al., 2017; BRACKMANN; WEBER; BOTH, 2015; THEWES et al., 2017a, 2017b; WEBER et al., 2015, 2017). O armazenamento de maçãs com pressões de CO_2 mais elevado reduziria custo com energia elétrica, porém é necessário estudar o efeito de pressões mais elevadas de CO_2 sobre a manutenção da qualidade dos frutos. Poucos trabalhos foram realizados avaliando o efeito de pressões mais elevadas de CO_2 no armazenamento de maçãs da cultivar Gala e mutantes na técnica da ACD-QR. Em trabalho realizado com maçã 'Galaxy', foi verificado que o armazenamento em ACD-QR 1,5 com 2,0 kPa de CO_2 reduziu a porcentagem de frutos sadios quando comparado com 1,6 e 1,2 kPa de CO_2 (BRACKMANN; WEBER; BOTH, 2015). No estudo anterior foi adotado o QR 1,5, talvez com o emprego de de um QR menor (1,3) seja possível armazenar maçãs com CO_2 mais elevado (2,0 kPa) sem causar perdas. Além disso, no trabalho citado acima, os autores utilizaram a temperatura de 1 °C, o emprego de uma temperatura maior (2,0 °C) poderia viabilizar o uso de CO_2 mais elevado em ACD-QR. Estudos mostraram que o armazenamento de maçãs em ACD-QR associado com

temperatura de 2,0 °C comparado a 1,0 e 1,5 °C resultou em maior qualidade dos frutos (BOTH et al., 2018). Outro fator importante a ser estudado é o efeito da baixa pressão parcial de CO_2 (0,4 kPa) na qualidade de maçãs da cultivar Gala e mutantes armazenadas em ACD, sendo o CO_2 absorvido por hidróxido de cálcio (cal hidratada) alocado no interior da câmara. O uso de hidróxido de cálcio se justifica por ser mais eficiente na absorção do CO_2 e por ter menor custo comparado com um equipamento de adsorção com carvão ativado que utiliza energia elétrica para o seu funcionamento, além de evitar a ocorrência de depressão na câmara que leva a entrada de ar e aumento do nível de oxigênio além do nível desejado. Por outro lado, deve ser considerado que o uso de cal não permite o controle do nível de CO_2 na câmara. Portanto, se for verificada melhor manutenção da qualidade de maçãs 'Gala' em baixo CO_2 pela absorção com hidróxido de cálcio comparado ao adsorvedor de carvão ativado, o uso de hidróxido de cálcio pode ser uma alternativa ao adsorvedor de carvão ativado pelo menor custo de adoção.

2.3.4 Aplicação de 1-metilciclopropeno em maçãs (1-MCP)

O 1-MCP é um fitorregulador que inibe a ação de etileno, por se ligar de forma irreversível aos receptores de etileno, impedindo que o etileno se ligue ao receptor (BLANKENSHIP; DOLE, 2003; SISLER; SEREK, 1997; WATKINS, 2006). A ligação do 1-MCP ao receptor de etileno evita a ocorrência dos eventos posteriores desencadeados pelo etileno, que irão culminar com a antecipação do amadurecimento e senescência dos frutos. O 1-MCP também atua na redução da expressão de genes que codificam as enzimas ACC oxidase (*MdACO1*), ACC sintase (*MdACS1*) e receptores de etileno (*MdERS1* e *MdERS2*) (TATSUKI et al., 2007), com isso, retarda a senescência e mantém a qualidade dos frutos (BULENS et al., 2012).

O 1-MCP é muito utilizado no armazenamento em AC e AR. Vários trabalhos têm mostrado os benefícios da sua aplicação. Entre eles, a manutenção da firmeza de polpa (BRACKMANN et al., 2013; THEWES et al., 2015; 2017a; WATKINS; NOCK, 2012, WEBER et al., 2017), a redução da taxa respiratória (BOTH et al., 2014; THEWES et al., 2017a; WATKINS; NOCK, 2012) da incidência de polpa farinácea (BRACKMANN et al., 2014; THEWES et al., 2017a), e a prevenção da formação de escaldadura (BLANKENSHIP; DOLE, 2003). Por outro lado, a aplicação de 1-MCP reduz a produção de compostos voláteis essenciais para o aroma da maçã (KONDO et al., 2005). Além disso, o 1-MCP é um produto químico com custo elevado e sua aplicação poderia ser substituída pelo armazenamento em

baixo nível de O_2, que estimula à produção de pequenas quantidades de etanol durante o armazenamento, que também reduz a síntese do etileno.

2.3.5 Produção de compostos voláteis durante o armazenamento

Os compostos voláteis (CVs) são importantes na formação do aroma e sabor da maçã, influenciando na aceitabilidade pelo consumidor (ECHEVERRÍA et al., 2008). Em maçãs estão presentes cerca de 400 compostos (FORNEY et al., 2009), sendo distribuídos em vários grupos. Os principais grupos que compõem o aroma de maçãs são os ésteres, álcoois e aldeídos (MEHINAGIC et al., 2006; SALAZAR et al., 2011). Entre esses três grupos, os ésteres são encontrados em maior quantidade (ECHEVERRÍA et al., 2004). Em maçãs 'Gala' e mutantes os principais ésteres são o acetato de butila, acetato de hexila e acetato de 2-metil butila (BOTH et al., 2017; PLOTTO, MCDANIEL; MATTHEIS, 2000; SALAZAR et al., 2011; THEWES et al., 2017a). Em maçãs 'Fuji' os principais ésteres são acetato de 2-metil butila; 2-metil butanoato de etila e acetato de hexila (ECHEVERÍA et al., 2004) e 'Fuji suprema' butanoato de 2-metil etila, butanoato de etila e hexanoato de etila (THEWES et al., 2017b).

A produção de compostos voláteis é afetada por vários fatores pré e pós-colheita como: aplicação de reguladores de crescimento (ANESE et al., 2019; SALAZAR et al., 2011); estádio de maturação (THEWES et al., 2017a); aplicação de 1-MCP (ANESE et al., 2020; AUBERT et al., 2015; LUMPKIN et al., 2014, THEWES et al., 2015); pressão parcial de O_2 na câmara (BOTH et al., 2014; BRACKMANN, STREIF; BANGERTH, 1993; RAFFO et al., 2009; THEWES et al., 2017c, DONADEL et al., 2019) e pressão parcial de CO_2 (BRACKMANN, STREIF; BANGERTH, 1993; LUMPKIN et al., 2014). Os compostos voláteis são produzidos principalmente a partir da degradação de lipídios e aminoácidos via da β-oxidação e da lipoxigenase (LOX) (CONTRERAS; BEAUDRY, 2013; SALAZAR et al., 2011). Os ésteres de cadeia linear são produzidos a partir de lipídios (em maçãs principalmente a partir do ácido linoleico) e os de cadeia ramificada, a partir de aminoácidos.

O armazenamento de maçãs em baixas pressões parciais de O_2 reduz a respiração e produção de etileno, mantendo assim a qualidade dos frutos por um maior período. Porém, a redução do metabolismo afeta a produção de compostos voláteis. Baixas pressões parciais de O_2 limitam β-oxidação de ácidos graxos e/ou a rota da LOX na síntese de precursores de compostos voláteis (BOTH et al., 2014; BRACKMANN et al., 1993; SONG; BANGERTH, 2003; YANG et al., 2016). Por outro lado, a redução na produção de etileno inibe a atividade da enzima AAT, que converte álcoois em ésteres (DEFILIPPI et al., 2005; QI et al., 2020;

YANG et al., 2016). Além da influência das baixas pressões parciais de O_2, a concentração de CO_2 também pode alterar a produção de compostos voláteis. Maçãs armazenadas em AC com 3% e 5% de CO_2 apresentaram menor produção de ésteres de cadeia linear (BRACKMANN; STREIF; BANGERTH, 1993; LUMPKIN et al., 2014, respectivamente).

A aplicação de 1-MCP reduz a produção de compostos voláteis em função de inibir a atividade de enzimas e receptores envolvidos na produção etileno (TATSUKI; ENDO, 2006; TATSUKI; ENDO; OHKAWA, 2007), além disso, reduz também a expressão do gene (*MdLOX*) (YANG et al., 2016). O armazenamento de maçã 'Royal Gala' em *ELO* (0,5 kPa) reduziu a emissão de ésteres de cadeia linear (BOTH et al., 2014), já em maçãs 'Galaxy' armazenadas em *ELO* (0,4 kPa) a produção de ésteres totais não diferiu dos frutos armazenados e AC (ANESE et al., 2020). O armazenamento em ACD-FC resulta em baixa produção de ésteres (ANESE et al., 2020; AUBERT; MATHIEU-HURTIGER; VAYSSE, 2015; BOTH et al., 2017). No armazenamento em ACD-QR foi verificado maior emissão de voláteis em QRs maiores (1,5 e 2,0) (ANESE et al., 2020; BOTH et al., 2017; DONADEL et al., 2019; THEWES et al., 2017a), segundo os autores, esse efeito é devido a maior indução do metabolismo anaeróbico em função de menores concentrações de O_2 empregadas quando comparado a QRs menores (QR 1,3). Nesse contexto, o armazenamento de maçãs em *ELO* com indução de estresses pela variação do O_2 e o armazenamento com níveis de CO_2 mais elevados em ACD-QR 1,3 podem favorecer emissão de compostos voláteis. Portanto, é necessário testar essas condições, pois não há estudos na literatura avaliando essas condições de armazenamento e seu efeito a produção de compostos voláteis.

3 ARTIGO 1

3.1 DYNAMIC CONTROLLED ATMOSPHERE STORAGE MONITORED BY THE RESPIRATORY QUOTIENT WITH HIGH CO_2 PARTIAL PRESSURES ON THE OVERALL QUALITY OF 'MAXI GALA' APPLE AFTER LONG-TERM STORAGE[1]

Abstract

The objective of this work was to evaluate different CO_2 partial pressures in dynamic controlled atmosphere monitored by the respiratory quotient (DCA-RQ 1.3) on the overall quality and the production of anaerobic compounds of 'Maxi Gala' apple stored at 2 ºC during 9 months plus 7 d of shelf life at 20 ºC. Two experiments were performed over two seasons. In the first season the apples were stored in: [1] Controlled atmosphere (CA 1.2 O_2 + 2.0 kPa CO_2), [2] CA + 0.625 µL-1 1-methylcyclopropene (1-MCP - 0.625 μL^{-1}), [3] DCA monitored by the emission of chlorophyll fluorescence (CF - 1.2 kPa CO_2), [4] DCA-RQ 1.3 + 0.4 kPa CO_2, [5] DCA-RQ 1.3 + 1.2 kPa CO_2, [6] DCA-RQ 1.3 + 1.6 kPa CO_2. In the second season, the same treatments were evaluate with addition of DCA-RQ 1.3 + 2.0 kPa CO_2. . This research showed that 'Maxi Gala' apple could be stored during 9 months in extreme low oxygen under DCA-RQ 1.3 at 2 °C with high pCO_2 (1.6 or 2.0) plus 7 d of shelf life at 20 °C, maintained fruit quality similar than those fruit under DCA-RQ 1.3 + 1.2 pCO_2. This allow, reduce costs with CO_2 adsorption during storage. 'Maxi Gala' apple stored under DCA-RQ 1.3 + 0.4 kPa of CO_2 and DCA-CF maintained healthy fruit percentage similar to DCA – RQ 1.3 + 1.2 and 1.6 pCO_2, but with lower flesh firmness. 1-MCP application maintained high flesh firmness, but this treatment reduced the healthy fruit amount. The storage of 'Maxi Gala' apple under DCA-RQ 1.3 + 1.6 pCO_2 is efficient in keeping quality.

Keywords: Conservation. Extremely low oxygen. High pCO_2. *Malus domestica*. Quality.

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

3.1.1 Introduction

Controlled atmosphere (CA) storage is the most widely used storage technique worldwide for apple conservation (Brackmann et al., 2008; Wright et al., 2015). This technique consists in reducing the O_2 and increasing the CO_2 partial pressures in the storage room, which reduces the fruit metabolism and extends fruit conservation. For 'Gala' apple, O_2 ranges from 1.0 to 1.2 kPa and CO_2 from 2.0 to 2.5 kPa and with temperature of 0.5 to 1.0 °C (Brackmann et al., 2008; Brackmann et al., 2013; Weber et al., 2013). Generally, 'Gala' apples are stored in CA for 6 to 7 months with good quality maintenance. For longer storage periods, 1-methylcyclopropene (1-MCP) can be applied. This compound ia a potent inhibitor of the ethylene action, which is the plant hormone responsible for fruit ripening and other physiological events (Sisler and Serek, 1997; Watkins, 2006; Watkins, Nock, 2012). 1-MCP reduces ethylene production, respiration rate, and flesh firmness loss (McCormick et al., 2012; Nock and Watkins, 2013; Thewes et al., 2017a). Furthermore, the 1-MCP use is not permitted for organic apples in Europe (Gabioud and Gasser, 2015). Thus, new technologies have emerged, like dynamic controlled atmosphere (DCA-storage) monitoring by ethanol production by apple fruit (DCA - Eth) (Schouten et al., 1995; Veltman et al., 2003), chlorophyll fluorescence emission (DCA - CF) (Prange et al., 2003; Prange, 2007), respiratory quotient (DCA - RQ) (Bessemans et al., 2016; Brackmann et al., 2015; Gasser et al., 2008, 2010; Weber et al., 2015) and based on carbon dioxide production (DCA - CD) (Thewes et al., 2020). All these technologies/procedures allow to monitor the lower oxygen limit (LOL) tolerated by the fruit during storage. The most widely used DCA is DCA-CF (Aubert et al., 2015; Both et al., 2017; Eren et al., 2015; Thewes et al., 2015; Tran et al., 2015; Wright et al., 2012; Zanella and Stürz, 2015). To monitor LOL with DCA-CF, several chlorophyll sensors are used in different places inside the storage room. A software records the chlorophyll stress level, and when LOL is detected, a chlorophyll fluorescence peak occurs and then the O_2 set point is increased 0.2 or 0.3 kPa above the LOL detected (Prange et al., 2007; Prange, 2018). DCA-CF maintains high fruit quality during long-term storage (Aubert et al., 2015; Both et al., 2017; Eren et al., 2015; Thewes et al., 2015; Tran et al., 2015).

In the last decade several studies with DCA-RQ have been carried out, and they confirmed that this method maintains higher fruit quality during long-term storage (Bessemans et al., 2016; Both et al., 2018; Brackmann et al., 2015; Gasser et al., 2008; Thewes et al., 2017b; Weber et al., 2019). The RQ is determined by the ratio between the CO_2 production and O_2 uptake, measured during determined time, in which, the gas control system is disabled

(Brackmann et al., 2015; Weber et al., 2015). Recent studies have found that the best RQ level for 'Gala' apple is between 1.3 and 1.5 (Anese et al., 2020; Both et al., 2018; Brackmann et al., 2015; Thewes et al., 2017c). The CO_2 partial pressure (pCO_2) adopted in DCA-RQ storage is generally 1.2 kPa or less (Anese et al., 2020; Both et al., 2018; Thewes et al., 2020; Weber et al., 2015). Previous study found that 'Galaxy' apple stored in DCA-RQ 1.5 with 1.6 kPa CO_2 maintain fruit quality similar to 1.2 kPa CO_2 (Brackmann, Weber and Both, 2015), but at the temperature 1°C. The same authors found that apple storage under DCA-RQ 1.5 with 2.0 kPa CO_2 reduced fruit quality at this temperature. However, no studies were found evaluating other apple cultivars stored in DCA with different pCO_2 at higher temperatures (Both et al., 2018; Anese et al., 2019)

During the storage, fruit uptake O_2 and release CO_2 through respiration. The excess of CO_2 in storage rooms must be eliminated, to avoid flesh breakdown and cavities formation (Corrêa et al., 2010; Lumpkin et al., 2014). On the other hand, studies report that high pCO_2 reduce ethylene production and respiration (Gorny and Kader, 1997; Mathooko et al., 1996). The adsorption of the excess CO_2 produced by fruit implies in storage costs, and this cost can be higher or lower, depending on the pCO_2 adopted during storage and the efficiency of the CO_2 adsorption system. Generally, CO_2 adsorption system with activated charcoal has greater efficiency at partial pressures higher than 1.0 kPa of CO_2. When pCO_2 adopted is lower than 1.0 kPa, generally, calcium hydroxide (lime) is put into the storage room to absorb CO_2, but it occupies space in the room. In this context, adoption of pCO_2 higher than 1.2 kPa in the storage of apples can reduce costs with the adsorption of CO_2 with a charcoal scrubber.

Therefore, the aim of this work was to evaluate the effect of higher CO_2 partial pressures combined with extremely low oxygen partial pressures in DCA-storage monitored by the respiratory quotient (DCA-RQ 1.3) on the quality of 'Maxi Gala' apple stored at 2 °C.

3.1.2 Material and methods

3.1.2.1 Fruit harvest, sample preparation and storage conditions

This research was conducted in two seasons (2017 and 2018) with 'Maxi Gala' apples harvested in a commercial orchard located at Vacaria - RS, Brazil. Immediately after harvest, the apples were transported to the Postharvest Research Center of the Federal University of Santa Maria - Brazil, where they were selected to remove those who presented any damage.

Each treatment consisted of four samples of 25 fruit. Three samples were submitted to initial analysis to evaluate the physicochemical conditions of the fruit at harvest (Table 1).

Table 1 - Metabolism and quality of 'Maxi Gala' apple at harvest plus one day at 20 °C.

Season	SPI* (1-10)	ACC oxidase (ng kg^{-1} s^{-1})	IEC (ug L^{-1})	Ethylene (ng kg^{-1} s^{-1})	Respiration (µg kg^{-1} s^{-1})	TA (% of malic acid)	Firmness (N)	SS (°Brix)
2017	6.43	16.86	4.13	1.67	4.49	0.33	74.57	12.83
2018	6.88	9.99	1.23	0.16	3.11	0.36	89.31	10.97

* SPI: Starch pattern index according Streif (1984)

Two experiments were carried out to evaluate pCO_2 under DCA-RQ storage technology. In the 1st experiment (2017) 6 storage conditions were evaluate: [1] controlled atmosphere (CA) 1.2 kPa O_2 + 2.0 kPa CO_2; [2] CA + 1-methylcyclopropene (0.625 µL L^{-1}); [3] DCA monitored by chlorophyll fluorescence emission (DCA-CF) 1.2 kPaCO_2; [4] DCA monitored by respiratory quotient (DCA-RQ) [4] DCA-RQ 1.3 + 0.4 kPa CO_2; [5] DCA-RQ 1.3 + 1.2 kPa CO_2; [6] DCA-RQ 1.3 + 1.6 kPa CO_2 and [7] DCA-RQ 1.3 + 2.0 kPa CO_2. In the 2nd experiment (2018), the same storage conditions were evaluated with addition of DCA-RQ 1.3 + 2.0 kPa CO_2 conditions.

3.1.2.2 Atmosphere establishment and monitoring

Four samples of each treatment were stored during 9 months into experimental chambers (230 L), kept inside a cold room at 2.0 °C. The temperature was decrease to 5 °C in the first storage day and, afterwards, it was gradually reduced during five days to 2 °C and remaining so during the entire storage period. The temperature was controlled with electronic thermostat and monitored daily by mercury thermometers, introduced into the apple pulp.

At the first day after the temperature establishment, the oxygen partial pressure was decreased to 5.0 kPa with N_2 flushing and, during the next 5 days, the oxygen was decreased to the desired partial pressure by fruit respiration. The pCO_2 were obtained by its accumulation in the chamber due to fruit respiration. pO_2 and pCO_2 were monitored and corrected daily with an automatic CA control system (Valis®, Lajeado, RS, Brazil) connected to a gas analyzer (Siemens®, Ultramat model, Germany). The equipment determines and performs the correction of pO_2 and pCO_2, comparing the actual level with the pre-established partial pressure (set point) for each treatment. If pCO_2 was above the set point, the chamber air was circulated through a

CO_2 absorber containing calcium hydroxide and, if pO_2 is below the set point, cold atmospheric air was injected until it reaches the level established for the treatment.

DCA-CF was installed and monitored according to Prange et al. (2007) and DCA-RQ was determined according to the methodology proposed by (Brackmann, 2015; Weber et al., 2015). The RQ was determined twice a week by the ratio between CO_2 production and O_2 uptake during 13 h, in which the chamber remained without O_2 injection and CO_2 absorption. When RQ was below or higher than 1.3, the pO_2 was changed in a range of 0.01-0.03 kPa in relation to the current pO_2. Relative humidity was maintained at 94 ± 2 % through the allocation of calcium chloride (10 g kg^{-1} of fruit), which absorbed excess moisture.

3.1.2.3 1-Methylcyclopropene (1-MCP) application

Four samples of fruit were put into a 230 L chamber, which was placed in a cold room at 2.0 ± 0.1 °C. A solution of 1-MCP (0.625 μL L^{-1}, SmartFresh®, 0.14 % active ingredient) was prepared. The solution was placed inside the chamber and hermetically closed over a period of 24 h. During this period, the air of the chamber was circulated with a fan, to homogenize the air. After 24 h, the fruit were removed from the chamber and stored in CA according to above reported.

3.1.2.4 Metabolism and quality analysis

These analyses were carried out after 9 months of storage plus 7 d shelf-life at 20 ± 1 °C and relative humidity at 80 % (± 2 %).

3.1.2.4.1 ACC oxidase activity

Determined according to the methodology proposed by Bufler (1986). Three replicates of 25 fruit each were evaluated per treatment. Results were expressed in ng C_2H_4 kg^{-1} s^{-1}.

3.1.2.4.2 Internal ethylene concentration (IEC)

Air from the fruit was removed with a vacuum pump at 565 mm Hg suction pressure, according to Mannapperuma et al. (1991). Two samples of 1 mL of the air were injected in to a Varian® gas chromatograph, Star 3400CX model, equipped with a flame ionization detector

(FID) and a Porapak N80/100 column. The temperature of the column, injector and detector were 90, 140 e 200 °C, respectively. Results were expressed in µg L^{-1}.

3.1.2.4.3 Ethylene production and respiration rate

Approximately 1.5 kg of fruit were placed in a 5 L glass that was hermetically sealed and maintained for one hour at 20 °C. After that, two samples of 1 mL of the headspace were withdrawn from the glass and immediately injected into the same gas chromatograph used to determine the IEC. The determination was performed at chamber opening, 2, 4 and 6 d shelf-life and expressed in ng kg^{-1} h^{-1}. The respiration rate was expressed by the CO_2 release from fruits. For respiration rate, the sample was prepared according ethylene production, after one hour was determined CO_2 production, by circulating the air through a gas analyzer (Isolcell® Italy, model Oxycarb 6). Evaluations were also made at chamber opening, 2, 4 and 6 d of shelf life and results expressed in µg CO_2 kg^{-1} h^{-1}.

3.1.2.4.4 Electrolyte leakage

Determined according to methodology proposed by Gago et al. (2015), with modifications. For this, 15 discs (with 5 mm diameter and thickness) from the pulp fruit were removed. Subsequently, the discs were immersed in a falcon tube with 25 mL of distilled water and left for one hour at 20 ± 1 °C. After that, the conductivity of the solution was determined. Then, the sample was boiled for 30 min at 100 ºC and then cooled to 20 °C in a freezer at -30 °C. Soon after, the conductivity was measured again. Results were expressed as percentage.

3.1.2.4.5 Flesh breakdown, mealiness, decay incidence and healthy fruit

For these parameters all the fruit from each replicate were evaluated. For flesh breakdown, mealiness and decay incidence evaluation, fruit were slicing in the equatorial region to observe any browning region, mealy pulp and decay symptoms. If fruit did not show any symptoms described above were counted as healthy fruit. Results were expressed as percentage in relation to the total fruit of each repetition.

3.1.2.4.6 Flesh firmness

Determined with a penetrometer, with a tip of 11 mm in diameter (Effegi, model FT 327, 3-27 lbs., Milan, Italy). The tip was inserted on two opposite sides of the pulp, in the equatorial region of the fruit, where previously the epidermis was removed. Results were expressed in Newton (N).

3.1.2.4.7 Titratable acidity and soluble solids

A juice sample was extracted from the 25 fruits of each repetition, with a juice extractor (Philips Walita®). For titratable acidity determination, 10 mL of juice were diluted in 100 mL of distilled water and titrated with NaOH at 0.1 N until pH 8.1. Results were expressed in % of malic acid. Some drops from the extracted juice were placed on the prism of a refractometer (Biobrix, Model 103, Curitiba, Brazil), in order to determined the soluble solids. Results were expressed in °Brix.

3.1.2.4.8 Anaerobic metabolism compounds

The samples preparation to determined acetaldehyde, ethanol and ethyl acetate concentrations were performed according to Both et. (2014). The anaerobic metabolism compounds were extracted from the juice via solid phase micro extraction (HS-SPME) and quantified with a DANI® (Dani Intruments Spa., Viale Brianza, Cologno Monzese, Italygas) gas chromatograph equipped with a flame ionization detector (FID) at 230 °C. The fiber was thermally desorbed into the injection port for 10 min at a temperature of 250 °C in a split less mode. The anaerobic metabolism compounds were separated with in a capillary column DN-5 (30 m 0.25 mm 0.25 µm). The flow rate of the carrier gas (nitrogen) was 1.0 mL min $^{-1}$. The temperature ramp used during the analysis was according Both et al. (2014). The linear retention index was determined according to Both et al. (2014).

Anaerobic metabolism compounds were identified with a Shimadzu QP2010 Plus gas chromatograph coupled to a mass spectrometer (GC/MS; Shimadzu Corporation, Kyoto, Japan). The extraction/desorption/injection procedures for identification of the anaerobic compounds, were the same as described above, with helium as carrier gas. The MS detector operated in electron ionization mode, with ionization energy of +70eV, a scan ranges from m/z 35‑350 and a temperature of 250 °C. The mass spectra of each anaerobic compound were identified comparing with mass spectra available in the National Institute of Standards and

Technology library and by comparing the linear retention index (LRI) with those available in the scientific literature. Results were expressed in μg L^{-1}.

3.1.2.4.9 Statistical analysis

Data were submitted to analysis of variance (ANOVA). Data that showed a significant difference by ANOVA ($p < 0.05$), were subjected by Scott Knott test at 5% of error probability. All data with significant difference 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).

3.1.3 Results

3.1.3.1 Multivariate analysis

For better understand de effect of the storage conditions and CO_2 partial pressure during storage in DCA, on the fruit metabolism and overall quality, a Principal Component Analysis (PCA) was performed (Fig. 1). On apples harvested on season 2017, the PC I and PCII explained 89.84 % of the total variable variance, but the main variance was explained by PC I (78.91 %).PC I discriminated well fruit stored on CA from DCA and CA + 1-MCP conditions (Fig. 1a). CA is associated with anaerobic metabolism compounds, mealiness, flesh breakdown incidence, ACC oxidase, IEC, respiration rate and ethylene production (Fig. 1b). On the other hand, DCAs and CA + 1-MCP conditions are associated with higher flesh firmness and healthy fruit (Fig. 1b). These results are related to the low pO_2 during storage (Fig. 3b) and 1-MCP application. An important result is that fruit storage under DCA-RQ 1.3, regardless of the pCO_2, showed high correlation with fruit quality. Fruit stored under DCA-RQ 1.3 + 1.6 CO_2 were separated from CA + 1-MCP, DCA-CF and DCA-RQ 1.3 with 0.4 and 1.2 pCO_2 in PC II (Fig. 1a). Flesh firmness, healthy fruit and anaerobic metabolism compounds are associated with DCA-RQ 1.3 + 1.6 CO_2 (Fig.1b).

At the season 2018 (Fig. 2), the PC I and PC II explained 80.68 % of the total variable variance. PC I separated again fruit under CA condition from the other storage conditions (Fig.

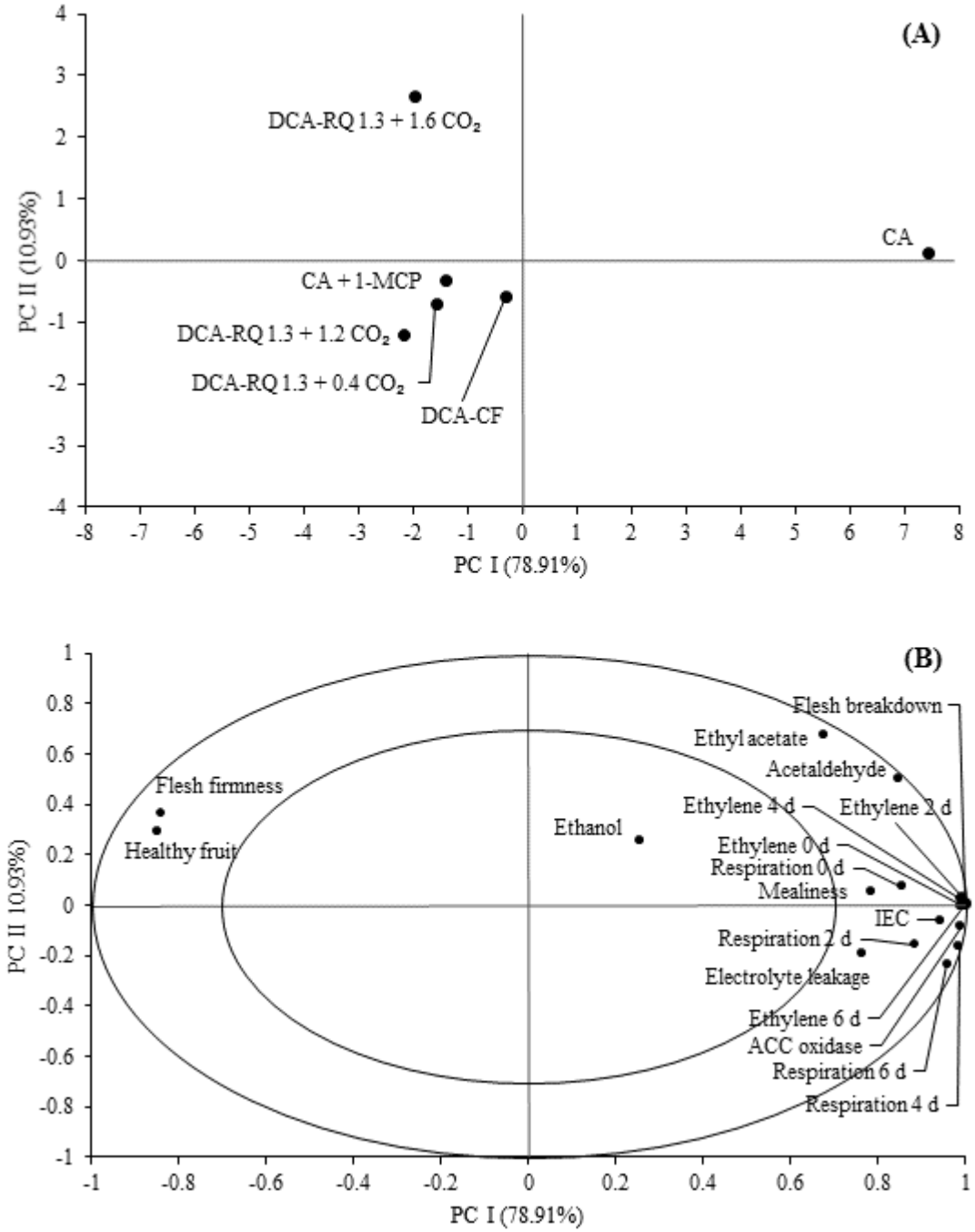


Figure 1. Principal component analysis (PCA) of the metabolism and quality of 'Maxi Gala' apples stored over 9 months under CA without or with 1-MCP treatment (0.650 µL L-1), DCA-CF, DCA-RQ 1.3 with 0.4, 1.2 and 1.6 kPa CO_2, plus 7 d of shelf life at 20 °C.

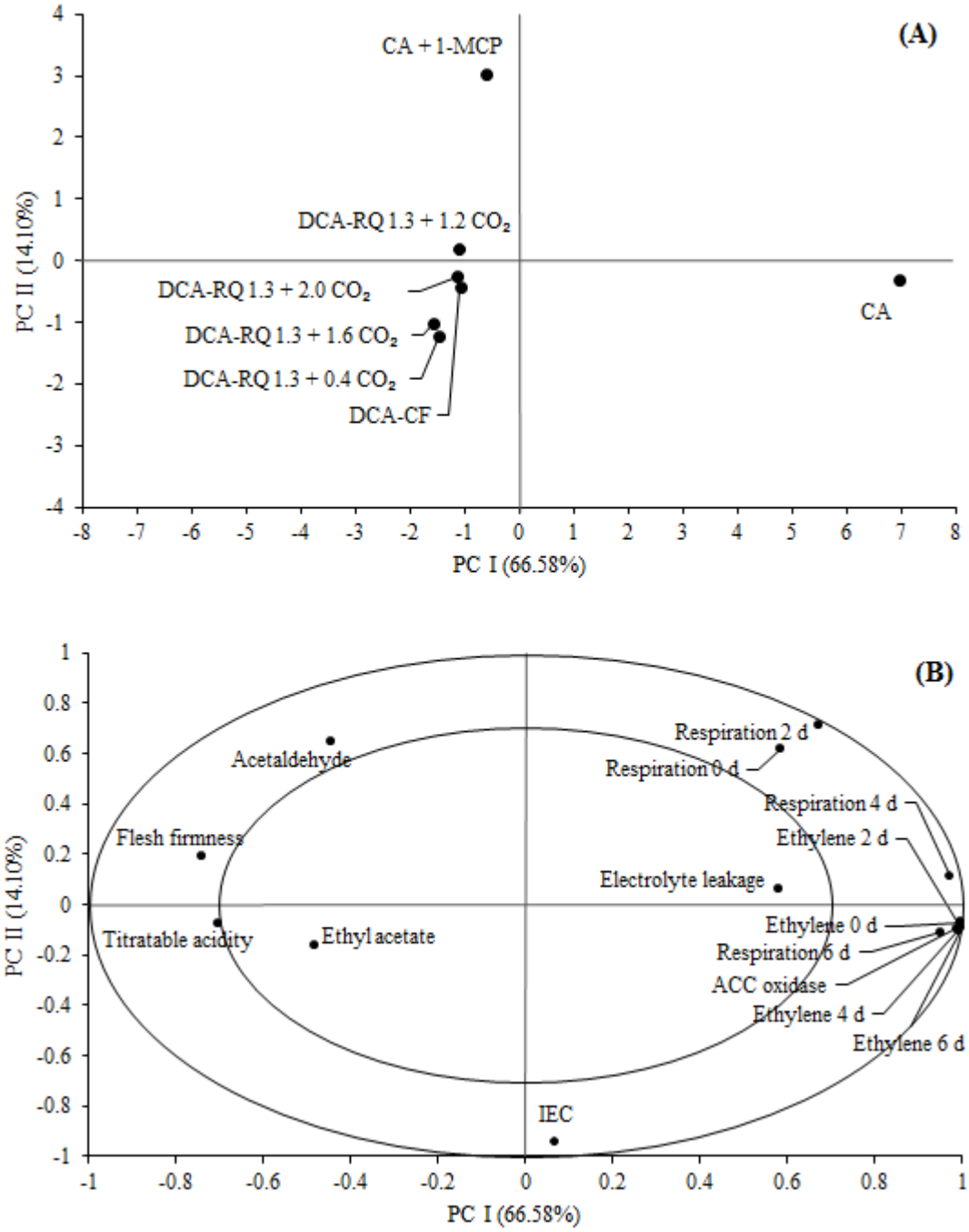


Figure 2. Principal component analysis (PCA) of the metabolism and quality of 'Maxi Gala' apples stored over 9 months under CA without or with 1-MCP treatment (0.650 µL L-1), DCA-CF, DCA-RQ 1.3 with 0.4, 1.2, 1.6 and 2.0 kPa CO_2, plus 7 d of shelf life at 20 °C.

2a). CA storage is associated with ACC oxidase activity, ethylene production, respiration rate and electrolyte leakage (Fig. 2b). DCA and CA + 1-MCP conditions are associated with higher flesh firmness, ethyl acetate and acetaldehyde (Fig. 2b). PC II separated CA + 1-MCP from

DCA conditions (Fig. 2a). Fruit storage in CA + 1-MCP are associated with acetaldehyde (Fig. 2b).

3.1.3.2 Oxygen set point variation during storage

The variation of oxygen during the storage of 'Maxi Gala' apple under CA, DCA-CF and DCA-RQ 1.3 with 0.4, 1.2, 1.6 and 2.0 kPa of CO_2 are shown in the Fig. 3. The pCO_2

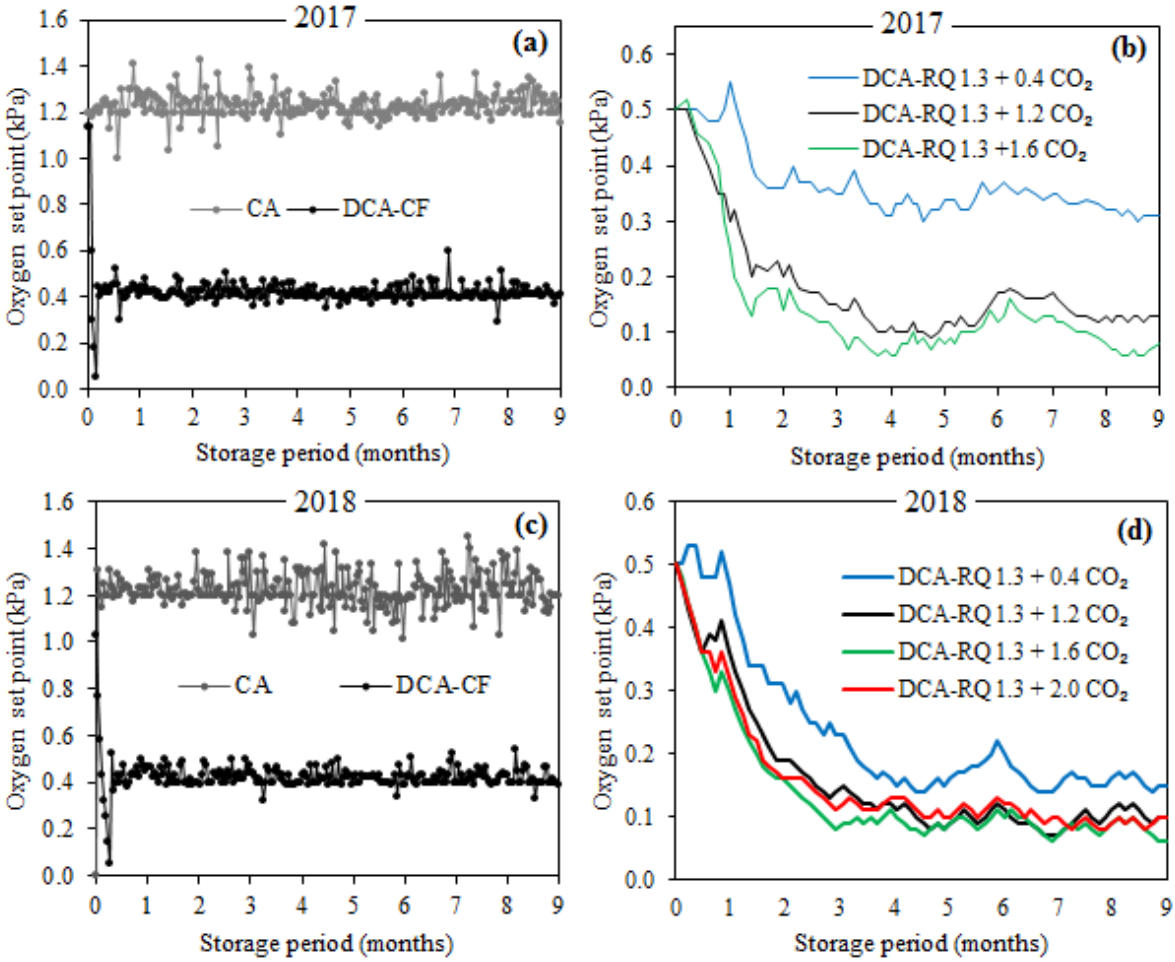


Figure 3. Oxygen set point variation of 'Maxi Gala' apple during 9 months stored under CA, DCA-CF (a, c) and DCA-RQ 1.3 with four pCO2 (0.4, 1.2, 1.6 and 2.0 kPa) (b, d), at 2.0 °C.

adopted in DCA-RQ 1.3 influenced the variation of oxygen partial pressure (pO_2) during storage in both seasons. As higher the pCO_2 adopted, lower was the pO_2 observed (Fig. 3b, d). Generally, pCO_2 of fruit pulp is higher than the pCO_2 maintained in the chamber atmosphere, duo to the CO_2 produced by fruit respiration. The different CO_2 set point adopted in the storage chamber may generates different gradient concentration between the fruit and surrounding

atmosphere, influencing on the diffusion of CO_2 and RQ determination. The mean of pO_2 during apple storage under DCA-RQ 1.3 with 0.4, 1.2 and 1.6 kPa of CO_2 at first season (2017) were 0.36, 0.17 and 0.14 kPa, respectively, and at the second season (2018) were 0.23, 0.16, 0.14 kPa, respectively and 0.15 kPa for pCO_2 2.0. Fruit stored under DCA-RQ 1.3 with pCO_2 0.4 showed higher O_2 mean at the first season compared to the fruit of the second season. This could be due to the growing season.

3.1.3.3 Anaerobic metabolism compounds

After 9 months of storage plus 7 d of shelf life at 20 °C, acetaldehyde concentration was higher in fruit stored under CA (season 2017) (Fig. 4a). When compared fruit stored under DCA-RQ 1.3 with different pCO_2, fruit stored with 1.6 kPa of CO_2 showed higher acetaldehyde concentration compared to pCO_2 0.4 and 1.2 (season 2017), but in 2018 fruit stored under DCA-RQ 1.3 + 1.2 CO_2 showed higher concentration, without differing from fruit stored under CA

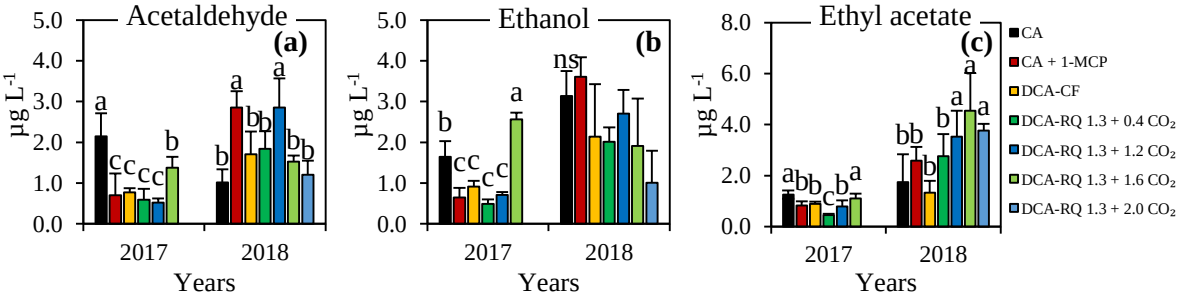


Figure 4. Acetaldehyde (a), ethanol (b) and ethyl acetate (c) concentration of 'Maxi Gala' apples stored over 9 months under CA, CA + 1-MCP, DCA-CF, DCA-RQ 1.3 with four pCO_2 (0.4, 1.2, 1.6 and 2.0 kPa), plus 7 d of shelf life at 20 °C. Means followed by the same letter do not differ by Scott Knott test at 5% of error probability. Error bars show the standard deviation. ns: not significant.

+ 1-MCP. Ethanol concentration in 2017 (Fig. 4b), was higher in fruit stored under DCA-RQ 1.3 with 1.6 pCO_2 compared to the fruit of the others conditions, but in 2018 there was no difference among the storage conditions. Regarding the ethyl acetate (Fig. 4c), fruit stored under CA and DCA-RQ 1.3 with 1.6 pCO_2 had higher concentration at 2017, but in 2018, the higher concentration is verified in fruit stored under DCA-RQ 1.3 with 1.2, 1.6 and 2.0 kPa of CO_2.

3.1.3.4 ACC oxidase activity, internal ethylene concentration, ethylene production and respiration rate

'Maxi Gala' apple stored under CA showed the highest ACC oxidase activity after 9 months of storage plus 7 d of shelf life in both seasons (Fig. 5a). Fruit stored in DCA-RQ 1.3, irrespective of pCO_2, result in the lowest ACC oxidase activity, but without differ from fruit stored under CA + 1-MCP and DCA-CF. The lower ACC oxidase activity resulted in lower internal ethylene concentration (IEC) (Fig. 5b) and ethylene production (Fig. 5c, d) in the two seasons, with exception of IEC in fruit stored under DCA-CF in 2017. There was no difference for IEC and ethylene production among the fruit stored under DCA-RQ 1.3 with different pCO_2 in both season.

Regarding respiration rate, fruit stored under DCAs and CA + 1-MCP showed lower respiration rate during the whole shelf life period in 2017, compared to CA (Fig. 5e). In 2018 (Fig. 5f), fruit stored under DCA-RQ 1.3 with pCO_2 0.4 and 1.2 showed lower respiration at chamber opening compared to the fruit on the other conditions. At 2, 4 and 6 d of shelf life, DCA-RQ 1.3, regardless of the pCO_2, showed low respiration rate. On the other hand, CA fruit showed high respiration during shelf life.

3.1.3.5 Overall quality

Electrolyte leakage is an important parameter to evaluate membrane permeability. Fruit stored in CA and DCA-CF showed higher electrolyte leakage in 2017 (Fig. 6a), but in 2018 the DCA-CF fruit showed lower electrolyte leakage. Different pCO_2 not influenced the electrolyte leakage in fruit stored under DCA-RQ 1.3.

'Maxi Gala' apple stored in CA showed a higher flesh breakdown percentage after 9 months of storage plus 7 d of shelf life at 20 °C in 2017, but in 2018 this disorder was not detected (Fig. 6b). Fruit from both seasons that were stored in DCA-RQ 1.3, regardless of the pCO_2, did not present any flesh breakdown incidence. Mealiness is an important disorder that occurs after long storage periods. In 2017, mealiness incidence was lower in fruit stored under DCA conditions, irrespective of the pCO_2 compared to CA with and without 1-MCP (Fig. 6c). However, in 2018 there was no difference among the conditions evaluated. Decay incidence is one of the main cause of losses during and after long-term storage. However, no differences among the conditions were observed in the two storage seasons (Fig 6d).

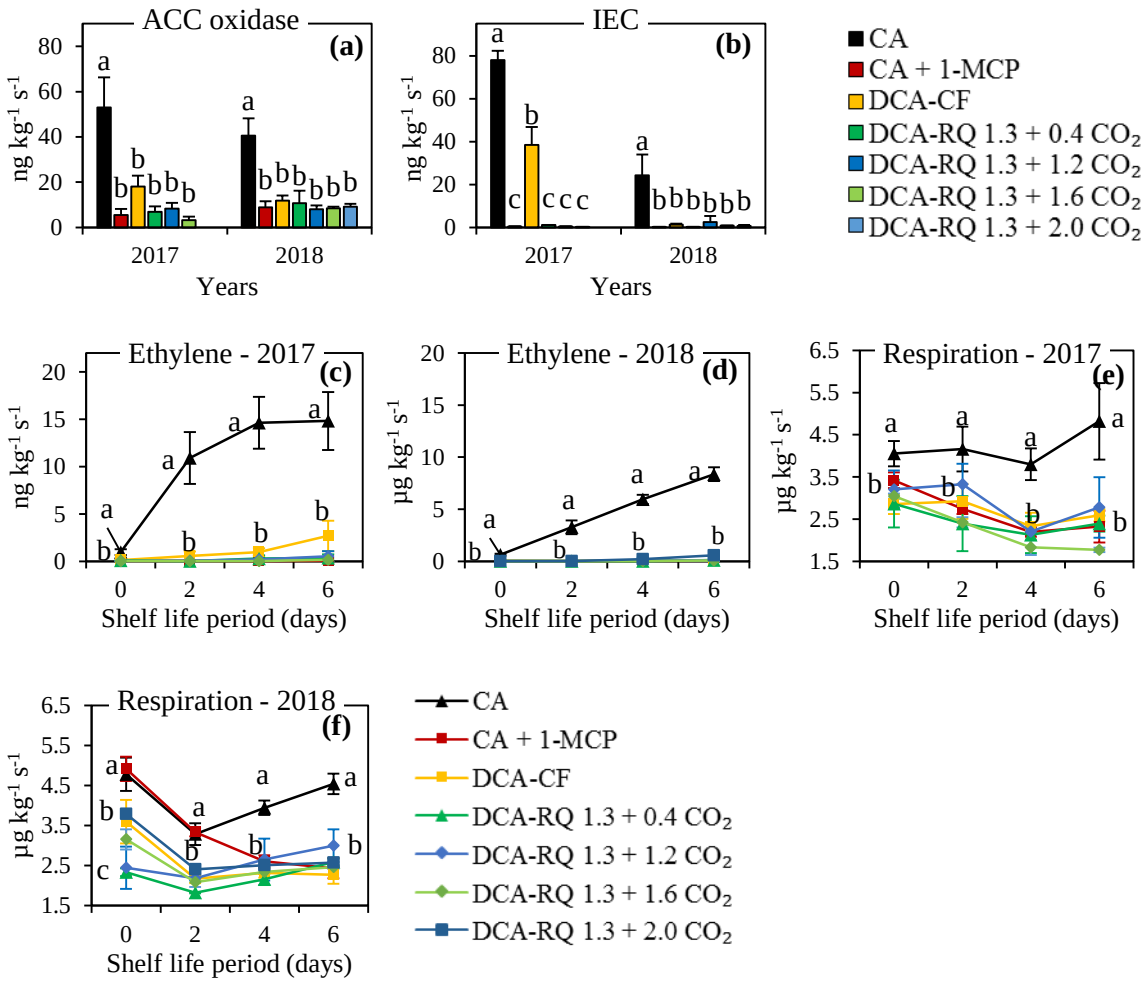


Figure 5. ACC oxidase activity (a), internal ethylene concentration (b) ethylene production (c, d) and respiration rate (e, f) of 'Maxi Gala' apples stored over 9 months under CA, CA + 1-MCP, DCA-CF DCA-RQ 1.3 with four pCO_2 (0.4, 1.2, 1.6 and 2.0 kPa), plus 7 d of shelf life at 20 °C. Means followed by the same letter do not differ by Scott Knott test at 5% of error probability. Error bars show the standard deviation. ns: not significant.

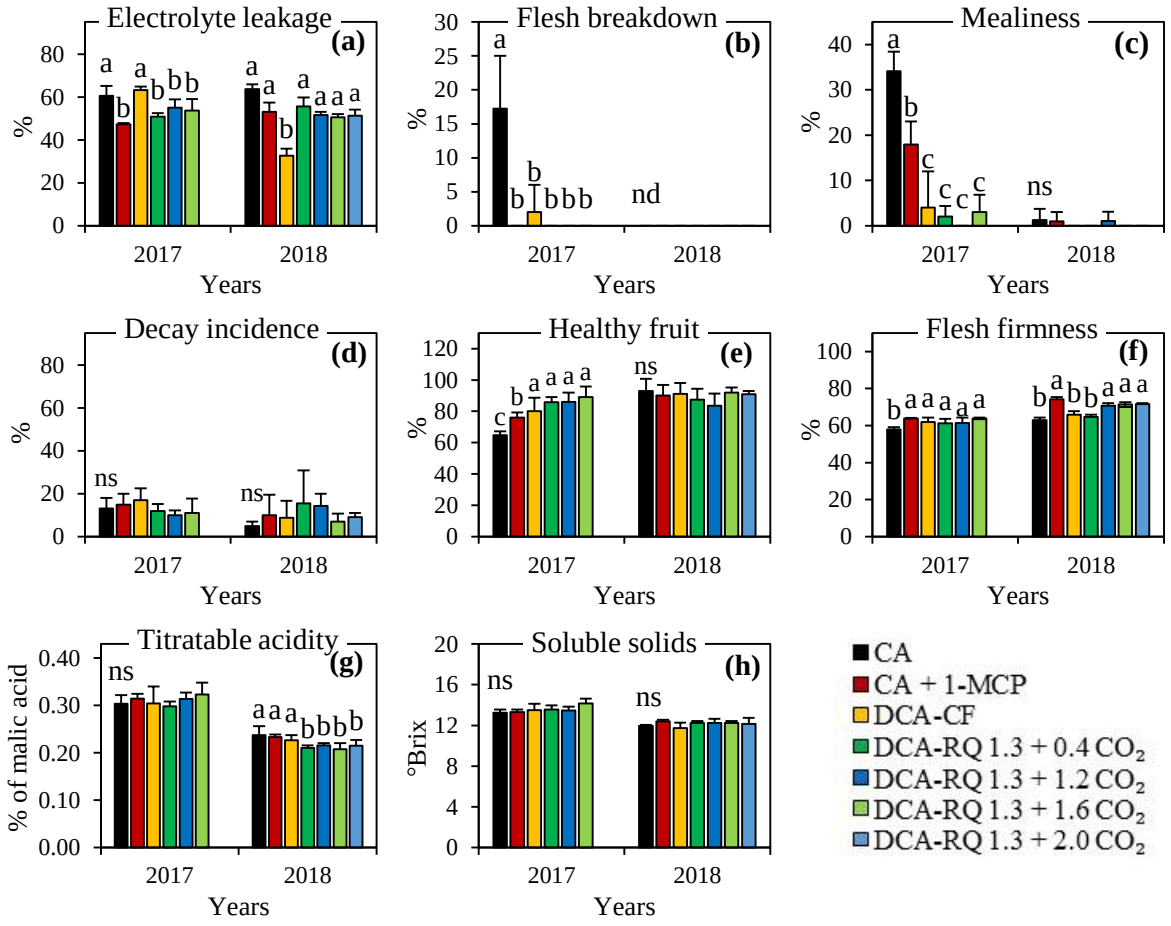


Figure 6. Electrolyte leakage (a), flesh breakdown (b), mealiness (c), decay incidence (d), healthy fruit (e), flesh firmness (f), titratable acidity (g) and soluble solids (h) of 'Maxi Gala' apples stored over 9 months under CA, CA + 1-MCP, DCA-CF, DCA-RQ 1.3 with four pCO_2 (0.4, 1.2, 1.6 and 2.0 kPa), plus 7 d of shelf life at 20 °C. Means followed by the same letter do not differ by Scott Knott test at 5% of error probability. Error bars show the standard deviation. ns: not significant. nd: not detected.

The main objective of the storers is obtain the higher amount of healthy fruit after storage. 'Maxi Gala' apples stored in DCA-CF and DCA-RQ 1.3 regardless of pCO_2 showed a higher healthy fruit amount compared to CA and CA + 1-MCP in 2017, but in 2018, no difference was observed (Fig. 6e). Other important quality parameter is the flesh firmness. DCA methods and CA + 1-MCP resulted in fruit with higher flesh firmness in the two seasons, with exception of DCA-RQ 1.3 with 0.4 of pCO_2 that had lower flesh firmness in 2018 (Fig. 6f). Titratable acidity and soluble solids (Fig. 6g, h), were less influenced by the storage conditions, with exception of titratable acidity in 2018, that was lower in fruit stored in DCA-RQ 1.3 regardless the pCO_2 adopted(Fig. 6g).

3.1.4 Discussion

3.1.4.1 Anaerobic metabolism compounds

Anaerobic metabolism compounds, such as acetaldehyde, ethanol and ethyl acetate when in higher concentration are correlated with off-flavors (Echeverría et al., 2008; Wright et al., 2015) and also can induce/participate on the development of physiological disorders, like flesh breakdown (Both et al., 2017). The increase in the production of these compounds are generally related to extremely low oxygen (Both et al., 2017; Thewes et al., 2020a; Wright et al., 2015) and high pCO_2 (Lumpkin et al., 2014) used during the storage. On the other hand, studies reported that the presence of ethanol in the storage environment reduced ethylene biosynthesis (Asoda et al., 2009; Jin et al., 2013; Weber et al., 2020, 2016), and thereby slow down fruit ripening. In our study, DCA-RQ 1.3 with 1.6 pCO_2 increased the anaerobic metabolism compounds production compared to fruit stored under DCA-RQ 1.3 with lower pCO_2, DCA-CF and CA + 1-MCP at the first season 2017, but this production stayed far below the odor threshold (OT) (Fig 4a, b and c). The OT reported in literature for acetaldehyde is 644 µg kg^{-1} (Komthong et al., 2006), ethyl acetate 13,500 µg kg^{-1} (Lopez et al., 2007) and ethanol 100,000 µg kg^{-1} (Leffingwell and Leffingwell, 1991). The higher anaerobic compounds in DCA-RQ 1.3 + 1.6 kPa CO_2 can be attributed to the combined effect of extremely low oxygen (0.14 kPa) and higher pCO_2. On the other hand, in 2018 higher pCO_2 (1.6 and 2.0 kPa) resulted in lower acetaldehyde concentration than pCO_2 1.2. In general, the treatments adopted did not cause excess fermentation products, even with such low O_2 (in DCA) and high CO_2.

3.1.4.2 ACC oxidase activity, IEC, ethylene production and respiration rate

ACC oxidase is the key enzyme in the last step to ethylene production and its activity depends on O_2 availability (Yang and Hoffmann 1984; Yang et al., 2016). Another factor that limits its activity is the 1-MCP application which reduced expression genes *MdACO1* (Thewes et al., 2020; Yang et al., 2016) and *MdACS1* (Tatsuki et al., 2007). For this reason, in the two experiments, ACC oxidase activity was lower in fruit storage under CA + 1-MCP, DCA-CF and DCA-RQ 1.3 regardless of pCO_2 when compared to static CA (Fig. 5a). This result was also reported by other studies (Anese et al., 2020; Both et al., 2017, 2018; Schmidt et al., 2020; Thewes et al., 2017c; Weber et al., 2020). Ethylene promotes the ripening process in climacteric fruit (Yang and Hoffmann 1984). The reduction of ethylene production and its internal

concentration is the key to prolong shelf life of these fruit. The 1-MCP application and decreased pO_2 (DCAs) reduced the ACC oxidase activity and consequently resulted in lower IEC, ethylene production and respiration rate during shelf life compared to CA (Fig. 5b to 5f). These results are already known in the literature for 1-MCP application (Thewes et al., 2017a, Schmidt et al., 2020; Watkins, 2006), DCA-CF and DCA-RQ (Anese et al., 2020; Both et al., 2017; Schmidt et al., 2020; Thewes et al., 2017c). Our results showed that the different pCO_2 adopted in extreme low oxygen with DCA-RQ 1.3 had no additional effect in reducing IEC, ethylene production and respiration rate on 'Maxi Gala' apple. A work evaluating 'Galaxy' apple storage in DCA-RQ 1.5 with 1.2, 1.6 and 2.0 kPa of CO_2, also found no difference for ethylene production (Brackmann, Weber and Both, 2015).

3.1.4.3 Overall quality analyses

High pCO_2 and low pO_2 can result in cell membrane damages due to the high anaerobic compounds accumulation (Saquet and Streif, 2008) and cause physiological disorders, like flesh breakdown. The cell damage can be evaluated by electrolyte leakage. The fruit stored in CA and DCA-CF conditions had higher electrolyte leakage in 2017 (Fig. 6a), but only CA fruit showed high anaerobic compounds concentration and high flesh breakdown incidence. On the other hand, fruit stored in DCA-RQ 1.3 with pCO_2 (1.6) showed high concentration of anaerobic compounds, but showed lower flesh breakdown incidence, evidencing that the anaerobic metabolism production was at adequate levels, contributing to the fruit quality preservation. At the second year (2018) (Fig. 6a), fruit stored in CA and DCA-RQ 1.3, regardless pCO_2 showed higher electrolyte leakage compared to DCA-CF fruit. However, no flesh breakdown was detected (Fig. 6b), even in fruit stored in CA without 1-MCP. A noteworthy fact is that, regardless pCO_2 adopted in DCA-RQ 1.3, no flesh breakdown was found.

Mealiness incidence occurs in fruit harvested with advanced maturity (Thewes et al., 2017c) and stored for a long-term in CA. This is characterized by a mealy aspect in the pulp, and occur due to pectins degradation, which are responsible for maintains the cells adhesion (Payasi et al., 2009; Prasanna et al., 2007). This disorder is accelerated by higher ethylene presence, which increases activity of pectin degradation enzymes (Prasanna et al., 2007). Fruit storage under DCA-CF and DCA-RQ 1.3, regardless of the pCO_2, showed lower mealiness incidence, compared to the CA storage in the 1st season 2017, mainly without 1-MCP application (Fig. 6c). This is correlated with the low fruit metabolism, and confirmed in the

PCA analysis (Fig. 6a, b). These results agree with other studies (Both et al., 2018; Thewes et al., 2017c). Fruit from the 2nd season (2018) did not differ among the storage conditions for mealiness occurrence (Fig. 6c) probably, because these fruits were harvested with higher flesh firmness (89.31 N) (Table 1) than fruits harvest in 2017 (74.57 N).

Despite decay incidence, did not differ among the storage conditions in the both seasons. DCA conditions maintained higher healthy fruit amount in the season 2017 compared to CA and CA+1-MCP. This result is correlated to the lower mealiness incidence (Fig. 6c), but in 2018, no difference was found among the storage conditions for healthy fruit. In contrast, other studies found higher healthy fruit amount in DCA-RQ compared to CA (Both et al., 2018; Thewes et al., 2017c; Weber et al., 2015). This study showed that, 'Maxi Gala' apple storage under DCA-RQ 1.3, regardless of the pCO_2, resulted in lower internal disorders and decay incidence and maintained higher amount of healthy fruit. The use of high pCO_2 in the apple storage is an advantage for stores, because can reduce costs with CO_2 adsorption.

Flesh firmness and titratable acidity are quality attribute well appreciated by apple consumers (Harker et al., 2008). 'Maxi Gala' apples stored under DCA-RQ 1.3, CA + 1-MCP and DCA-CF showed higher flesh firmness compare to CA in season 2017 (Fig. 6f). This is related to the lower fruit metabolism and physiological disorders measured in these conditions. The firmness loss occurs with the ethylene action, which increases cell wall degrading enzymes activity (Nishiyama et al., 2007; Payasi et al., 2009; Prassana et al., 2007). In the season 2018, DCA-RQ 1.3 with 1.2, 1.6 and 2.0 pCO_2 and CA + 1-MCP maintained higher flesh firmness. In relation to titratable acidity, there was no difference among the storage conditions at season 2017, but in 2018, fruit storage under CA, CA + 1-MCP and DCA-CF showed higher titratable acidity compared to fruit in DCA-RQ 1.3 (Fig. 6g). In contrast, Both et al. (2018) found higher titratable acidity in 'Galaxy' apple stored in DCA-RQ 1.5 compared to CA. There was no difference among different pCO_2. Similar result was found for 'Galaxy' apple stored under DCA-RQ 1.5 with 1.2, 1.6 and 2.0 kPa CO_2 (Brackmann, Weber and Both, 2015).

3.1.5 Conclusions

'Maxi Gala' apple stored in in DCA-RQ 1.3, with extreme low oxygen, associated to high pCO_2 (1.6 or 2.0) and at temperature of 2 ºC maintains fruit quality similar than DCA-RQ 1.3 with 1.2 pCO_2. This implies lower costs with CO_2 adsorption during storage.

DCA-RQ 1.3 with 0.4 kPa of CO_2 and DCA-CF maintain the percentage of healthy fruit similar to DCA-RQ 1.3 with 1.2, 1.6 and 2.0 pCO_2, but with lower flesh firmness,

suggesting that high CO_2 brings beneficial effect even in DCA-RQ storage. 1-MCP application, despite maintain high flesh firmness, reduces healthy fruit amount.

3.1.6 References

Anese, R. de O., Brackmann, A., Thewes, F. R., Schultz, E. E., Ludwig, V., Klein, B. 2020. Impact of dynamic controlled atmosphere storage and 1-methylcyclopropene treatment on quality and volatile organic compounds profile of 'Galaxy' apple. Food Packag. Shelf Life, 23, 100-443. https://doi.org/10.1016/j. fpsl.2019.100443.

Anese, R. de O., Brackmann, A., Wendt, L.M., Thewes, F.R., Schultz, E.E., Ludwig, V., Berghetti, M.R.B., 2018. Interaction of 1-methylcyclopropene, temperature and dynamic controlled atmosphere by respiratory quotient on 'Galaxy' apples storage. Food Packag. Shelf Life 20, 100-246. https://doi.org/10.1016/j.fpsl.2018.07.004.

Asoda, T., Terai, H., Kato, M., Suzuki, Y., 2009. Effects of postharvest ethanol vapor treatment on ethylene responsiveness in broccoli. Postharvest Biol. Technol. 52, 216‑220. https://doi.org/10.1016/j.postharvbio.2008.09.015.

Aubert, C., Mathieu-Hurtiger, V., Vaysse, P., 2015. Effects of dynamic atmosphere on volatile compounds, polyphenolic content, overall fruit quality, and sensory evaluation of "Pink Lady®" apples. Acta Hortic. 275-280. https://doi.org/10.17660/ActaHortic.2015.1071.34.

Bessemans, N., Verboven, P., Verlinden, B.E., Nicolaï, B.M., 2016. A novel type of dynamic controlled atmosphere storage based on the respiratory quotient (RQ-DCA). Postharvest Biol. Technol. 115, 91-102. https://doi.org/10.1016/j.postharvbio.2015.12.019.

Both, V., Brackmann, A., Thewes, F.R., Ferreira, D. de F., Wagner, R., 2014. Effect of storage under extremely low oxygen on the volatile composition of 'Royal Gala' apples. Food Chem. 156, 50-57. https://doi.org/10.1016/j.foodchem.2014.01.094.

Both, V., Brackmann, A., Thewes, F.R., Weber, A., Schultz, E.E., Ludwig, V., 2018. The influence of temperature and 1-MCP on quality attributes of 'Galaxy' apples stored in controlled atmosphere and dynamic controlled atmosphere. Food Packag. Shelf Life, 16, 168‑177. https://doi.org/10.1016/j.fpsl.2018.03.010.

Both, V., Thewes, F.R., Brackmann, A., de Oliveira Anese, R., de Freitas Ferreira, D., Wagner, R., 2017. Effects of dynamic controlled atmosphere by respiratory quotient on some quality parameters and volatile profile of 'Royal Gala' apple after long-term storage. Food Chem. 215, 483-492. https://doi.org/10.10 16/j.foodchem.2016.08.009.

Brackmann, A., Anese, R.O., Weber, A., Both, V., Gasperin, A.R., Pavanello, E.P., 2013. Efeito do estresse inicial por baixo O_2 combinado com 1-metilciclopropeno na conservação de maçãs 'Royal Gala' armazenadas com ultrabaixo O_2. Semina: Cienc. Agrárias 34, 1185-1194. DOI: 10.5433/1679-0359.2013v34n3p1185.

Brackmann, A. Control apparatus for controlled atmosphere cells for storing perishable items. ***U.S. Patent***, n.US2015/0257401A1. 2015.

Brackmann, A., Weber, A., Both, V., 2015. CO_2 partial pressure for respiratory quotient and Harwest WatchTM dynamic controlled atmosphere for 'Galaxy' apple storage. Acta Hort. 1079, 435-440. https://doi.org/10.17660/ActaHortic.2015.1079.56.

Brackmann, A., Weber, A., Pinto, J.A.V., Neuwald, D.A., Steffens, C.A., 2008. Manutenção da qualidade pós-colheita de maçãs 'Royal Gala' e 'Galaxy' sob armazenamento em atmosfera controlada. Cienc. Rural 38, 2478-2484. https://doi.org/10.1590/S0103-84782008000900010.

Bufler, G., 1986. Ethylene-promoted conversion of 1-aminocyclopropane-1-carboxylic to ethylene in peel of apple at various stages of fruit development. Plant Physiol. 80, 539-543. https://doi.org/10.1104/pp.80.2.539.

Corrêa, T.R., Steffens, C.A., Amaranta, C.V.T do., Brackmann, A., Silveira, J.P.G., Tanaka, H., Vanderlei Both, V., 2010. Qualidade de maçãs 'Fuji'armazenadas em atmosfera controlada e influência do clima na degenerescência da polpa. Pesq. Agropec. Bras. 45, (6), 531-538. https://doi.org/10.1590/S0100-204X2010000600001.

Echeverría, G., Graell, J., Lara, I., López, M.L., 2008. Physicochemical measurements in 'Mondial Gala®' apples stored at different atmospheres: influence on consumer acceptability. Postharvest Biol. Technol. 50, 135 - 144. https://doi.org/10.1016/j.postharvbio.2008.05.002.

Eren, I., Çalhan, O., Onursal, C.E., Güneyli, A., 2015. Effects of controlled atmosphere, dynamic controlled atmosphere and 1-MCP on quality of "Granny Smith" apples. Acta Hortic. 495-502. https://doi.org/10.17660/ActaHortic.2015.1071.64.

Gabioud Rebeaud, S., Gasser, F., 2015. Fruit quality as affected by 1-MCP treatment and DCA storage - a comparison of the two methods. Europ. J. Hort. Sci. 80, 18-24. https://doi.org/10.17660/eJHS.2015/80.1.3.

Gago, C.M.L., Guerreiro, A.C., Miguel, G., Panagopoulos, T., Sánchez, C., Antunes, M. D. C. 2015. Effect of harvest date and 1-MCP (SmartFresh^{TM}) treatment on 'Golden Delicious' apple cold storage physiological disorders. Postharvest Biol. Technol. 110, 77-85. https://doi.org/10.1016/j.postharvbio.2015.07.018.

Gasser, F., Eppler, T., Naunheim, W., Gabioud, S., Hoehn, E., 2008. Control of the critical oxygen level during dynamic CA storage of apples by monitoring respiration as well as chlorophyll fluorescence. Acta Hortic. 69-76. https://doi.org/10.17660/ActaHortic.2008.796.6.

Gasser, F., Eppler, T., Naunheim, W., Gabioud, S., Nising, A.B., 2010. Dynamic CA storage of apples: monitoring of the critical oxygen concentration and adjustment of optimum conditions during oxygen reduction. Acta Hortic. 876, 39 - 46. https://doi.org/10.17660/actahortic.2010.876.3.

Gorny, J.R., Kader, A.A., 1997. Low Oxygen and Elevated Carbon Dioxide Atmospheres Inhibit Ethylene Biosynthesis and Preclimacteric and Climacteric Apple Fruit. J. Am. Soc. Hortic. Sci. 122, 542-546. https://doi.org/10.21273/JASHS.122.4.542.

Harker, F.R., Kupferman, E.M., Marin, A.B., Gunson, F.A., Triggs, C.M., 2008. Eating quality standards for apples based on consumer preferences. Postharvest Biol. Technol. 50, 70-78. https://doi.org/10.1016/j.postharvbio.2008.03.020.

Jin, Y.Z., Lv, D.Q., Liu, W.W., Qi, H.Y., Bai, X.H., 2013. Ethanol vapor treatment maintains postharvest storage quality and inhibits ethylene biosynthesis during storage of oriental sweet melons. Postharvest Biol. Technol. 86, 372-380. https://doi.org/10.1016/j.postharvbio.2013.07.019.

Komthong, P., Hayakawa, S., Katoh, T., Igura, N., Shimoda, M., 2006. Determination of potent odorants in apple by headspace gas dilution analysis. Food Sci. Technol. LWT 39, 472-478.

Leffingwell, J.C., Leffingwell, D., 1991. GRAS flavor chemicals. Detection thresholds. Perfumer Flavorist 16, 2-19.

López, M.L., Villatoro, C., Fuentes, T., Graell, J., Lara, I., Echeverría, G., 2007. Volatile compounds, quality parameters and consumer acceptance of 'Pink Lady1' apples stored in different conditions. Postharvest Biol. Technol. 43, 55-66.Lumpkin, C., Fellman, J.K., Rudell, D.R., Mattheis, J., 2014. ' Scarlett Spur Red Delicious ' apple volatile production accompanying physiological disorder development during low pO_2 controlled atmosphere storage. J. Agric. Food Chem. 62, 1741-1754. https://doi.org/10.1021/jf405267b.

Mannapperuma, J.D., Singh, R.P., Montero, M.E., 1991. Simultaneous gas diffusion and chemical reaction in foods stored in modified atmospheres. J. Food Eng. 14, 167-183.

Mathooko, F.M., 1996. Regulation of ethylene biosynthesis in higher plants by carbon dioxide. Postharvest Biol. Technol. 7, 1-26. https://doi.org/10.1016/0925-5214(95)00026-7.

McCormick, R., Neuwald, D. A., & Streif, J. (2012). Commercial apple CA storage temperature regimes with 1-MCP (SmartFreshTM): Benefits and risks. Acta Hortic. 934, 263-270. https://doi.org/10.17660/ActaHortic.2012.934.32.

Nishiyama, K., Guis, M., Rose, J.K.C., Kubo, Y., Bennett, K.A.B., Lu, W., Kato, K., Koichiro, U., Ryohei, N., Akitsugu, I., Mondher, B., Alain, L., Jean-Claude, P., Bennett, A.B., 2007. Ethylene regulation of fruit softening and cell wall disassembly in charentais melon. J. Exp. Bot. 58, 1281-1290. https://doi.org/10.1093/jxb/erl283.

Nock, J. F., Watkins, C. B., 2013.Repeated treatment of apple fruit with 1-methylcyclopropene (1-MCP) prior to controlled atmosphere storage. Postharvest Biol. Technol. 79, 73-79. https://doi.org/10.1016/j.postharvbio.2013.01.002.

Payasi, A., Mishra, N.N., Chaves, A.L.S., Singh, R., 2009. Biochemistry of fruit softening: an overview. Physiol. Mol. Biol. Plants 15, 103-113. https://doi.org/10.1007/s12298-009-0012-z.

Prange, R.K., DeLong, J. M., Harrison, P.A., Leyte, J.C., McLean, S.D., 2003. Oxygen concentration affects chlorophyll fluorescence in chlorophyll-containing fruit and vegetable. J. Am. Soc. Hortic. Sci. 128, 603-607. https://doi.org/10.21273/JASHS.128.4.0603.

Prange, R.K., Delong, J.M., Harrison, P., Mclean, S., Scrutton, J., Cullen, J., 2007. Method and apparatus for monitoring a condition in chlorophyll containing matter. U.S. Patent, n.WO/2002/006795.

Prasanna, V., Prabha, T.N., Tharanathan, R.N., 2007. Fruit ripening phenomena-an overview. Crit. Rev. Food Sci. Nutr. 47, 1-19. https://doi.org/10.1007/s12298-009-0012-z.

Saquet, A.A., Streif, J., 2008. Fermentative metabolism in ‘Jonagold’ apples under controlled atmosphere storage. Europ. J. Hortic. Sci. 73, 4.

Schouten, S. P. 1995. Dynamic control of the oxygen content during CA storage of fruits and vegetables. Preprints of “Control Applications in Post-Harvest and Processing Technology CAPPT’9”, Ostend (Belgium) 131-136.

Schmidt, S.F.P., Schultz, E.E., Ludwig, V., Berghetti, M.R.P., Thewes, F.R., Anese, R., de, O., Both, V., Brackmann, A., 2020. Volatile compounds and overall quality of ‘Braeburn’ apples after long-term storage: interaction of innovative storage technologies and 1-MCP treatment. Sci. Hortic. 262, 109039. https://doi.org/10.1016/j.scienta.2019.109039.

Sisler, E.C., Serek, M., 1997. Inhibitors of ethylene responses in plants at the receptor level: recent developments. Physiol. Plant. 100, 577-582. https://doi.org/10.1111/j.1399-3054.1997.tb03063.x.

Tatsuki, M., Endo, A., Ohkawa, H., 2007. Influence of time from harvest to 1-MCP treatment on apple fruit quality and expression of genes for ethylene biosynthesis enzymes and ethylene receptors. Postharvest Biol. Technol. 43, 28-35. https://doi.org/10.1016/j.postharvbio.2006.08.010.

Thewes, F.R., Brackmann, A., Anese, R., de, O., Ludwig, V., Schultz, E.E., dos Santos, L.F., Wendt, L.M., 2017a. Effect of dynamic controlled atmosphere monitored by respiratory quotient and 1-methylcyclopropene on the metabolism and quality of ‘Galaxy’ apple harvested at three maturity stages. Food Chem. 222, 84-93. https://doi.org/10.1016/j.foodchem.2016.12.009.

Thewes, F.R., Both, V., Brackmann, A., Ferreira, D.F., Wagner, R., 2015. 1-Methylcyclopropene effects on volatile profile and quality of ‘Royal Gala’ apples produced in Southern Brazil and stored in controlled atmosphere. Ciência Rural. 12, 2259-2266. http://dx.doi.org/10.1590/0103-8478cr20141613.

Thewes, F.R., Brackmann, A., Both, B., Anese, de O.R., Schultz, E.E., Ludwig, V., Wendt, L.M., Berghetti, M.R.P., Thewes, F.R., 2020a. Dynamic controlled atmosphere based on carbon dioxide production (DCA-CD): Lower oxygen limit establishment, metabolism and overall quality of apples after long-term storage. Postharvest Biol. Technol. 168, 111-285. https://doi.org/10.1016/j.postharvbio.2020.111285.

Thewes, F.R., Brackmann, A., Both, V., Weber, A., Anese, R., de, O., Ferrão, T., dos, S., Wagner, R., 2017b. The different impacts of dynamic controlled atmosphere and controlled atmosphere storage in the quality attributes of ‘Fuji Suprema’ apples. Postharvest Biol. Technol. 130, 7-20. https://doi.org/10.1016/j.postharvbio.2017.04.003.

Thewes, F.R., Brackmann, A., de Oliveira Anese, R., Bronzatto, E.S., Schultz, E.E., Wagner, R., 2017c. Dynamic controlled atmosphere storage suppresses metabolism and enhances volatile concentrations of ‘Galaxy’ apple harvested at three maturity stages. Postharvest Biol. Technol. 127, 1-13. https://doi.org/10.1016/j.postharvbio.2017.01.002.

Thewes, F.R., Brackmann, A., de Oliveira Anese, R., Ludwig, V., Schultz, E.E., Berghetti, M.R.P., 2018. 1-Methylcyclopropene suppresses anaerobic metabolism in apples stored under dynamic controlled atmosphere monitored by respiratory quotient. Sci. Hortic. 227, 288-295. https://doi.org/10.1016/j.scienta.2017.09.028.

Thewes, F. R., Anese, R. O., Thewes, F. R., Ludwig, V., Klein, B., Wagner, R., Nora, F. R., Rombaldi, C. V., Brackmann, A., 2020b. Dynamic controlled atmosphere (DCA) and 1-

MCP: Impact on volatile esters synthesis and overall quality of 'Galaxy' apples. Food Packag. Shelf Life. 26, 100-563. https://doi.org/10.1016/j.fpsl.2020.100563.

Tran, D.T., Verlinden, B.E., Hertog, M., Nicolaï, B.M., 2015. Monitoring of extremely low oxygen control atmosphere storage of 'Green star' apples using chlorophyll fluorescence. Sci. Hortic. 184, 18-22. https://doi.org/10.1016/j.scienta.2014.11.

Veltman, R.H., Verschoor, J.A., van Dugteren, J.H.R., 2003. Dynamic control system (DCS) for apples (Malus domestica Borkh. Cv 'Elstar'): optimal quality through storage based on product response. Postharvest Biol. Technol. 27, 79-86. https://doi.org/10.1016/S0925-5214(02)00186-2.

Watkins, C.B., 2006. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. Biotechnol. Adv. 24, 389‑409. https://doi.org/10.1016/j.biotechadv.2006.01.005.

Watkins, C.B., Nock, J.F., 2012. Rapid 1-methylcyclopropene (1-MCP) treatment and delayed controlled atmosphere storage of apples. Postharvest Biol. Technol. 69, 24-31. https://doi.org/10.1016/j.postharvbio.2012.02.010.

Weber, A., Brackmann, A., Anese, R. de O., Both, V. Pavanello, E.P., 2013. Atmosfera controlada para o armazenamento da maçã 'Maxi Gala'. Rev. Cien. Agron. 44, 294-301. https://doi.org/10.1590/S1806-66902013000200011.

Weber, A., Brackmann, A., Both, V., Pavanello, E.P., Anese, R.O., Schorr, M.R.W., 2016. Ethanol reduces ripening of "Royal Gala" apples stored in controlled atmosphere. An. Acad. Bras. Ciênc. 88, 403‑410. https://doi.org/10.1590/0001-3765201620140181.

Weber, A., Brackmann, A., Both, V., Pavanello, E.P., De O., Anese, R., Thewes, F.R., 2015. Respiratory quotient: innovative method for monitoring 'Royal Gala' apple storage in a dynamic controlled atmosphere. Sci. Agric. 72, 28-33. https://doi.org/10.1590/0103-9016-2013-0429. https://doi.org/10.1590/0103-9016-2013-0429.

Weber, A., Neuwald, D.A., Kittemann, D., Thewes, F.R., Both, V., Brackmann, A., 2020. Influence of respiratory quotient dynamic controlled atmosphere (DCA‑RQ) and ethanol application on softening of Braeburn apples. Food Chem. 303, 125346. https://doi.org/10.1016/j.foodchem.2019.125346.

Weber, A., Thewes, F.R., Sellwig, M., Brackmann, A., Wünsche, J.N., Kittemann, D., Neuwald, D.A., 2019. Dynamic controlled atmosphere: impact of elevated storage temperature on anaerobic metabolism and quality of 'Nicoter' apples. Food Chem. 298, 125017. https://doi.org/10.1016/j.foodchem.2019.125017.

Wright, A.H., Delong, J.M., Arul, J., Prange, R.K., 2015. The trend toward lower oxygen levels during apple (Malus×domestica Borkh) storage. J. Hortic. Sci. Biotechnol. 90, 1-13. https://doi.org/10.1080/14620316.2015.11513146.

Wright, A. H., DeLong, J. M., Gunawardena, A.H.L. A. N., Prange, R K., 2012. Dynamic controlled atmosphere (DCA): Does fluorescence reflect physiology in storage? Postharvest Biol. Technol. 64, 19-30. https://doi.org/10.1016/j.postharvbio.2011.09.015.

Yang, S.F., Hoffman, N.E., 1984. Ethylene biosynthesis and its regulation in higher plants. Annu. Rev. Plant Biol. 35, 155-189. https://doi.org/10.1146/annurev.pp.35.060184.001103.

Yang, X., Song, J., Lina, D., Forney, C., Campbell-Palmer, L., Fillmore, S., Wismer, P., Zhang, Z., 2016. Ethylene and 1-MCP regulate major volatile biosynthetic pathwaysin apple fruit. Food Chem. 194, 325-336. https://doi.org/10.1016/j.foodchem.2015.08.018.

Zanella, A., Cazzanelli, P., Rossi, O., 2008. Dynamic controlled atmosphere (DCA) storage by the means of chlorophyll fluorescence response for firmness retention in apples. Acta Hortic. 796, 77-82. https://doi.org/10.17660/ActaHortic.2008.796.7.

Zanella, A., Stürz, S., 2015. Optimizing postharvest life of horticultural products by means of dynamic ca: fruit physiology controls atmosphere composition during storage. Acta Hortic. 59-68. https://doi.org/10.17660/ActaHortic.2015.1071.4.

4 ARTIGO 2

4.1 DIFFERENT CO_2 LEVELS IN DYNAMIC CONTROLLED ATMOSPHERE MONITORED BY RESPIRATORY QUOTIENT: EFFECT ON OVERALL QUALITY AND VOLATILE ORGANIC COMPOUNDS PROFILE OF 'MAXI GALA' APPLES AFTER STORAGE AND EXTENDED SHELF LIFE[2]

Abstract

The objective of this work was to evaluate the effect of different pCO_2 on the overall quality and volatile profile of 'Maxi Gala' apple stored under dynamic controlled atmosphere (DCA-storage) monitored by the respiratory quotient (RQ 1.3) during 9 months plus 7, 14 and 21 d of shelf life at 20 ºC and compared them with CA, CA + 1-MCP and DCA-CF. Apples were stored at 2 °C and in: [1] Controlled atmosphere (CA - 1.2 O_2 + 2.0 kPa CO_2), [2] CA + 1-methylcyclopropene (1-MCP - 0.625 µL L^{-1}), [3] DCA monitored by the emission of chlorophyll fluorescence (CF - 1.2 kPa C^2O_2), [4] DCA-RQ 1.3 + 0.4 kPa CO_2, [5] DCA-RQ 1.3 + 1.2 kPa CO_2, [6] DCA-RQ 1.3 + 1.6 kPa CO_2 and [7] DCA-RQ 1.3 + 2.0 kPa CO_2. 'Maxi Gala' apple could be adequately stored under DCA-RQ 1.3 with 1.6 pCO_2, because it maintained fruit quality similar or better than 1.2 pCO_2 after 9 months of storage plus 21 d of shelf life at 20 °C. Low pCO_2 (0.4 kPa) under DCA-RQ maintained healthy fruit percentage similar to 1.2 and 1.6 pCO_2, but flesh firmness loss was higher during the entire shelf life evaluation. The storage in DCA-RQ 1.3 with 2.0 kPa of CO_2 maintained fruit quality similar to pCO_2 1.2 and 1.6 up to 14 d of shelf life, but not until 21 d. CA storage, without 1-MCP application, maintained higher volatile organic compounds (VOCs) characteristic of apple aroma, mainly from ester class, which increased until 14 d shelf life, and then, decreased. At 21 d shelf life DCA-RQ 1.3, mainly with 1.2, 1.6 and 2.0 kPa CO_2, resulted in increased esters production, without differing from CA stored fruit, but with greater overall quality, suggesting a good physic and sensorial quality of these fruit with extended shelf life. 1-MCP application and DCA-CF affect negatively the VOCs production, even with extended shelf life.

Keywords: Extreme low oxygen. High pCO_2. *Malus domestica*. Physiological disorders. Volatile compounds.

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

4.1.1 Introduction

Apples need to be stored to supply this fruit during the off-season worldwide, because it presents a short harvest window. In Brazil, 'Gala' apples and their mutants accounted for more than 60 % of the production (Kist et al., 2019). 'Maxi Gala', a mutant of 'Gala' apple, is one of the most produced in the country, because it displays a good red skin coverage and due to its organoleptic quality, increasing the consumer preference for this apple cultivar.

The great part of apples are stored in controlled atmosphere (CA), where oxygen partial pressures (pO_2) of 1.2 kPa and carbon dioxide (pCO_2) of 2.0 kPa are recommended for 'Gala' apple and mutants (Brackmann et al., 2008; Brackmann et al., 2013; Weber et al., 2013). However, extending the storage beyond 7 months may result in significant quality losses, mainly due to physiological disorders incidence and flesh firmness loss (Thewes et al., 2015; Thewes et al., 2017a; Both et al., 2017). To prolong the storage time, companies usually apply 1-methylcyclopropene (1-MCP), a potent inhibitor of the ethylene action, a plant hormone responsible for ripening of climacteric fruits (Sisler and Serek, 1997; Watkins, 2006; Watkins, Nock, 2012). Another technique already commercially employed is the dynamic controlled atmosphere monitored by the emission of chlorophyll fluorescence (DCA-CF) (Prange et al., 2003; Prange et al., 2007; Wright et al., 2012). DCA-CF estimates the lower pO_2 limit (LOL) tolerated by the fruit during storage, and allow to adjust the pO_2 set point in 0.2 to 0.3 kPa above the LOL detected. DCA-CF storage provides higher fruit quality compared to storage in CA (Zanella et al., 2008; Tran et al., 2015; Thewes et al., 2015; Weber et al., 2015; Both et al., 2017), but hinders the volatile compounds production, related to the characteristic apple aroma (Both et al., 2017; Raffo et al., 2009; Thewes et al., 2017).

Over the last decade a new DCA technique based on the respiratory quotient (RQ) has been developed. This technique also monitors the LOL tolerated by fruit during storage (Gasser et al., 2008; Brackmann, 2015; Weber et al., 2015; Wright et al., 2012; Bessemans et al., 2016). The RQ measures the ratio between CO_2 release by the fruit and O_2 uptake during storage. When ratio is 1.0 or below, fruit are predominantly in aerobic metabolism and, when the RQ is above, indicates the onset of anaerobic metabolism (Boersig; Kader and Romani, 1988). The objective of this technique is to keep the fruit always with RQ above 1.0, so that a small ethanol production is induced. Ethanol at low concentrations helps in the fruit quality maintenance, because it inhibits the ethylene production (Asoda et al., 2009; Jin et al., 2013; Weber et al., 2016, 2020).

Several studies have shown that DCA-RQ maintains high apples quality after long-term storage (Thewes et al., 2017c; Anese et al., 2019; Both et al., 2017; Weber et al., 2015, 2020). According to previous studies, RQ 1.3 is the most appropriate for maintaining the quality of 'Gala' apples for a 9 month of storage (Thewes et al., 2017a, 2018; Anese et al., 2018). In DCA-RQ storage, with extremely low pO_2 'Gala' apples are usually stored with pCO_2 of 1.2 kPa (Thewes et al., 2017a, 2018; Both et al., 2018; Anese et al., 2020). Few studies were conducted using pCO_2 higher than 1.2 kPa in DCA-RQ. According to Brackmann, Weber and Both, (2015), 'Galaxy' apple with 1.6 kPa CO_2 maintains fruit quality similar to 1.2 kPa CO_2, but these fruit were stored in DCA-RQ 1.5 and at temperature of 1 °C.

The storage of apples with higher pCO_2 (>1.2 kPa) is desired because it increases the efficiency of CO_2 adsorption systems, reducing energy costs by reducing the adsorption time. For adsorption system with activated charcoal, theefficiency is higher with pCO_2 (>1.0 kPa). When pCO_2 adopted is < 1.0 kPa, generally, calcium hydroxide it put into the storage to absorb CO_2. Another benefit of increased pCO_2 is the reduction in ethylene production and respiration (Gorny and Kader, 1997; Mathooko et al., 1996) delaying the fruit ripening and senescence. CO_2 inhibit some enzymes of respiratory pathway such as succinate dehydrogenase, isocitrate dehydrogenase, cytochrome c oxidase and phosphofructokinase (LIU et al., 2004). On the other hand, excessive pCO_2 in the storage chamber can be toxic to apples, causing physiological disorders such as flesh breakdown and cavities (Corrêa et al., 2010; Lumpkin et al., 2015). According to Zanella et al. (2005) and Neuwald et al. (2012), the pCO_2 for DCA must be keep lower than CA, to avoid browning pulp by high CO_2. For 'Galaxy' apple, the pCO_2 recommended for CA is 2.0 kPa and for DCA-RQ 1.5 is 1.2 kPa (Brackmann, Weber and Both, 2015). Therefore, it is necessary to carry out studies to verify the effect of high pCO_2 (> 1.2 kPa) on 'Maxi Gala' apples during a long storage period on DCA-RQ 1.3. Furthermore, it is important to verify the effect of pCO_2 on the volatile profile.

Volatile organic compounds (VOCs) contribute to the apples aroma and flavor and esters are the main class, but also other compounds such as alcohols, aldehydes, ketones and terpenes may contribute (Mehinagic et al., 2006). The main esters in 'Gala' apple are butyl acetate, 2-methylbutyl acetate and hexyl acetate (Both et al., 2017; Plotto, McDaniel and Mattheis, 2000; Salazar et al., 2011; Thewes et al., 2017c). It has been demonstrated that the VOCs production in apples its affected by several factors such: maturity stage (Thewes et al., 2017c); growth regulators application (Anese et al., 2019; Salas et al., 2011); 1-MCP application (Anese et al., 2020; Lumpkin et al., 2014, Thewes et al., 2015); pO_2 during storage

(Both et al., 2014, 2017; Brackmann, Streif and Bangerth 1993; Raffo et al., 2009; Thewes et al., 2017c, Donadel et al., 2019), pCO_2 in 'Fuji' apples stored in CA (Lumpkin et al., 2014).

After storage, apples can take several days or weeks until being marketed. Thus, the effect of the storage conditions on apple quality after longer period of shelf life, is of paramount importance. Almost all studies conducted with apples storage consider a shelf life period of 7 d at 20 °C (Anese et al., 2020; Both et al., 2018; Thewes et al., 2020; Weber et al., 2020).

Therefore, the aim of this work was to evaluate the effect of different pCO_2 on the overall quality and volatile profile of 'Maxi Gala' apple stored under DCA monitored by the respiratory quotient (RQ 1.3) during 9 months and compared them with the storage in CA, CA + 1-MCP and DCA-CF. Evaluations were made after 7, 14 and 21 d shelf life, in order to verify the best storage condition for extended shelf life.

4.1.2. Material and methods

4.1.2.1 Fruit harvest and sample preparation

'Maxi Gala' apple were harvested in a commercial orchard located at Vacaria - RS, Brazil. The apples were transported to the Postharvest Research Centre of the Federal University of Santa Maria – Brazil, and selected to remove those with damages. After that, samples of 25 fruit were prepared, with each treatment containing four samples (replicates). Three samples were also submitted to initial analysis, to verify the physicochemical quality at harvest. In this evaluation the fruit presented a starch pattern index, according Streif (1984), of 6.88 (1 – 10); ACC oxidase activity of 9.99 ng C_2H_4 kg^{-1} s^{-1}; internal ethylene concentration – IEC of 1.23 µg C_2H_4 L^{-1}; ethylene production of 0.16 ng C_2H_4 kg^{-1} s^{-1}; respiration rate of 3.11 µg CO_2 kg^{-1} s^{-1}; titratable acidity of 0.36 % of malic acid; flesh firmness of 89.31 N and soluble solids of 10.97 °Brix.

4.1.2.2 Storage conditions

The storage conditions evaluated were: [1] controlled atmosphere (CA) 1.2 kPa O_2 + 2.0 kPa CO_2; [2] CA + 1-methylcyclopropene (0.625 µL L^{-1}); [3] dynamic controlled atmosphere (DCA) monitored by chlorophyll fluorescence emission (DCA-CF) + 1.2 kPa CO_2; [4] DCA monitored by the respiratory quotient (DCA-RQ); DCA-RQ 1.3 + 0.4 kPa CO_2 [5] DCA-RQ 1.3 + 1.2 kPa CO_2; [6] DCA-RQ 1.3 + 1.6 kPa CO_2 and [7] DCA-RQ 1.3 + 2.0 kPa CO_2. DCA-

CF was managed according to Prange et al. (2007) and DCA-RQ was determined according to the methodology proposed by Brackmann (2015) and Weber et al. (2015). The RQ was determined twice a week by the ratio between the CO_2 production and O_2 uptake during a period of 13 h, in which the chamber remained disabled. When de RQ was below 1.3, the pO_2 was decrease 10 % and when RQ was above 1.3, the pO_2 was increase 10 % in relation to the current set point of O_2.

4.1.2.3 Atmosphere installation and maintenance

Four samples of each treatment (for each evaluation time) were allocated inside experimental CA-rooms (0.18 m^3), hermetically sealed and kept inside of a 45 m^3 cold room, in which the temperature was controlled. On the 1st day the temperature was reduced to 5 °C, and in the following 5 d, the temperature was reduced 1 ºC day^{-1} to reach the storage temperature. After reaching the desired storage temperature, the chambers were flushed with nitrogen to reduce the pO_2 to 5.0 kPa, and, in the following 5 d, pO_2 was gradually reduced to the pre-established condition (0.5 kPa for DCAs and 1.2 kPa in CA) by fruit respiration. The pCO_2 was also obtained by fruit respiration. The pO_2 and pCO_2 were monitored and corrected by an automatic control system (Valis®, Lajeado, RS, Brazil) connected to a gas analyzer (Siemens®, Ultramat model, Germany). If pCO_2 was above the set point, the air of the experimental chamber was circulated through a CO_2 absorber containing calcium hydroxide and, if pO_2 was below the set point, air was injected until it reached the level established for the treatment.

4.1.2.4 Temperature and relative humidity

'Maxi Gala' apple were stored at 2.0 ± 0.1 °C. The temperature was automatically maintained by the cold room control system with the aid of electronic thermostats. Temperature was monitoring daily with a mercury thermometer inserted in the pulp of a fruit. The relative humidity (RH) inside the experimental chambers was maintained at 94 ± 2 % using calcium chloride (10.0 g kg^{-1} of fruit), which absorbed excess moisture.

4.1.2.5 1-methylcyclopropene application

Before storage, a sample of fruit was treated with 1-MCP, by put the fruit into an experimental chamber of 0.18 m^3, at 2.0 ± 0.1 °C. A solution with released a concentration of 0.625 µL L^{-1} 1-MCP (SmartFresh®, 0.14 % active ingredient) was prepared, andplaced in a petri dish, inside the chamber that was immediately closed and remained sealed during 24 h. During this period, the air of the chamber was circulated with a fan, to homogenize the 1-MCP distribution. After 24 h, the fruit were removed from the chamber and stored in CA.

4.1.2.6 Physicochemical and biochemical analyses

The analyses of the physicochemical variables were performed after 9 months of storage plus 7, 14 and 21 d shelf-life at 20 ± 2 °C and relative humidity of 80 ± 2 %. The following variables were evaluated:

4.1.2.6.1 ACC oxidase activity

Determined according to Bufler (1986). Skin samples from 10 fruits (3 g) of each replicate were dipped into a solution containing 0.1 mol L^{-1} ACC and 10 mmol L^{-1} MES (2-(N-morpholino) ethanesulfonic acid) buffer at pH 6.0; after 30 min, samples were transferred to hermetic 50 mL syringes, with 2 % CO_2. After 30 min, two aliquots (1 mL) of the air from the syringe was withdrawn and injected in to a gas chromatograph (described below) to ethylene concentration determination. Results were expressed in ng C_2H_4 kg^{-1} s^{-1}.

4.1.2.6.2 Ethylene production and respiration rate

Approximately 1.5 kg fruit from each replicate were placed in a 5 L glass jar which were hermetically sealed for one hour. After that, two air samples (1 mL) were removed from each glass jar and injected into the gas chromatograph Varian® (Star CX3400 model, Palo Alto, CA, USA), equipped with flame ionization detector (FID) and Porapak N80/100 column. Column, injector and detector temperatures were 90, 140 and 200 °C, respectively. The results were expressed as ng C_2H_4 kg^{-1} s^{-1}. The respiration rate was expressed by the CO_2 release of fruits. To determine respiration rate, an electronic gas analyzer (Schele, model GA S1, version 2015, Germany), was connected to the same glass jars to determine ethylene production. The

air in the glass jar was circulated through the analyzer, which determined the CO_2 concentration. Respiration rate was expressed in μg CO_2 kg^{-1} s^{-1}.

4.1.2.6.3 Internal ethylene concentration (IEC)

Determined according to the methodology proposed by Mannapperuma et al. (1991), with adaptations. Slices of fruits were allocated in a desiccator with water and on the fruit was placed an inverted funnel with a septum at the thinner end, then with a vacuum pump a suction pressure of 565 mm Hg was applied to remove the air from fruit. Then, two samples of 1 mL of this air were taken and injected into the same gas chromatograph used to determine ethylene production. Results were expressed in μg C_2H_4 L^{-1}.

4.1.2.6.4 Electrolyte leakage

Determined according to Gago et al. (2015), with modifications. For this, 15 discs (with 5 mm diameter and thickness) from the pulp fruit were removed. Subsequently, the discs were immersed in a falcon tube with 25 mL of distilled water and left for one hour at 20 ± 1 °C. After that, the conductivity of the solution was determined. Then, the sample was boiled for 30 min at 100 ºC and then cooled to 20 °C in a freezer at -30 °C. Soon after, the conductivity was measured again. Electrolyte leakage was obtained by the formula EL= (cb/ca)*100, being; EL = Electrolyte leakage; cb= conductivity before and ca = conductivity after. Results were expressed as percentage.

4.1.2.6.5 Flesh breakdown, mealiness, decay incidence and healthy fruit amount

For flesh breakdown, mealiness and decay incidence evaluation, fruit were sliced in the equatorial region to visualization any browning region, mealy pulp and decay symptoms. Decayed apples were also evaluated by external symptoms. A ll fruit that did not show any symptoms described above were counted as healthy fruit. Results evaluations were expressed as percentage in relation to the total fruit of each repetition.

4.1.2.6.6 Flesh firmness

Determined with a penetrometer, (Effegi, model FT 327, 3-27 lbs., Milan, Italy) with a tip of 11 mm in diameter was inserted on two opposite sides of the fruit, in the equatorial region, where previously was removed the epidermis. Results were presented in Newton (N).

4.1.2.6.7 Soluble solids and titratable acidity

A juice sample was extracted from the 25 fruits of eachreplicate, with a juice extractor (Philips Walita®). The soluble solids of the juice was determined with a refractometer (Biobrix, Model 103, Curitiba, Brazil). Results were expressed in °Brix. Titratable acidity was determined with the same juice extracted for soluble solids; 10 mL of juice was diluted in 100 mL of distilled water and titrated with NaOH at 0.1 N until pH 8.1. Results were expressed in % of malic acid.

4.1.2.6.8 Volatile organic compounds analysis

The volatile organic compounds (VOCs) were analyzed in the juice previously extracted from the apples. Three replicates containing 10 apples each were cooled to 0.5 °C and juice of slices of equatorial region without seeds and endocarp were extracted with a juicer (Philips-Walitta®). The juice was put into 100 mL amber glasses and immediately frozen at -30 °C until analysis.

At the day of VOCs quantification, frozen samples were thawed in tap water, 10 min before the sample preparation. Then, 10 mL of juice were put inside a 20 mL vial flask, with 3 g NaCl and 10 µL of 3-octanol standard solution (82.2 µg mL^{-1}) and sealed with a PTFE-coated silicone lid. The vial was put in a water bath, at 35 °C with constant shaking, during five min to reach temperature equilibrium. Then a fiber (Supelco, 50/30 µm x 10 mm, covered with divinylbenzene/carboxen/polydimethylsiloxane polymers), was exposed to the headspace of the vial for 60 min, for volatile adsorption. The fiber was then desorbed at the injector of a gas chromatograph (Dani®, Dani Instruments Spa., Viale Brianza, Cologno Monzese, Italy), at 250 °C for 10 min, adopting a split-less mode in the first minute. The gas chromatograph was equipped with a flame ionization detector (FID), kept at 230 °C. A capillary column DN-5 (30 m 0.25 mm 0.25 µm) was used to separate the compounds. The flow rate of the carrier gas (nitrogen) was 1.0 mL min^{-1}. The temperature ramp used during the analysis was according Both et al. (2014). The linear retention index was determined according to the same authors.

VOCs were identified with a Shimadzu QP2010 Plus gas chromatograph coupled to a mass spectrometer (GC/MS; Shimadzu Corporation, Kyoto, Japan). The extraction/desorption/injection procedures for identification of the volatile compounds, was the same as described above, with helium as carrier gas. The MS detector operated in electron ionization mode, with ionization energy of +70eV, a scan ranges from m/z 35‑350 and a temperature of 250 °C. The mass spectra of each VOCs were identified comparing with mass spectra available in the National Institute of Standards and Technology library and by comparing the linear retention index (LRI) with those available in the scientific literature.

4.1.2.6.9 Statistical analysis

The experiments were conducted in a completely randomized design. The data were submitted to the error normality and variance homogeneity tests, by the Shapiro-Wilk and Bartlett tests, respectively. The variables that did not present normality of errors were transformed by the formula arc.sin $\sqrt{x/100}$. The data were submitted to analysis of variance (ANOVA). Data that showed a significant difference by ANOVA ($p < 0.05$), were compared by Scott Knott test at 5 % of error probability. 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. The data matrix was auto scaled for each variable in order to obtain the same weight (mean = 0 and variance = 1), before performing the PCA.

4.1.3 Results and discussion

4.1.3.1 Variation of the oxygen set point during storage

The variation of oxygen partial pressure during the storage of 'Maxi Gala' apple under CA, DCA-CF and DCA-RQ 1.3 with 0.4, 1.2, 1.6 and 2.0 kPa of CO_2 are shown in the Fig. 1. The pCO_2 adopted in DCA-RQ 1.3 influenced the variation of oxygen partial pressure (pO_2) during storage. As higher the pCO_2 adopted, lower was the pO_2 observed (Fig. 1b), but with minor differences between 1.2, 1.6, and 2.0 kPa. It is known that the pCO_2 of fruit pulp is higher than the pCO_2 maintained in the chamber atmosphere, and this generates a gradient concentration between them, causing a diffusion of CO_2 from the fruit pulp to the chamber atmosphere. The higher the gradient concentration (lower pCO_2, in the chamber), higher is the diffusion rate of CO_2 from the fruit pulp to the chamber atmosphere and this may increase the

RQ calculated. Therefore, the storage system order to increase the oxygen set point (Fig 1b - see blue line of 0.4 kPa CO_2). The mean pO_2 during apple storage in DCA-RQ 1.3 with 0.4, 1.2, 1.6 and 2.0 kPa of CO_2 were: 0.23, 0.16, 0.14 and 0.15 kPa, respectively.

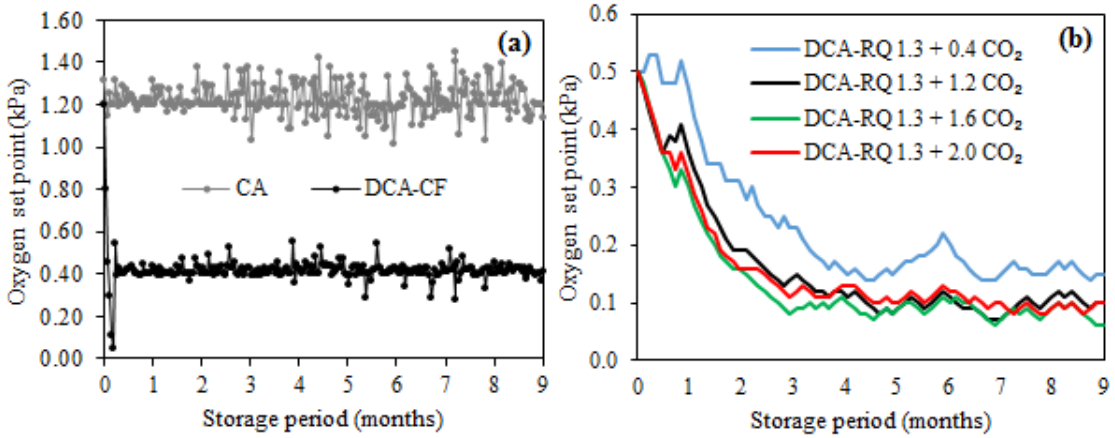


Figure 1. Variation of the oxygen set point of 'Maxi Gala' apple during 9 months stored under CA and DCA-CF (a), DCA-RQ 1.3 with four pCO_2 (0.4, 1.2, 1.6 and 2.0 kPa) (b) stored at 2.0 °C.

4.1.3.2 ACC oxidase activity, internal ethylene concentration, ethylene production and respiration rate

The fruit assessment was carried out after 9 months of storage under 2.0 °C plus 7, 14 and 21 d of shelf life at 20 °C. Fruit stored in DCA-RQ 1.3, irrespective of pCO_2, in CA+1-MCP, and DCA-CF showed lower ACC oxidase enzyme activity compared to these stored in CA, without difference between them at 7th d shelf life evaluation. This result can be attributed to the lower pO_2 maintained during storage and 1-MCP application (Fig. 1a, b). ACC oxidase is the key enzyme in the last step to ethylene production and its activity is limited by O_2 availability (Yang and Hoffmann 1984; Yang et al., 2016). 1-MCP application reduced the gene expression of *MdACO1* (Yang et al., 2016) and *MdACS1* (Tatsuki et al., 2007). This result corroborate with others works (Anese et al., 2020; Both et al., 2017, 2018; Schmidt et al., 2020; Thewes et al., 2017c; Weber et al., 2020). However, at 14 d of shelf life, fruit stored in DCA-RQ 1.3 +1.2 kPa of CO_2 showed the lowest ACC oxidase activity, followed by CA + 1-MCP and DCA-RQ 1.3 + 0.4 kPa of CO_2. Significantly, higher ACC oxidase activity was observed in apple stored in CA without 1-MCP, which peaked at this evaluation time. After 21 d, fruit kept in CA showed lower ACC oxidase enzyme activity compared to CA + 1-MCP and DCA-

CF fruit, but without difference from DCA-RQ 1.3, irrespective of pCO_2 (Fig. 2a). The reduction in ACC oxidase activity of fruit stored in CA can be related to the advanced ripening with extending shelf life. Different pCO_2 in storage under DCA-RQ 1.3 did not influence in ACC oxidase activity.

The internal ethylene concentration (IEC) at 7th d of shelf life was the highest in CA stored fruit, without difference between all other storage conditions (Fig. 2b). This same response was observed for ACC oxidase activity (Fig. 2a). At 14 d of shelf-life fruit stored in DCA-RQ 1.3 + 2.0 kPa of CO_2 showed a sharp increase in IEC (Fig. 2b), with the highest value but without differing from CA, followed by DCA-RQ 1.3 + 1.6 kPa of CO_2. Fruit stored in DCA-RQ with 0.4 and 1.2 pCO_2 resulted in a lower IEC, when compared to 1.6 and 2.0 kPa CO_2. The lowest IEC was verified in fruit stored under CA + 1-MCP. After 21 d of shelf life, only fruit stored under CA + 1-MCP and DCA-RQ 1.3 + 0.4 kPa of CO_2 presented the lowest IEC, compared to the other conditions (Fig. 2b).

Apples stored under CA peaked in ethylene production at 14 d of shelf life (Fig. 2c) and decreased thereafter as can be observed for ACC oxidase activity (Fig 2a). On the other hand, fruit of the other treatments showed an increase in ethylene production up to 20 d of shelf life, without reaching the climacteric peak, suggesting that these fruits were yet not overripe even with extended shelf life. Fruit stored under DCA-RQ 1.3 with different pCO_2 presented lower ethylene production at chamber opening and 6 d compared to CA fruit, but without difference for DCA-CF and CA + 1-MCP (Fig. 2c). This result is related to the lower ACC oxidase and IEC (2a, b). Another works also found lower ethylene production in fruit under CA + 1-MCP (Bekele et al., 2015; Thewes et al., 2017c) and DCA-RQ (Anese et al., 2020; Thewes et al., 2017c; Weber et al., 2015, 2020) compared to CA. Comparing the fruit stored among the different pCO_2 at 14 d of shelf life, fruit stored under DCA-RQ 1.3 + 2.0 kPa of CO_2 showed higher production. At 21 d, only fruit stored under CA + 1-MCP showed lower ethylene production. Previous studies found that high pCO_2 (20 kPa) inhibit ethylene biosynthesis in apple (Gorny and Kader, 1996), and ethylene action, because competes with ethylene at the receptor-binding site (Mathooko, 1996), but in this work the pCO_2 was far below to the above mentioned, and so, was not able to inhibit the ethylene production during long-term of shelf life. On the other hand, the fruit exposure to high pO_2 (20.9 kPa) during shelf life, increased O_2 availability to the ACC oxidase enzyme convert 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene.

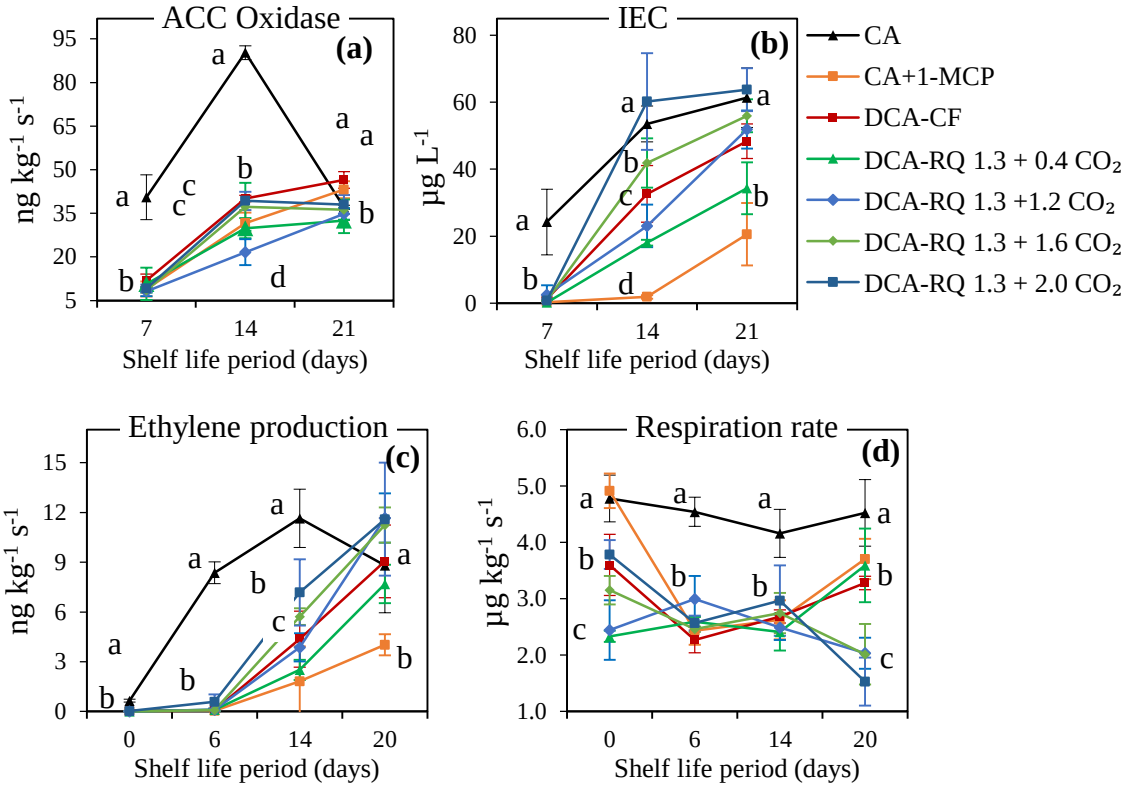


Figure 2. ACC oxidase activity (a), internal ethylene concentration (IEC), (b) ethylene production (c) and, respiration rate (d) of 'Maxi Gala' apple stored during 9 months in CA, CA + 1-MCP, DCA-CF DCA-RQ 1.3 with four pCO_2 (0.4, 1.2, 1.6 and 2.0 kPa), plus 7, 14 and 21 d of shelf life at 20 °C. Means followed by the same letter between storage conditions at same shelf life time do not differ by Scott Knott test at 5 % of error probability. Error bars show the standard deviation. ns: not significant.

The high ACC oxidase activity, IEC and ethylene production resulted in high respiration rate in fruit stored under CA during entire shelf-life (Fig. 2d), which can be assigned to the higher pO_2 (1.2 kPa) employed during storage. Previous studies have also found higher respiration rate in CA stored fruit as compared with DCA-RQ (Anese et al., 2020; Thewes et al., 2017c). At the chamber opening, fruit stored under DCA-RQ 1.3 with 0.4 and 1.2 kPa CO_2 showed the lowest respiration rate, but after 20 d of shelf life, fruit kept at DCA-RQ 1.3 with 1.2, 1.6 and 2.0 kPa of CO_2 maintained the lowest respiration rate. On the other hand, fruit stored in DCA-RQ 1.3 with 0.4 kPa of CO_2 showed intermediate respiration rate at 20 d of shelf life, without difference for the fruit stored under DCA-CF and CA + 1-MCP.

4.1.3.3 Overall quality

The stability and permeability of cell membrane permeability can be evaluated by electrolyte leakage (efflux). A storage condition that result in excess of anaerobic metabolism products accumulation, such as acetaldehyde, ethanol and ethyl acetate, may damage and result in higher electrolyte leakage (Saquet, Streif, and Bangerth, 2000). The low energy status to repair cell membranes damage (Saquet et al., 2003; Fan et al., 2011; Franck et al., 2007), can be involved in the development of internal disorders, like flesh breakdown. Another factor that contributed to this disorder is the advanced ripening. At 7 d of shelf life (Fig. 3a), fruit stored in CA showed the highest percentage of electrolyte leakage. On the other hand, fruit stored in DCA-CF showed the lowest electrolyte leakage. All other conditions, showed intermediate values, without difference among them, even with different pCO_2. However, at 14 and 21 d of shelf life (Fig. 3a), there was no difference in electrolyte leakage between fruit of all storage conditions. This can be explained by the lower anaerobic compounds production (4c, 5b and 6b).

Flesh breakdown is a physiological disorder that may occurs during long-term apple storage and pCO_2 very high. After 9 months storage plus 7 d of shelf life at 20 °C, there was no incidence of this disorder (Fig 3b). However, at 14 d of shelf life, a noteworthy fact is that apples stored under DCA-RQ 1.3 regardless of the pCO_2 showed a lower incidence of this disorder compared to CA fruit (Fig. 3b). Previous work related that high pCO_2 may cause oxidative stress and reactive oxygen species formation, which leads to cell membrane damage and internal browning (Castro et al., 2008; Herremans et al., 2013), which is also classified as flesh breakdown. In the present research, the adoption of higher pCO_2 (until 2.0 kPa in DCA-RQ 1.3) did not induce flesh breakdown incidence, suggesting that pCO_2 up to 2.0 kPa may be adopted for 'Maxi Gala' apples long-term storage in DCA, without causing internal damage. At 21 d of shelf life, there was no difference among the fruit on the conditions evaluated (Fig. 3b).

Mealiness is another important disorder common after long-term storage of 'Gala' mutants and increases when the fruit are harvested with advanced maturity (Thewes et al., 2017c). This disorder is characterized by a mealy aspect in the pulp, and occurs due to the degradation of the pectins, which maintains the cells adhesion (Payasi et al., 2009; Prasanna et al., 2007). At 7 d of shelf life, there was no difference among the storage conditions (Fig. 3c), that can be explained by the high flesh firmness at harvest (89.31 N). However, after 14 d shelf life there was a drastic increase in mealiness occurrence in fruit storage in CA (Fig. 3c). Fruit stored under DCA-RQ 1.3 with different pCO_2 showed a lower mealiness compared to CA. This is related to the lower pO_2 employed during storage, retarding fruit ripening, and supported

by the lower metabolism (Fig. 2a, b, c, d). At 21 d of shelf life, apples stored under DCA-RQ 1.3 with 1.6 and 2.0 kPa of CO_2 showed lower mealiness incidence, followed by apples stored in DCA-RQ 1.3 with 0.4 and 1.2 kPa of CO_2. This result showed that association of low pO_2 and higher pCO_2 (< 1.2 kPa under DCA-RQ 1.3 storage) were efficient to maintain low mealiness incidence after prolonged shelf life. DCA-RQ 1.3, irrespective of pCO_2 was more efficient in maintaining lower mealiness incidence at extended shelf life, compared to DCA-CF and CA even with 1-MCP application (Fig. 3c).

Decay incidence causes economic losses for storers and hinder the marketing. Decayed apples and can cause health problems because the presence of patulin, a mycotoxin produced by *Penicillium* and *Aspergillus* fungus (dos Santos et al., 2018). Generally, infection occurs on the orchard, in which the control is not adequate, and progress during storage and shelf life, if the storage conditions are inadequate. Results evidenced that after 7 and 14 d of shelf life the decay incidence varies from 5 to 17.5 %, but without differences among conditions (Fig. 3d). However, after 21 d of shelf life at 20 °C, DCA-RQ with 1.2 and 1.6 kPa of CO_2 showed lower decay incidence compared to fruit in the others storage conditions (Fig. 3d). This is an important result for consumers, because can decrease losses during commercialization at longer period after storage, which occurs mainly in places away from the production centers.

Beyond of evaluate individual disorders as related above, it is desirable to obtain the highest healthy fruit amount after long-term storage and extended shelf life. Healthy fruit represent the amount of apple without decay and physiological disorders. After 9 months of storage plus 7 d of shelf life, there was no difference in the healthy fruit percentage among the storage conditions (Fig. 3e). In contrast, others researches reported higher healthy fruit in DCA-RQ compared to CA (Both et al., 2018; Thewes et al., 2017a; Weber et al., 2015). Brackmann, Weber and Both (2015) found higher healthy fruit when 'Galaxy' apple were stored under DCA-RQ 1.5 with 1.2 and 1.6 compared to 2.0 pCO_2, but in lower storage temperature. After 14 d of shelf life (Fig. 3e), fruit stored under DCA-RQ 1.3 with 1.2, 1.6 and 2.0 kPa CO_2 and DCA-CF maintained the highest healthy fruit percentage. On the other hand, storage in CA drastically reduced the healthy fruit amount by extending the shelf life from 7 to 14 d. After 21 d of shelf life, DCA-RQ 1.3 with 0.4, 1.2 and 1.6 kPa CO_2 maintained higher healthy fruit (Fig. 3e). CA stored fruit remain with lower healthy fruit, even with 1-MCP application. Storage in DCA-CF and DCA-RQ 1.3 + 2.0 kPa CO_2 drastically reduced healthy fruit amount by extending the shelf life from 14 to 21 d. The lower pCO_2 (0.4) maintain higher healthy fruit percentage after 21 d shelf life, but increase costs with CO_2 absorption, or required another CO_2 management strategy, by insert lime in the storage rooms.

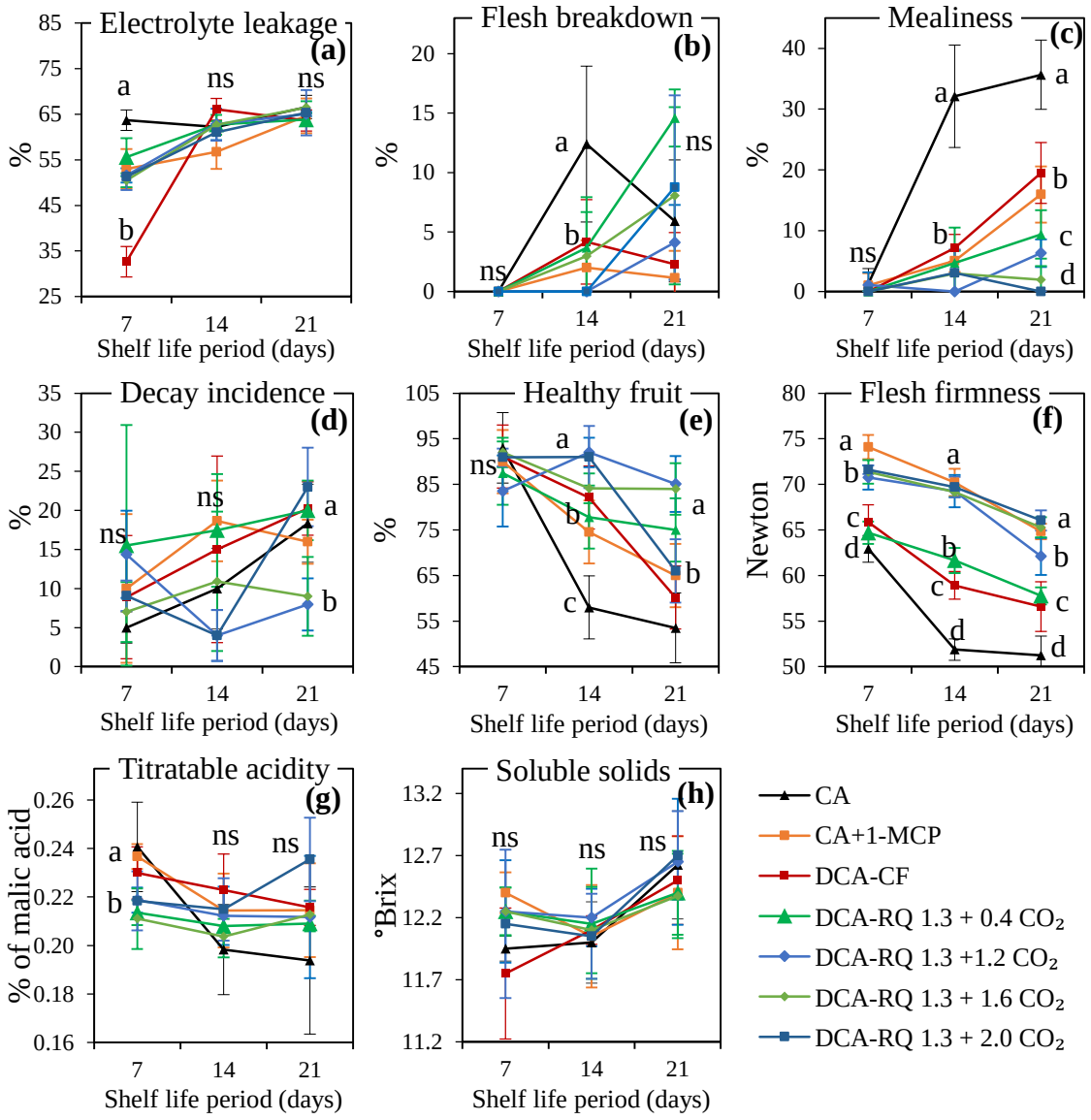


Figure 3. Electrolyte leakage (a), flesh breakdown (b), mealiness (c), decay incidence (d), healthy fruit (e), flesh firmness (f), titratable acidity (g) and soluble solids (h) of 'Maxi Gala' apples stored during 9 months under CA, CA + 1-MCP, DCA-CF, DCA-RQ 1.3 with four pCO_2 (0.4, 1.2, 1.6 and 2.0 kPa), plus 7, 14 and 21 d of shelf life at 20 °C. Means followed by the same letter between storage conditions at same shelf life time do not differ by Scott Knott test at 5 % of error probability. Error bars show the standard deviation. ns: not significant.

Flesh firmness is one of the main quality factors evaluated by apple consumers (Harker et al., 2008). The firmness loss is attribute to pectin methylesterase polygalacturonase and β-galactosidase activity, which degrade the cell wall and their activity is ethylene dependent (Nishiyama et al., 2007; Payasi et al., 2009; Prassana et al., 2007). 1-Methylcyclopropene

application, a potent inhibitor of ethylene action (Blankenship and Dole, 2003; Sisler and Serek, 1997), maintains high flesh firmness during the entire shelf life period (Fig. 3f). On the other hand, fruit storage under DCA-RQ 1.3 with 1.2, 1.6 and 2.0 pCO_2 maintained good flesh firmness after 7 d shelf life, but lower as 1-MCP treated fruit. CA storage resulted in significantly lower flesh firmness, yet at the 7th day shelf life. The high flesh firmness under DCA-RQ is related to low pO_2 employed (Fig. 1b). High flesh firmness was reported in 'Galaxy' apples stored under DCA-RQ 1.5 with 1.2, 1.6 and 2.0 kPa of CO_2 compared to CA and DCA-CF fruit, without difference among pCO_2 (Brackmann, Both and Weber et al., 2015). At 14 d of shelf life, fruit stored in CA plus 1-MCP, DCA-RQ 1.3 with 1.2, 1.6 and 2.0 kPa CO_2 showed higher flesh firmness. These stored in CA undergo a drastic reduce in firmness and remained so until 21 days of shelf life (Fig. 3f). After 21 d of shelf life, only the fruit stored in DCA-RQ 1.3 with 1.6, 2.0 kPa CO_2 and CA plus 1-MCP application showed higher flesh firmness, followed by fruit stored under DCA-RQ 1.3 with 1.2 kPa CO_2 (Fig. 3f). These result show that extremely low O_2 (0.14 and 0.15 kPa in average, respectively) during storage in DCA-RQ 1.3 linked to higher pCO_2, and 1-MCP application has a prolonged effect on flesh firmness maintenance during extended shelf life.

Soluble solids and titratable acidity of apples are important quality parameters on consumer's acceptance (Harker et al., 2008). After 7 d shelf life, 'Maxi Gala' apples showed higher titratable acidity under CA, CA + 1-MCP and DCA-CF compare to fruit on DCA-RQ 1.3 (Fig. 3g). There was no difference among different pCO_2. Similar result was found in 'Galaxy' storage under DCA-RQ 1.5 with 1.2, 1.6 and 2.0 kPa of CO_2 (Brackmann, Weber and Both, 2015). From 7 to 14 d, fruit stored in CA, CA + 1-MCP and DCA-CF showed a reduction in acidity compared to DCA-RQ 1.3. However, there was no difference among conditions evaluated at 14 and 21 d. The storage conditions not affected the soluble solids during all period of shelf life (Fig. 3h).

4.1.3.4 Volatile organic compounds (VOCs)

After 9 months of storage plus 7, 14 and 21 d of shelf life at 20 °C VOCs were evaluated and 13 esters, 14 alcohols, 12 aldehydes, 1 ketone and 2 acids were identified (Fig. 4, 5 and 6), in all storage conditions.

4.1.3.4.1 Principal esters

2-Methylbutyl acetate, hexyl acetate and butyl acetate have the highest impact on aroma and flavor of 'Gala' apples (Plotto, McDaniel and Mattheis, 2000; Thewes et al., 2017c). These same esters were detected in higher amounts during all shelf life evaluations in the present research (Fig. 4f, g and l). After 7 and 14 d of shelf life at 20 °C, fruit storage under CA showed the highest total esters concentration compared to the others conditions, which did not differ among them (Fig. 4a). Fruit storage under DCA-RQ 1.3 with different pCO_2 showed no difference on total esters. However, at 21 d of shelf life, fruit storage under CA and DCA-RQ 1.3 with pCO_2 1.2 had higher concentration compared with the others conditions (Fig. 4a). Fruit storage under DCA-RQ 1.3 with pCO_2 0.4, 1.6 and 2.0 showed an intermediate concentration and fruit under CA + 1-MCP and DCA-CF the lowest total esters. Fruit storage under CA had a higher increment from 7 to 14 d of shelf life in total esters concentration compared with the others conditions. However, at 21 d, fruit under CA decrease and the others condition had an increment in total esters concentration.

Butyl acetate concentration was higher in fruit from CA storage, after 7, 14 and 21 d of shelf life compared to the other conditions, without difference between CA + 1-MCP and DCAs conditions (Fig. 4f). This result is related to the lower precursor (1-butanol) verified under CA + 1-MCP and DCAs conditions (5 d). Lower pO_2 limits the β-oxidation of fatty acids and/or via the lypoxygenase (LOX) pathway in the precursor synthesis of butyl acetate (Brackmann et al., 1993; Song and Bangerth, 2003). Lower ethylene during storage reduced the alcohol acyltransferase (AAT) activity that convert alcohols to esters (Defilippi et al., 2005; Qi et al., 2020), which could explain the lower butyl acetate in 1-MCP treated fruit. Lower butyl acetate also was verified by others authors in CA + 1-MCP and DCAs conditions (Both et al., 2014; Thewes et al., 2015; Yang et al., 2016). Lower butyl acetate under extremely low oxygen also was verified in Royal 'Gala' (Both et al., 2014; Both et al., 2016) 'Scartlett Spur Red Delicious' apples (Lumpkin et al., 2014). In contrast, Thewes et al. (2017c) found higher butyl acetate in 'Galaxy' apples under DCA-RQ 1.5 storage.

Fruit stored under CA had higher hexyl acetate at 7 and 14 compared to fruit stored in CA + 1-MCP and DCAs conditions (Fig. 4l). Similar to butyl acetate, hexyl acetate production is ethylene dependent, because ethylene increased alcohol acyltransferase (AAT) enzyme activity by the increased of the expression of *MdAAT2* gene (Yang et al., 2016). 1-MCP application decrease expression of *MdAAT1* and *MdAAT2* genes, decreasing ATT enzyme activity, resulting in lower hexyl acetate production. 'Royal Gala' apple stored under extremely low pO_2 (< 0.5 kPa), resulted in lower hexyl acetate concentration (Both et al., 2016; Both et al., 2014). Fruit stored in DCA-RQ 1.3 with 0.4 kPa of CO_2 showed higher hexyl acetate

precursor (1-hexanol) at 7 d of shelf life, but low hexyl acetate concentration. On this case, the low ethylene production (Fig 2c), may have inhibit the ATT enzyme activity. At 21 d of shelf life, contrary as observed to butyl acetate, the hexyl acetate concentration of fruit stored in CA reduced drastically however remained in the highest concentration at this point, as well as DCA-RQ 1.3 with 1.2 and 1.6 kPa of CO_2.

2-Methylbutyl acetate was the ester most abundant in 'Maxi Gala', wihich increase until 21 d of shelf life. Fruit stored under CA, and DCA-RQ 1.3 with 1.2, 1.6 and 2.0 kPa of CO_2 showed higher concentration of 2-methylbutyl acetate at 7 d of shelf life. This ester is formed from L-isoleucine via 2-methyl-1-butanol (Wyllie and Fellman, 2000). Higher amounts of 2-methyl-1-butanol was found in CA, and DCA-RQ 1.3 with 1.2, 1.6 and 2.0 kPa of CO_2 at 7 d of shelf life, which explain this high ester concentration. Low pO_2 had low effect on branched-chain esters, such 2-methylbuthyl acetate (Both et al., 2014; Brackmann et al., 1993; Echeverría et al., 2008). Thewes et al. (2017c) found higher 2-methylbuthyl acetate in 'Galaxy' apple harvested in three maturity stage and storage under DCA-RQ 1.5, but when fruit were storage under CA + 1-MCP, the lowest 2-methylbutyl acetate concentration was detected. This corroborates with result found at the present research, where fruit treated with 1-MCP showed low production during the entire shelf life period. 'Royal Gala' apples stored under CA and DCA-RQ 2.0 had higher 2-methylbutyl acetate concentration compared to fruit storage under DCA-CF (Both et al., 2017). At 14 d of shelf life fruit in CA produced higher amount of 2-methylbuthyl acetate compared to the others conditions, which is in accordance to the alcohol precursor (2-methyl-1-butanol). At 21 d, CA condition reduced 2-methylbuthyl acetate concentration and fruit under DCA-RQ 1.3 with 1.2 kPa of CO_2 had the highest amount, evidencing an increasing concentration with prolonged shelf life in this condition. Storage in DCA-CF and CA + 1-MCP had the lowest concentration. No difference was found for the precursor concentration.

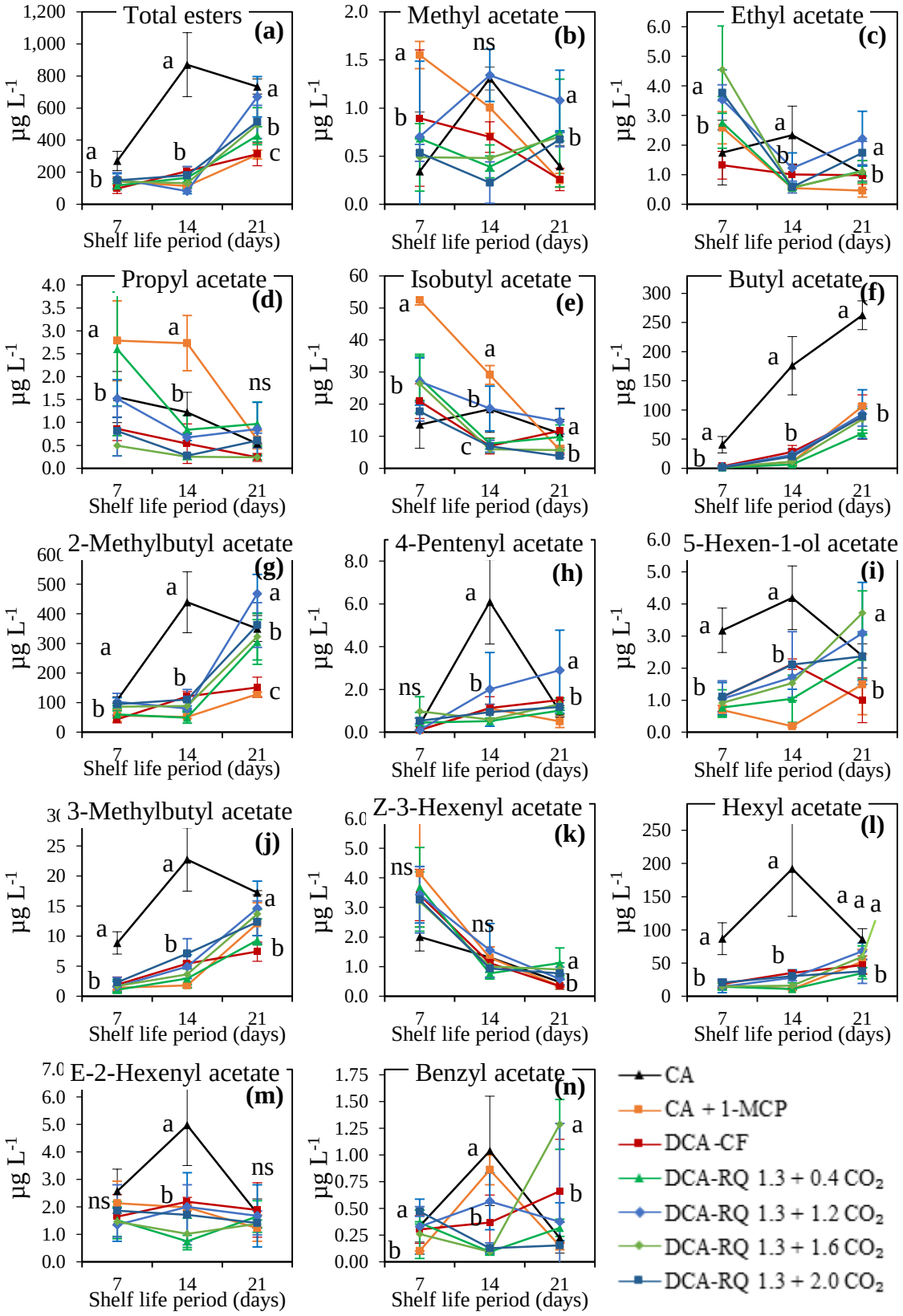


Figure 4. Esters concentration (µg L^{-1}) of 'Maxi Gala' apple after 9 months of storage under CA, CA + 1-MCP, DCA-CF and DCA-RQ 1.3 with 0.4, 1.2, 1.6 and 2.0 kPa of CO_2 plus 7 d of shelf life at 20 °C. Means followed by the same letter between storage conditions at

same shelf life time do not differ by Scott Knott test at 5 % of error probability. Error bars show the standard deviation. ns: not significant.

Anaerobic metabolism compound such as ethyl acetate, when in high concentrations, are strongly related to off-flavor (Echeverría et al., 2008; Forney et al., 2000; Wright et al., 2015). Fruit storage under DCA-RQ 1.3 with higher pCO_2 (1.2, 1.6 and 2.0) had a higher concentration of ethyl acetate at 7 d of shelf life compared to fruit storage under CA, CA + 1-MCP, DCA-CF and DCA-RQ 1.3 with 0.4 kPa of CO_2 (Fig. 4c) However, its precursor, ethanol (Fig 5b), had a low concentration, without difference among the conditions evaluated. The ethyl acetate concentration remained far below to its odor threshold (13,500 µg kg^{-1}), even under higher pCO_2 and, thus, did not cause physiological disorders, like flesh. Thus, even during the storage in the lowest pO_2 and highest pCO_2 there was no production of excessive anaerobic metabolism compounds, which could affected on the fruit tissues. After 14 d of shelf life, there was no difference of ethyl acetate concentration among fruit storage under higher pCO_2, but with reduction compared to 7 d and compared to CA storage. At 21 d, fruit storage under DCA-RQ 1.3 with 1.2 and 2.0 kPa of CO_2 had higher concentration compared with the others conditions, but the concentration was lower than at 7 d of shelf life.

4.1.3.4.2 Principal alcohols

The total alcohols concentration did not differ among the storage conditions at 7 d shelf life (Fig. 5a), but at 14 d, CA and DCA-RQ 1.3 with 0.4 kPa of CO_2 showed higher concentration compared to the fruit of the others conditions. The most abundant alcohol that contributes more to the total alcohols was 1-hexanol (Fig. 5k), the precursor of hexyl acetate. The 1-hexanol confers a green and grassy flavor to fruit (Mehinagic et al., 2006). At 7 d, DCA-RQ 1.3 with 0.4 kPa CO_2 had higher 1-hexanol concentration, but at 14 and 21 d DCA-RQ 1.3 with 1.2 kPa CO_2 showed higher concentration. Hexanal is a precursor of 1-hexanol, but hexanal at 7 d in DCA-RQ 1.3 with 0.4 kPa CO_2 did not differ from the others conditions. At 14 and 21 d, fruit stored in DCA-RQ 1.3 with 1.2 kPa CO_2 showed higher concentration of 1-hexanol that could be related to the higher hexanal concentration (Fig 6f). Fruit storage in CA resulted in higher 1-butanol concentration at 7, 14 and 21 d of shelf life (Fig. 5d). 1-butanol confers to apple a sweet aroma (Mehinagic et al., 2006). Both et al. (2017), reported higher 1-butanol concentration in CA and DCA-RQ 2.0. Raffo et al. (2009) found that the 1-butanol was inhibit by low pO_2. In this research, both the 1-MCP application and low pO_2 affect the 1-

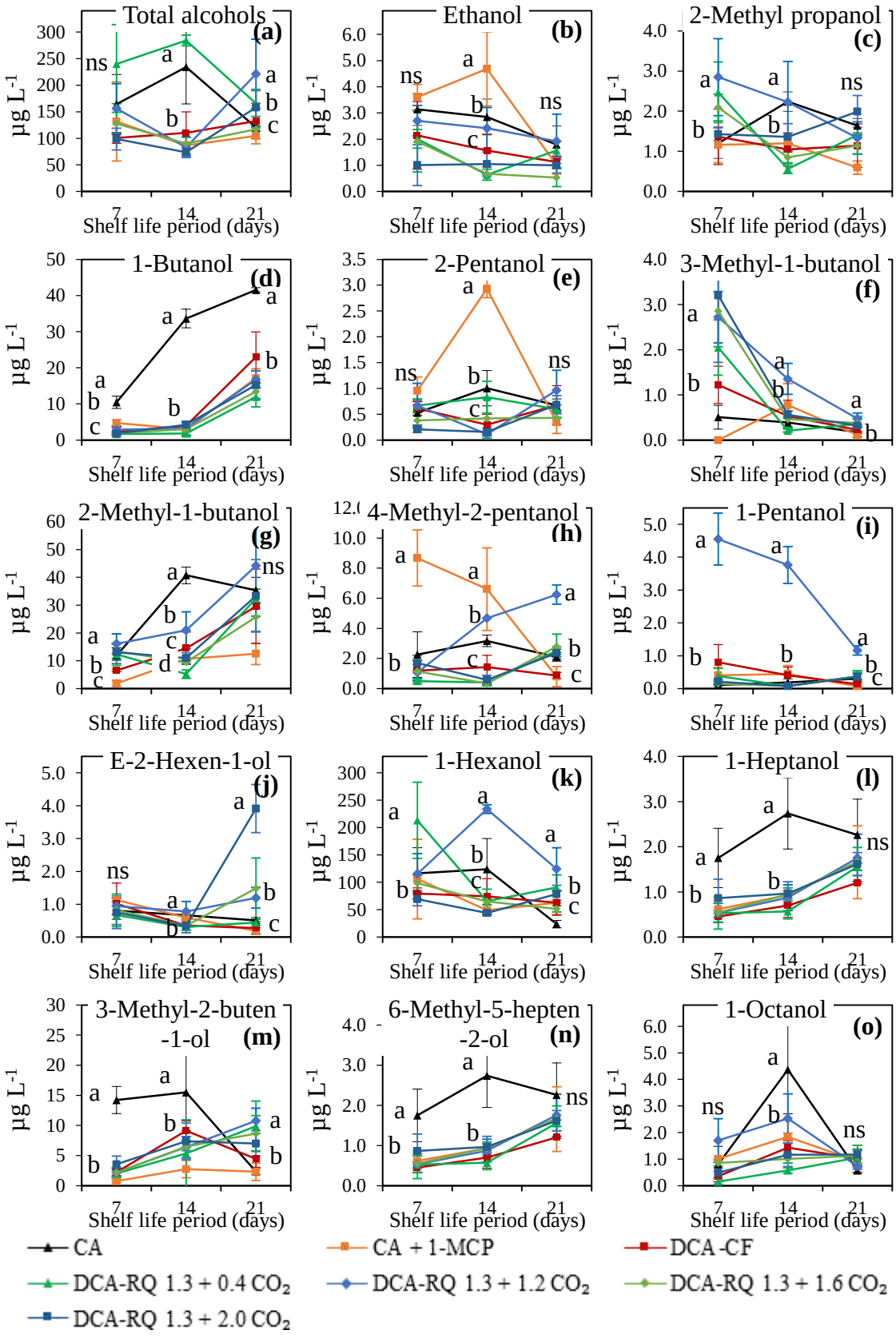


Figure 5. Alcohols concentration (µg L^{-1}) of 'Maxi Gala' apple after 9 months of storage under CA, CA + 1-MCP, DCA-CF and DCA-RQ 1.3 with 0.4, 1.2, 1.6 and 2.0 kPa of CO_2

plus 7 d of shelf life at 20 °C. Means followed by the same letter between storage conditions at same shelf life time do not differ by Scott Knott test at 5 % of error probability. Error bars show the standard deviation. ns: not significant.

butanol production throughout the shelf life. Thewes et al. (2017c) also verified lower 1-butanol production in 'Galaxy' apple stored in CA + 1-MCP and DCA-RQ 1.3 harvested in ripe and overripe maturity stage. Fruit storage under CA and DCA-RQ 1.3 produced higher 2-methyl-1-butanol at the 7th d shelf life, but at 14 d fruit stored in CA experienced a sharp increase of this alcohol production, which reduced at 21 d, a result similar to was observed for the ester 2-methylbutyl acetate (Fig 4g). After 21 d shelf life there was no difference between storage condition for 2-methyl-1-butanol, because in CA stored fruit there was a reduction and in the other storage conditions an increase in this alcohol concentration, related to the 14th d (Fig. 5g). A high 2-methyl-1-butanol concentration was observed in overripe 'Galaxy' apples under CA (Thewes et al., 2017c), that may explain the sharp increase in fruit stored in CA in the evaluation made at 14 d, while in the other conditions this trend was observed only at 21 d shelf life. Ethanol concentration was little affected by the storage conditions. This may be due to the low acetaldehyde concentration, a precursor of ethanol (Fig. 5b).

4.1.3.4.3 Main aldehydes

The total aldehydes were higher in fruit stored under DCA-RQ 1.3 with 1.2 kPa of CO_2 compared to the fruit on the others conditions after 7 d shelf life (Fig. 6a), fruit stored under DCA with pCO_2 1.6 and 2.0 showed an intermediate concentration and fruit under pCO_2 0.4 a lower, without differing from fruit in CA storage. At 14 d, total aldehydes concentration reduced, without difference among the conditions evaluated. However, at 21 d, fruit stored under CA, DCA-RQ 1.3 with pCO_2 1.2 and 1.6 showed higher aldehydes concentration. The most abundant aldehyde identified was E-2-hexenal (Fig. 6g), that is produced by LOX from linolenic acid (Salazar et al., 2011). The compound confers a green leafy sensorial attribute to apple flavor (Mehinagic et al., 2006). E-2-hexenal had higher concentration under DCA-RQ 1.3 with 1.2 kPa CO_2 at 7 d of shelf life and then occur a drastic reduction until 14 d and a small increase at 21 days of shelf life. 'Royal Gala' apples stored under DCA-RQ 1.5 and 2.0 showed a lower E-2-hexenal compared to CA fruit (Both et al., 2017). Hexanal is another important aldehyde (Fig. 6f), which confer green aroma to apple (Mehinagic et al., 2006). Only at 21 days of shelf life, there was difference among the conditions, in which fruit stored under CA and

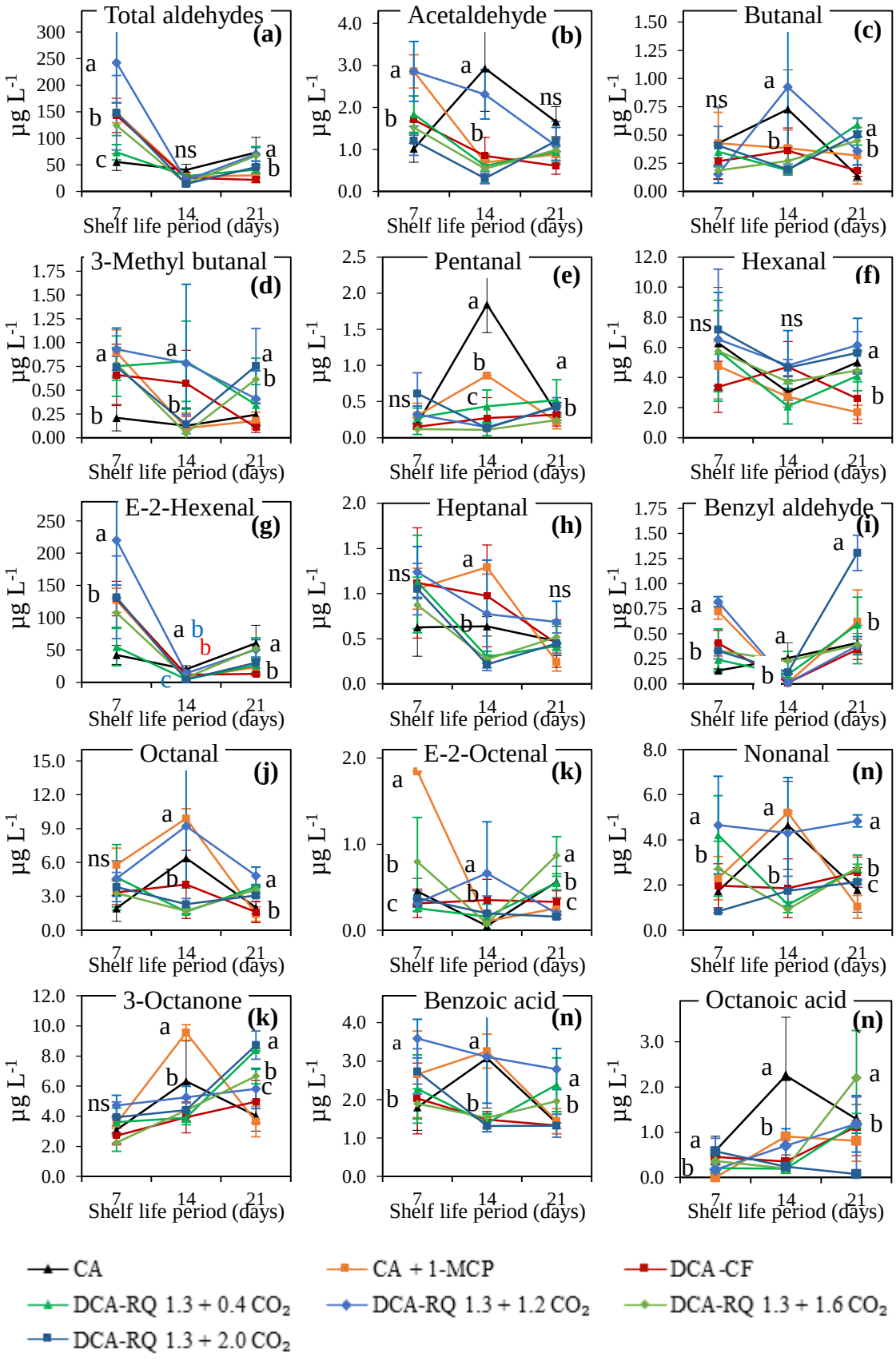


Figure 6. Aldehydes, ketone and acid concentration (µg L^{-1}) of 'Maxi Gala' apple after 9 months of storage under CA, CA + 1-MCP, DCA-CF and DCA-RQ 1.3 with 0.4, 1.2,

1.6 and 2.0 kPa of CO_2 plus 7 d of shelf life at 20 °C. Means followed by the same letter between storage conditions at same shelf life time do not differ by Scott Knott test at 5 % of error probability. Error bars show the standard deviation. ns: not significant.

DCA-RQ conditions had higher concentration compared to storage in CA + 1-MCP and DCA-CF.

Acetaldehyde is an important aldehyde, which it formed on fermentative pathway. Acetaldehyde is toxic metabolic for fruit tissue, which may trigger physiological disorders, like flesh breakdown. Acetaldehyde concentration was low (< 3.0 µg L^{-1}) on all conditions during shelf life (Fig. 6b). This result reflects in any physiological disorder at 7 days of shelf life. In 'Royal Gala' apples, flesh breakdown it attributed to the higher acetaldehyde concentration (67.5 µg L^{-1}) under DCA-RQ 1.5 storage (Both et al., 2017). Thus, in the present research the flesh breakdown incidence may be related to advanced maturity, as observed for CA on 14 d shelf life and all other conditions only at 21 d (Fig 3b), and not to higher acetaldehyde from fermentative pathway, even in the lower pO_2.

4.1.3.4.4 Principal component analysis

For better understand the relations among the storage conditions and variables evaluated at 7 d shelf life was performed a Principal component analysis (PCA) (Fig. 7). The principal component one (PC I) and two (PC II) explained 73.54 % of the total variable variance. PC I separated CA condition from the other conditions (Fig. 7a). Fruit storage in CA were associated with higher ACC oxidase activity, IEC, ethylene production, respiration rate, electrolyte leakage, but also to main esters as hexyl acetate, 2-methylbutyl acetate, 3-methylbutyl acetate, butyl acetate, and other VOCs like 1-butanol (Fig. 7b). DCAs and CA + 1-MCP conditions are associated with higher flesh firmness, ethyl acetate, E-2-hexenal, 2-methyl-1-butanol, acetaldehyde and others VOCs less important (Fig. 7b). PC II separated CA + 1-MCP from DCAs storage conditions (Fig. 7a). Fruit storage in CA + 1-MCP are associated with methyl acetate, isobutyl acetate, n-propyl acetate, ethanol, 4-methyl-2-pentanol and acetaldehyde (Fig. 7b). DCAs are associated with IEC, ethyl acetate, 2-methylbutyl acetate, 2-methyl-1-butanol, 3-methyl-1-butanol, 2-methyl-1-propanol (Fig. 7a, b).

At 14 d of shelf life PC I and PC II explained 79.34 % of the total variable variance. PC I separated CA condition from the other conditions (Fig. 8a). Fruit storage in CA are associated with ACC oxidase enzyme activity, IEC, ethylene production, respiration rate, mealiness, flesh

breakdown and the most esters, alcohol and aldehydes compounds (Fig.8a, b). On the other hand, DCAs and CA + 1-MCP are associated with flesh firmness and healthy fruit (Fig. 8a, b). PC II was important to separate the fruit stored under CA + 1-MCP and DCA-RQ 1.3 with 1.2 kPa of CO_2 from fruit stored under DCA-CF, DCA-RQ 1.3 with 0.4, 1.6, 2.0 kPa of CO_2 and CA conditions (Fig. 8a). Fruit storage in CA + 1-MCP and DCA-RQ 1.3 with 1.2 kPa of CO_2 are associated with flesh firmness, most alcohols and aldehydes (Fig. 8b).

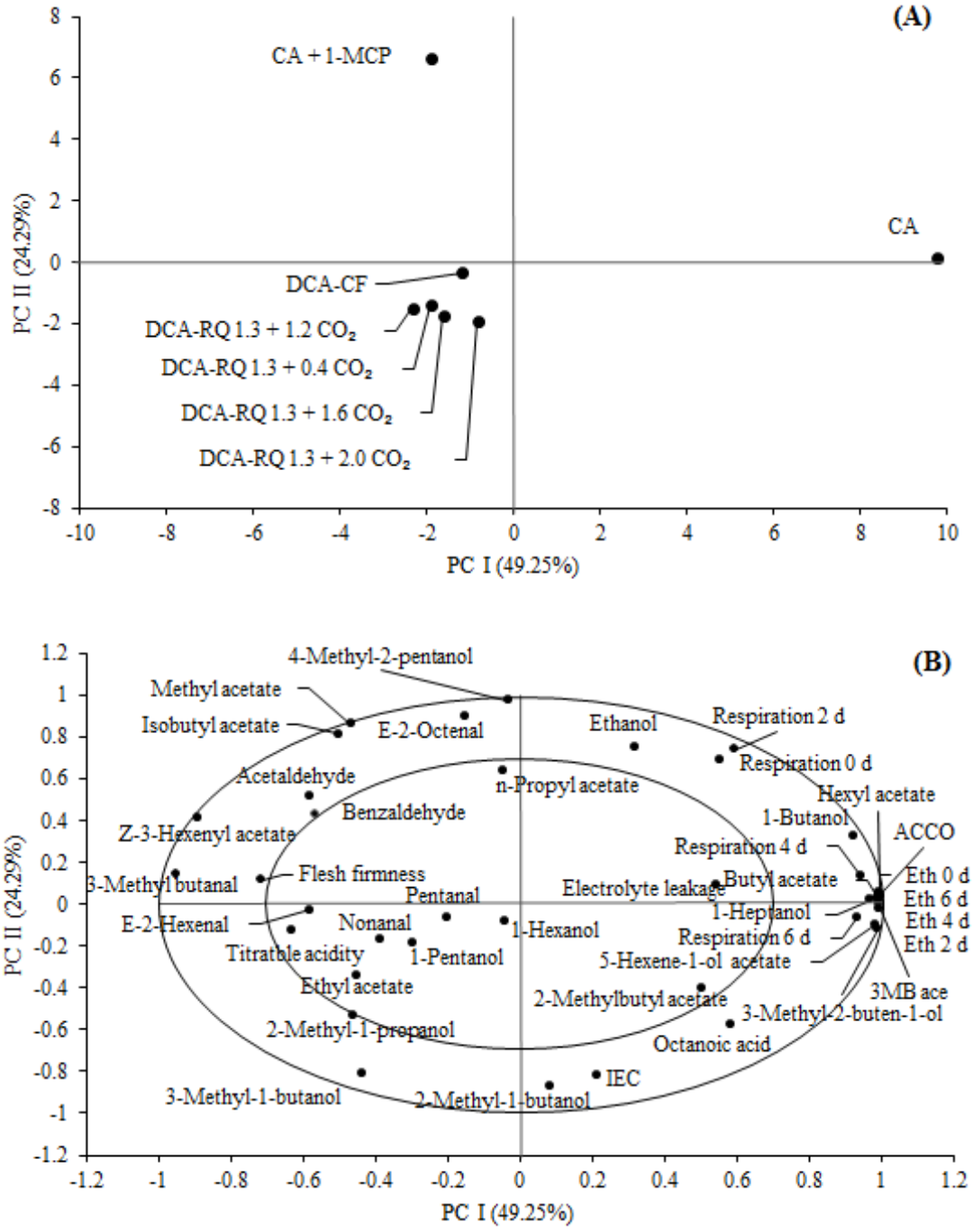


Figure 7. Principal component analysis (PCA) showing the principal component one (PC I) and two (PC II) of 'Maxi Gala' apple after 9 months of storage under CA without or with 1-MCP treatment (0.650 µL L^{-1}), DCA-CF, DCA-RQ 1.3 with 0.4, 1.2, 1.6 and

2.0 kPa CO_2, plus 7 d of shelf life at 20 °C. 3MB ace: 3-methylbutyl acetate, Eth: ethylene at 0, 2, 4 and 6 d, ACCO: ACC oxidase.

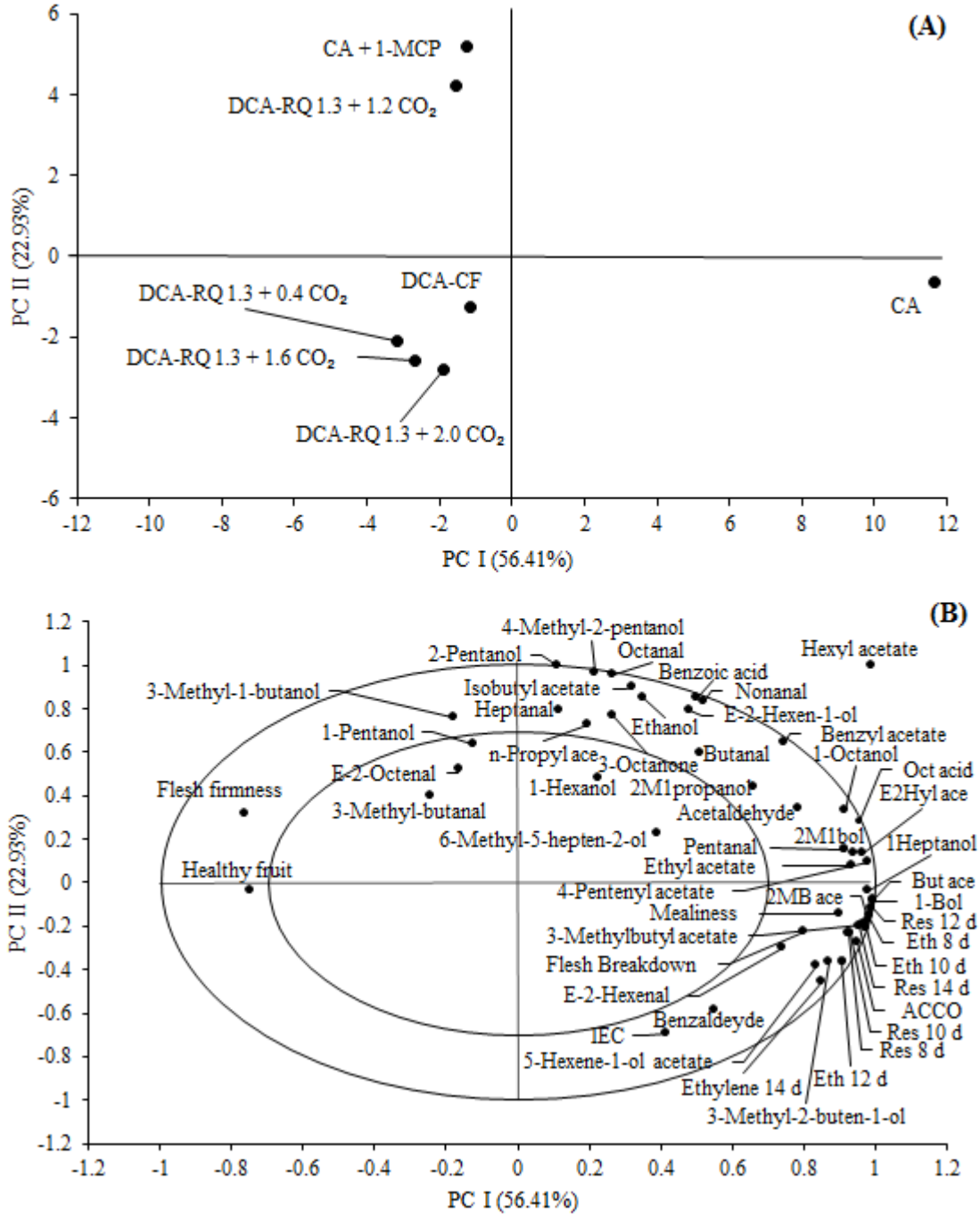


Figure 8. Principal component analysis (PCA) in 'Maxi Gala' apple after 9 months of storage under CA without or with 1-MCP treatment (0.650 µL L^{-1}), DCA-CF, DCA-RQ 1.3 with 0.4, 1.2, 1.6 and 2.0 kPa CO_2, plus 14 d of shelf life at 20 °C. n-propyl ace: n-propyl acetate, 2M1propanol: 2-methyl-1-propanol, E2Hyl ace: E-2-hexenyl acetate, 2M1bol: 2-mehyl-1-butanol, But ace: butyl acetate, 2MB ace: 2-methylbutyl acetate,

1-Bol: 1-butanol, Eth: ethylene at 8, 10 and 12 d, Res: respiration at 8, 10, 12 and 14 d, ACCO: ACC oxidase.

At 21 d of shelf life PC I and PC II explained 64.81 % of the total variable variance. PC I separated fruit stored under CA, CA + 1-MCP and DCA-CF from those stored under DCA-RQ conditions (Fig. 9a). Respiration rate, ACC oxidase enzyme activity, mealiness, decay, butyl acetate, hexyl acetate and 1-butanol were related to apples stored under CA (Fig. 9a, b). Fruit stored under DCA-RQ conditions are related to flesh firmness, healthy fruit, and the most VOCs compounds, evidencing the ability of fruit stored in DCA-RQ to recover the VOCs production after extended shelf life, maintaining fruit overall quality. PC II separated fruit stored under CA and DCA-RQ 1.3 with 1.2 kPa of CO_2 condition from those stored under CA + 1-MCP, DCA-CF, DCA-RQ 1.3 with 0.4, 1.6 and 2.0 kPa of CO_2 (Fig. 9a). Fruit storage in CA and DCA-RQ 1.3 with 1.2 kPa of CO_2 are associated with ethylene production, IEC, respiration rate and the majority esters compounds. Flesh firmness, Z-3-Hexenyl acetate, 1-hexanol, E-2-hexen-1-ol, butanal and 3-methylbutanal are related to fruit stored under DCA-RQ 1.3 with 0.4, 1.6 and 2.0 kPa of CO_2.

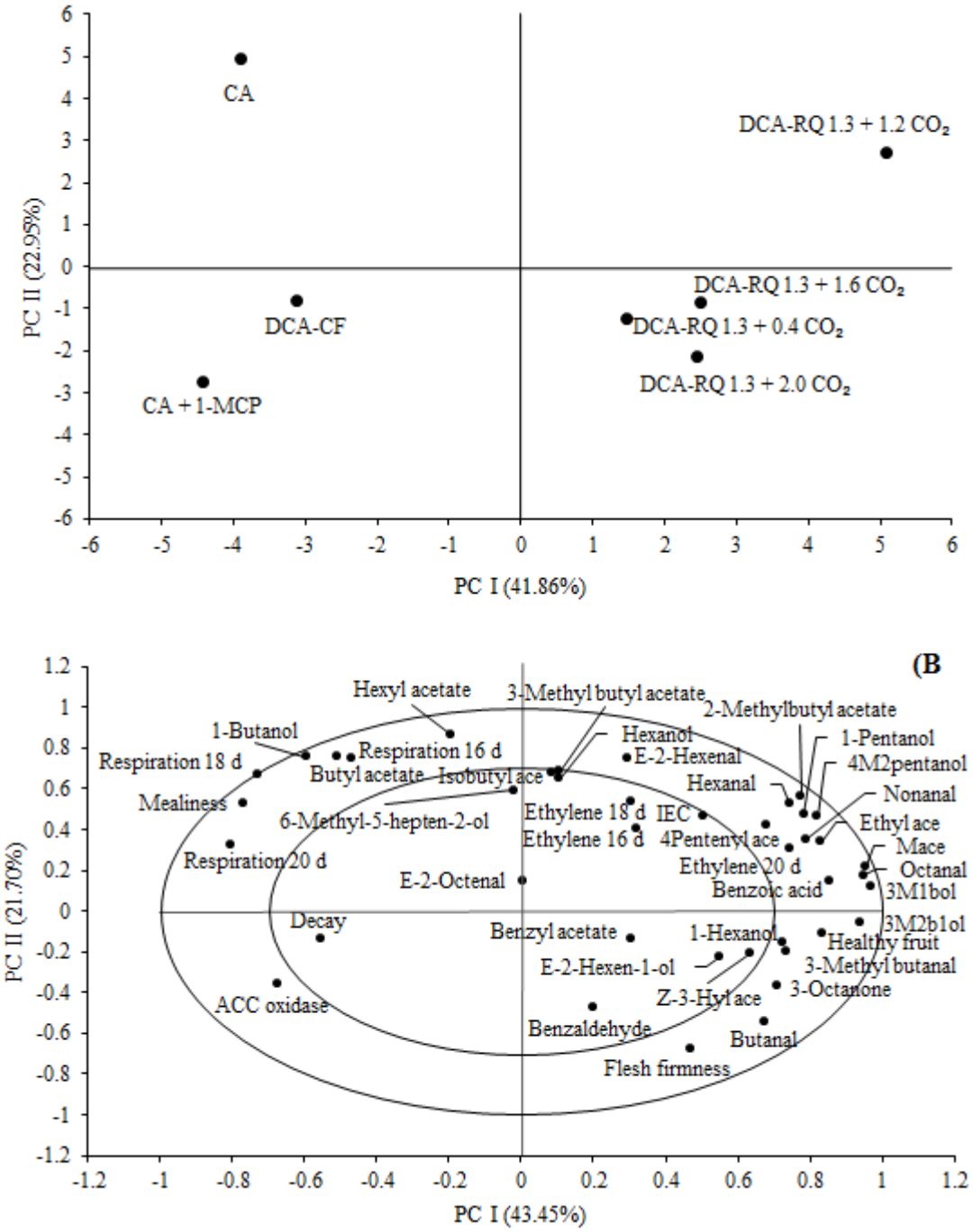


Figure 9. Principal component analysis (PCA) in 'Maxi Gala' apples after 9 months of storage under CA without or with 1-MCP treatment (0.650 µL L^{-1}), DCA-CF, DCA-RQ 1.3 with 0.4, 1.2, 1.6 and 2.0 kPa CO_2, plus 21 d of shelf life at 20°C. 4Pentenyl ace: 4-pentenyl acetate, Isobutyl ace: isobutyl acetate, Z-3-Hyl ace: Z-3-hexenyl acetate, Ethyl ace: ethyl acetate, Mace: methyl acetate, 3M1bol: 3-methyl-1-butanol and 3M2b1ol: 3-methyl-2-buten-1-ol.

4.1.4 Conclusions

DCA-RQ 1.3 with 1.2 or 1.6 kPa CO_2 maintained good quality of 'Maxi Gala' apples, but fruit stored in CA and DCA-CF resulted in less quality after 7 d shelf life. CA + 1-MCP may not keep quality until 21 d, mainly due lower healthy fruit amount, as well as for DCA-RQ 1.3 with higher pCO_2 (2.0 kPa).

'Maxi Gala' apple could be stored under DCA-RQ 1.3 with 1.6 pCO_2, because it maintained fruit quality similar or better than 1.2 pCO_2 after 9 months of storage plus 21 d of shelf life at 20 °C.

'Maxi Gala' apple stored under DCA-RQ 1.3 with 0.4 kPa of CO_2 maintained percentage of healthy fruit similar to 1.2 and 1.6 pCO_2, but flesh firmness loss was higher during the entire shelf life evaluation period. The storage in DCA-RQ 1.3 with 2.0 kPa of CO_2 maintained fruit quality similar to pCO_2 1.2 and 1.6 up to 14 d of shelf life, but not until 21 d.

CA storage, without 1-MCP application, maintained higher VOCs characteristic of apple aroma, mainly from the ester class, which increased until 14 d shelf life, and then, decreased. At 21 d shelf life DCA-RQ 1.3, mainly with 1.2, 1.6 and 2.0 kPa CO_2, resulted in increased esters production, without differing from CA stored fruit, but with greater overall quality, suggesting a good physic and sensory quality of these fruit with extended shelf life. 1-MCP application and DCA-CF affected negatively the VOCs production, even with extended shelf life.

4.1.5 References

Anese, R. de O., Brackmann, A., Wendt, L.M., Thewes, F.R., Schultz, E.E., Ludwig, V., Berghetti, M.R.B., 2018. Interaction of 1-methylcyclopropene, temperature and dynamic controlled atmosphere by respiratory quotient on 'Galaxy' apples storage. Food Packa. Shelf Life 20, 100-246. https://doi.org/10.1016/j.fpsl.2018.07.004.

Anese, R., de, O., Thewes, F.R., Brackmann, A., Schultz, E.E., Wagner, R., Klein, B., Berghetti, M.R.P., Wendt, L.M., 2020. Growth regulators on quality traits and volatile organic compounds profile of 'Royal Gala' apple at harvest and after dynamic controlled atmosphere storage. Postharvest Biol. Technol. 164, 111-158. https://doi.org/10.1016/j.postharvbio.2020.111158.

Asoda, T., Terai, H., Kato, M., Suzuki, Y., 2009. Effects of postharvest ethanol vapor treatment on ethylene responsiveness in broccoli. Postharvest Biol. Technol. 52, 216–220. https://doi.org/10.1016/j.postharvbio.2008.09.015.

Bessemans, N., Verboven, P., Verlinden, B.E., Nicolaï, B.M., 2016. A novel type of dynamic controlled atmosphere storage based on the respiratory quotient (RQ-DCA). Postharvest Biol. Technol. 115, 91-102. https://doi.org/10.1016/j.postharvbio.2015.12.019.

Boersig, M.R., Kader, A.A., Romani, R.J., 1988. Aerobic-anerobic respiratory transition in pear fruit and cultured pear fruit cells. J. Am. Soc. Hortic. Sci. 113, 869-873.

Both, V., Brackmann, A., Thewes, F.R., Ferreira, D., de, F., Wagner, R., 2014. Effect of storage under extremely low oxygen on the volatile composition of 'Royal Gala' apples. Food Chem. 156, 50-57. https://doi.org/10.1016/j.foodchem.2014.01.094.

Both, V., Brackmann, A., Thewes, F.R., Weber, A., Schultz, E.E., Ludwig, V., 2018. The influence of temperature and 1-MCP on quality attributes of 'Galaxy' apples stored in controlled atmosphere and dynamic controlled atmosphere. Food Packag. Shelf Life, 16, 168‑177. https://doi.org/10.1016/j.fpsl.2018.03.010.

Both, V., Thewes, F.R., Brackmann, A., de Oliveira Anese, R., de Freitas Ferreira, D., Wagner, R., 2017. Effects of dynamic controlled atmosphere by respiratory quotient on some quality parameters and volatile profile of 'Royal Gala' apple after long-term storage. Food Chem. 215, 483-492. https://doi.org/10.1016/j.foodchem.2016.08.009.

Brackmann, A., Anese, R.O., Weber, A., Both, V., Gasperin, A.R., Pavanello, E.P., 2013. Efeito do estresse inicial por baixo O_2 combinado com 1-metilciclopropeno na conservação de maçãs 'Royal Gala' armazenadas com ultrabaixo O_2. Semina: Cienc. Agrárias 34, 1185-1194. DOI: 10.5433/1679-0359.2013v34n3p1185.

Brackmann, A. Control apparatus for controlled atmosphere cells for storing perishable items. ***U.S. Patent***, n.US2015/0257401A1. 2015.

Brackmann, A., Weber, A., Both, V., 2015. CO_2 partial pressure for respiratory quotient and Harwest WatchTM dynamic controlled atmosphere for 'Galaxy' apple storage. Acta Hort. 1079, 435-440. https://doi.org/10.17660/ActaHortic.2015.1079.56.

Brackmann, A., Weber, A., Pinto, J.A.V., Neuwald, D.A., Steffens, C.A., 2008. Manutenção da qualidade pós-colheita de maçãs 'Royal Gala' e 'Galaxy' sob armazenamento em atmosfera controlada. Ciência Rural 38, 2478-2484. https://doi.org/10.1590/S0103-84782008000900010.

Brackmann, A., Streif, J., Bangerth, F. 1993. Relationship between a reduced aroma production and lipid metabolism of apples after long-term controlled atmosphere storage. J. Am. Soc. Hortic. Sci., 118, 243-247. https://doi.org/10.21273/JASHS.118.2.243.

Bufler, G., 1986. Ethylene-promoted conversion of 1-aminocyclopropane-1-carboxylic to ethylene in peel of apple at various stages of fruit development. Plant Physiol. 80, 539-543. https://doi.org/10.1104/pp.80.2.539.

Bufler, G., and Streif, J. 1986. Ethylene Biosynthesis of 'Golden Delicius' Apples Stored in Different Mixtures of Carbon Dioxide and Oxygen. Sci. Hortic. 30, 177-185. https://doi.org/10.1016/0304-4238(86)90096-8.

Contreras, C., Tjellström, H., & Beaudry, R. M. (2016). Relationships between free and esterified fatty acids and LOX-derived volatiles during ripening in apple. Postharvest Biol. Technol. 112, 105-113. https://doi.org/10.1016/j.postharvbio.2015.10.009.

Donadel, J.Z., Thewes, F.R., de Oliveira Anese, R., Schultz, E.E., Berghetti, M.R.P., Ludwig, V., Klein, B., Cichoski, A.J., Barin, J.S., Both, V., Brackmann, A., Wagner, R., 2019. Key volatile compounds of 'Fuji Kiku' apples as affected by the storage conditions and shelf life: correlation between volatile emission by intact fruit and juice extracted from the fruit. Food Res. Int. 108-625.https://doi.org/10.1016/j.foodres. 2019.108-625.

dos Santos, I.D., Pizzutti, I.R., Dias, J.V., Fontana, M.E.Z., Brackmann, A., Anese, R.O.,Thewes, F.R., Marques, L.N., Cardoso, C.D., 2018. Patulin accumulation in apples under dynamic controlled atmosphere storage. Food Chem. 255, 275 – 281. https://doi.org/10.1016/j.foodchem.2018.02.022.

Echeverría, G., Graell, J., Lara, I., López, M.L., 2008. Physicochemical measurements in 'Mondial Gala®' apples stored at different atmospheres: influence on consumer acceptability. Postharvest Biol. Technol. 50, 135 – 144. https://doi.org/10.1016/j.postharvbio.2008.05.002.

Franck, C., Lammertyn, J., Ho, Q.T., Verboven, P., Verlinden, B., Nicolai, B.M., 2007. Browning disorders in pear fruit. Postharvest Biol. Technol. 43, 1-13. https://doi.org/10.1016/j.postharvbio.2006.08.008.

Gago, C.M.L., Guerreiro, A.C., Miguel, G., Panagopoulos, T., Sánchez, C., & Antunes, M. D. C. 2015. Effect of harvest date and 1-MCP (SmartFresh^{TM}) treatment on 'Golden Delicious' apple cold storage physiological disorders. Postharvest Biol. Technol. 110, 77-85. https://doi.org/10.1016/j.postharvbio.2015.07.018.

Gasser, F., Eppler, T., Naunheim, W., Gabioud, S., Nising, A.B., 2010. Dynamic CA storage of apples: monitoring of the critical oxygen concentration and adjustment of optimum conditions during oxygen reduction. Acta Hortic. 876, 39 – 46. https://doi.org/10.17660/actahortic.2010.876.3.

Gorny, J.R., Kader, A.A., 1997. Low Oxygen and Elevated Carbon Dioxide Atmospheres Inhibit Ethylene Biosynthesis and Preclimacteric and Climacteric Apple Fruit. J. Am. Soc. Hortic. Sci. 122, 542-546. https://doi.org/10.21273/JASHS.122.4.542.

Jin, Y.Z., Lv, D.Q., Liu, W.W., Qi, H.Y., Bai, X.H., 2013. Ethanol vapor treatment maintains postharvest storage quality and inhibits ethylene biosynthesis during storage of oriental sweet melons. Postharvest Biol. Technol. 86, 372-380. https://doi.org/10.1016/j.postharvbio.2013.07.019.

Harb, J., Lara, I., Saleh, O., Streif, J., Khraiwesh, B., 2011. Treatments that suppress ethylene production or ethylene action modify ADH and AAT gene expression and aroma-related enzyme activities in 'Delbard Estivale' apple: consequences for the aroma profiles of fruits. J. Hort. Sci. Biotech. 86, 182-188. https://doi.org/10.1080/14620316.2011.11512745.

Harker, F.R., Kupferman, E.M., Marin, A.B., Gunson, F.A., Triggs, C.M., 2008. Eating quality standards for apples based on consumer preferences. Postharvest Biol. Technol. 50, 70-78. https://doi.org/10.1016/j.postharvbio.2008.03.020.

Kist, B.B. et al., 2019. Anuário brasileiro da maçã 2019 /– Santa Cruz do Sul: Editora Gazeta Santa Cruz, 2019. 56 p.: il. Avaliable in: http://www.editoragazeta.com.br/sitewp/wp-content/uploads/2019/06/MA%C3%A7%C3%A2_2019_DUPLA.pdf.

Liu, S., Yang, Y., Murayama, H., Taira, S., Fukushima, T. 2004. Effects of CO_2 on respiratory metabolism in ripening banana fruit. Postharvest Biol. Technol. 33, 27-34. https://doi:10.1016/j.postharvbio.2004.01.006.

Lumpkin, C., Fellman, J.K., Rudell, D.R., Mattheis, J., 2014. 'Fuji' apple (Malus domestica Borkh.) volatile production during high pCO_2 controlled atmosphere storage. Postharvest Biol. Technol. 100, 234-243. https://doi.org/10.1016/j.postharvbio.2014.10.008.

Lumpkin, C., Fellman, J.K., Rudell, D.R., Mattheis, J., 2014. 'Scarlett Spur Red Delicious' apple volatile production accompanying physiological disorder development during low pO_2 controlled atmosphere storage. J. Agric. Food Chem. 62, 1741-1754. https://doi.org/10.1021/jf405267b.

Maguire, K.M., Banks N.G., Lang, A., Gordon I.L., 2000. Harvest date, cultivar, orchard and tree effects on water vapor permanence in apples. J. Am. Soc. Hort. Sci. 125, 100-104. https://doi.org/10.21273/JASHS.125.1.100.

Mannapperuma, J.D., Singh, R.P., Montero, M.E., 1991. Simultaneous gas diffusion and chemical reaction in foods stored in modified atmospheres. J. Food Eng. 14, 167-183.

Mathooko, F.M., 1996. Regulation of ethylene biosynthesis in higher plants by carbon dioxide. Postharvest Biol. Technol. 7, 1-26. https://doi.org/10.1016/0925-5214(95)00026-7.

Mehinagic, E., Royer, G., Symoneaux, R., Lourjon, F., Prost, C., 2006. Characterization of odor-active volatiles in apples: influence of cultivars and maturity stage. J. Agric. Food Chem. 54, 2678‑2687. https://doi.org/10.1021/jf052288n.

Nishiyama, K., Guis, M., Rose, J.K.C., Kubo, Y., Bennett, K.A.B., Lu, W., Kato, K., Koichiro, U., Ryohei, N., Akitsugu, I., Mondher, B., Alain, L., Jean-Claude, P.,Bennett, A.B., 2007. Ethylene regulation of fruit softening and cell wall disassembly in charentais melon. J. Exp. Bot. 58, 1281-1290. https://doi.org/10.1093/jxb/erl283.

Payasi, A., Mishra, N.N., Chaves, A.L.S., Singh, R., 2009. Biochemistry of fruit softening: an overview. Physiol. Mol. Biol. Plants 15, 103-113. https://doi.org/10.1007/s12298-009-0012-z.

Plotto, A., McDaniel, M. R., & Mattheis, J. P. (2000). Characterization of changes in 'Gala' apple aroma during storage using Osme analysis, a gas chromatography olfactometry technique. J. Am. Soc. Horti. Sci. 125, 714-722. https://doi.org/10.21273/JASHS.125.6.714.

Prasanna, V., Prabha, T.N., Tharanathan, R.N., 2007. Fruit ripening phenomena-an overview. Crit. Rev. Food Sci. Nutr. 47, 1-19. https://doi.org/10.1007/s12298-009-0012-z.

Prange, R.K., DeLong, J. M., Harrison, P.A., Leyte, J.C., McLean, S.D., 2003. Oxygen concentration affects chlorophyll fluorescence in chlorophyll-containing fruit and vegetable. J. Am. Soc. Hortic. Sci. 128, 603-607. https://doi.org/10.21273/JASHS.128.4.0603.

Prange, R.K., Delong, J.M., Harrison, P., Mclean, S., Scrutton, J., & Cullen, J., 2007. Method and apparatus for monitoring a condition in chlorophyll containing matter. U.S. Patent, n.WO/2002/006795.

Raffo, A., Kelderer, M., Paoletti, F., Zanella, A., 2009. Impact of innovative controlled atmosphere storage technologies and postharvest treatments on volatile compound

production in Cv. pinova apples. J. Agric. Food Chem. 57, 915-923. https://doi.org/10.1021/jf802054y.

Salas, N.A., Molina-Corral, F.J., González-Aguilar, G.A., Otero, A., Sepulveda, D.R., Olivas, G. I. 2011. Volatile production by 'Golden Delicious' apples is affected by preharvest application of aminoethoxyvinylglycine. Sci. Hortic. 130, 436-444. https://doi.org/10.1016/j.scienta.2011.07.017.

Salazar, S., Aideé, N., Orozco, O., Isela, G., 2016. El aroma de la manzana. Interciencia 36 365-271.

Saquet, A.A., Streif, J., Bangerth, F., 2000. Changes in ATP, ADP and pyridine nucleotide levels related to the incidence of physiological disorders in 'Conference' pears and 'Jonagold' apples during controlled atmosphere storage. J. Hort. Sci. Biotechnol. 75, 243-249. https://doi.org/10.1080/14620316.2000.11511231.

Schmidt, S.F.P., Schultz, E.E., Ludwig, V., Berghetti, M.R.P., Thewes, F.R., Anese, R., de, O., Both, V., Brackmann, A., 2020. Volatile compounds and overall quality of 'Braeburn' apples after long-term storage: interaction of innovative storage technologies and 1-MCP treatment. Sci. Hortic. 262, 109039. https://doi.org/10.1016/j.scienta.2019.109039.

Sisler, E.C., Serek, M., 1997. Inhibitors of ethylene responses in plants at the receptor level: recent developments. Physiol. Plant. 100, 577-582. https://doi.org/10.1111/j.1399-3054.1997.tb03063.x.

Saquet, A. A., Streif, J., & Bangerth, F. 2003. Impaired aroma production of CA stored 'Jonagold' apples as affected by adenine and pyridine nucleotide levels and fatty acid concentration. J. Hortic. Sci. Biotechnol. 78, 695-705.

Song, J., & Bangerth, F. 2003. Fatty acids as precursor for aroma volatile biosynthesis in pre-climacteric and climacteric apple fruit. Postharvest Biol. Technol. 30, 113-121. https://doi.org/10.1016/S0925-5214(03)00098-X.

Stanger, M.C., Steffens, C.A., Soethe, C., Moreira, M.A., Amarante, C.V.T., Both, V., Brackmann, A. 2018. Phenolic compounds content and antioxidant activity of 'Galaxy' apples stored in dynamic controlled atmosphere and ultralow oxygen conditions. https://doi.org/10.1016/j.postharvbio.2018.05.014.

Tatsuki, M., Endo, A., Ohkawa, H., 2007. Influence of time from harvest to 1-MCP treatment on apple fruit quality and expression of genes for ethylene biosynthesis enzymes and ethylene receptors. Postharvest Biol. Technol. 43, 28-35. https://doi.org/10.1016/j.postharvbio.2006.08.010.

Thewes, F.R., Brackmann, A., Both, B., Anese, de O.R., Schultz, E.E., Ludwig, V., Wendt, L.M., Berghetti, M.R.P., Thewes, F.R., 2020. Dynamic controlled atmosphere based on carbon dioxide production (DCA-CD): Lower oxygen limit establishment, metabolism and overall quality of apples after long-term storage. Postharvest Biol. Technol. 168, 111-285. https://doi.org/10.1016/j.postharvbio.2020.111285.

Thewes, F.R., Brackmann, A., Anese, R., de, O., Ludwig, V., Schultz, E.E., dos Santos, L.F., Wendt, L.M., 2017a. Effect of dynamic controlled atmosphere monitored by respiratory quotient and 1-methylcyclopropene on the metabolism and quality of 'Galaxy' apple

harvested at three maturity stages. Food Chem. 222, 84-93. https://doi.org/10.1016/j.foodchem.2016.12.009.

Thewes, F.R., Both, V., Brackmann, A., Ferreira, D.F., Wagner, R., 2015. 1-Methylcyclopropene effects on volatile profile and quality of 'Royal Gala' apples produced in Southern Brazil and stored in controlled atmosphere. Ciência Rural. 12, 2259-2266. http://dx.doi.org/10.1590/0103-8478cr20141613.

Thewes, F.R., Brackmann, A., Both, V., Weber, A., Anese, R., de, O., Ferrão, T., dos, S., Wagner, R., 2017b. The different impacts of dynamic controlled atmosphere and controlled atmosphere storage in the quality attributes of 'Fuji Suprema' apples. Postharvest Biol. Technol. 130, 7-20. https://doi.org/10.1016/j.postharvbio.2017.04.003.

Thewes, F.R., Brackmann, A., de Oliveira Anese, R., Bronzatto, E.S., Schultz, E.E., Wagner, R., 2017c. Dynamic controlled atmosphere storage suppresses metabolism and enhances volatile concentrations of 'Galaxy' apple harvested at three maturity stages. Postharvest Biol. Technol. 127, 1-13. https://doi.org/10.1016/j.postharvbio.2017.01.002.

Thewes, F.R., Brackmann, A., de Oliveira Anese, R., Ludwig, V., Schultz, E.E., Berghetti, M.R.P., 2018. 1-Methylcyclopropene suppresses anaerobic metabolism in apples stored under dynamic controlled atmosphere monitored by respiratory quotient. Sci. Hortic. 227, 288-295. https://doi.org/10.1016/j.scienta.2017.09.028.

Tran, D.T., Verlinden, B.E., Hertog, M., Nicolaï, B.M., 2015. Monitoring of extremely low oxygen control atmosphere storage of 'Green star' apples using chlorophyll fluorescence. Sci. Hortic. 184, 18-22. https://doi.org/10.1016/j.scienta.2014.11.

Zanella, A., Cazzanelli, P., Rossi, O., 2008. Dynamic controlled atmosphere (DCA) storage by the means of chlorophyll fluorescence response for firmness retention in apples. Acta Hortic. 796, 77-82. https://doi.org/10.17660/ActaHortic.2008.796.7.

Watkins, C.B., 2006. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. Biotechnol. Adv. 24, 389‑409. https://doi.org/10.1016/j.biotechadv.2006.01.005.

Watkins, C.B., Nock, J.F., 2012. Rapid 1-methylcyclopropene (1-MCP) treatment and delayed controlled atmosphere storage of apples. Postharvest Biol. Technol. 69, 24-31. https://doi.org/10.1016/j.postharvbio.2012.02.010.

Weber, A., Brackmann, A., Anese, R. de O., Both, V. Pavanello, E.P., 2013. Atmosfera controlada para o armazenamento da maçã 'Maxi Gala'. Rev. Cien. Agron. 44, 294-301. https://doi.org/10.1590/S1806-66902013000200011.

Weber, A., Brackmann, A., Both, V., Pavanello, E.P., De O., Anese, R., Thewes, F.R., 2015. Respiratory quotient: innovative method for monitoring 'Royal Gala' apple storage in a dynamic controlled atmosphere. Sci. Agric. 72, 28-33. https://doi.org/10.1590/0103-9016-2013-0429. https://doi.org/10.1590/0103-9016-2013-0429.

Weber, A., Brackmann, A., Both, V., Pavanello, E.P., Anese, R.O., Schorr, M.R.W., 2016. Ethanol reduces ripening of "Royal Gala" apples stored in controlled atmosphere. An. Acad. Bras. Cienc. 88, 403‑410. https://doi.org/10.1590/0001-3765201620140181.

Weber, A., Neuwald, D.A., Kittemann, D., Thewes, F.R., Both, V., Brackmann, A., 2020. Influence of respiratory quotient dynamic controlled atmosphere (DCA‑RQ) and ethanol

application on softening of Braeburn apples. Food Chem. 303, 125346. https://doi.org/10.1016/j.foodchem.2019.125346.

Weber, A., Thewes, F.R., Sellwig, M., Brackmann, A., Wünsche, J.N., Kittemann, D., Neuwald, D.A., 2019. Dynamic controlled atmosphere: impact of elevated storage temperature on anaerobic metabolism and quality of ‘Nicoter’ apples. Food Chem. 298, 125017. https://doi.org/10.1016/j.foodchem.2019.125017.

Wild, H.P.J., Balk P.A., Fernandes, E.C.A., Peppelenbos, H.W., 2005. The action site of carbon dioxide in relation to inhibition of ethylene production in tomato fruit. Postharvest Biol. Technol. 36, 273-280. https://doi.org/10.1016/j.postharvbio.2005.02.004.

Wright, A.H., Delong, J.M., Arul, J., Prange, R.K., 2015. The trend toward lower oxygen levels during apple (Malus×domestica Borkh) storage. J. Hortic. Sci. Biotechnol. 90, 1-13. https://doi.org/10.1080/14620316.2015.11513146.

Wright, A. H., DeLong, J. M., Gunawardena, A.H.L. A. N., Prange, R K., 2012. Dynamic controlled atmosphere (DCA): Does fluorescence reflect physiology in storage? Postharvest Biol. Technol. 64, 19-30. https://doi.org/10.1016/j.postharvbio.2011.09.015.

Wyllie, S., Fellman, J., 2000. Formation of volatile branched chain esters in bananas (*Musa sapientum* L.). J. Agric. Food Chem. 48, 3493-3496. https://doi.org/10.1021/jf070464h.

Yang, S.F., Hoffman, N.E., 1984. Ethylene biosynthesis and its regulation in higher plants. Annu. Rev. Plant Biol. 35, 155-189. https://doi.org/10.1146/annurev.pp.35.060184.001103.

Yang, X., Song, J., Lina, D., Forney, C., Campbell-Palmer, L., Fillmore, S., Wismer, P., Zhang, Z., 2016. Ethylene and 1-MCP regulate major volatile biosynthetic pathways in apple fruit. Food Chem. 194, 325‑336. https://doi.org/10.1016/j.foodchem.2015.08.018.

5 ARTIGO 3

5.1 EXTREME LOW OXYGEN WITH DIFFERENT HYSTERESIS AND DYNAMIC CONTROLLED ATMOSPHERE (DCA): IMPACT ON OVERALL QUALITY AND VOLATILE PROFILE OF 'MAXI GALA' APPLE[3]

Abstract

This study aimed to evaluate 'Maxi Gala' apple storage using a novel extreme low oxygen (ELO) technique and compare it with traditional controlled atmosphere (CA) and dynamic controlled atmosphere (DCA) methods. The overall quality and volatile compounds were evaluated after 9 months of storage plus 7, 14, and 21 d shelf life. Two experiments were performed in different years evaluating controlled atmosphere (CA), CA + 1-methylcyclopropene, dynamic controlled atmosphere monitored by chlorophyll fluorescence emission (DCA-CF), DCA monitored by the respiratory quotient (RQ 1.3), and different ELO conditions. 'Maxi Gala' apple storage under ELO, with or without hysteresis, maintained fruit quality similar to CA + 1-MCP, DCA-CF, and was better than CA after 9 months of storage plus 7 d shelf life at 20 °C. Regardless of hysteresis, ELO maintained high amounts of healthy apples and low physiological disorders incidence, although flesh firmness decreased compared to DCA-RQ 1.3 and CA + 1-MCP after 9 months of storage plus 14 d shelf life. 'Maxi Gala' apple stored for 9 months under 0.4 kPa O_2 with 0.4 and 0.6 hysteresis plus 21 d of shelf life at 20 °C led to higher amount of healthy fruit than DCA-CF, CA+1-MCP, and was similar to DCA-RQ 1.3, albeit the apples had lower flesh firmness compared to DCA-RQ 1.3 and CA + 1-MCP. Irrespective of the storage condition, ELO suppressed 2-methylbutyl acetate and hexyl acetate ester at 7 and 14 d shelf life, and butyl acetate was high under ELO conditions at 7 d but reduced at 14 d. Fruit stored under CA had higher 2-methylbutyl acetate and hexyl acetate concentrations after 7 and 14 d shelf life and lower values until 21 d shelf life. Butyl acetate increased from 7 until 21 d of shelf life under CA storage, and CA + 1-MCP. DCA-CF suppressed the most important esters (2-methylbutyl acetate, butyl acetate, and hexyl acetate) in 'Maxi Gala' apples during the entire shelf life.

Keywords: Esters. Healthy fruit. *Malus domestica*. Physiological disorders. Shelf life.

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

5.1.1 Introduction

The apple harvest period is concentrated in a few months of the year. In order to provide apples to the market during the off-season, efficient storage techniques must be employed to supply the demand, including refrigerated atmosphere, controlled atmosphere (CA), 1-methylcyclopropene (1-MCP) application, ultra-low oxygen (ULO), and dynamic controlled atmosphere (DCA). The CA is the most commonly employed storage method for 'Gala' mutants, the pO_2 is maintained between 1.0 and 1.2 kPa and the pCO_2 at 2.0 kPa (Brackmann et al., 2008; Weber et al., 2013). However, storage longer than 7-8 months may reduce flesh firmness and cause physiological disorder incidence (Both et al., 2017; Bessemans et al., 2016; Wright et al., 2015). Generally, 1-MCP is applied along with CA conditions to maintain high fruit quality, suppress ethylene action, and reduce flesh firmness loss (Lu et al., 2018; Sisler and Serek, 1997; Watkins, 2006; Watkins, Nock, 2012), although 1-MCP is not allowed in organic apple production (Gabioud Rebeaud and Gasser, 2015).

Recently, the DCA method was developed to prolong storage period for up to 9 10 months keeping high fruit quality and without using chemical products. This technique changes the pO_2 in the cold room during storage according to the lower oxygen limit (LOL) tolerated by the fruit. The main methods of monitoring the LOL are through ethanol measurements (Schouten, 1995; Veltmann et al., 2003), chlorophyll fluorescence emission (DCA-CF) (Prange et al., 2003; Prange et al., 2007; Wright et al., 2012), and the respiratory quotient (DCA-RQ) (Brackmann, 2015; Weber et al., 2015; Bessemans et al., 2016; Gasser et al., 2010, 2008). Fruits stored under DCA-CF have higher quality compared to conventional CA-stored apples (Zanella et al., 2008; Tran et al., 2015; Both et al., 2017), and this DCA method is the most commercially used despite requiring additional equipment to monitor chlorophyll fluorescence emission.

The method based on RQ measures the ratio between CO_2 production and O_2 uptake during storage, and the most recommended RQ values for 'Gala' apple storage are 1.3 and 1.5 (Thewes et al., 2017a; Both et al., 2018; Anese et al., 2020). To obtain these values, pO_2 partial pressure must be reduced below the anaerobic compensation point (ACP) to increase anaerobic compound production, maintaining ethylene production at low concentrations and preserving high fruit quality during long-term storage (Asoda et al., 2009; Jin et al., 2013; Thewes et al., 2021; Stanger et al., 2018; Weber et al., 2020). The RQ values are determined using additional equipment installed inside the cold room to determine the uptakeO_2 and CO_2 production.

Ultra-low oxygen (ULO) storage, with pO_2 below 1.0 kPa and extreme low oxygen (ELO), with pO_2 below 0.5 kPa (Both et al., 2014), did not require additional apparatus in storage rooms. Several authors have reported the benefits of ULO in apple quality, including lower superficial scald incidence and higher flesh firmness (Zanella, 2003; Sabban-Amin et al., 2011). 'Royal Gala' apple stored under ELO (0.4 kPa) had similar firmness compared to DCA-CF, and 'Galaxy' apple showed higher firmness than DCA-CF ones (Thewes et al., 2015b). Moreover, 'Royal Gala' and 'Galaxy' apples presented similar fruit quality when comparing ULO to DCA-CF conditions (Anese et al., 2020). To study new storage techniques without increasing storage costs, this trial aimed to investigate an additional technique for ELO that consists of inducing stress induction during the storage by varying the pO_2 around a set point and thus maintain higher fruit quality for long-term storage periods.

Apple storage under low pO_2 and 1-MCP application reduces ethylene production and respiration and maintains higher fruit quality in long-term storage than CA (Both et al., 2017; Lafer, 2008; Zanella et al., 2008). However, volatile organic compound (VOC) reduction occurs because ethylene is necessary for lipoxygenase (LOX), alcohol dehydrogenase (ADH), and alcohol acyltransferase (AAT) activity, which are involved in producing VOCs (Harb et al., 2011; Schiller et al., 2015). Several studies demonstrated that DCA-CF (Both et al., 2017; Raffo et al., 2009) and 1-MCP application (Anese et al., 2020; Aubert et al., 2015) reduce VOC concentrations in apple (Both et al., 2017; Raffo et al., 2009; Anese et al., 2020; Aubert et al., 2015). In 'Galaxy' apple storage under ULO, DCA-RQ 1.3 caused low ester concentrations (Anese et al., 2020), while DCA-RQ 1.5 (Anese et al., 2020; Thewes et al., 2020) and DCA-RQ 2.0 (Both et al., 2017) led to high ester levels. The pO_2 adopted in DCA-RQ 1.5 and 2.0 is lower than ULO, DCA-CF, and DCA-RQ 1.3, resulting in higher ester production, and this may be attributed to the lower pO_2 levels that caused anaerobic metabolism, producing ethanol, increasing the ester production in the fruit. Apples stored under ELO with stress induced by low pO_2 (0.2 until 0.0 kPa) may be an alternative to induce VOC production.

Therefore, this experiment aimed to evaluate the overall quality and volatile profile of 'Maxi Gala' apple storage under different ELO conditions with stress induction by low oxygen and compare to CA + 1-MCP, DCA-CF, and DCA-RQ 1.3 after 9 months of storage plus 7, 14, and 21 d of shelf life at 20 °C.

5.1.2 Material and methods

5.1.2.1 Fruit harvest and sample preparation

This study was carried out in two seasons (2017 and 2018) with 'Maxi Gala' apple harvested in a commercial orchard in Vacaria (Rio Grande do Sul State, Brazil). The apples were transported to the Postharvest Research Centre of the Federal University of Santa Maria (Brazil). Replicates of 25 fruit were performed, and each treatment was composed of four replicates.Three samples were submitted to initial analysis to verify the physicochemical conditions of the fruit at harvest (Table 1).

Table 1 - Metabolism and quality of 'Maxi Gala' apple at harvest plus one day at 20 °C.

Season	SPI* (1-10)	ACC oxidase (ng C2H4 kg^{-1} s^{-1})	IEC (ug L^{-1})	Ethylene (ng kg^{-1} s^{-1})	Respiration (µg kg^{-1} s^{-1})	TA (% of malic acid)	Firmness (N)	SS (°Brix)
2017	6.43	16.86	4.13	1.67	4.49	0.33	74.57	12.83
2018	6.88	9.99	1.23	0.16	3.11	0.36	89.31	10.97

* SPI: Starch pattern index according to Streif (1984).

5.1.2.2 Storage conditions

Two experiments were carried out in two years. In the first experiment (2017), nine storage conditions were evaluated: [1] CA (1.2 kPa O_2 + 2.0 kPa CO_2); [2] CA + 1-MCP (0.625 µL L^{-1}); [3] DCA-CF (1.2 kPaCO_2); [4] DCA-RQ 1.3 + 1.2 kPa CO_2; [5] 0.4 O_2 + 0.4 hysteresis (HY); [6] 0.4 O_2 + 0.6 HY; [7] 0.4 O_2 + 0.8 HY; [8] 0.5 O_2 + 0.6 HY; [9] 0.5 O_2 + 0.8 HY. In the second experiment (2018), ten treatments were tested: [1] CA (1.2 kPa O_2 + 2.0 kPa CO_2); [2] CA + 1-MCP (0.625 µL L^{-1}); [3] DCA-CF; [4] DCA-RQ 1.3; [5] 0.4 O_2 ; [6] 0.4 O_2 + 0.4 HY; [7] 0.4 O_2 + 0.6 HY; [8] 0.5 O_2; [9] 0.5 O_2 + 0.4 HY; [10] 0.5 O_2 + 0.6 HY. The pCO_2 was 1.2 kPa in all storage conditions.

5.1.2.3 Atmosphere installation and maintenance

Four samples from each treatment were placed inside 180-L CA-rooms inside a cold room (45 m^3), and the chambers were then hermetically sealed. The temperature was reduced to 5 °C on the first day, decreasing by 1 ºC day^{-1} for the following 5 d until the desired storage temperature (2 °C). On the first day after reaching the desired temperature, the chambers were flushed with nitrogen to reduce pO_2 levels to 5.0 kPa, and, in the following 5 d, pO_2 was gradually reduced to the pre-established condition (0.5 kPa for DCA, 1.2 kPa in CA, 0.4 and

0.5 for ELO) by fruit respiration. The storage pCO_2 was obtained by fruit respiration. The gas concentration was monitored and adjusted by an automatic control system (Valis, Lajeado, RS, Brazil) connected to a gas analyzer (Ultramat 23, Siemens, Germany) to determine and adjust the pO_2 and pCO_2 by comparing the reading with the pre-established partial pressure (setpoint) of each treatment. If the pCO_2 was above the setpoint, the chamber gas was circulated through a CO_2 absorber containing calcium hydroxide and, if the pO_2 was below the setpoint, cold atmospheric air was injected until it reached pO_2 established for the treatment.

5.1.2.4 DCA-CF and DCA-RQ installation and determination

The DCA-CF was installed and monitored according to Prange et al. (2007), and DCA-RQ determination was performed according to Brackmann (2015) and Weber et al. (2015). The RQ was determined twice a week by the ratio between CO_2 production and O_2 uptake after 13 h, in which the chamber remained closed. When the RQ was below 1.3, the pO_2 was decreased by 10 %, and when RQ was above 1.3, the pO_2 was increased by 10 % in relation to the current set point.

5.1.2.5 Extreme low oxygen treatment establishment

The pO_2 (0.4 or 0.5 kPa) and hysteresis (HY; 0.4, 0.6, or 0.8) were set in the software that automatically corrects the gas partial pressure. For example, in the 0.4 O_2 + 0.4 HY treatment (Fig. 1a), the pO_2 varied from 0.2 to 0.6 kPa. The reduction of the oxygen until 0.2 occurred by fruit respiration and when the O_2 arrives at 0.2 kPa, the automatic gas control system injects O_2 up to 0.6; afterward, the oxygen is once again uptaken until 0.2 kPa by fruit respiration. This process occurs throughout the storage period in all treatments with hysteresis.

5.1.2.6 Temperature and relative humidity (RH)

'Maxi Gala' apple was stored at 2.0 ± 0.1 °C. The cold room control system automatically maintained the temperature using electronic thermostats. Temperature monitoring was performed daily with a mercury thermometer inserted in the pulp of a fruit. The RH inside the chambers was maintained at 94 ± 2 % using calcium chloride to absorb excess moisture.

5.1.2.7 1-Methylcyclopropene application

The fruit were packed in a 180-L chamber inside a cold room (2.0 ± 0.1 °C). A 0.625-μL L^{-1} solution of 1-MCP (SmartFresh, 0.14 % active ingredient) was prepared, and soon after, the solution was placed in a petri dish inside the chamber. The chamber was immediately closed for 24 h while the chamber air was circulated with a fan to homogenize 1-MCP. After 24 h, the fruit were removed from the chamber and stored in CA.

5.1.2.8 Physicochemical and biochemical analysis

The analyses of the physicochemical variables were performed after 9 months of storage plus 7, 14 and 21 d of shelf life at 20 ± 2 °C and RH of 80 ± 5 %.

5.1.2.8.1 ACC oxidase activity

The ACC oxidase activity was determined according to Bufler (1986). Skin samples from 10 fruits (3 g) were dipped in a solution containing 0.1 mol L^{-1} of ACC and 10 mmol L^{-1} of 2(n-morpholino)ethanesulfonic acid (MES) buffer at pH 6.0. After 30 min, the samples were transferred to hermetic syringes (50 mL) with 2% CO_2, and after another 30 min, two aliquots (1 mL) of the air in the syringe were withdrawn and injected into a gas chromatograph to measure ethylene concentrations. The results were expressed in ng kg^{-1} s^{-1}.

5.1.2.8.2 Ethylene production and respiration rate

Approximately 1.5 kg of fruit from each replicate were placed in a 5-L bottle that was sealed hermetically for one hour. Two air samples (1 mL) were then removed from the bottle and injected into a gas chromatograph (Varian Star 3400 CX, Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and a Porapak N80/100 column. Column, injector, and detector temperatures were 90, 140, and 200 °C, respectively, and the results are expressed as ng kg^{-1} s^{-1}. Respiration rate was determined using an electronic gas analyzer (Schele, model GA S1, version 2015, Germany) connected to the same bottle where the gas samples were taken to determine ethylene production. The air in the bottle was circulated through the analyzer and the CO_2 concentration was determined. Respiration rate were expressed in μg kg^{-1} s^{-1}.

5.1.2.8.3 Internal ethylene concentration

Internal ethylene concentrations (IEC) were determined according to the method proposed by Mannapperuma et al. (1991), with adaptations. Slices of fruit were placed in a container with water and an inverted funnel with a septum at the thinner end was placed on fruits. Then, a vacuum pump with a suction pressure of 565 mm Hg was applied to remove the air from the fruits, and two samples (1 mL) of this air were taken and injected into the same gas chromatograph used to determine ethylene production. The results were expressed in $\mu g\ L^{-1}$.

5.1.2.8.4 Electrolyte leakage

Electrolyte leakage was determined according to Gago et al. (2015), with modifications, and 15 slices (5 mm diameter and thickness) were removed from the pulp of 15 fruits. The slices were immersed in a falcon tube with 25 mL of distilled water and left exposed to 20 ± 1 °C for one hour to determine the conductivity of the solution. Afterward, the sample was boiled for 30 min at 100 ºC and cooled to 20 °C in a freezer at -30 °C. Finally, the conductivity was measured again, and the results were expressed as a percentage.

5.1.2.8.5 Flesh breakdown, mealiness, decay incidence, and healthy fruit

To evaluate the flesh breakdown, mealiness, and decay incidence, the fruits were sliced in the equatorial region in order to identify signs of browning, mealy pulp, and decay. For healthy fruit evaluation, all fruits without these symptoms were considered healthy, and the results of all evaluations were expressed as a percentage of the total fruit of each repetition.

5.1.2.8.6 Flesh firmness

Flesh firmness was determined using a FT-327 penetrometer with an 11 mm tip (Effegi, Milan, Italy). The probe was inserted on two opposite sides of the pulp in the equatorial region of the fruit where the epidermis had been previously removed. The results were expressed in Newton (N).

5.1.2.8.7 Soluble solids and titratable acidity

A juice sample was extracted from the 25 fruit of each repetition with a juice extractor (Philips Walita). The soluble solids of the juice were determined with a refractometer (Model 103, Biobrix, Curitiba, Brazil), and the results were expressed in °Brix. Next, titratable acidity was determined with the same juice extracted for the soluble solids, and 10 mL of juice was diluted in distilled water (100 mL) and titrated with NaOH (0.1 N) until pH 8.1. The results were expressed in % of malic acid.

5.1.2.8.8 Volatile organic compound analysis

After 9 months of storage plus 7, 14, and 21 days of shelf life at 20 °C, the VOCs in the juice extracted from the apples were analyzed. For sample preparation, three replicates containing ten apples each were cooled to 0.5 °C, and slices of the equatorial whithout seeds region and endocarp, were extracted and centrifuged in a juicer (Philips Walita). Immediately after extraction, the juice was placed in 100 mL amber glasses and frozen at -30 °C for further analysis.

For VOC quantification, frozen samples were thawed in a water bath at 20 °C for 10 min before sample preparation. For the samples, 10 mL of juice was placed in a 20-mL vial with NaCl (3 g) and 10 µL of 3-octanol standard solution (82.2 µg mL^{-1}) and sealed with a PTFE-coated silicone lid. The vial was then heated for 5 min with constant shaking in a water bath at 35 °C. The volatile compounds were extracted by solid-phase microextraction (HS-SPME) by exposing a fiber (50/30 µm x 10 mm, Supelco) covered with divinylbenzene/carboxen/polydimethylsiloxane polymers in the headspace of the vial for 60 min while the vial remained in the water bath at 35 °C. The fiber was then desorbed at the injector of a gas chromatograph (Dani Instruments SpA., Viale Brianza, Cologno Monzese, Italy) at 250 °C for 10 min using splitless mode in the first minute.

A DN-5 capillary column (30 m, 0.25 mm, 0.25 mm) was used to separate the compounds, and nitrogen was the carrier gas with a flow rate (nitrogen) of 1.0 mL min^{-1}. The temperature ramp used during the analyses was the same as described by Both et al. (2014). The flame ionization detector (FID) was kept at 230 °C, and the linear retention index was determined according to Both et al. (2014).

The VOCs were identified using a gas chromatograph coupled to a mass spectrometer (GC/MS; QP2010 Plus, Shimadzu, Kyoto, Japan). The extraction/desorption/injection procedures to identify the volatile compounds were the same as described above, with helium as the carrier gas. The MS detector operated in electron ionization mode, with ionization energy

of +70eV, scan ranges between 35‑350 m/z, and temperature of 250 °C. The mass spectra of each VOC were identified by comparing with mass spectra available in the National Institute of Standards and Technology library and the linear retention index with those available in the scientific literature.

5.1.2.8.9 Statistical analysis

The experiments were conducted in a completely randomized design. The data were submitted to the error normality and variance homogeneity tests by the Shapiro-Wilk and Bartlett tests, respectively. The variables that did not present normality of errors were transformed by the formula arc.sin $\sqrt{x/100}$, and data were submitted to analysis of variance (ANOVA). In data that showed a significant difference by ANOVA ($p < 0.05$), the means were compared by the Scott Knott test at 5% of error probability. Moreover, the data were submitted to principal component analysis (PCA) using the Unscrambler X software (version 9.7, CAMO A/S, Trondheim, Norway) to obtain an overview of the results. Before the PCA, the data matrix was auto-scaled to obtain the same weight for all variables (mean = 0 and variance = 1).

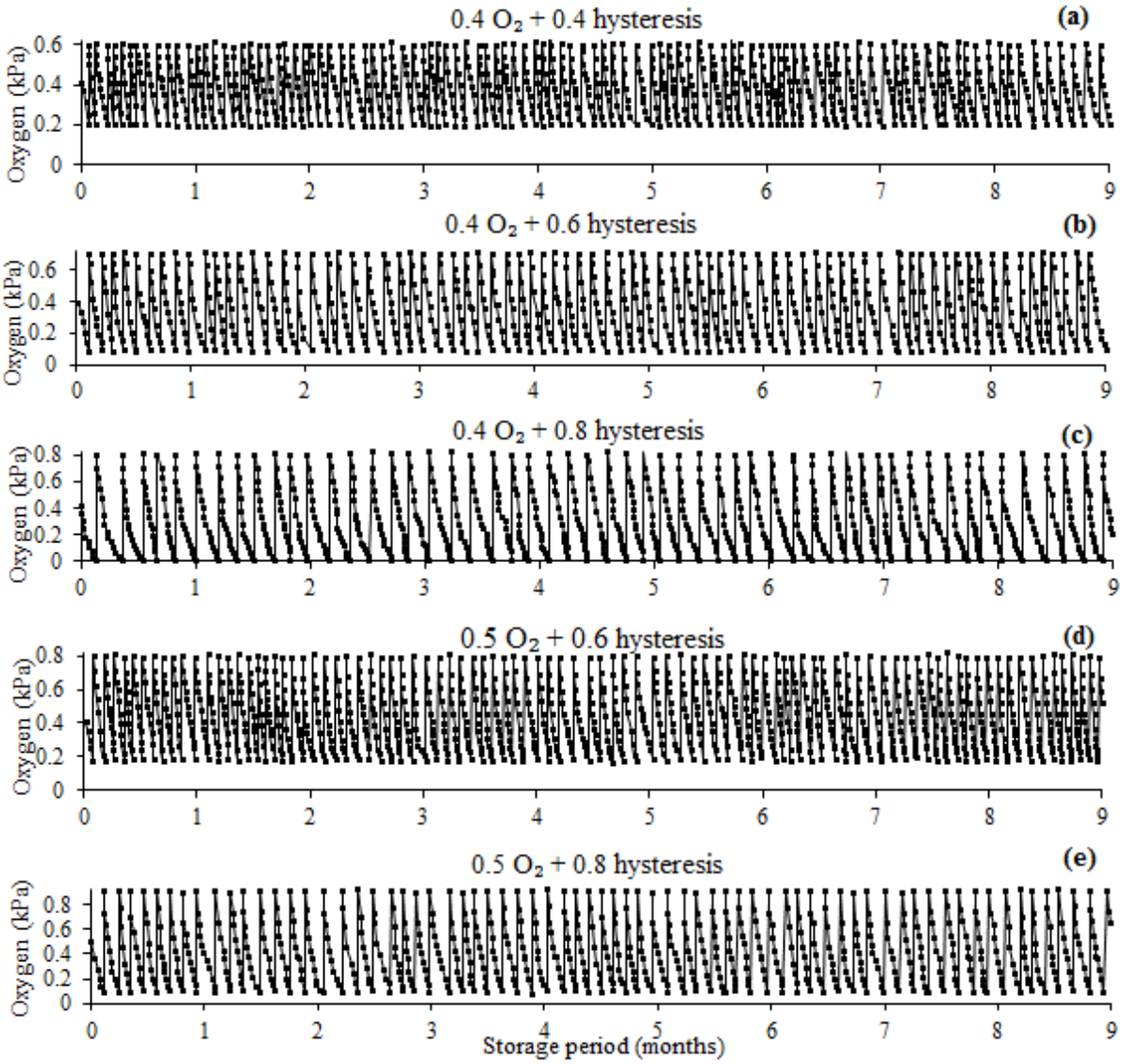
5.1.3 Results and discussion

5.1.3.1 Oxygen setpoint variation during storage

The oxygen setpoint variation (hysteresis) during 9 months of CA storage of ‘Maxi Gala’ apple under ELO at 0.4 kPa O_2 + 0.4, 0.6, and 0.8 HY, and ELO with 0.5 kPa O_2 + 0.6 and 0.8 HY, and CA, DCA-CF, and DCA-RQ 1.3 (season 2017) are shown in Figure 1. In 2018, the ‘Maxi Gala’ apple were stored under ELO at 0.4 and 0.5 kPa O_2 + 0.4 and 0.6 HY, ELO with 0.4 and 0.5 kPa O_2 without HY, and CA, DCA-CF, and DCA-RQ 1.3. Variation of oxygen partial pressure are illustrated in Figure 2. The amount in the frequency of low oxygen stresses during the storage period followed the sequence: 0.5 O_2 + 0.4 HY > 0.4 O_2 + 0.4 HY > 0.5 O_2 + 0.6 HY > 0.4 O_2 + 0.6 HY > 0.5 O_2 + 0.8 HY > 0.4 O_2 + 0.8 HY.

5.1.3.2 ACC oxidase enzyme activity, internal ethylene concentration, ethylene production, and respiration rate

In 2017, all fruit stored under ELO conditions with hysteresis (except for 0.4 O_2 + 0.8 HY) had low ACC oxidase activity after 7 d of shelf life, without differing from fruit stored



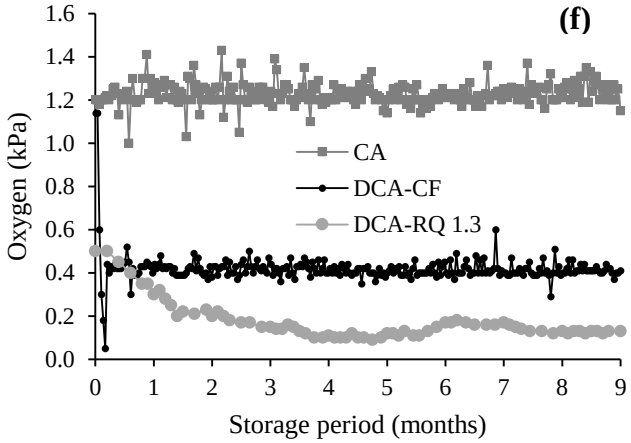
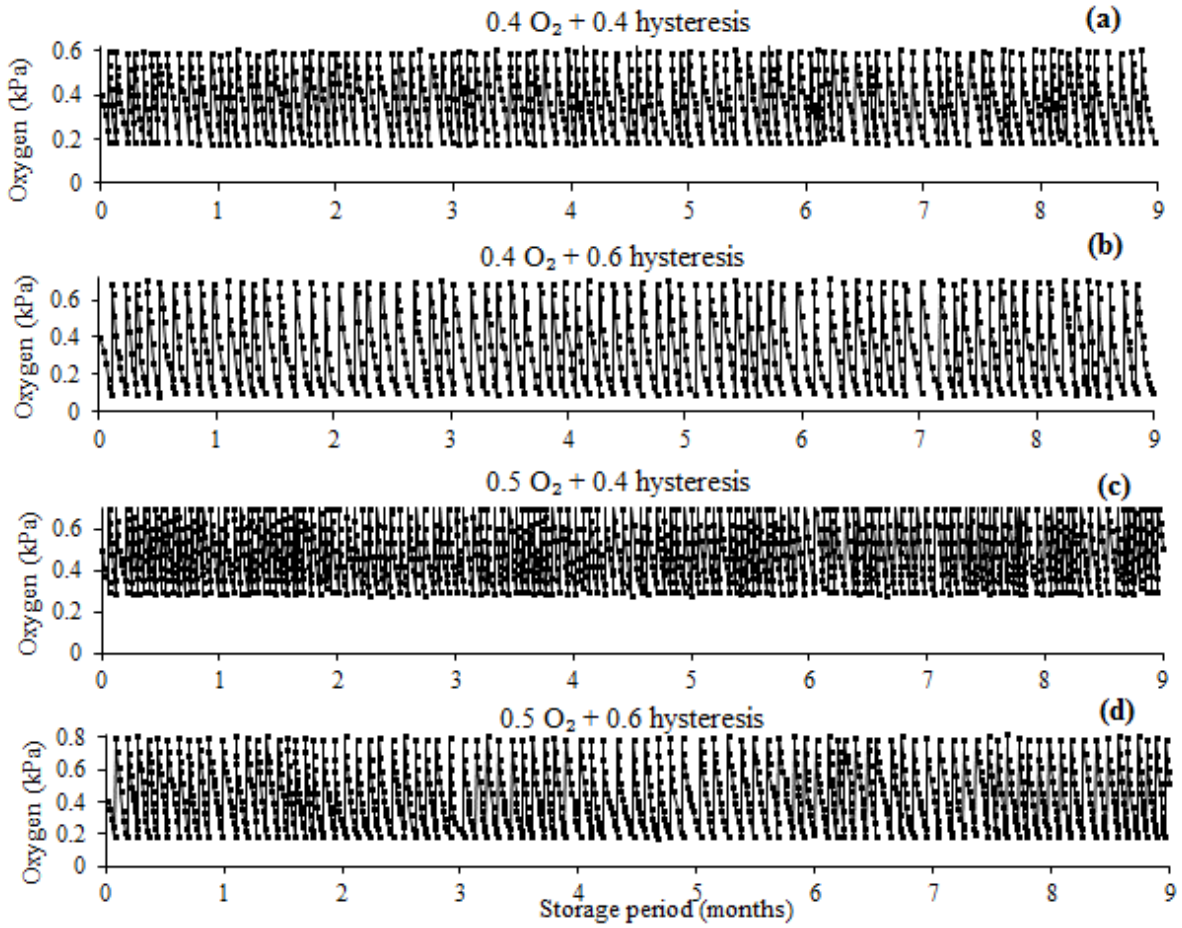


Figure 1. Oxygen set point variation of 'Maxi Gala' apple during 9 months of storage, at 2 °C under Extreme low oxygen (ELO) 0.4 and 0.5 kPa of O_2 with hysteresis 0.4, 0.6 and 0.8 (a, b, c, d and e), CA, DCA-CF and DCA-RQ 1.3 (f), on season 2017.



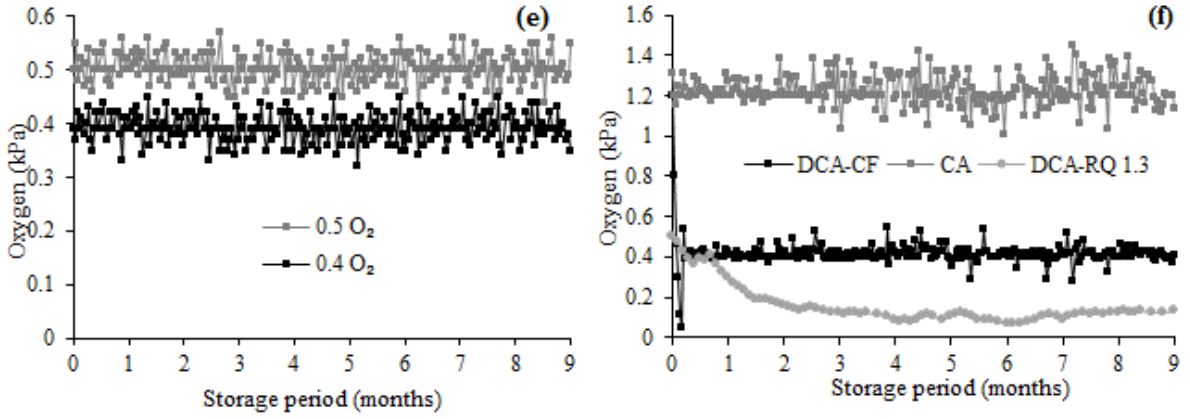


Figure 2. Oxygen set point variation of 'Maxi Gala' apple during 9 months of storage, at 2 °C, under Extreme low oxygen (ELO) 0.4 and 0.5 kPa of O_2 (e) with hysteresis 0.4 and 0.6 (a, b, c, and d), CA, DCA-CF and DCA-RQ 1.3 (f), on the season 2018.

under DCA-RQ and 1-MCP (Fig. 3a). Fruit storage and DCA-CF and 0.4 O_2 + 0.8 HY presented intermediate ACC oxidase activity. In 2018 (Fig. 3b), fruit stored under 0.4 O_2 + 0.6 HY had lower ACC oxidase compared to fruits under other conditions with hysteresis, albeit not differing from DCA and 1-MCP application.

Thewes et al. (2015b) also reported lower ACC oxidase in fruit stored under ELO (0.4 kPa O_2) compared to DCA-CF and CA, and Both e al. (2014) compared ELO with CA. The lower pO_2 (≤0.1 kPa) induced with hysteresis at 0.4 O_2 + 0.6 HY may explain the low ACC oxidase. When higher pO_2 were adopted (0.5 O_2 + 0.4 and 0.6 HY), ACC oxidase was higher. At 14 d of shelf life, CA stored fruit undergo a strong increase in ACC oxidase activity (Fig. 3b). The DCA-RQ maintained lower activity, which can be attributed to the lower pO_2 (0.16 kPa), specially on the end of storage period. After 21 d of shelf life (Fig. 3b), fruit stored under 0.4 O_2 + 0.6 HY and 0.5 O_2 + 0.6 HY showed higher ACC oxidase activity than the other samples under conditions with hysteresis. In general, CA-stored fruit showed elevated ACC oxidase for up to 14 d and lower ACC oxidase activity until 21 d of shelf life because the samples were in advanced ripening stages. Nevertheless, on the others storage conditions the fruit presented lower ACC oxidase activity on the 7 d shelf, with increment until 14 d and maintenance thereafter.

The IEC was lower under all ELO conditions with hysteresis (except for 0.4 O_2 + 0.8 HY) compared to CA, although there were no differences compared to DCA-RQ and CA + 1-MCP after 7 d of shelf life, regardless of the season (Figs. 3c and 3d). Higher IEC under DCA-CF and 0.4 O_2 + 0.8 HY in 2017 is related to the higher ACC oxidase activity (Fig. 3a). At 14

d of shelf life (Fig. 3d), fruit stored under 0.4 O_2 + 0.6 HY, 0.5 O_2, 0.5 O_2 + 0.4 HY, and CA showed higher IEC than the other conditions. After 21 d (Fig. 3d), the highest IEC was found

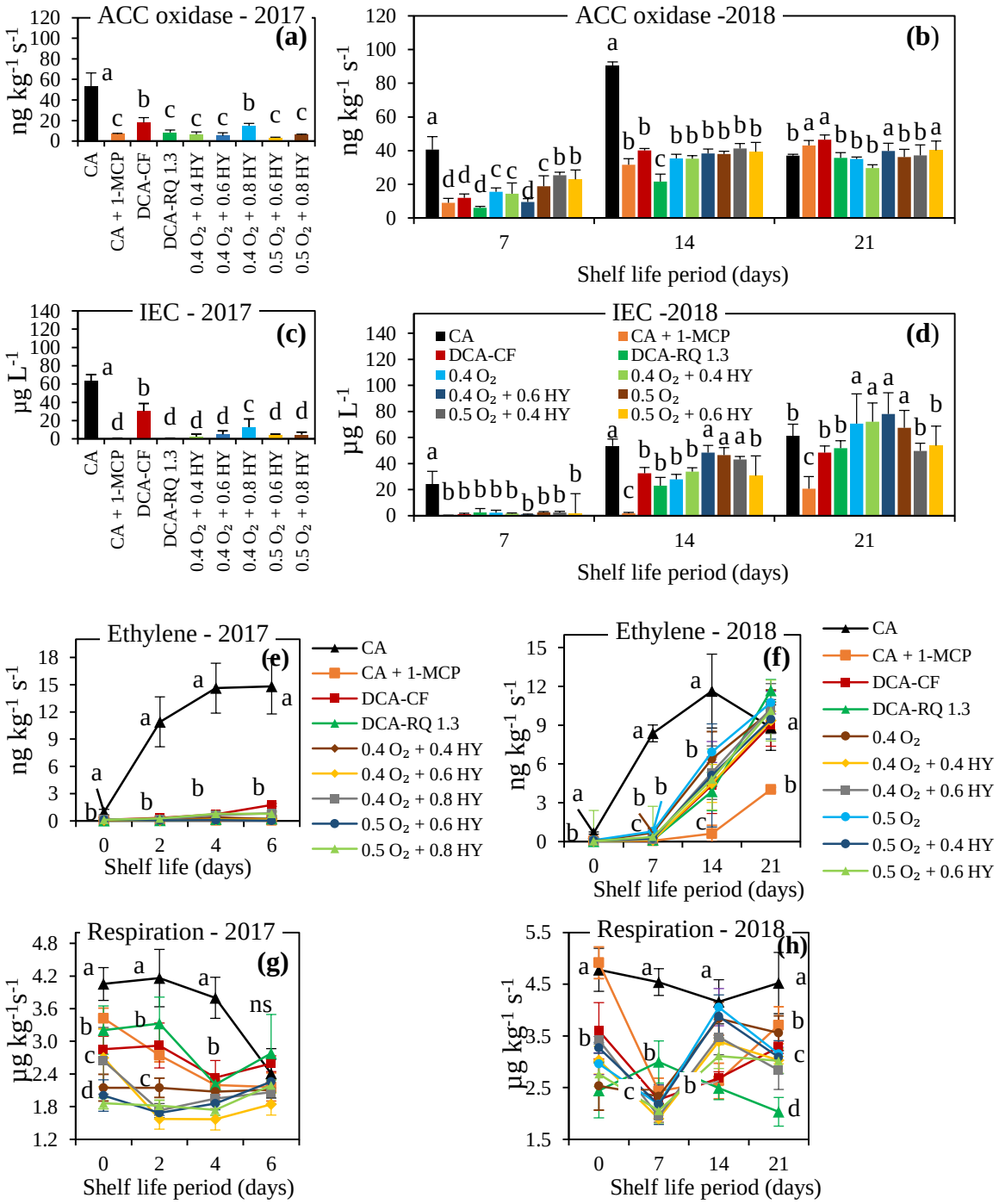


Figure 3. ACC oxidase activity (a, b), internal ethylene concentration (c, d) ethylene production (e, f) and respiration rate (g, h) of 'Maxi Gala' apples stored over 9 months under CA, CA + 1-MCP, DCA-CF DCA-RQ 1.3, Extreme low oxygen with 0.5 and 0.4 kPa static and with hysteresis (HY) 0.8, 0.6 and 0.4 after 9 months of storage plus 7 d (2017) and 7, 14 and 21 d of shelf life at 20 °C (2018). Means followed by the same letter at the same

day of shelf life do not differ by Scott Knott test at 5% of error probability. Error bars show the standard deviation. ns: not significant.

in fruit stored under 0.4 O_2, 0.4 O_2 + 0.4 HY, 0.4 O_2 + 0.6 HY, and 0.5 O_2. Nonetheless, 1-MCP application maintained lower IEC at 14 and 21 d of shelf life in 2018. Ethylene production is the main factor that induces apple ripening, and its reduction during storage is necessary to extend shelf life. All ELO conditions with hysteresis maintained low ethylene production after 9 months plus 7 d of shelf life in both years and only differed from CA-stored fruit (Figs. 3e and 3f), and this finding is related to the low ACC oxidase activity (Figs. 3a and 3b). In other studies, lower ethylene production was also found when comparing ULO and ELO with CA (Both et al., 2014, Thewes et al., 2015b, Weber et al., 2013). After 7 until 21 d of shelf life, all ELO conditions showed higher ethylene production, although without reaching the climacteric peak, unlike CA-stored apples, and fruit with 1-MCP application presented the lowest ethylene production throughout the shelf life.

Respiration is a key indicator of fruit metabolism (Steffens et al., 2007), and higher ethylene production generally increases fruit respiration rates (Pre-Aymard, Weksler, and Lurie, 2003). High respiration rate was verified in fruits stored under CA in both years throughout the shelf life (Figs. 3g and 3h), which is related to the high ethylene production (Figs. 3e and 3f), although fruit submitted to ELO (with or without hysteresis) presented low respiration rate until the seventh day of shelf life (Figs, 3g and 3h) due to the reduced metabolism induced by low pO_2 levels. In addition, previous studies have also reported that oxygen reduction decreases respiration rate (Both et al., 2014; Thewes et al., 2015b; Weber et al., 2013). After 7 d up to 14 d (Fig. 3h), fruit stored under ELO (with or without hysteresis) showed higher respiration rate and remained stable for up to 21 d of shelf life, and the DCA-RQ samples had the lowest respiration rate at 21 d of shelf life, and this is likely because the fruit adapted to the low pO_2 employed during storage (average 0.16 kPa).

5.1.3.3 Electrolyte leakage, mealiness, flesh breakdown and decay incidence

Cell damage can be identified by electrolyte leakage and associated with high anaerobic compound accumulation, causing flesh breakdown incidence (Saquet and Streif, 2008). In 2017 (Fig. 4a), all fruit stored under ELO + HY showed lower electrolyte leakage compared to CA and DCA-CF fruit, although only CA showed high flesh breakdown, which may be due to high pO_2 levels (1.2 kPa; Fig. 1f), and the result of higher metabolism during storage (Figs. 2a, 2c,

2e, and 2g). In 2018, higher electrolyte leakage occurred in fruit stored under CA and 0.4 O_2 + 0.6 HY, followed by other ELO + HY conditions, albeit without flesh breakdown incidence after 7 d of shelf life (Fig. 4d). The DCA-CF fruit showed lower electrolyte leakage, and at 14 and 21 d of shelf life, no differences were found among storage conditions (Fig. 4b). At 14 d of shelf life (Fig 4 d), CA fruit had higher flesh breakdown, and no differences among conditions were noted at 21 d.

Mealiness is a physiological disorder related to the advanced ripening stages of apples. In 2017, all fruit stored under ELO + HY and DCA conditions had lower mealiness incidence after 7 d of shelf life (Fig. 4e) in comparison to CA with and without 1-MCP. The lower mealiness incidence may be related to low fruit metabolism, such as IEC and ethylene production (Fig. 3c, and 3e), which is reduced by the low pO_2 employed under these conditions. Similar experiments have also reported low mealiness incidence under ULO (Anese et al., 2020; Thewes et al., 2015b) and DCA conditions (Both et al., 2018; Weber e al., 2015; Zanella and Rossi, 2015) compared to CA. The highest mealiness incidence in CA-stored fruit may be related to high IEC and ethylene production (Fig. 3c, and 3e).

Ethylene is necessary for triggering enzymatic activities, including pectin methylesterase, which degrades pectin from the middle layer of the cell wall (Nishiyama et al., 2007; Payasi et al., 2009), leading to cell separation and mealiness. In 2018, there was low incidence and no differences were observed among storage conditions after 7 d of shelf life (Fig. 4f), and this may be a result of the high flesh firmness (89.31 N) at harvest. However, at 14 and 21 d of shelf life, CA-stored fruits showed higher mealiness incidence, which is related to the high pO_2 levels employed and leads to high ethylene production and respiration rate (Fig. 3f and 3h, respectively). Fruit stored under ELO with hysteresis conditions did not differ from fruit stored under DCA and CA with 1-MCP application throughout the shelf life.

Decay incidence is a major cause of postharvest losses, and decayed fruit still produce mycotoxins, such as patulin (dos Santos et al., 2018), which are harmful to human health. No difference in decay was noted among the storage conditions throughout the shelf life period evaluated in both years (Fig. 4g and 4h), corroborating with Anese et al. (2020), which did not found difference for ‘Galaxy’ apples stored under CA, CA + 1-MCP, ULO, DCA-CF, and DCA-RQ conditions.

5.1.3.4 Healthy fruit, flesh firmness, titratable acidity, and soluble solids

Higher amount of healthy fruit after 9 months of storage plus 7 d of shelf life were found under ELO + HY than CA and CA + 1-MCP, in the season 2017 (Fig. 5a), which is related to the lower flesh breakdown and mealiness incidence (Fig. 4c and 4e). Another significant result is that ELO + HY showed a similar quantity of healthy fruit as DCA-CF and DCA-RQ 1.3.

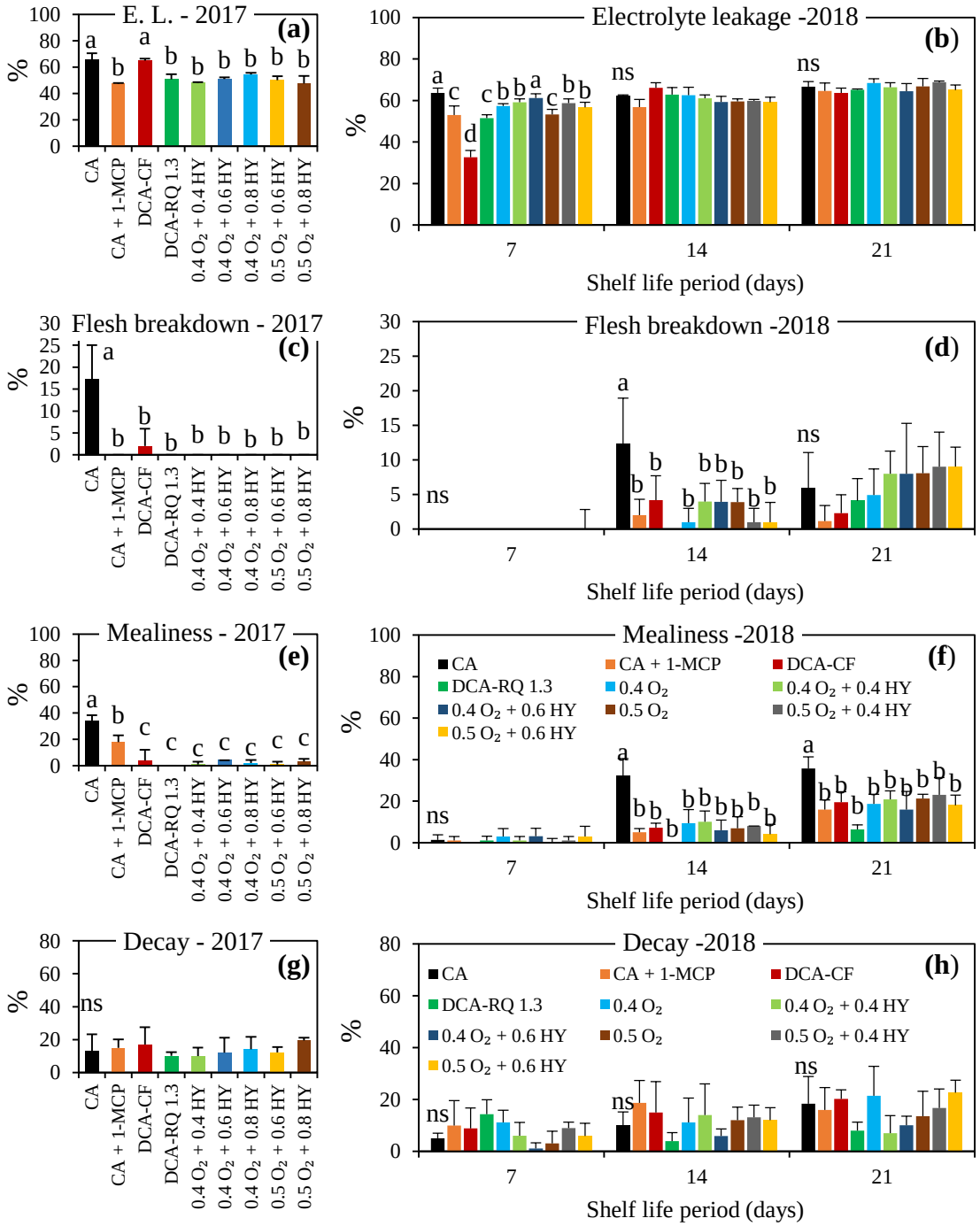


Figure 4. Electrolyte leakage (a, b), flesh breakdown (c, d) mealiness (e, f) and decay incidence (g, h) of 'Maxi Gala' apples stored over 9 months under CA, CA + 1-MCP, DCA-CF DCA-RQ 1.3, Extreme low oxygen 0.5 and 0.4 kPa static and with hysteresis (HY.) 0.8, 0.6 and 0.4 after 9 months of storage plus 7 d (2017) and 7, 14 and 21 d

of shelf life at 20 °C (2018). Means followed by the same letter at the same shelf life day do not differ by Scott Knott test at 5% of error probability. Error bars show the standard deviation. ns: not significant

Other authors have also reported similar findings by comparing static ELO (0.4 kPa) with DCA-CF and DCA-RQ 1.3 (Anese et al., 2020) and DCA-CF (Thewes et al., 2015b). In 2018, the healthy fruit percentage did not differ significantly among storage conditions at 7 d of shelf life (Fig. 5b). After 14 d of shelf life, there were more healthy fruit after storage under ELO, DCA-CF and DCA-RQ 1.3 than CA and CA + 1-MCP. In fact, ELO yielded a similar amount of healthy fruit as DCA-CF and DCA-RQ 1.3, although ELO (0.4 O_2) + HY (0.4 and 0.6) and DCA-RQ 1.3 maintained a higher amount of healthy fruit compared to the other conditions after 21 d of shelf life. These data show that ELO (0.4 O_2) + HY (0.4 or 0.6) may be an alternative to DCA-CF and CA + 1-MCP storage, given there is no need for additional equipment, thus reducing storage costs and maintaining a higher healthy fruit amount.

Flesh firmness is another critical quality parameter for consumers. In 2017, flesh firmness was higher in fruits stored under CA + 1-MCP, ELO 0.4 O_2 + 0.4 HY, and ELO 0.5 O_2 + 0.6 and 0.8 HY compared to the other conditions (Fig. 5c). The DCA-CF, DCA-RQ 1.3 and 0.4 O_2 + 0.6 and 0.8 HY showed intermediate flesh firmness, while CA-stored fruit had the lowest. Lower flesh firmness in CA-stored fruit results from higher pO_2 during storage, increasing ethylene production and respiration and causing higher membrane degradation and flesh firmness loss (Yang and Hoffman, 1984). In 2018, fruit stored under DCA-RQ and CA with 1-MCP showed higher firmness than ELO (with or without hysteresis) and CA conditions showed the lowest flesh firmness (Fig. 5d). Fruit stored under ELO and DCA-CF condition had intermediate values of flesh firmness. Fruit with 1-MCP treatment have higher flesh firmness due to the ethylene action inhibition (Blankenship and Dole, 2003; Sisler and Serek, 1997). The low pO_2 levels inhibit ethylene production and reduce flesh firmness loss in apples (Yang and Hoffman, 1984). The gradual reduction of the oxygen setpoint during storage at pO_2 below 0.2 kPa, as in DCA-RQ 1.3 leads to lower fruit metabolism and higher flesh firmness maintenance. Titratable acidity did not differ among storage conditions after 7 d of shelf life, in the season 2017 (Fig. 5e), although fruit stored under DCA-RQ 1.3 and ELO (with or without hysteresis, except for 0.4 O_2 + 0.4 HY) showed lower titratable acidity in 2018 (Fig. 5f). At 14 and 21 d of shelf life, no differences were observed among the storage conditions. Regarding to soluble solids, no difference were found among storage conditions in both years and different shelf life

periods evaluated (Fig. 5g and 5h). In addition, several authors have also reported little to no difference in titratable acidity or soluble solids compared to CA, ULO, ELO, DCA-CF, and DCA-RQ 1.3 (Thewes et al., 2015b; Anese et al., 2020).

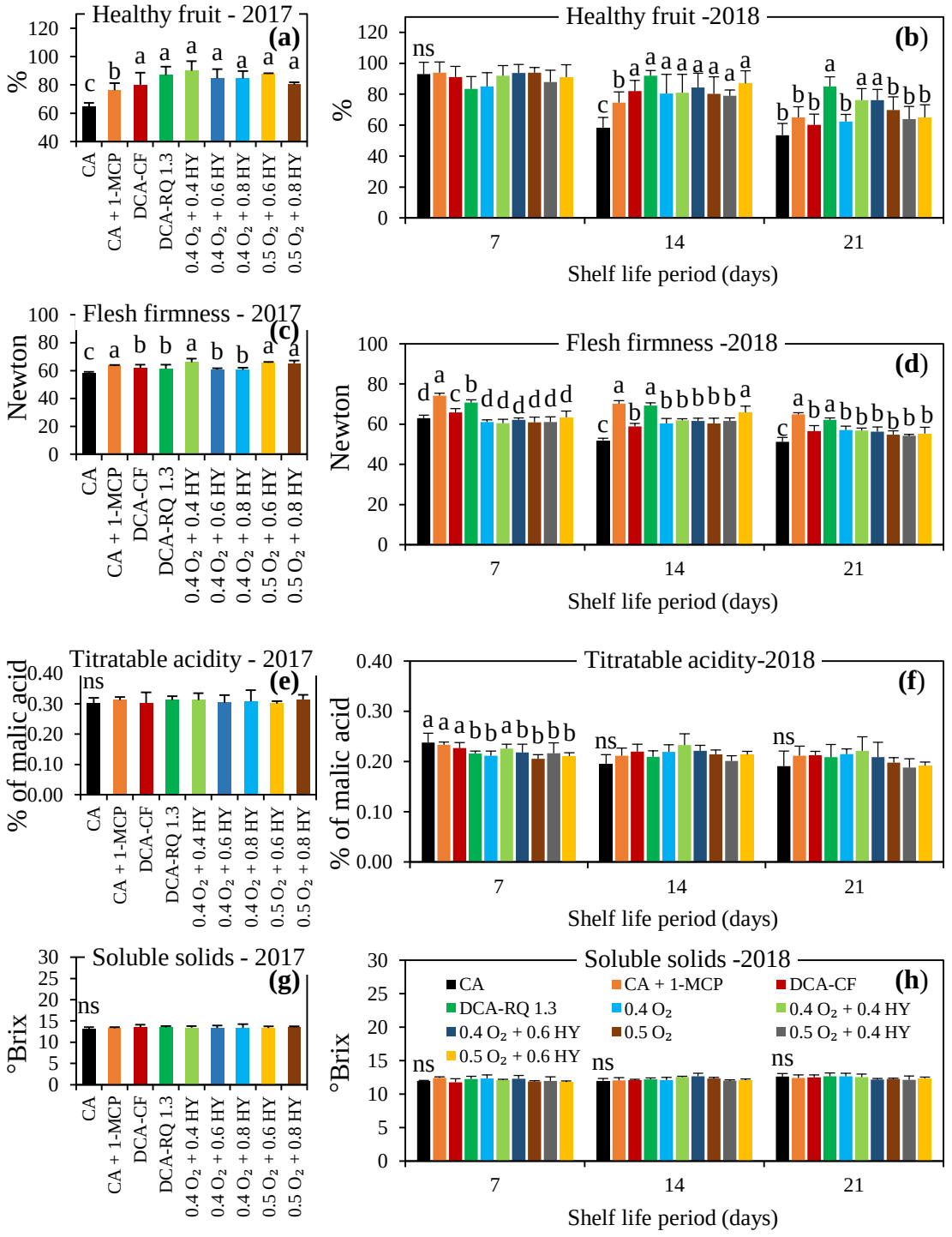


Figure 5. Healthy fruit (a, b), flesh firmness (c, d) titratable acidity (e, f) and soluble solids (g, h) of 'Maxi Gala' apples stored over 9 months under CA, CA + 1-MCP, DCA-CF DCA-RQ 1.3, Extreme low oxygen with 0.5 and 0.4 kPa static and with hysteresis

(HY.) 0.8, 0.6 and 0.4 after 9 months of storage plus 7 d (2017) and 7, 14 and 21 d of shelf life at 20 °C (2018). Means followed by the same letter at the same shelf life day do not differ by Scott Knott test at 5% of error probability. Error bars show the standard deviation. ns: not significant.

5.1.3.5 Volatile organic compounds (VOCs)

The VOCs were evaluated in 'Maxi Gala' apples harvested in 2018 after 9 months of storage plus 7, 14, and 21 d of shelf life at 20 °C. A total of 41 VOCs were identified: 13 esters (Fig. 6), 14 alcohols (Fig. 7), 12 aldehydes, 1 ketone and 2 acids (Fig. 8).

5.1.3.5.1 Main ester compounds

Fruit stored under ELO 0.4 and 0.4 O_2 + 0.6 HY showed higher total esters at 7 d of shelf life (Fig. 6a), and fruit under CA + 1-MCP, DCA-CF, and DCA-RQ 1.3 presented the lowest concentrations. At 14 d, CA-stored fruit had the highest total ester concentration, 0.5 O_2 had intermediate values, and the other conditions showed lower concentrations. After 21 d, fruit stored under CA + 1-MCP and DCA-CF had the lowest total ester concentrations, while ELO (0.5 O_2 with or without hysteresis and 0.4 O_2 + 0.6 HY) showed the highest total ester values. Fruit stored in DCA-RQ 1.3 also showed a sharp increase of total esters after 21d shelf life. In this study, the most abundant esters found during the entire shelf life were butyl acetate, 2-methylbutyl acetate, and hexyl acetate (Fig. 6f, 6g, and 6l, respectively). Esters biosynthesis are ethylene dependent, because this plant hormone acts in AAT expression (Defilippi et al., 2005). ATT catalyzes the esterification of alcohol and acyl-CoAs to form esters, and low ethylene concentrations are known to reduce butyl acetate biosynthesis (Both et al., 2014; Thewes et al., 2015a; Yang et al., 2016), albeit fruit stored under 0.4 and 0.4 O_2 + 0.6 HY showed the highest butyl acetate concentrations at 7 d of shelf life (Fig. 6f) and low ethylene production (Fig. 3f). The highest butyl acetate concentration in 0.4 O_2 can be attributed to the highest concentration of the precursor 1-butanol (Fig. 7d). The lowest butyl acetate concentration was observed under CA, CA + 1-MCP, DCA-CF, and DCA-RQ 1.3 storage conditions. At 14 and 21 d of shelf life, CA had the highest butyl acetate concentration, which can be associated with high ethylene production and 1-butanol concentrations (Fig 3f and 7d). Fruit stored under 0.5 O_2 and 0.5 O_2 + 0.4 HY showed an intermediate concentration, and the fruit under the other conditions had the lowest concentration.

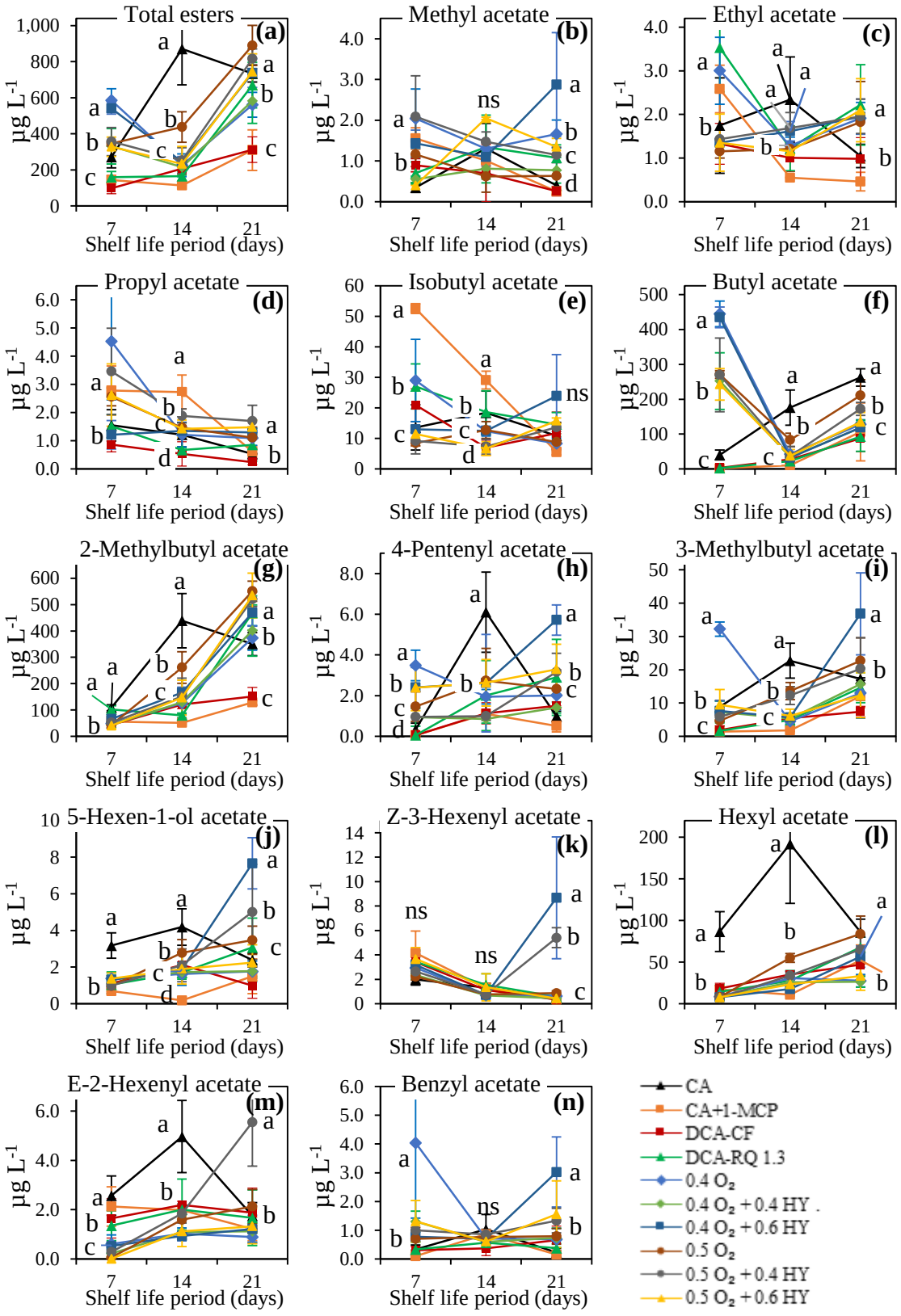


Figure 6. Esters concentration (µg L^{-1}) of 'Maxi Gala' apples stored under CA, CA + 1-MCP, DCA-CF, DCA-RQ 1.3, static and extreme low oxygen with 0.5 and 0.4 kPa O_2 and with hysteresis (HY.) 0.6 and 0.4, after 9 months of storage plus 7, 14 and 21 d of

shelf life at 20 °C. Means followed by the same letter at the same shelf life day do not differ by Scott Knott test at 5% of error probability. Error bars show the standard deviation. ns: not significant.

2-Methylbutyl acetate concentrations were higher under CA and DCA-RQ 1.3 at 7 d of shelf life compared to the other conditions (Fig. 6g). High pO_2 (1.2 kPa) levels during storage favored higher ethylene production under CA, and this may have elevated AAT activity, resulting in high 2-methylbutyl acetate concentrations. Nevertheless, fruit under DCA-RQ 1.3 with the lowest pO_2 (0.16 kPa on average) and low ethylene production would have probably had sufficient acetyl-CoA and precursor levels to stimulate the AAT to produce these ester. Fruit storage under ELO + HY showed a low concentration at 7 and 14 d but increased until 21 d of shelf life, which can be attributed to increased ethylene production and the precursor 2-methyl-1-butanol (Fig. 7g). Additionally, DCA-CF and CA+1-MCP showed the lowest 2-methylbutyl acetate concentration, and this may have likely occurred due to the low 2-methyl-1-butanol concentration. CA storage resulted in the highest 2-methylbutyl acetate at 14 d and thereafter decreased.

Hexyl acetate is another important ester in ‘Gala’ apples and presented higher concentrations in CA-stored fruit during the entire shelf life, despite decreased from 14 to 21 d (Fig. 6l). This finding can be explained by the high ethylene production verified and confirmed in other experiments (Anese et al., 2020; Both et al., 2014; Thewes et al., 2017a). However, all other conditions showed lower hexyl acetate concentrations until 14 d of shelf life, which is closely related to lower ethylene production (Fig. 3f). After 21 d, CA + 1-MCP, DCA-CF, ELO with 0.4 and 0.4 O_2 + 0.4 HY, and 0.5 O_2 + 0.6 HY maintained lower concentrations compared to the other conditions. At high concentrations, ethyl acetate is related to off-flavors (Echeverría et al., 2008; Thewes et al., 2018; Wright et al., 2015). This anaerobic compound is induced by oxygen shortage, in which the glycolytic pathway increases in order to produce low quantities of ATP for cell maintenance, resulting in anaerobic metabolism compounds, such as ethyl acetate. Fruit stored under DCA-RQ 1.3, CA + 1-MCP, and ELO with 0.4 O_2 showed higher concentrations than the samples under the other storage conditions at 7d of shelf life (Fig 6c).

Higher ethyl acetate under DCA-RQ 1.3 can be attributed to lower pO_2 levels employed (0.16 on average). In fact, Thewes et al. (2017a) reported elevated ethyl acetate concentrations under DCA-RQ 1.5 (pO_2 0.21 kPa on average). Fruit under ELO with 0.4 O_2 showed higher ethanol levels, which can explain the higher ethyl acetate concentration. At 14 d, higher ethyl acetate concentrations were observed in fruit stored under CA, ELO 0.4 O_2 + 0.6 HY, and ELO 0.5 O_2 + 0.4 HY. Nonetheless, all ELO-stored fruit (with or without hysteresis) showed high

ethyl acetate concentrations compared to CA, CA + 1-MCP, and DCA-CF at 21 d of shelf life. Anaerobic compounds, including ethyl acetate which in this study was far below the odor threshold (13,500 µg kg^{-1}), when at high concentrations, can trigger physiological disorders (Saquet, Streif, and Bangerth, 2000), although no differences were found among the conditions for physiological disorders in the present study.

5.1.3.5.2 Main alcohols

Alcohols also contribute to the apple aroma, besides being important precursors of esters. Total alcohol concentrations at 7 d of shelf life were higher in fruit under ELO 0.4 O_2 without hysteresis compared to other conditions (Fig. 7a); this is related to the higher total aldehydes detected (Fig. 8a). At 14 and 21 d, the highest total alcohol content was in fruit stored under 0.4 O_2 + 0.6 HY. Nevertheless, the lowest total alcohol concentration was observed in CA, CA + 1-MCP, and DCA-CF fruits. The most abundant alcohol detected in the juice of 'Maxi Gala' apples after the storage at 7, 14, and 21 d of shelf life was 1-hexanol (Figure 7k). 1-Hexanol was in higher concentrations in apple stored under ELO 0.4 O_2 at 7 d of shelf life, and this may be related to a higher concentration of the precursor hexanal (Fig. 8f).

In 'Royal Gala apples, 1-hexanol concentrations decreased with pO_2 reductions (1.0 to 0.5 kPa) (Both et al., 2014). At 14 d of shelf life, all fruit stored under ELO conditions and DCA-RQ 1.3 showed higher 1-hexanol concentrations than those under CA, CA + 1-MCP, and DCA-CF. Once again, higher hexanal concentrations were found with the exception of DCA-RQ 1.3. Fruit storage under ELO 0.4 O_2 + 0.6 HY had high 1-hexanol concentrations at 21d of shelf life. On the other hand, the lowest concentration was found in fruits from CA, CA + 1-MCP, and DCA-CF.

1-Butanol was high in CA and 0.4 O_2 at 7 d of shelf life (Fig. 7d), and the highest 1-butanol content in 0.4 O_2 can be related to high butanal concentrations (Fig. 8c). At 14 d, fruit stored under ELO conditions showed intermediate 1-butanol concentrations, although these levels were high at 21 d and only differed from CA + 1-MCP, DCA-CF, and DCA-RQ 1.3. The high concentration at 21 d in fruit stored under ULO conditions can be attributed to high butanal concentrations. 2-Methyl-1-butanol showed an increment in concentration from 7 to 21 d in all ELO conditions (Fig. 7g), while ethanol concentrations reduced from 7 to 21 d of shelf life (Fig. 7 b). Ethanol concentrations were higher in fruit stored under 0.4 O_2 at 7 d of shelf life, which is related to the high acetaldehyde concentration (Fig. 8b), the precursor of ethanol.

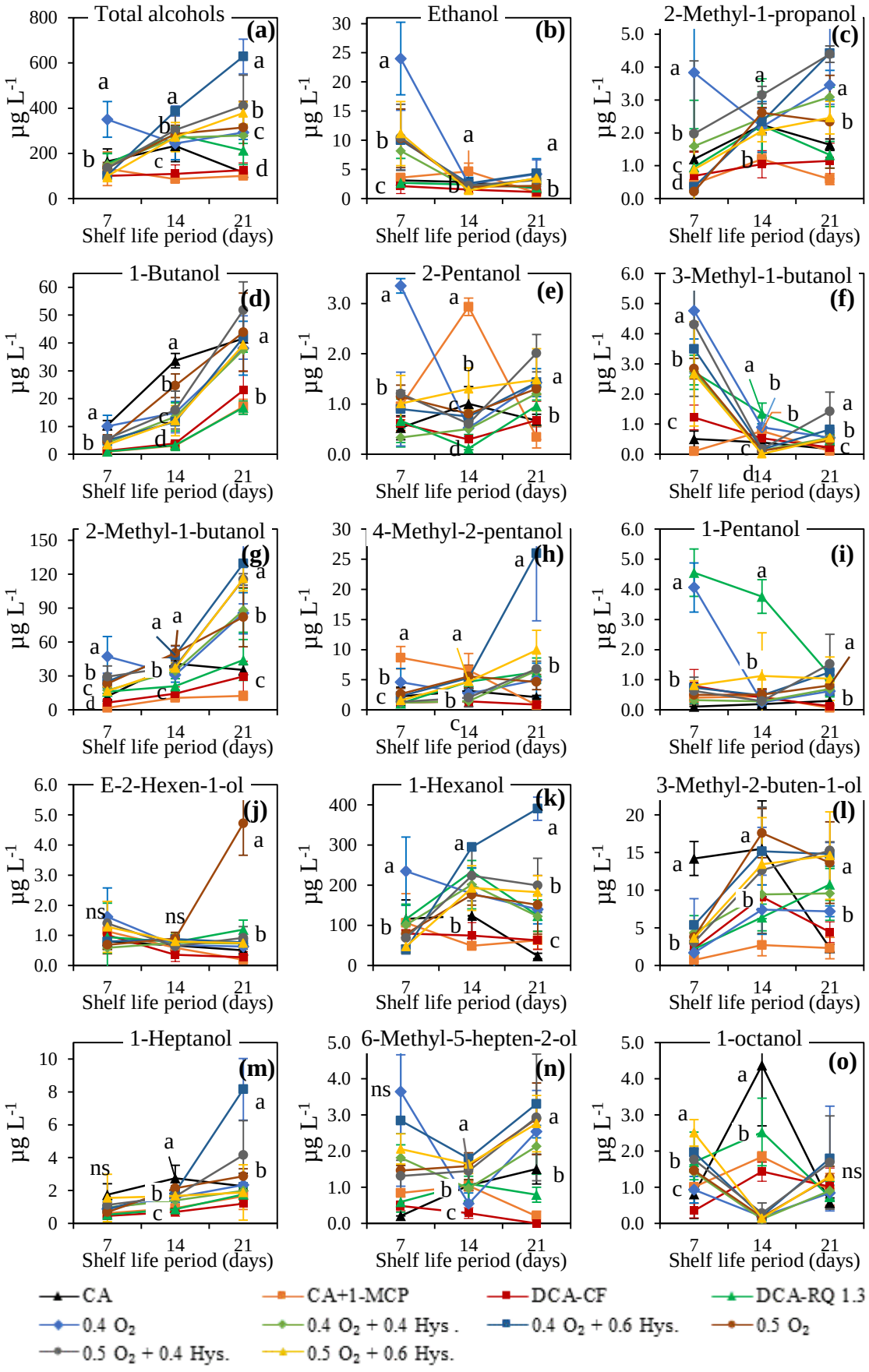


Figure 7. Alcohols concentration (µg L^{-1}) of 'Maxi Gala' apples after 9 months of storage under CA, CA + 1-MCP, DCA-CF and DCA-RQ 1.3, Extreme low oxygen with 0.5 and 0.4

kPa static and with hysteresis (HY.) 0.6 and 0.4 plus 7, 14 and 21 d of shelf life at 20 °C. Means followed by the same letter at the same shelf life day do not differ by Scott Knott test at 5% of error probability. Error bars show the standard deviation. ns: not significant.

5.1.3.5.3 Main aldehydes

Total aldehyde content at 7 d of shelf life was higher in fruit stored under ELO 0.4 O_2 (Fig. 8a). At 14 d, 0.5 and 0.4 O_2 + 0.6 HY showed the highest concentration, and samples stored under 0.4 O_2 + 0.4 and 0.6 HY had the highest total aldehyde concentrations at 21 d of shelf life, and the lowest content was observed in fruit stored under CA + 1-MCP and DCA-CF. The most abundant aldehydes were E-2-hexenal and hexanal, although were detected in lower concentrations also other aldehydes such as butanal and acetaldehyde, which are precursors of 1-butanol and ethanol, respectively. In general, ULO conditions presented higher concentrations of these aldehydes at 14 and 21 d compared to fruit stored under other conditions.

5.1.3.5.4 Principal component analysis (PCA)

The PCA was performed to obtain an overview of the results. Four PCAs were performed, one for the 2017 experiment and three for the 2018 experiment (one for each shelf-life period, 7, 14, and 21 d). For 2017 season, the principal component 1 (PCI) and two (PC II) explained 86.22 % of the total variable variance, being that PC I explained 76.01% of the variance. The PC I separated CA and DCA-CF conditions from the other conditions (Fig. 9a). Fruit storage under CA is associated with high ACC oxidase activity, IEC, ethylene production, mealiness, and flesh breakdown (Fig. 9b), while CA + 1-MCP, DCA-RQ 1.3, and ELO conditions are associated with higher flesh firmness and healthy fruit (Fig. 9b).

The PCA of apples from season 2018, stored for 9 months plus 7 d of shelf life at 20 °C (Fig. 10), discriminated on PC I (45.53%) the storage condition CA, CA +1-MCP, DCA-CF, and DCA-RQ 1.3 from the ELO conditions (Fig. 10a). Fruit stored under CA are associated with ACC oxidase activity, IEC, ethylene production, respiration rate, hexyl acetate, and 2-methylbutyl acetate, while CA + 1-MCP, DCA-CF, and DCA-RQ 1.3 are correlated to high flesh firmness (Fig. 10b). Nevertheless, ELO conditions are associated with butyl acetate and the more significant amounts of alcohols and aldehydes. The PC II (19.06 %) separated CA

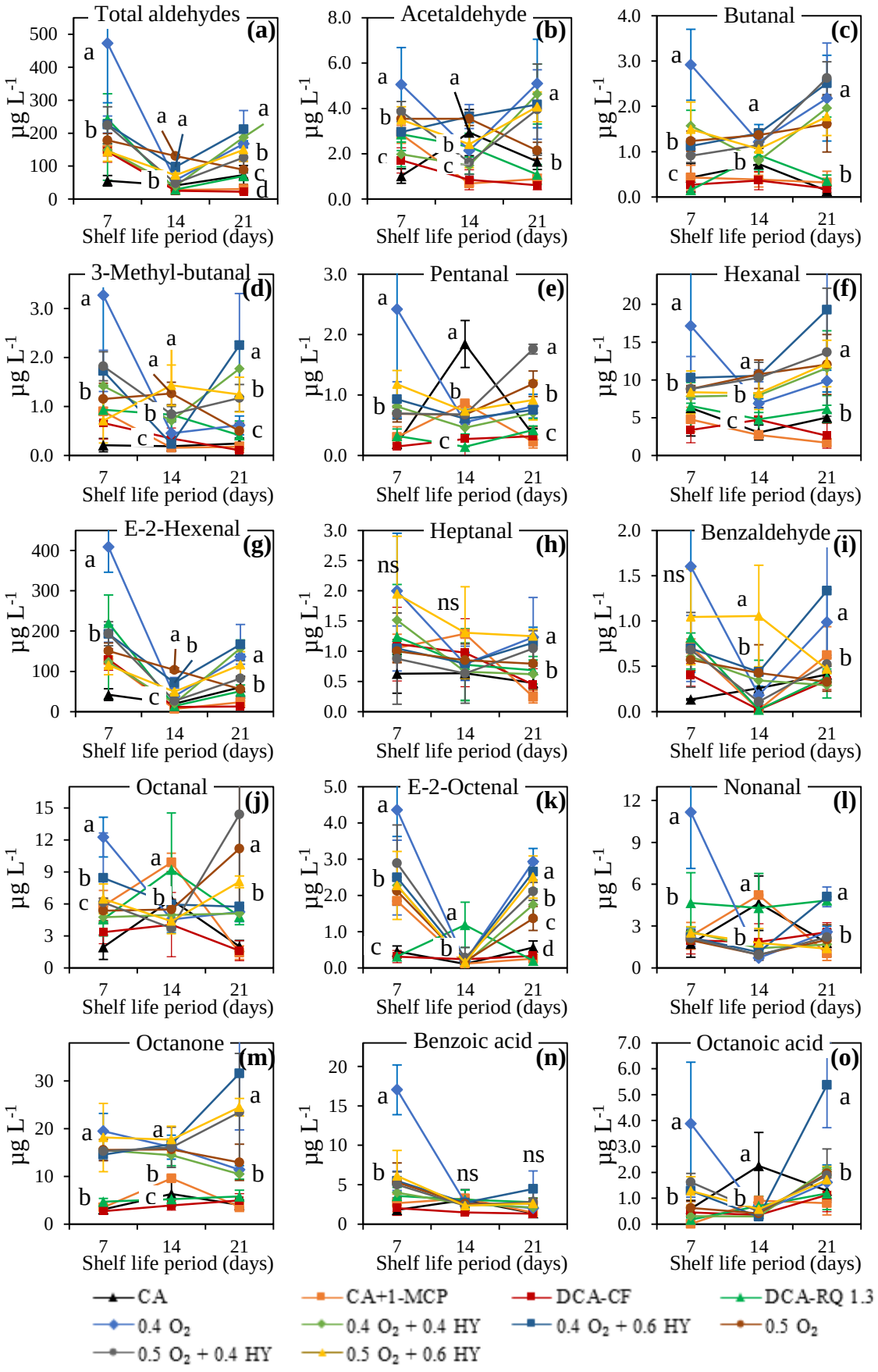


Figure 8. Aldehydes, ketone and acid concentration (µg L^{-1}) of 'Maxi Gala' apples after 9

months of storage under CA, CA + 1-MCP, DCA-CF and DCA-RQ 1.3, Extreme low oxygen with 0.5 and 0.4 kPa static and with hysteresis (HY) 0.6 and 0.4 plus 7, 14 and 21 d of shelf life at 20 °C. Means followed by the same letter do not differ by Scott Knott test at 5% of error probability. Error bars show the standard deviation. ns: not significant.

and ELO 0.4 O_2 from the DCA, CA + 1-MCP, and other ELO conditions, and fruit stored under 0.4 O_2 are associated with 2-methyl-1-butanol, 1-hexanol, ethanol, butanal, E-2-hexenal, hexanal, and acetaldehydes (Fig. 10b).

At 14 d of shelf life, the PC I and PC II explained 69.15% of the total variable variance (Fig. 11), and the PC I (46.72%) separated fruit under CA from those stored under CA + 1-MCP, DCA-CF, and DCA-RQ 1.3 (Fig. 11a). Fruit stored under CA are closely associated with ACC oxidase activity, IEC, ethylene production, mealiness, flesh breakdown, 1-butanol, and the main esters, including butyl acetate, hexyl acetate, and 2-methylbutyl acetate (Fig. 11b). At 14 days of shelf life, flesh firmness and healthy fruit are related with fruit stored in CA + 1-MCP, DCA-CF and DCA-RQ 1.3. The PC II (22.43%) separated the fruit stored under ELO from the other conditions, and ELO conditions are correlated to healthy fruit, 1-hexanol, hexanal, butanal, and acetaldehyde.

At 21 d of shelf life, the PC I and PC II explained 75.98% of the total variable variance (Fig. 12). The PC I (51.34%) separated fruit stored under CA, CA + 1-MCP, DCA-CF, and DCA-RQ 1.3 from the samples stored under ELO conditions (Fig. 12a). Ethylene production, IEC, and a significant amount of VOCs were related to apples stored under ELO conditions (Fig. 12b). Moreover, fruit stored under CA were correlated to mealiness, respiration rate at 20 d and hexyl acetate, and DCA and CA + 1-MCP with flesh firmness. The PC II was important for separating samples stored under CA, ELO 0.5 O_2 with or without hysteresis, and 0.4 O_2 from those stored under DCA-RQ 1.3 and ELO 0.4 O_2 + 0.4 and 0.6 HY. Finally, healthy fruit were correlated with DCA-RQ 1.3 and ELO 0.4 O_2 + 0.4 and 0.6 HY (Fig. 5b).

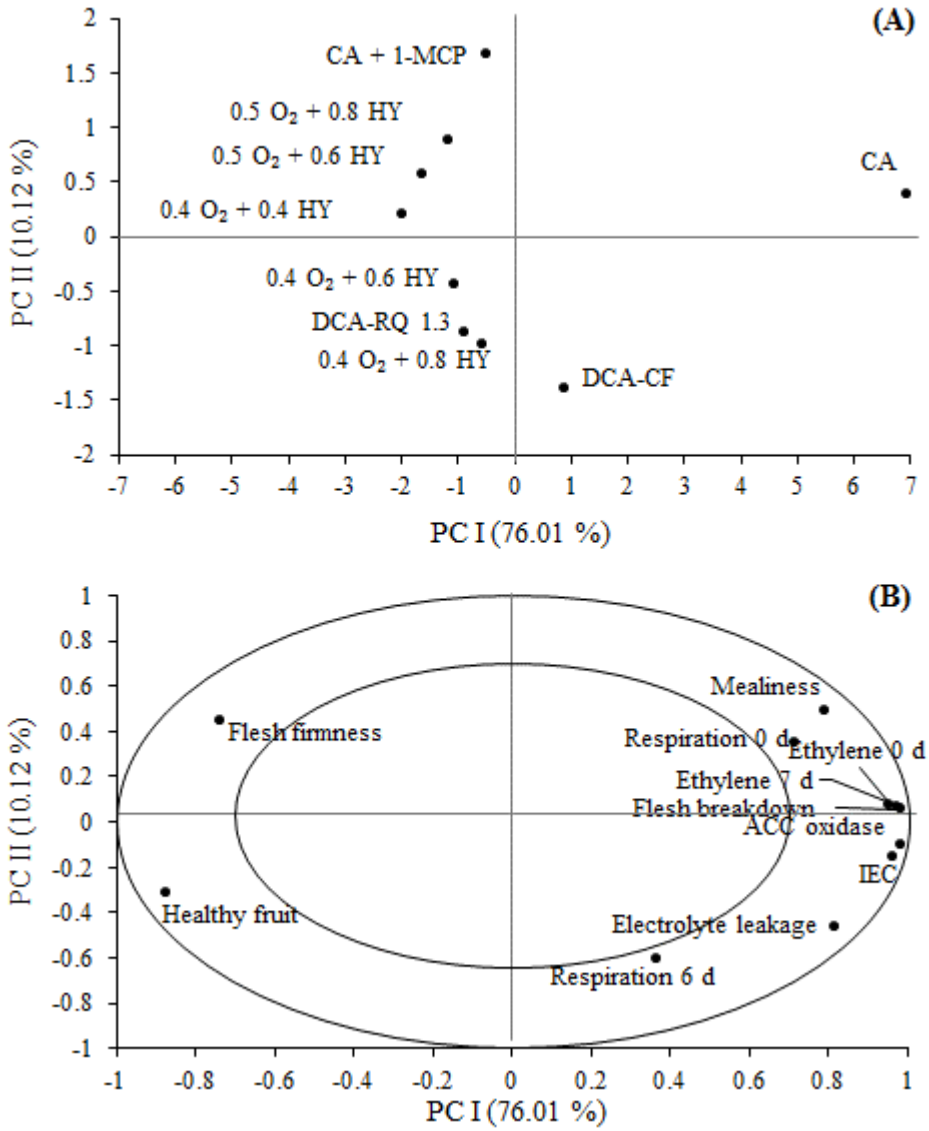


Figure 9. Principal component analysis (PCA) in 'Maxi Gala' apples after 9 months of storage under CA, CA + 1-MCP, DCA-CF, DCA-RQ 1.3, Extreme low oxygen with 0.4 O_2 with hysteresis (Hys.) 0.4, 0.6 and 0.8 and 0.5 O_2 kPa with 0.6 and 0.8 Hys. plus 7 d of shelf life at 20 °C. IEC: internal ethylene concentration. PCA from experiment 2017.

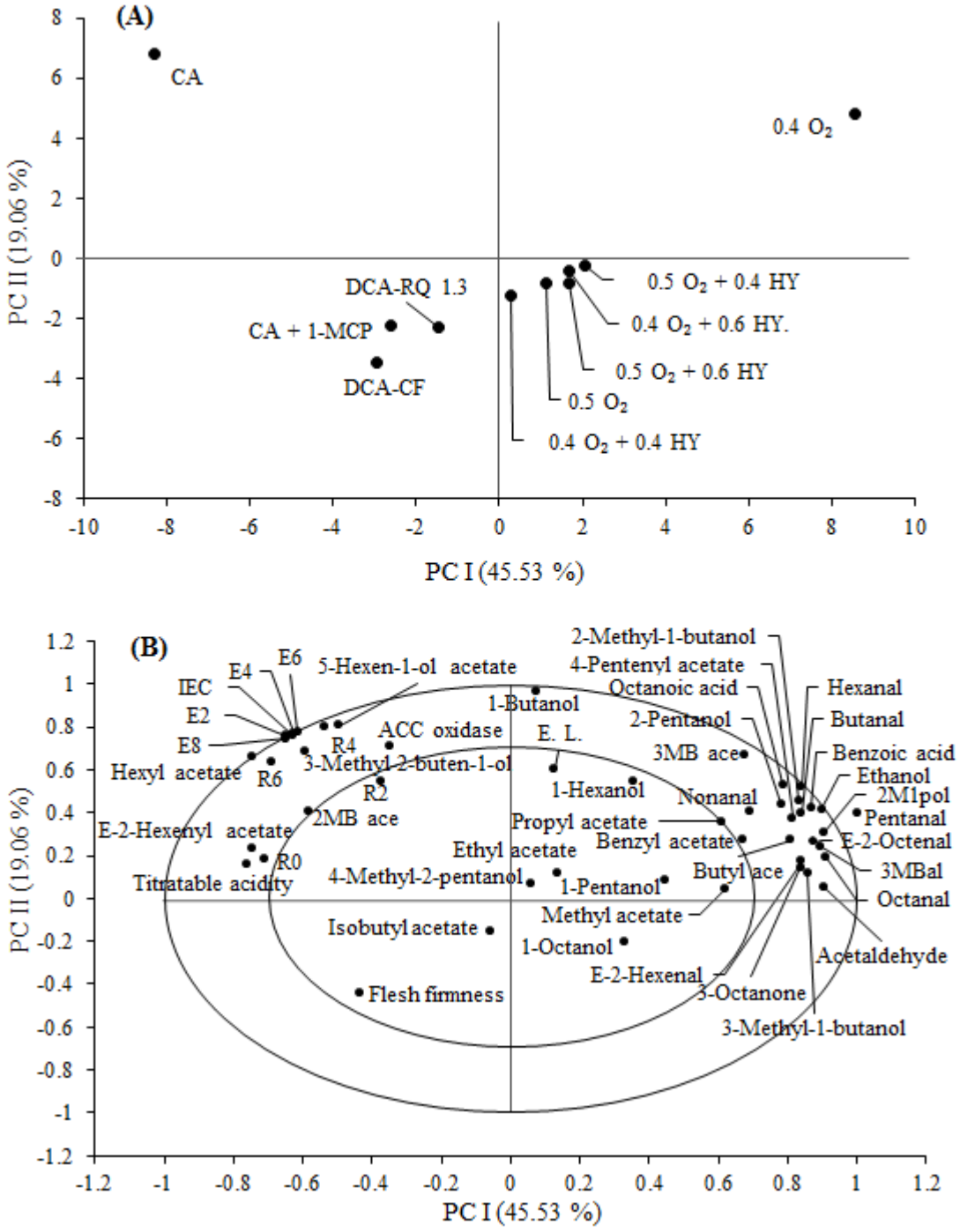


Figure 10. Principal component analysis (PCA) in 'Maxi Gala' apples after 9 months of storage under CA, CA + 1-MCP, DCA-CF, DCA-RQ 1.3, Extreme low oxygen with 0.5 and 0.4 kPa static and with hysteresis (HY) 0.6 and 0.4 plus 7 d of shelf life at 20 °C. 2-MB ace: 2-Methylbutyl acetate, 3-MB ace: 3-Methylbutyl acetate, 2M1propanol: 2-methyl-1-propanol, Butyl ace: Butyl acetate, 3MBal: 3-Methylbutanal, R0-R2-R4-R6: Respiration, 0, 2, 4 and 6 days, E0, E2, E4, E6: Ethylene 0, 2, 4 and 6 days. E. L.: Electrolyte leakage. PCA from experiment 2018.

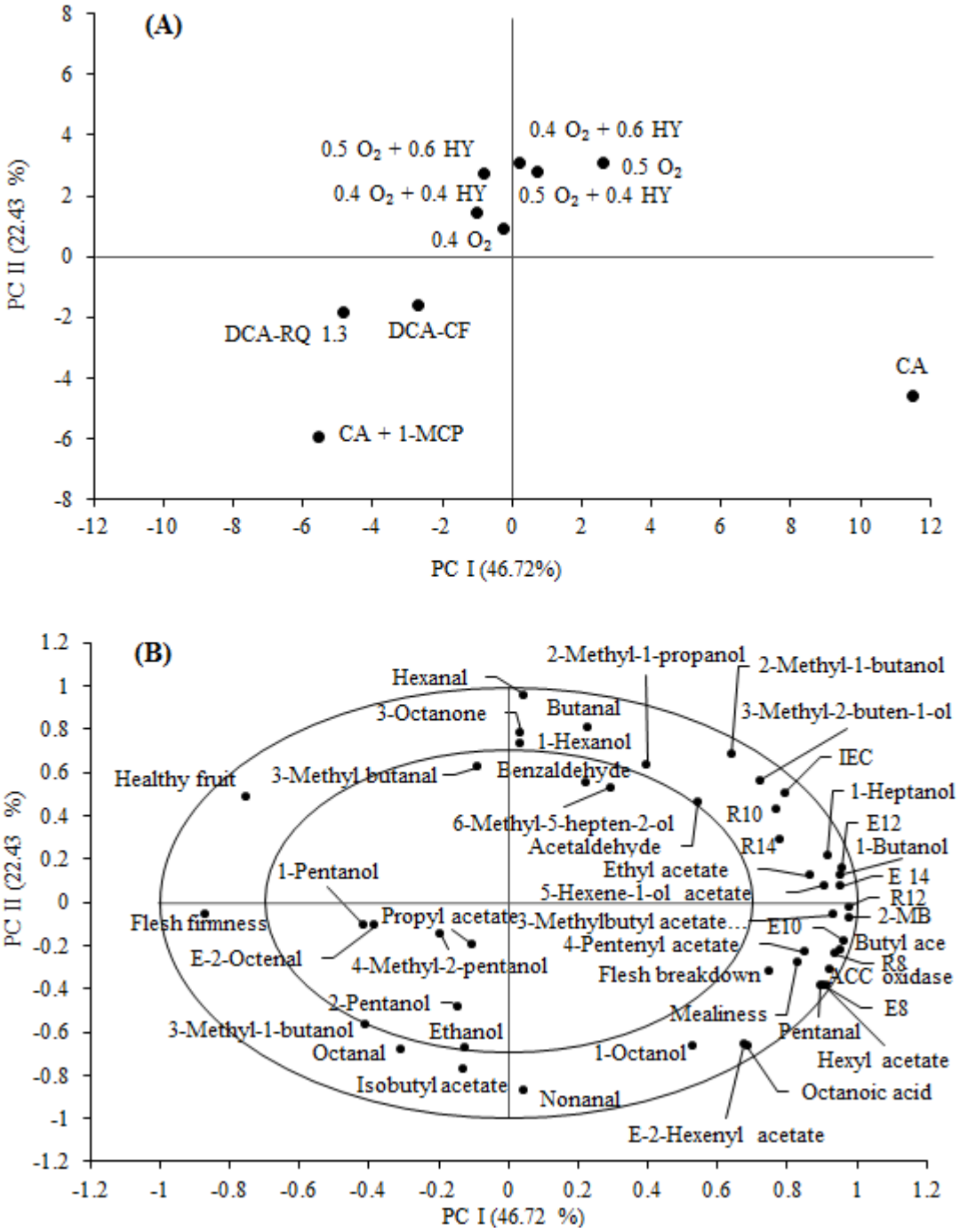


Figure 11. Principal component analysis (PCA) in 'Maxi Gala' apples after 9 months of storage under CA, CA + 1-MCP, DCA-CF, DCA-RQ 1.3, Extreme low oxygen with 0.5 and 0.4 kPa static and with hysteresis (Hys.) 0.6 and 0.4 plus 14 d of shelf life at 20 °C. 2-MB ace: 2-Methylbutyl acetate, Butyl ace: Butyl acetate, R8-R10-R12-R14: Respiration, 8, 10, 12 and 14 days, E8, E10, E12, E14: Ethylene 8, 10, 12 and 14 days.

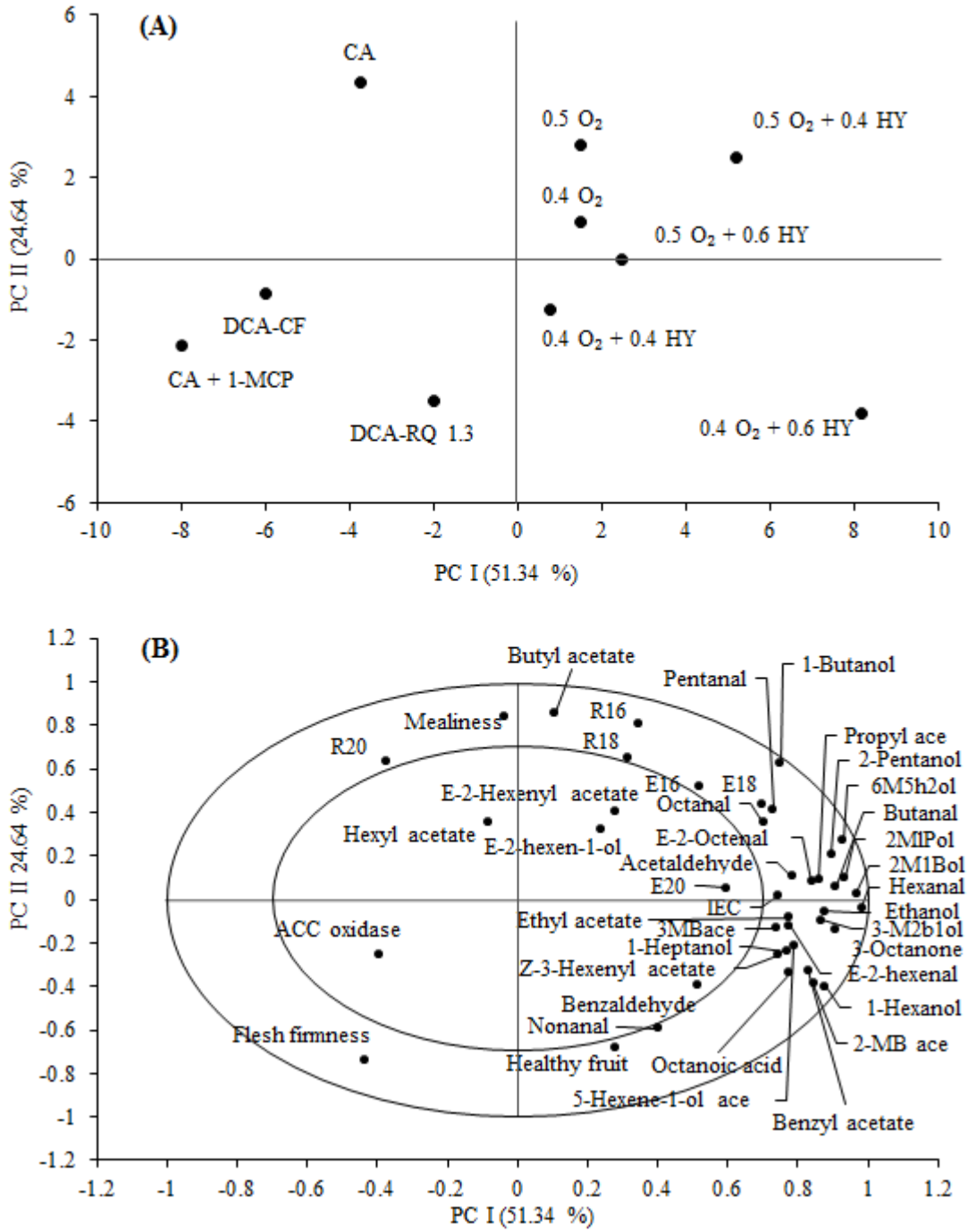


Figure 12. Principal component analysis (PCA) in 'Maxi Gala' apples after 9 months of storage under CA, CA + 1-MCP, DCA-CF, DCA-RQ 1.3, Extreme low oxygen with 0.5 and 0.4 kPa static and with hysteresis (HY.) 0.6 and 0.4 plus 21 d of shelf life at 20 °C. 2-MB ace: 2-Methylbutyl acetate, Z-3-Hyl ace: Z-3-hexenyl acetate, 3M2b1ol: 3-methyl-2-buten-1-ol, 6M5h2-ol: 6-Methyl-5-hepten-2-ol, 2M1Pol: 2-Methyl-1-propanol, R16-R18: Respiration 16 and 18 days, E16, E18, E20: Ethylene 16, 18 and 20 days.

5.1.4 Conclusions

Storage of ‘Maxi Gala’ apple under Extreme low oxygen with or without hysteresis maintained fruit quality similar to CA + 1-MCP, DCA-CF, and DCA-RQ 1.3 and better than CA after 9 months of storage plus 7 d of shelf life at 20 °C.

The ‘Maxi Gala’ apple stored under ELO, regardless of hysteresis, for 9 months plus 14 d of shelf life maintained high amounts of healthy fruit, low physiological disorder incidence and ethylene production, although flesh firmness decreased compared to DCA-RQ 1.3 and CA + 1-MCP.

‘Maxi Gala’ apples stored for 9 months under ELO at 0.4 O_2 + 0.4 and 0.6 HY led to a higher amount of healthy fruit than DCA-CF, CA+1-MCP, and similar to DCA-RQ 1.3 in extended shelf life (21 d), albeit fruit had lower flesh firmness compared to DCA-RQ 1.3 and CA + 1-MCP.

Irrespective of the condition, ELO suppressed 2-methylbutyl acetate and hexyl acetate ester at 7 and 14 d of shelf life, and butyl acetate was high under ELO conditions at 7 d but reduced at 14 d. Fruit stored under CA had high 2-methylbutyl acetate and hexyl acetate concentrations after 7 and 14 d of shelf life, which reduced until 21 d. In ELO 2-methylbutyl acetate increased at 14 to 21 d. Butyl acetate increased from 7 until 21 d of shelf life in apple stored under CA. Lastly, the CA + 1-MCP, and DCA-CF conditions suppressed the most important esters (2-methylbutyl acetate, butyl acetate, and hexyl acetate) in ‘Maxi Gala’ apple during the entire shelf life.

5.1.5 References

Anese, R. de O., Brackmann, A., Wendt, L.M., Thewes, F.R., Schultz, E.E., Ludwig, V., Berghetti, M.R.B., 2018. Interaction of 1-methylcyclopropene, temperature and dynamic controlled atmosphere by respiratory quotient on ‘Galaxy’ apples storage. Food Packag. Shelf Life. 20, 100-246. https://doi.org/10.1016/j.fpsl.2018.07.004.

Anese, R., de, O., Thewes, F.R., Brackmann, A., Schultz, E.E., Wagner, R., Klein, B., Berghetti, M.R.P., Wendt, L.M., 2020. Growth regulators on quality traits and volatile organic compounds profile of ‘Royal Gala’ apple at harvest and after dynamic controlled atmosphere storage. Postharvest Biol. Technol. 164, 111-158. https://doi.org/10.1016/j.postharvbio.2020.111158.

Asoda, T., Terai, H., Kato, M., Suzuki, Y., 2009. Effects of postharvest ethanol vapor treatment on ethylene responsiveness in broccoli. Postharvest Biol. Technol. 52, 216 – 220. https://doi.org/10.1016/j.postharvbio.2008.09.015.

Bessemans, N., Verboven, P., Verlinden, B.E., Nicolaï, B.M., 2016. A novel type of dynamic controlled atmosphere storage based on the respiratory quotient (RQ-DCA). Postharvest Biol. Technol. 115, 91-102. https://doi.org/10.1016/j.postharvbio.2015.12.019.

Both, V., Brackmann, A., Thewes, F.R., Ferreira, D., de, F., Wagner, R., 2014. Effect of storage under extremely low oxygen on the volatile composition of 'Royal Gala' apples. Food Chem. 156, 50-57. https://doi.org/10.1016/j.foodchem.2014.01.094.

Both, V., Brackmann, A., Thewes, F.R., Weber, A., Schultz, E.E., Ludwig, V., 2018. The influence of temperature and 1-MCP on quality attributes of 'Galaxy' apples stored in controlled atmosphere and dynamic controlled atmosphere. Food Packag. Shelf Life, 16, 168‑177. https://doi.org/10.1016/j.fpsl.2018.03.010.

Both, V., Thewes, F.R., Brackmann, A., de Oliveira Anese, R., de Freitas Ferreira, D., Wagner, R., 2017. Effects of dynamic controlled atmosphere by respiratory quotient on some quality parameters and volatile profile of 'Royal Gala' apple after long-term storage. Food Chem. 215, 483-492. https://doi.org/10.1016/j.foodchem.2016.08.009.

Brackmann, A. Control apparatus for controlled atmosphere cells for storing perishable items. ***U.S. Patent***, n.US2015/0257401A1. 2015.

Brackmann, A., Weber, A., Pinto, J.A.V., Neuwald, D.A., Steffens, C.A., 2008. Manutenção da qualidade pós-colheita de maçãs 'Royal Gala' e 'Galaxy' sob armazenamento em atmosfera controlada. Cienc. Rural 38, 2478-2484. https://doi.org/10.1590/S0103-84782008000900010.

Bufler, G., 1986. Ethylene-promoted conversion of 1-aminocyclopropane-1-carboxylic to ethylene in peel of apple at various stages of fruit development. Plant Physiol. 80, 539-543. https://doi.org/10.1104/pp.80.2.539.

Dos Santos, I.D., Pizzutti, I.R., Dias, J.V., Fontana, M.E.Z., Brackmann, A., Anese, R.O., Thewes, F.R., Marques, L.N., Cardoso, C.D., 2018. Patulin accumulation in apples under dynamic controlled atmosphere storage. Food Chem. 255, 275 ‑ 281. https://doi.org/10.1016/j.foodchem.2018.02.022.

Echeverría, G., Graell, J., Lara, I., López, M.L., 2008. Physicochemical measurements in 'Mondial Gala®' apples stored at different atmospheres: influence on consumer acceptability. Postharvest Biol. Technol. 50, 135 ‑ 144. https://doi.org/10.1016/j.postharvbio.2008.05.002.

Gabioud Rebeaud, S., Gasser, F., 2015. Fruit quality as affected by 1-MCP treatment and DCA storage - a comparison of the two methods. Europ. J. Hort. Sci. 80, 18–24. https://doi.org/10.17660/eJHS.2015/80.1.3.

Gago, C.M.L., Guerreiro, A.C., Miguel, G., Panagopoulos, T., Sánchez, C., Antunes, M. D. C. 2015. Effect of harvest date and 1-MCP (SmartFresh^{TM}) treatment on 'Golden Delicious' apple cold storage physiological disorders. Postharvest Biol. Technol. 110, 77-85. https://doi.org/10.1016/j.postharvbio.2015.07.018.

Gasser, F., Eppler, T., Naunheim, W., Gabioud, S., Hoehn, E., 2008. Control of the critical oxygen level during dynamic CA storage of apples by monitoring respiration as well as chlorophyll fluorescence. Acta Hortic. 69-76. https://doi.org/10.17660/ActaHortic.2008.796.6.

Gasser, F., Eppler, T., Naunheim, W., Gabioud, S., Nising, A.B., 2010. Dynamic CA storage of apples: monitoring of the critical oxygen concentration and adjustment of optimum conditions during oxygen reduction. Acta Hortic. 876, 39-46. https://doi.org/10.17660/actahortic.2010.876.3.

Jin, Y.Z., Lv, D.Q., Liu, W.W., Qi, H.Y., Bai, X.H., 2013. Ethanol vapor treatment maintains postharvest storage quality and inhibits ethylene biosynthesis during storage of oriental sweet melons. Postharvest Biol. Technol. 86,372-380. https://doi.org/10.1016/j.postharvbio.2013.07.019.

Harb, J., Lara, I., Saleh, O., Streif, J., Khraiwesh, B., 2011. Treatments that suppress ethylene production or ethylene action modify ADH and AAT gene expression and aroma-related enzyme activities in 'Delbard Estivale' apple: consequences for the aroma profiles of fruits. J. Hort. Sci. Biotech. 86, 182-188. https://doi.org/10.1080/14620316.2011.11512745.

Harker, F.R., Kupferman, E.M., Marin, A.B., Gunson, F.A., Triggs, C.M., 2008. Eating quality standards for apples based on consumer preferences. Postharvest Biol. Technol. 50, 70-78. https://doi.org/10.1016/j.postharvbio.2008.03.020.

Lafer, G. (2008). Storability and fruit quality of 'Braeburn' apples as affected by harvest date, 1-MCP treatment and different storage conditions. Acta Hortic., 796, 179-184.

Lumpkin, C., Fellman, J.K., Rudell, D.R., Mattheis, J., 2014. 'Fuji' apple (Malus domestica Borkh.) volatile production during high pCO_2 controlled atmosphere storage. Postharvest Biol. Technol. 100, 234-243. https://doi.org/10.1016/j.postharvbio.2014.10.008.

Mannapperuma, J.D., Singh, R.P., Montero, M.E., 1991. Simultaneous gas diffusion and chemical reaction in foods stored in modified atmospheres. J. Food Eng. 14, 167-183.

Mazzurana, E.R., Argenta, L.C., Amarante, C.V.T., Steffens, C. A. 2016. Potenciais benefícios do aumento da temperatura de armazenagem em atmosfera controlada de maçãs 'Gala' tratadas com 1-MCP. Rev. Bras. Frutic. 38(1), 043-052. http://dx.doi.org/10.1590/0100-2945-237/14.

Mehinagic, E., Royer, G., Symoneaux, R., Lourjon, F., Prost, C., 2006. Characterization of odor-active volatiles in apples: influence of cultivars and maturity stage. J. Agric. Food Chem. 54, 2678‑2687. https://doi.org/10.1021/jf052288n.

Nishiyama, K., Guis, M., Rose, J.K.C., Kubo, Y., Bennett, K.A.B., Lu, W., Kato, K., Koichiro, U., Ryohei, N., Akitsugu, I., Mondher, B., Alain, L., Jean-Claude, P.,Bennett, A.B., 2007. Ethylene regulation of fruit softening and cell wall disassembly in charentais melon. J. Exp. Bot. 58, 1281-1290. https://doi.org/10.1093/jxb/erl283.

Payasi, A., Mishra, N.N., Chaves, A.L.S., Singh, R., 2009. Biochemistry of fruit softening: an overview. Physiol. Mol. Biol. Plants 15, 103-113. https://doi.org/10.1007/s12298-009-0012-z.

Prange, R.K., DeLong, J.M., Harrison, P.A., Leyte, J.C., McLean, S.D., 2003. Oxygen concentration affects chlorophyll fluorescence in chlorophyll-containing fruit and

vegetable. J. Am. Soc. Hortic. Sci. 128, 603-607. https://doi.org/10.21273/JASHS.128.4.0603.

Prange, R.K., Delong, J.M., Harrison, P., Mclean, S., Scrutton, J., Cullen, J., 2007. Method and apparatus for monitoring a condition in chlorophyll containing matter. U.S. Patent, n.WO/2002/006795.

Pre-Aymard, C., Weksler, A., Lurie, S. 2003. Responses of 'Anna', a rapidly ripening summer apple, to 1-methylcyclopropene. Postharvest Biol. Technol. 27, 163-170. https://doi.org/10.1016/S0925-5214(02)00069-8.

Sabban-Amin, R., Feygenberg, O., Belausov, E., Pesis, E. 2011. Low oxygen and 1-MCP pretreatment delay superficial scald development by reducing reactive oxygen species (ROS) accumulation in stored 'Granny Smith' apples. Postharvest Biol. Tech. 62, 295-304. https://doi.org/10.1016/j.postharvbio.2011.06.016.

Salas, N. A., Molina-Corral, F. J., González-Aguilar, G. A., Otero, A., Sepulveda, D. R., Olivas, G. I. 2011. Volatile production by 'Golden Delicious' apples is affected by preharvest application of aminoethoxyvinylglycine. Sci. Hortic. 130, 436 - 444. https://doi.org/10.1016/j.scienta.2011.07.017.

Saquet, A.A., Streif, J., 2008. Fermentative metabolism in 'Jonagold' apples under controlled atmosphere storage. Eur. J. Hortic. Sci. 73, 4.

Saquet, A.A., Streif, J., Bangerth, F., 2000. Changes in ATP, ADP and pyridine nucleotide levels related to the incidence of physiological disorders in 'Conference' pears and 'Jonagold' apples during controlled atmosphere storage. J. Hort. Sci. Biotechnol. 75, 243-249. https://doi.org/10.1080/14620316.2000.11511231.

Salazar, S., Aideé, N., Orozco, O., Isela, G., 2016. El aroma de la manzana. Interciencia 36 365-271.

Schiller, D., Contreras, C., Vogt, J., Dunemann, F., Defilippi, B. G., Beaudry, R., et al. (2015). A dual positional specific lipoxygenase functions in the generation of flavor compounds during climacteric ripening of apple. Hortic. Research, 2, 15003.

Sisler, E.C., Serek, M., 1997. Inhibitors of ethylene responses in plants at the receptor level: recent developments. Physiol. Plant. 100, 577-582. https://doi.org/10.1111/j.1399-3054.1997.tb03063.x.

Stanger, M.C., Steffens, C.A., Soethe, C., Moreira, M.A., Amarante, C.V.T., Both, V., Brackmann, A. 2018. Phenolic compounds content and antioxidant activity of 'Galaxy' apples stored in dynamic controlled atmosphere and ultralow oxygen conditions. Postharvest Biol. Technol. https://doi.org/10.1016/j.postharvbio.2018.05.014.

Tatsuki, M., Endo, A., Ohkawa, H., 2007. Influence of time from harvest to 1-MCP treatment on apple fruit quality and expression of genes for ethylene biosynthesis enzymes and ethylene receptors. Postharvest Biol. Technol. 43, 28-35. https://doi.org/10.1016/j.postharvbio.2006.08.010.

Thewes, F.R., Balkees, B.M., Büchele, F., Wünsche, J.N., Neuwald, D.A, Brackmann, A. 2021. Ethanol vapor treatment inhibits apple ripening at room temperature even with the

presence of ethylene. Postharvest Biol. Technol. 173 (2021) 111-415. https://doi.org/10.1016/j.postharvbio.2020.111415.

Thewes, F.R., Brackmann, A., Both, B., Anese, de O.R., Schultz, E.E., Ludwig, V., Wendt, L.M., Berghetti, M.R.P., Thewes, F.R., 2020. Dynamic controlled atmosphere based on carbon dioxide production (DCA-CD): Lower oxygen limit establishment, metabolism and overall quality of apples after long-term storage. Postharvest Biol. Technol. 168, 111-285. https://doi.org/10.1016/j.postharvbio.2020.111285.

Thewes, F.R., Brackmann, A., Anese, R., de, O., Ludwig, V., Schultz, E.E., dos Santos, L.F., Wendt, L.M., 2017a. Effect of dynamic controlled atmosphere monitored by respiratory quotient and 1-methylcyclopropene on the metabolism and quality of 'Galaxy' apple harvested at three maturity stages. Food Chem. 222, 84-93. https://doi.org/10.1016/j.foodchem.2016.12.009.

Thewes, F.R., Both, V., Brackmann, A., Ferreira, D.F., Wagner, R., 2015. 1-Methylcyclopropene effects on volatile profile and quality of 'Royal Gala' apples produced in Southern Brazil and stored in controlled atmosphere. Cienc. Rural. 12, 2259-2266. http://dx.doi.org/10.1590/0103-8478cr20141613.

Thewes, F.R., Both, V., Brackmann, A., Weber, A., Anese, R.O. 2015. Dynamic controlled atmosphere and ultralow oxygen storage on 'Gala' mutants quality maintenance. Food Chemistry, 188, 62-70. http://dx.doi.org/10.1016/j.foodchem.2015.04.128.

Thewes, F.R., Brackmann, A., de Oliveira Anese, R., Ludwig, V., Schultz, E.E., Berghetti, M.R.P., 2018. 1-Methylcyclopropene suppresses anaerobic metabolism in apples stored under dynamic controlled atmosphere monitored by respiratory quotient. Sci. Hortic. 227, 288-295. https://doi.org/10.1016/j.scienta.2017.09.028.

Tran, D.T., Verlinden, B.E., Hertog, M., Nicolaï, B.M., 2015. Monitoring of extremely low oxygen control atmosphere storage of 'Green star' apples using chlorophyll fluorescence. Sci. Hortic. 184, 18-22. https://doi.org/10.1016/j.scienta.2014.11.

Zanella, A., Rossi, O. 2015. Post-harvest retention of apple fruit firmness by 1 methylcyclopropene (1-MCP) treatment or dynamic CA storage with chlorophyll fluorescence (DCA-CF). Eur. J. Hortic. Sci. 80 (1), 11-17.

Zanella, A. 2003. Control of apple superficial scald and ripening – a comparison between 1-methylcyclopropene and diphenylamine postharvest treatments, initial low oxygen stress and ultra low oxygen storage. Postharvest Biol. Technol. 27, 69 – 78. https://doi.org/10.1016/S0925-5214(02)00187-4.

Zanella, A., Cazzanelli, P., Rossi, O., 2008. Dynamic controlled atmosphere (DCA) storage by the means of chlorophyll fluorescence response for firmness retention in apples. Acta Hortic. 796, 77-82. https://doi.org/10.17660/ActaHortic.2008.796.7.

Watkins, C.B., 2006. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. Biotechnol. Adv. 24, 389–409. https://doi.org/10.1016/j.biotechadv.2006.01.005.

Watkins, C.B., Nock, J.F., 2012. Rapid 1-methylcyclopropene (1-MCP) treatment and delayed controlled atmosphere storage of apples. Postharvest Biol. Technol. 69, 24-31. https://doi.org/10.1016/j.postharvbio.2012.02.010.

Weber, A., Brackmann, A., Anese, R. de O., Both, V. Pavanello, E.P., 2013. Atmosfera controlada para o armazenamento da maçã 'Maxi Gala'. Rev. Cienc. Agron. 44, 294-301. https://doi.org/10.1590/S1806-66902013000200011.

Weber, A., Brackmann, A., Both, V., Pavanello, E.P., De O., Anese, R., Thewes, F.R., 2015. Respiratory quotient: innovative method for monitoring 'Royal Gala' apple storage in a dynamic controlled atmosphere. Sci. Agric. 72, 28-33. https://doi.org/10.1590/0103-9016-2013-0429. https://doi.org/10.1590/0103-9016-2013-0429.

Weber, A., Brackmann, A., Both, V., Pavanello, E.P., Anese, R.O., Schorr, M.R.W., 2016. Ethanol reduces ripening of "Royal Gala" apples stored in controlled atmosphere. Ann. Acad. Bras. Cienc. 88, 403‑410. https://doi.org/10.1590/0001-3765201620140181.

Weber, A., Neuwald, D.A., Kittemann, D., Thewes, F.R., Both, V., Brackmann, A., 2020. Influence of respiratory quotient dynamic controlled atmosphere (DCA‑RQ) and ethanol application on softening of Braeburn apples. Food Chem. 303, 125346. https://doi.org/10.1016/j.foodchem.2019.125346.

Wright, A.H., Delong, J.M., Arul, J., Prange, R.K., 2015. The trend toward lower oxygen levels during apple (*Malus x domestica* Borkh) storage. J. Hortic. Sci. Biotechnol. 90, 1-13. https://doi.org/10.1080/14620316.2015.11513146.

Wright, A. H., DeLong, J. M., Gunawardena, A.H.L. A. N., Prange, R K., 2012. Dynamic controlled atmosphere (DCA): Does fluorescence reflect physiology in storage? Postharvest Biol. Technol. 64, 19-30. https://doi.org/10.1016/j.postharvbio.2011.09.015.

Yang, S.F., Hoffman, N.E., 1984. Ethylene biosynthesis and its regulation in higher plants. Annu. Rev. Plant Biol. 35, 155-189. https://doi.org/10.1146/annurev.pp.35.060184.001103.

Yang, X., Song, J., Lina, D., Forney, C., Campbell-Palmer, L., Fillmore, S., Wismer, P., Zhang, Z., 2016. Ethylene and 1-MCP regulate major volatile biosynthetic pathways in apple fruit. Food Chem. 194, 325‑336. https://doi.org/10.1016/j.foodchem.2015.08.018.

6 DISCUSSÃO GERAL

A busca por melhorias constantes nas técnicas de armazenamento de maçãs se faz necessária para melhorar a conservação dos frutos no período da entressafra. Para reduzir perdas é necessário desenvolver novas técnicas de armazenamento e aperfeiçoar as já existentes, para que se consiga uma melhor conservação da qualidade dos frutos durante todo período de armazenamento e também buscar a otimização das técnicas existentes para reduzir custos para os armazenadores.

No primeiro artigo desta tese foi estudado o efeito de diferentes pressões parciais de CO_2 em atmosfera controlada dinâmica, monitorada pelo quociente respiratório (ACD-QR 1,3), sobre a qualidade de maçãs 'Maxi Gala' armazenadas a 2 ºC durante nove meses seguido de sete 7 dias de vida de prateleira a 20 ºC, em dois anos de experimentos. Foi comprovado que o armazenamento de maçãs 'Maxi Gala' em ACD-QR 1,3 com pressões parciais de CO_2 de 1,6 kPa manteve a qualidade dos frutos nos dois anos de experimento similar a ACD-QR 1,3 com 1,2 kPa de CO_2, que é a pressão parcial padrão utilizada comercialmente. Um estudo anterior que foi realizado com o objetivo de verificar a pCO_2 mais adequada para o armazenamento de maçãs 'Gala' e mutantes em ACD-QR, observou que as pCO_2 de 1,2 e 1,6 kPa resultaram em frutos com melhor qualidade, porém naquele estudo foram armazenadas maçãs 'Galaxy' em ACD-QR 1,5 e em temperatura mais baixa (1 °C) (BRACKMANN; WEBER; BOTH, 2015). Naquele estudo os autores verificaram maior incidência de polpa farinácea em ACD-QR com 2,0 kPa de CO_2, ao contrário do que foi observado na presente tese, onde a incidência foi quase nula nos dois experimentos. A adoção de 0,4 kPa de CO_2, resultou em menor firmeza de polpa quando comparado com os frutos mantidas nas outras pCO_2. Este resultado está relacionado com a maior pO_2 (0,23 kPa) em que estes frutos permaneceram durante o armazenamento comparado com as outras condições de ACD-QR, que foram armazenados em pO_2 inferior ($\leq$ 0,16 kPa) e também pela menor pCO_2. Outro resultado importante observado foi a baixa produção de compostos resultantes do metabolismo anaeróbico mesmo com a maior pCO_2 (2,0 kPa) em ACD-QR 1,3. Segundo Argenta et al. (2004), altas pCO_2 estão relacionados com acúmulo de compostos do metabolismo anaeróbico (acetato de etila, etanol e acetaldeído) e podem causar a incidência de distúrbios fisiológicos. Vários estudos reportaram que elevados pressões parciais de CO_2 causaram distúrbios fisiológicos como a degenerescência de polpa (DE CASTRO et al., 2008; CORRÊA et al., 2010; KITTEMANN; NEUWALD; STREIF, 2015 LUMPKIN et al., 2014; HERREMANS et al., 2013) e ocorrência de cavernas (DE CASTRO et al., 2008; SAQUET; STREIF; BANGERTH, 2000). Por fim, o armazenamento em ACD-QR

1,3 com 1,2, 1,6 e 2,0 kPa CO_2 mantém maior quantidade de frutos sadios comparado a AC + 1-MCP e maior firmeza de polpa que os frutos da ACD-FC. Esse resultado está relacionado com a adaptação dos frutos as baixas pO_2 (≤ 0,16 kPa) em que eles foram armazenados em ACD-QR, reduzindo ao máximo o metabolismo e assim, conservando maior qualidade.

No segundo artigo foram estudados os efeitos das diferentes pCO_2 (0,4, 1,2, 1,6 e 2,0 kPa) em ACD-QR 1,3 na qualidade geral e perfil volátil, avaliando um longo período de vida de prateleira (7, 14 e 21 dias). Aos sete dias de vida de prateleira pôde se observar que os frutos das condições de armazenamento ACD-QR 1.3 + 0,4 kPa de CO_2, ACD-FC e AC apresentaram menor firmeza de polpa comparado as outras condições, que está associado a maior pO_2 em que esses frutos foram armazenados (0,23, 0,4 e 1,2 kPa). Em ACD-QR 1.3 + 0,4 kPa de CO_2, o baixo CO_2 teve efeito na redução da firmeza de polpa. Um estudo que avaliou ULO com 0,4 kPa de O_2 combinado com 1,0 e 0,0 kPa de CO_2 observou menor firmeza de polpa com 0,4 O_2 + 0,0 kPa de CO_2, mostrando que menores pCO_2 reduzem a qualidade da maçã (THEWES et al., 2021). Em relação aos frutos sadios, que é o principal objetivo dos armazenadores, não houve diferença entre as condições avaliadas, um fator que contribui com esse resultado é o ponto de maturação em que esses frutos foram colhidos e a firmeza de polpa, que nesse caso foi de 89,31 N, é um importante parâmetro. Estudo recente também não observou diferença para frutos sadios, quando estes foram colhidos num estádio inicial de maturação, com firmeza de 85,8 N (THEWES et al., 2017a). Porém, aos 14 dias, houve uma maior perda de qualidade dos frutos armazenados ACD-QR 1.3 + 0,4 kPa de CO_2, ACD-FC e AC, com menor firmeza de polpa e frutos sadios.

A diferença entre as condições de armazenamento foi maior aos 21 dias. As condições ACD-QR 1.3 + 1,2 e 1,6 kPa de CO_2 foram mais adequadas para a conservação da qualidade de maçãs ‘Maxi Gala’, resultando em maior quantidade de frutos sadios, firmeza de polpa e menor ocorrência de podridões. A ACD-QR 1.3 + 2,0 kPa de CO_2 mantém alta firmeza de polpa, porém apresentou baixa porcentagem de frutos sadios, devido à alta incidência de podridões. Um estudo também observou menor quantidade de frutos sadios em ACD-QR 1,5 + 2,0 kPa de CO_2 (BRACKMANN; WEBER; BOTH, 2015). O 1-MCP foi eficaz na manutenção da firmeza de polpa semelhante as condições de ACD-QR 1,3 + 1,2 e 1,6 kPa de CO_2, mas resultou em menor quantidade de frutos sadios, que está relacionado a maior incidência de polpa farinácea e podridões. O efeito do 1-MCP na conservação da firmeza de polpa é em função da inibição da ação do etileno, hormônio que responsável pelo amadurecimento e senescência dos frutos (BLANKENSHIP; DOLE, 2003; SISLER; SEREK, 1997; WATKINS, 2006; YANG et al., 2016). Com relação à podridão, alguns autores observaram maior ocorrência de podridões

com a aplicação de 1-MCP (DOS SANTOS et al., 2018). As condições de ACD-QR 1,3 + 0,4 kPa de CO_2 e ACD-FC não são adequadas para o armazenamento de maçãs 'Maxi 'Gala' durante nove meses mais 21 dias de vida de prateleira a 20 °C, pois apresentaram baixa firmeza de polpa, porcentagem de frutos sadios e alta incidência de podridões.

Com relação à emissão de compostos voláteis, o 1-MCP e a ACD-FC reduziram a emissão destes compostos durante todo o período de vida de prateleira. A aplicação de 1-MCP reduziu a expressão de genes *MdAAT1* and *MdAAT2*, reduzindo assim a atividade da enzima Álcool acil transferase, que atua na conversão de álcoois em ésteres (DEFILIPPI et al., 2005; QI et al., 2020; YANG et al., 2016). Vários autores observaram redução na emissão de voláteis em AC + 1-MCP (THEWES et al., 2015; YANG et al., 2016). Em relação a ACD-FC, a baixa produção de compostos voláteis está relacionada com a menor produção de precursores (DONADEL et al., 2019; THEWES et al., 2017c), o que está de acordo com o resultado encontrado neste artigo da tese, por exemplo, menor produção álcoois precursores de ésteres em ACD-FC. Segundo estudo recente, a baixa produção de voláteis pode estar relacionada com a baixa expressão da enzima lipoxigenase (*MdLOX1*) e da enzima AAT (*MdAAT1*) (THEWES et al., 2020).

A produção de compostos voláteis, entre eles os principais ésteres em maçãs 'Maxi Gala' (acetato de 2-metil butila, acetato de butila e acetato de hexila) são baixos aos 7 e 14 dias (com exceção do acetato de 2-metil butila aos 7 dias) em ACD-QR 1,3 + 1,2, 1,6 e 2,0 kPa CO_2. Porém, aos 21 dias de vida de prateleira ocorreu um aumento na emissão desses ésteres. A baixa produção desses ésteres aos 7 e 14 dias está relacionada com a menor concentração de precursores (1-butanol, 1-hexanol) e a menor atividade da AAT, que é reduzida em baixas pO_2 (BOTH et al., 2014; 2016; BRACKMANN; STREIF; BANGERTH, 1993; LOPEZ et al., 2007; LUMPKIN et al., 2014). Ésteres de cadeia ramificada como o acetato de 2-metil butila, que teve alta concentração aos sete dias são pouco afetados pelas baixas pO_2 (BOTH et al., 2014; BRACKMANN et al., 1993; ECHEVERRÍA et al., 2008). O aumento na concentração dos principais ésteres aos 21 dias de vida de prateleira está relacionado com o aumento na produção de etileno, que favorece a atividade da ATT, na conversão de álcoois em ésteres (DEFILIPPI; KADER; DANDEKAR, 2005; SOULEYRE et al., 2005; YANG et al., 2016). O aumento dos principais ésteres até os 21 dias em maçãs armazenadas em ACD-QR é muito importante para estimular o armazenamento de maçãs nesse método, tendo em vista que em AC ocorre a redução na emissão desses ésteres com o prolongamento da vida de prateleira. O armazenamento em AC sem aplicação de 1-MCP, manteve alta concentração de compostos voláteis, principalmente os ésteres mais importantes, com incremento na emissão até os 14 dias e seguido de um

decréscimo até os 21 dias, com exceção do acetato de butila. A maior produção de ésteres está relacionada com a maior indução da *MdAAT1* em AC pela maior disponibilidade de etileno (THEWES et al., 2020). Com relação aos compostos da fermentação, houve pouca diferença entre as condições avaliadas com baixa produção desses compostos, mesmo em ACD-QR 1.3 com a maior pCO_2 (2,0 kPa).

No terceiro artigo foram estudadas condições de *ELO* (0,4 e 0,5 kPa) com indução de estresses pela variação do oxigênio (0,4, 0,6, 0,8 de histerese), comparando com ACD-QR 1,5, ADC-FC, AC + 1-MCP e AC. Foram conduzidos dois experimentos em anos distintos para avaliar a qualidade geral e perfil volátil após nove meses de armazenamento mais 7, 14 e 21 dias de vida de prateleira. No geral, o armazenamento em condições de *ELO* com ou sem histerese mantiverem a porcentagem de frutos sadios similar as condições AC + 1-MCP, ACD-FC e ACD-QR 1,3, mas com menor firmeza de polpa após 7 dias de vida de prateleira a 20 °C. Alguns trabalhos não encontraram diferença entre *ELO* (0,4 kPa) e ACD-FC em maçãs 'Royal Gala' (THEWES et al., 2015) e maçãs 'Galaxy' armazenadas em *ELO* (0,4 kPa) comparado à ACD-FC, ACD-QR 1,3 e 1,5 (ANESE et al., 2020). Aos 14 dias, a quantidade de frutos sadios nas condições de *ELO* não diferiu da AC + 1-MCP, ACD-FC e ACD-QR 1,3, mas a firmeza de polpa foi menor que AC + 1-MCP e ACD-QR 1,3. Porém, aos 21 dias de vida de prateleira a 20 °C as condições de *ELO* 0,4 O_2 + 0,4 e 0,6 de histerese mantiveram uma maior porcentagem de frutos sadios que a ACD-FC e AC+1-MCP, e similar ACD-RQ 1,3, mas com menor firmeza de polpa que a ACD-RQ 1,3 e AC + 1-MCP. Apesar da menor firmeza de polpa (56,8 e 56,3 N), ela ainda está bem acima do mínimo permitido para comercialização que é de 40 N (IN n° 5/2006, MAPA). Com relação à produção de compostos voláteis, se destaca a baixa produção de acetato de 2-metilbutila e acetato de hexila nas diferentes condições de *ELO* aos sete e 14 dias de vida de prateleira, porém houve uma maior produção de acetato de butila aos 7 dias nas condições de *ELO* comparado a ACD e AC. Maior produção de acetato de butila foi observada em maçãs 'Galaxy' armazenadas em *ELO* (0,4 kPa) comparado a AC, ACD-QR 1,3 e 1,5 (ANESE et al., 2020). Frutos armazenados em AC tiveram alta emissão de acetato de 2-metilbutila e acetato de hexila aos sete e 14, com redução aos 21dias, já o acetato de butila aumenta dos sete até os 21 dias, estando relacionado com o aumento na produção do precursor 1-butanol. AC + 1-MCP, ACD-FC, e ACD-QR 1,3 apresentaram baixa produção dos principais ésteres durante toda a vida de prateleira.

7 CONSIDERAÇÕES FINAIS

Os resultados obtidos nesta tese são importantes para a melhoria no armazenamento de maçãs. Um dos principais resultados é a possibilidade do aumento da pCO_2 no armazenamento de maçãs 'Maxi Gala' em ACD-QR 1,3 que atualmente é de 1,2 kPa para 1,6 kPa. Assim, pode-se otimizar o uso do adsorvedor de carvão ativado, reduzindo o tempo de adsorção e, por fim, reduzir o custo dessa operação e manter uma alta qualidade dos frutos armazenados ACD-QR 1,3 após 21 dias de vida de prateleira. A comprovação da manutenção da qualidade com período de vida de prateleira estendido é um resultado importante do ponto de vista prático, pois garante a oferta de maçãs com alta qualidade em locais mais distantes dos centros de produção durante todo ano com redução nas perdas pós armazenamento, obtendo-se altos porcentuais de frutos sadios, com boa firmeza de polpa. O armazenamento de maçãs em *ELO* com 0,4 kPa O_2 com 0,4 e 0,6 de histerese apresentou um bom resultado na conservação da qualidade dos frutos após 21 dias de vida de prateleira. Essa técnica de armazenamento pode ser uma alternativa para a ACD-FC e a aplicação de 1-MCP, tendo como vantagem o menor custo de adoção, por não necessitar de equipamentos adicionais, e evitar a aplicação de produto químico nos frutos. Além disso, como a produção orgânica de frutas não permite o uso de produtos químicos, o armazenamento em condições de oxigênio extremamente baixo ou em ACD-QR viabiliza este tipo de produção. Deve ser destacado as condições de oxigênio extremamente baixo permitem uma satisfatória produção de compostos voláteis que compõe o aroma, ao contrário dos frutos tratados com 1-MCP. A partir dos resultados obtidos nos artigos desta tese, as melhores condições de armazenamento para conservação da qualidade geral de maçãs após longo período de armazenamento e vida de prateleira estendido (21 dias), seguem a seguinte ordem: ACD-QR > *ELO* ≥ AC +1-MCP = ACD-FC > AC.

8 REFERÊNCIAS BIBLIOGRÁFICAS

ANESE, R.O. et al. Interaction of 1-methylcyclopropene, temperature and dynamic controlled atmosphere by respiratory quotient on 'Galaxy' apples storage. **Food Packaging and Shelf Life**, [s. l.], v. 20, p. 100-246, 2019.

ANESE de R.O. et al. Impact of dynamic controlled atmosphere storage and 1-methylcyclopropene treatment on quality and volatile organic compounds profile of 'Galaxy' apple. **Food Packaging and Shelf Life**, [s. l.], v. 23, p. 100-443, 2020.

ASODA, T. et al. Effects of postharvest ethanol vapor treatment on ethylene responsiveness in broccoli. **Postharvest Biology and Technology**, [s. l.], v. 52, n. 2, p. 216-220, 2009.

AUBERT, C.; MATHIEU-HURTIGER, V.; VAYSSE, P. Effects of dynamic atmosphere on volatile compounds, polyphenolic content, overall fruit quality, and sensory evaluation of "pink lady®" apples. **Acta Horticulturae**, [s. l.], n. 1071, p. 275-280, 2015.

BEAUDRY, R. M. Effect of O_2 and CO_2 partial pressure on selected phenomena affecting fruit and vegetable quality. **Postharvest Biology and Technology**, [s. l.], v.15 p. 293-303, 1999.

BERGHETTI, M. R. P. et al. Extremely low oxygen partial pressures for the storage of 'Royal Gala' apple. **Bragantia,** [s. 1], v. 79, n. 1, p.145-154, 2020.

BESSEMANS, N. et al. A novel type of dynamic controlled atmosphere storage based on the respiratory quotient (RQ-DCA). **Postharvest Biology and Technology**, [s. l.], v. 115, p. 91-102, 2016.

BLANKENSHIP, S. M.; DOLE, J. M. 1-Methylcyclopropene: a review. **Postharvest Biology and Technology**, [s. l.], v. 28, n. 1, p. 1-25, 2003.

BOTH, V. et al. Effect of storage under extremely low oxygen on the volatile composition of 'Royal Gala' apples. **Food Chemistry**, [s. l.], v. 156, p. 50-57, 2014.

BOTH, V. et al. Effect of low oxygen conditioning and ultralow oxygen storage on the volatile profile, ethylene production and respiration rate of 'Royal Gala' apples. **Scientia Horticulturae**, [s. l.], v. 209, p. 156-164, 2016.

BOTH, V. et al. Effects of dynamic controlled atmosphere by respiratory quotient on some quality parameters and volatile profile of 'Royal Gala' apple after long-term storage. **Food Chemistry**, [s. l.], v. 215, p. 483-492, 2017.

BOTH, V. et al. The influence of temperature and 1-MCP on quality attributes of 'Galaxy' apples stored in controlled atmosphere and dynamic controlled atmosphere. **Food Packaging and Shelf Life**, [s. l.], v. 16, p. 168-177, 2018.

BRACKMANN, A. et al. Manutenção da qualidade pós-colheita de maçãs "Royal Gala" e "Galaxy" sob armazenamento em atmosfera controlada. **Ciência Rural**, [s. l.], v. 38, n. 9, p. 2478-2484, 2008.

BRACKMANN, A. et al. Efeito do estresse inicial por baixo O2 combinado com 1-metilciclopropeno na conservação de maçãs 'Royal Gala' armazenadas com ultrabaixo O2. **Semina: Ciências Agrárias**, [s. l.], v. 34, n. 3, p. 1185-1194, 2013.

BRACKMANN, A. Control apparatus for controlled atmosphere cells for storing perishable items. **U.S. Patent**, n.US/2015/0257401, 2015.

BRACKMANN, A.; STREIF, J.; BANGERTH, F. Relationship between a Reduced Aroma Production and Lipid Metabolism of Apples after Long-term Controlled-atmosphere Storage. **Journal of the American Society for Horticultural Science**, [s. l.], v. 118, n. 2, p. 243-247, 1993.

BRACKMANN, A.; WEBER, A.; BOTH, V. CO2 partial pressure for respiratory quotient and harvestwatchtm dynamic controlled atmosphere for "galaxy" apples storage. **Acta Horticulturae**, [s. l.], n. 1079, p. 435-440, 2015.

BRACKMANN, A. et al. Aplicação de 1-metilciclopropeno, estresse inicial com baixo oxigênio
e armazenamento em ultrabaixo oxigênio na qualidade de maçã 'Fuji'. **Ciência Rural**, v.42, n.8, p.1386-1391, 2012.

CONTRERAS, C.; BEAUDRY, R. Lipoxygenase-associated apple volatiles and their relationship with aroma perception during ripening. **Postharvest Biology and Technology**, [s. l.], v. 82, p. 28-38, 2013.

CORRÊA, T. R. et al. Qualidade de maçãs "Fuji" armazenadas em atmosfera controlada e influência do clima na degenerescência da polpa. **Pesquisa Agropecuária Brasileira**, [s. l.], v. 45, n. 6, p. 531-538, 2010.

DE CASTRO, E. et al. Biochemical factors associated with a CO2-induced flesh browning disorder of Pink Lady apples. **Postharvest Biology and Technology**, [s. l.], v. 48, n. 2, p. 182-191, 2008.

DELONG, J.M. et al. A new technology that determines low-oxygen thresholds in controlled-atmosphere-stored apples. **HortTechnology**, [s. l.], v. 14, p. 262-266, 2004.

DEFILIPPI, B. G.; KADER, A. A.; DANDEKAR, A. M. Apple aroma: alcohol acyltransferase, a rate limiting step for ester biosynthesis, is regulated by ethylene. **Plant Science**, [s. l.], v. 168, n. 5, p. 1199-1210, 2005.

DONADEL, J. Z. et al. Key volatile compounds of 'Fuji Kiku' apples as affected by the storage conditions and shelf life: Correlation between volatile emission by intact fruit and juice extracted from the fruit. **Food Research International**, [s. l.], p. 108-625, 2019.

DOS SANTOS, I. D. et al. Patulin accumulation in apples under dynamic controlled atmosphere storage. **Food Chemistry**, [s. l.], v. 255, p. 275-281, 2018.

ECHEVERRÍA, G. et al. Relationships between volatile production, fruit quality and sensory evaluation of Fuji apples stored in different atmospheres by means of multivariate analysis. **Journal of the Science of Food and Agriculture**, [s. l.], v. 84, n. 1, p. 5-20, 2004.

ECHEVERRÍA, G. et al. Physicochemical measurements in ‘Mondial Gala®’ apples stored at different atmospheres: Influence on consumer acceptability. **Postharvest Biology and Technology**, [s. l.], v. 50, n. 2-3, p. 135-144, 2008.

FAOSTAT, Food and Agriculture Organization of the United Nations Statistics. 2018. Disponível em: <http://www.fao.org/faostat/en/#compare>. Acesso em: 20 Jun. 2021.

FORNEY CF, MATTHEIS JP, BALDWIN AE (2009). Effects on flavor. En Yahia EM (Ed) *Modified and Controlled Atmospheres for the Storage, Transportation, and Packaging of Horticultural Commodities.* CRC. Boca Ratón, FL, EEUU. p.119-159, 2009.

GASSER, F. et al. Control of the critical oxygen level during dynamic ca storage of apples by monitoring respiration as well as chlorophyll fluorescence. **Acta Horticulturae**, [s. l.], n. 796, p. 69-76, 2008.

GASSER, F. et al. Effects of decreasing oxygen levels in the storage atmosphere on the respiration and production of volatiles of Idared apples. **Acta Horticulturae**, [s. 1.], n.682, p.1585-1592, 2005.

HERREMANS, E. et al. Characterisation of ‘Braeburn’ browning disorder by means of X-ray micro-CT. **Postharvest Biology and Technology**, [s. l.], v. 75, p.114-124, 2013.

JIN, Y. Z. et al. Ethanol vapor treatment maintains postharvest storage quality and inhibits internal ethylene biosynthesis during storage of oriental sweet melons. **Postharvest Biology and Technology**, [s. l.], v. 86, p. 372-380, 2013.

KIST, B. B. Anuário brasileiro da maçã 2018. Santa Cruz do Sul, **Editora Gazeta Santa Cruz**, p. 56, 2019.

KIST, B. B. Anuário brasileiro da maçã 2016. Santa Cruz do Sul, **Editora Gazeta Santa Cruz**, p. 64, 2016.

KITTEMANN, D.; NEUWALD, D. A.; STREIF, J. Internal browning in “Kanzi®” apples - reasons and possibilities to reduce the disorder. **Acta Horticulturae**, [s. l.], n. 1079, p. 409-414, 2015.

KONDO, S. et al. Aroma volatile biosynthesis in apples affected by 1-MCP and methyl jasmonate. **Postharvest Biology and Technology**, [s. l.], v. 36, n. 1, p. 61-68, 2005.

KÖPCKE, D. 1-Methylcyclopropene (1-MCP) and dynamic controlled atmosphere (DCA) applications under elevated storage temperatures: Effects on fruit quality of “Elstar”, “Jonagold” and “Gloster” apple (Malus domestica Borkh.). **European Journal of Horticultural Science**, [s. l.], v. 80, n. 1, p. 25-32, 2015.

KWEON, H. et al. Fruit maturity, controlled atmosphere delays and storage temperature affect fruit quality and incidence of storage disorders of ‘Fuji’ apples. **Scientia Horticulturae**, [s. l.], v. 157, p. 60-64, 2013.

LAFER, G. Storability and fruit quality of ‘Braeburn’ apples as affected by harvest date, 1-MCP treatment and different storage conditions. **Acta Horticulturae**, [s. l.], n. 796, v.179-183, 2008.

LÓPEZ, M. L. et al. Volatile compounds, quality parameters and consumer acceptance of ‘Pink Lady’ apples stored in different conditions. **Postharvest Biology and Technology,** [s. l.], v. 43, p. 55-66, 2007.

LUMPKIN, C. et al. ‘Fuji’ apple (Malus domestica Borkh.) volatile production during high pCO2 controlled atmosphere storage. **Postharvest Biology and Technology**, [s. l.], v. 100, p. 234-243, 2015.

MAPA, Instrução Normativa Nº 5, de 9 de Fevereiro de 2006. Disponível em: https://www.gov.br/agricultura/pt-br/assuntos/inspecao/produtos-vegetal/legislacao-1/normativos-cgqv/pocs/instrucao-normativa-no-5-de-09-de-fevereiro-de-2006-maca/view>. Acesso em: 18 fev. 2021.

MATTÉ, P. et al. ILOS + ULO as a practical technology for apple scald prevention. **Acta Horticulturae**, [s. l.], n.682, p.1543-1550, 2005.

MEHINAGIC, E. et al. Characterization of Odor-Active Volatiles in Apples: Influence of Cultivars and Maturity Stage. **Journal of Agricultural and Food Chemistry**, [s. l.], v. 54, n. 7, p. 2678-2687, 2006.

PLOTTO, A.; Mc DANIEL, M. R.; MATTHEIS, J. P. Characterisation of changes in ‘Gala’ apple aroma during storage using Osme analysis, a gas chromatographyolfactometry technique. **Journal of the American Society for Horticultural Science,** v.125, 714-722, 2000.

PRANGE, R. K. et al. Oxygen Concentration Affects Chlorophyll Fluorescence in Chlorophyll-containing Fruit and Vegetables. **Journal of the American Society for Horticultural Science**, [s. l.], v. 128, n. 4, p. 603- 607, 2003.

PRANGE, R. K. et al. Method and apparatus for monitoring a condition in chlorophyll containing matter. **U.S. Patent**, n.WO/2002/006795, 2007.

PRE-AYMARD, C.; WEKSLER, A.; LURIE, S. Responses of ‘Anna’, a rapidly ripening summer apple, to 1-methylcyclopropene. **Postharvest Biology and Technology**, [s. l.], v. 27, n. 2, p. 163-170, 2003.

QI, W. et al. Ethylene Emission as a Potential Indicator of Fuji Apple Flavor Quality Evaluation Under Low Temperature. **Horticultural Plant Journal**, v.6 n.4, p.231-239, 2020.

REBEAUD, S.G., GASSER, F., fruit quality as affected by 1-MCP treatment and DC A storage- a comparison of the two methods. **Europen Journal and Horticultural Science**, [s. l.], v. 80, n. 1, p.18-24, 2015.

RAFFO, A. et al. Impact of Innovative Controlled Atmosphere Storage Technologies and Postharvest Treatments on Volatile Compound Production in Cv. Pinova Apples. **Journal of Agricultural and Food Chemistry**, [s. l.], v. 57, n. 3, p. 915-923, 2009.

SABBAN-AMIN, R. et al. Low oxygen and 1-MCP pretreatment delay superficial scald development by reducing reactive oxygen species (ROS) accumulation in stored 'Granny Smith' apples. **Postharvest Biology and Technology**, [s. l.], v. 62, p. 295-304, 2011.

SALAZAR, N. S.; OROZCO, G. O. El aroma de la manzana. [s. l.], v. 36, p. 8, 2011.

SAQUET, A. A.; STREIF, J.; BANGERTH, F. Changes in ATP, ADP and pyridine nucleotide levels related to the incidence of physiological disorders in 'Conference' pears and 'Jonagold' apples during controlled atmosphere storage. **The Journal of Horticultural Science and Biotechnology**, [s. l.], v. 75, n. 2, p. 243-249, 2000.

SAQUET, A.; ALMEIDA, D. Internal disorders of 'Rocha' pear affected by oxygen partial pressure and inhibition of ethylene action. **Postharvest Biology and Technology**, [s. l.], v. 128, p. 54-62, 2017.

SCHOUTEN, S. P. et al. Improvement of Quality of Elstar Apples by Dynamic Control of ULO Conditions. **IFAC Proceedings Volumes**, [s. l.], v. 31, n. 9, p. 25-29, 1998.

SISLER, E. C.; SEREK, M. Inhibitors of ethylene responses in plants at the receptor level: Recent developments. **Physiologia Plantarum**, [s. l.], v. 100, n. 3, p. 577-582, 1997.

SOULEYRE, E. J. F. et al. An alcohol acyl transferase from apple (cv. Royal Gala), MpAAT1, produces esters involved in apple fruit flavor. **Federation of European Biochemical Societies Journal**, [s. l.], v. 272, p.3132-3144, 2005.

SONG, J.; BANGERTH, F. Fatty acids as precursors for aroma volatile biosynthesis in pre-climacteric and climacteric apple fruit. **Postharvest Biology and Technology**, [s. l.], v. 30, n. 2, p. 113-121, 2003.

TAIZ, L.; ZEIGER, E. **Fisiologia Vegetal**, 5. ed. Porto Alegre: Artmed, 2013. 918 p.

TATSUKI, M.; ENDO, A. Analyses of Expression Patterns of Ethylene Receptor Genes in Apple (Malus domestica Borkh.) Fruits Treated with or without 1-Methylcyclopropene (1-MCP). **Journal of the Japanese Society for Horticultural Science**, [s. l.], v. 75, n. 6, p. 481-487, 2006.

TATSUKI, M.; ENDO, A.; OHKAWA, H. Influence of time from harvest to 1-MCP treatment on apple fruit quality and expression of genes for ethylene biosynthesis enzymes and ethylene receptors. **Postharvest Biology and Technology**, [s. l.], v. 43, n. 1, p. 28-35, 2007.

THEWES, F. R. et al. Dynamic controlled atmosphere and ultralow oxygen storage on 'Gala' mutants quality maintenance. **Food Chemistry**, [s. l.], v. 188, p. 62-70, 2015. a.

THEWES, F. R. et al. Effect of dynamic controlled atmosphere monitored by respiratory quotient and 1-methylcyclopropene on the metabolism and quality of 'Galaxy' apple harvested at three maturity stages. **Food Chemistry**, [s. l.], v. 222, p. 84-93, 2017. a.

THEWES, F.R., et al. 1- Methylcyclopropene effects on volatile profile and quality of 'Royal Gala' apples produced in Southern Brazil and stored in controlled atmosphere. **Ciência Rural**. [s. l.], v. 45, n. 12, p.2259-2266, 2015b.

THEWES, F. R. et al. Dynamic controlled atmosphere storage suppresses metabolism and enhances volatile concentrations of ‘Galaxy’ apple harvested at three maturity stages. **Postharvest Biology and Technology**, [s. l.], v. 127, p. 1-13, 2017. b.

THEWES, F.R. et al. Dynamic controlled atmosphere (DCA) and 1-MCP: Impact on volatile esters synthesis and overall quality of ‘Galaxy’ apples. **Food Packaging and Shelf Life**, [s. l.], v. 26, p.100-563, 2020.

THEWES, F. R. et al. The different impacts of dynamic controlled atmosphere and controlled atmosphere storage in the quality attributes of ‘Fuji Suprema’ apples. **Postharvest Biology and Technology**, [s. l.], v. 130, p. 7-20, 2017. c.

THEWES, F. R. et al. Static × dynamic controlled atmosphere: Impacts of aerobic and anaerobic metabolism on physiological disorders and overall quality of ‘Royal Gala’ apples. **LWT**, v. 141, p.110-922, 2021.

VELTMAN, R. H.; VERSCHOOR, J. A.; VAN DUGTEREN, J. H. R. Dynamic control system (DCS) for apples (Malus domestica Borkh. cv ‘Elstar’): optimal quality through storage based on product response. **Postharvest Biology and Technology**, [s. l.], v. 27, n. 1, p. 79-86, 2003.

WANG, Z.; DILLEY, D. R. Initial low oxygen stress controls superficial scald of apples. **Postharvest Biology and Technology**, [s. 1.], v.18, p.201-21, 2000.

WATKINS, C. B.; NOCK, J. F. Rapid 1-methylcyclopropene (1-MCP) treatment and delayed controlled atmosphere storage of apples. **Postharvest Biology and Technology**, [s. 1.], v.69, p.24-31, 2012.

WATKINS, C. B. Postharvest ripening regulation and innovation in storage technology. **Acta Horticulturae**, [s. l.], n. 796, p.51-58, 2008.

WATKINS, C. B. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. **Biotechnology Advances**, [s. l.], v. 24, n. 4, p. 389-409, 2006.

WEBER, A. et al. Respiratory quotient: innovative method for monitoring ‘Royal Gala’ apple storage in a dynamic controlled atmosphere. **Scientia Agricola**, [s. l.], v. 72, n. 1, p. 28-33, 2015.

WEBER, A. et al. Ethanol reduces ripening of “Royal Gala” apples stored in controlled atmosphere. **Anais da Academia Brasileira de Ciências**, [s. l.], v. 88, n. 1, p. 403-410, 2016.

WEBER, A. et al. Dynamic controlled atmosphere (DCA): interaction between DCA methods and 1-methylcyclopropene on ‘Fuji Suprema’ apple quality. **Food Chemistry**, [s. l.], v. 235, p. 136-144, 2017.

WRIGHT, A. H. et al. The trend toward lower oxygen levels during apple (Malus x domestica Borkh) storage – A review. **Journal of Horticultural Science & Biotechnology**, [s. l.], v. 90, n. 1, p. 14, 2015.

YANG, X. et al. Ethylene and 1-MCP regulate major volatile biosynthetic pathways in apple fruit. **Food Chemistry**, [s. l.], v. 194, p. 325-336, 2016.

ZANELLA, A. et al. Fruit fluorescence response to low oxygen stress: modern storage technologies compared to 1-mcp treatment of apple. **Acta Horticulturae**, [s. l.], n. 682, p. 1535-1542, 2005.

ZANELLA, A.; CAZZANELLI, P.; ROSSI, O. Dynamic controlled atmosphere (dca) storage by the means of chlorophyll fluorescence response for firmness retention in apple. **Acta Horticulturae**, [s. l.], n. 796, p. 77-82, 2008.