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

Suele Fernanda Prediger Schmidt

PONTO DE COMPENSAÇÃO ANAERÓBICO PARA O ARMAZENAMENTO DE MAÇÃS 'MAXI GALA' EM ATMOSFERA CONTROLADA DINÂMICA E SUA RELAÇÃO COM O NÍVEL DE CO_2

Santa Maria, RS
2020

Suele Fernanda Prediger Schmidt

PONTO DE COMPENSAÇÃO ANAERÓBICO PARA O ARMAZENAMENTO DE MAÇÃS 'MAXI GALA' EM ATMOSFERA CONTROLADA DINÂMICA E SUA RELAÇÃO COM O NÍVEL DE CO_2

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

Orientador: Prof. D.r Auri Brackmann

Ficha catalográfica elaborada através do Programa de Geração Automática da Biblioteca Central da UFSM, com dados fornecidos pelo (a) autor (a).

Schmidt, Suele Fernanda Prediger
PONTO DE COMPENSAÇÃO ANAERÓBICO PARA O ARMAZENAMENTO DE MAÇÃS 'MAXI GALA' EM ATMOSFERA CONTROLADA DINÂMICA E SUA RELAÇÃO COM O NÍVEL DE CO_2/ Suele Fernanda Prediger Schmidt. 2020.
93 p.; 30cm

Orientador: Auri Brackmann
Dissertação (mestrado) – Universidade Federal de Santa Maria, Centro de Ciências Rurais, Programa de Pós-graduação em Agronomia,Santa Maria, RS, 2020

1. Malus domestica 2. Aroma 3. Quociente respiratório 4. Vida de prateleira 5. Limite mínimo de oxigênio I. Brackmann, Auri II. Título.

Suele Fernanda Prediger Schmidt

PONTO DE COMPENSAÇÃO ANAERÓBICO PARA O ARMAZENAMENTO DE MAÇÃS 'MAXI GALA' EM ATMOSFERA CONTROLADA DINÂMICA E SUA RELAÇÃO COM O NÍVEL DE CO_2

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

Aprovado em 21 de fevereiro de 2020:

_____
Auri Braackmann, Prof. Dr. (UFSM)
(Presidente/Orientador)

_____
Vanderlei Both, Prof. Dr. (UFSM)

_____
Anderson Weber, Prof. Dr. (Unipampa)

DEDICATÓRIA

Aos meus pais Clacio Schmidt e Etla Ivete Prediger Schmidt, pelo exemplo de dedicação e que não mediram esforços para proporcionar meu acesso aos estudos. Aos meus irmãos Joce Micheli Prediger Schmidt e Vitor Erseno Prediger Schmidt pelo apoio e companheirismo durante toda minha jornada até aqui. Dedico também à minha avó Amália Dreyer Prediger (*in memoriam*) pelo exemplo de vida e mulher forte.

Dedico.

AGRADECIMENTOS

À Deus pela vida e força que me concedera.

Aos meus pais pelo exemplo de vida e por não exitarem em momento algum no apoio aos meus estudos.

Aos meus irmãos Joce Micheli Prediger Scmidt e Vitor Erseno Prediger Scmidt pelo apoio durante toda minha jornada acadêmica.

À Universidade Federal de Santa Maria, RS pela estrutura de ensino concendida.

À CAPES pelo financiamento de minha bolsa de estudos.

À COOPAGRO pela concessão das maçãs, objeto de minha pesquisa.

Um especial agradecimento ao meu orientador Professor Dr. Auri Brackmann por abrir as portas para a realização do meu estágio final de graduação em julho de 2017 e a continuar como orientada no mestrado.

Ao Professor Dr. Vanderlei Both pelo apoio, pelo auxílio na condução de experimentos e diálogo aberto na discussão de resultados.

Gostaria de agradecer a todos meus colegas de Núcleo de Pesquisa em Pós-Colheita: Erani, Vagner, Magno, Fabiane, Lucas, Flávio, Fábio e Thays pela parceria e companheirismo no dia-a-dia.

RESUMO

PONTO DE COMPENSAÇÃO ANAERÓBICO PARA O ARMAZENAMENTO DE MAÇÃS 'MAXI GALA' EM ATMOSFERA CONTROLADA DINÂMICA E SUA RELAÇÃO COM O NÍVEL DE CO_2

AUTORA: Suele Fernanda Prediger Schmidt
ORIENTADOR: Auri Brackmann

No armazenamento de maçãs por longos períodos, o método de atmosfera controlada dinâmica (ACD) monitorada pelo quociente respiratório (ACD-QR) teve sua eficácia atestada quando comparado à atmosfera controlada (AC) convencional, com e sem a aplicação de 1-metilciclopropeno, com maior concentração de compostos voláteis que compõe o aroma da maçã se comparado a ACD monitorada pela fluorescência de clorofilas (ACD-FC). Porém, este método é dependente de um equipamento para determinação do QR, o que onera esta técnica de armazenamento. Assim, objetivou-se desenvolver uma tecnologia mais prática e de menor custo que possa ser aplicada a nível comercial. Para tanto, foram conduzidos dois experimentos testando um método baseado no ponto de compensação anaeróbico (PCA), onde apenas a respiração das maçãs é utilizada para determinar o limite mínimo de oxigênio (LMO) tolerado pelos frutos. Este método foi comparado ao método do ACD-QR 1,3, ACD-FC e AC convencional (1,2 kPa de O_2 + 2,0 kPa de CO_2), com e sem a aplicação de 1-metilciclopropeno (1-MCP). Também foi avaliado o efeito de diferentes pressões parciais de CO_2, combinados à tecnologia de PCA. No experimento I, com pressão parcial de 1,2% de CO_2 nas ACDs, foram comparados AC convencional, com e sem a aplicação de 1-MCP, ACD-FC e ACD-QR 1,3, com o tratamento de ACD-PCA. No experimento II, foram comparados os efeitos das pressões parciais de 0,4; 1,2; 1,6; e, 2,0 kPa de CO_2 utilizadas no armazenamento em ACD-PCA com AC convencional e ACD-FC (1,2 kPa CO_2) sobre a conservação das maçãs. Os frutos foram avaliados após nove meses de armazenamento em 2,0 °C, mais 7, 14 e 21 dias de vida de prateleira à 20 °C. No artigo 1, as condições de ACD-FC, ACD-QR, ACD-PCA e AC +1-MCP mantiveram baixa produção de etileno e maior percentual de frutos sadios até os 14 dias de vida de prateleira. Após 21 dias, ACD-QR 1,3 e ACD-PCA mantiveram maior número de frutos sadios. Após 7 dias de vida de prateleira, maçãs armazenadas em AC mantiveram maior concentração de importantes compostos do aroma de mutantes 'Gala' como o de acetato de 2-metila e acetato de hexila, a ACD-PCA manteve maior concentração acetato de hexila se comparado a AC+1-MCP, ACD-FC e ACD-QR 1,3, porém inferior a AC. ACD-PCA manteve maior concentração de (E)-2-hexenal, e ACD-QR 1.3 e ACD-PCA manteviveram maior concentração de 2-metil-butanal após 14 dias de vida de prateleira. No artigo 2, o armazenamento em ACD-PCA com 1,2 e 1,6 kPa CO_2 resultou em maior manutenção da firmeza de polpa, frutos sadios, menor incidência de polpa farinácea após 14 e 21 dias de vida de prateleira e menor concentração interna de etileno aos 7 dias de vida de prateleira quando comparados a AC, ACD-FC, ACD-PCA com 0,4 e 2,0 kPa de CO_2. Maçãs armazenadas em apresentaram maior incidência de polpa farinácea, em consequência da alta respiração, concentração interna de etileno e atividade da enzima ACC oxidase, além da menor mautenção de frutos sadios.

Palavras-chave: Aroma, Limite mínimo de oxigênio, *Malus domestica*, Quociente respiratório, Vida de prateleira.

ABSTRACT

ANAEROBIC COMPENSATION POINT TO THE STORAGE OF 'MAXI GALA' APPLES UNDER DANAMIC CONTROLLED ATMOSPHERE AND ITS RELATION WITH CO_2 LEVEL

AUTHOR: Suele Fernanda Prediger Schmidt
ADVISER: Auri Brackmann

At prolonged apple storage, dynamic controlled atmosphere monitored by the respiratory quotient (DCA-RQ) condition has its efficient attested when compared to conventional controlled atmosphere (CA), whetter with or without 1-methylcyclopropene application on overall quality maintenance, with higher aroma volatile compounds maintenance, also when compared to DCA monitored by chlorophyll fluorescence (DCA-CF). Although, DCA-RQ is a hardware dependent for the RQ determination, which encumbers this technic. Thus, we proposed to develop a simple and low cost methology which can be comercialy applied. Therefore, two experiments were conduced to test the apple storage under a DCA monitored by the anaerobic compensation point (DCA-ACP), where only the apple respiration is required to monitore the lowest oxygen limit (LOL) tolerated by the fruit. This storage condition was comared to DCA-RQ 1.3, DCA-CF, and to conventional CA (1.2 kPa O_2 + 2.0 kPa CO_2) with or without 1-methylcyclopropene (1-MCP) application. Also, the different carbon dioxide concentrations (0.4, 1.2, 1.6 and 2.0 kPa CO_2) effect on DCA-ACP storage condition was evaluated. On the first experiment, DCA conditions were conduced with 1.2 kPa CO_2, where compared to DCA-ACP was compared to conventional CA wit and without 1-MCP application, DCA-CF and DCA-RQ 1.3. On the second experiment, the effect of the CO_2 concentration of 0.4, 1.2, 1.6, and 2.0 kPa on DCA-ACP storage were compared to conventional CA and DCA-CF (with 1.2 kPa CO_2) on overall apple quality concervation. Apples of the 'Maxi Gala' apples were evaluated after 9 months of storage at 2.0 °C plus 7, 14, and 21 days of shelf life at 20 °C. At the first experiment, the conditions of DCA-CF, DCA-RQ 1.3, DCA-ACP and CA + 1-MCP maintained lower ethylene productions and higher healphy fruit after 14 days of shelf life. After 21 days, DCA-ACP and DCA-RQ 1.3 stored apple mainteined higher number of healphy fruit. After 7 days of shelf life, apples stored under CA maintained higher 'Gala' important aroma volatile compounds like 2methylbutyl acetate and hexyl acetate. The DCA-ACP maintained higher hexyl acetate concentration when compared to CA + 1-MCP, DCA-CF, and DCA-RQ 1.3, although lower than CA. DCA-ACP maintained higher (E)-2-hexenal concentration, and DCA-RQ 1.3 and DCA-ACP maintained higher 2-metyl butanal after 14 days of shelf life. On the second experiment, 'Maxi Gala' apple storage under DCA-ACP with 1.2 and 1.6 kPa CO_2 resulted in higher flesh firmness maintenance, healphy fruit, and lower mealiness incidence after 14 and 21 days of shelf life when compared to CA, DCA-CF, and DCA-ACP with 0.4 and 2.0 kPa CO_2. Apples stored under CA presented higher fruit senescence as a result of the higher mealiness incidence, as a responce to the higher respiration rate, internal ethylene concentration and higher ACC oxidase activity, besides lower healphy fruit maintenance.

Keywords: Aroma, *Malus domestica*, Lowest oxygen limit, Respiratory quotient, Shelf life.

LISTA DE FIGURAS

Artigo 1

LISTA DE TABELAS

Artigo 2

LISTA DE ABREVIATURAS

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-CF	Atmosfera controlada dinâmica monitorada pela fluorescência de clorofilas
ACD-QR	Atmosfera controlada dinâmica monitorada pelo quociente respiratório
ACP	Anaerobic compensation point
ADH	Álcool desidrogenase
ANOVA	Análise de variância
AR	Atmosfera refrigerada
ATP	Adenosina trifosfato
CA	Controlled atmosphere
C_2H_4	Fórmula molecular do etileno
CO_2	Dióxido de carbono (gás carbônico)
DCA	Dynamic controlled atmosphere
DCA-ACP	Dynamic controlled atmosphere – anaerobic compensation point
DCA-CF	Dynamic controlled atmosphere – chlorophyll fluorescence
DCA-RQ	Dynamic controlled atmosphere – respiratory quotient
DCS	Dynamic Controlled System
FID	Flame ionization detector
GC-FID	Cromatógrafo a gás com detector por ionização em chama
ha	Hectare
kg	Quilograma
kPa	Quilograma Pascal (unidade de medida internacional para a concentração de gases)
LMO	Limite mínimo de oxigênio
LOL	Lowest oxygen limit
MdACS	Gene que expressa a ACC sintase em maçã

mg	Miligrama
mL	Mililitro
mm	Milímetro
N	Newton
N_2	Nitrogênio
NaCl	Cloreto de sódio
NaOH	Hidróxido de sódio
O_2	Oxigênio/oxygen
OTH	Odor threshold
PC	Principal Component
PCA	Ponto de compensação anaeróbica
QR	Quociente respiratório
s	Segundo
ULO	Ultra low oxygen

SUMÁRIO

1 INTRODUÇÃO E JUSTIFICATIVA

Após a abscisão da planta mãe, a maçã continua o processo de amadurecimento, desta forma a conservação dos atributos qualitativos fica reduzida a curtos períodos se mantida em ambiente sem refrigeração e oxigênio ambiente (20,9 kPa). Assim, o armazenamento se faz necessário porque a colheita das principais cultivares produzidas no Brasil, dentre elas a 'Maxi Gala', possuem janela de colheita muito curta, gerando um volume muito grande de frutos colhidos em poucas semanas. Assim, para que empresas do setor possam fornecer o produto no mercado durante o ano todo, são requeridas técnicas para a conservação por longos períodos de grandes volumes.

Perdas durante o armazenamento refrigerado (AR) chegam à 35%, quando armazenados por longos períodos (ANTONIOLLI, 2011), assim, as principais empresas de armazenamento de maçãs, para prolongar a manutenção da qualidade de maçãs, adotam a aplicação de produtos químicos como o 1-metilciclopropeno (1-MCP) e o armazenamento em atmosfera controlada (AC) (CHERVIN et al., 2001; WATKINS; NOCK; WHITAKER, 2000; WATKINS, 2006). O armazenamento sob AC possibilita armazenar os frutos por até 8 a 9 meses, porém perdas de firmeza e maior inidência de polpa farinácea ainda estão presentes. A aplicação de 1-MCP auxilia na manutenção de alguns atributos de qualidade após longos períodos de armazenamento, embora tenha efeito negativo na manutenção de compostos voláteis que compõe o aroma da maçã (YOUNG, 2016). Assim, foram desenvolvidas metodologias que prolongam a manutenção da qualidades de maçãs que dispensam o uso do fitorregulador 1-MCP, pelo monitoramento do limite mínimo do oxigênio (LMO). Dentre essas, as mais estudadas são a AC dinâmica (ACD) monitorada pela fluorescência de clorofilas (ACD-FC) e pelo quociente respiratório (ACD-QR).

A ACD-QR preconiza o acompanhamento da respiração anaeróbica dos frutos armazenados por meio da relação do CO_2 produzido e o O_2 consumido, permitindo a diminuição do O_2 na câmara a níveis extremamente baixos após algumas semanas de armazenamento. Resultados recentes atestaram que frutos armazenados pelo QR 1,3 e pelo QR 1,5 obtiveram drástica redução da produção de etileno (THEWES et al., 2017c) e redução de distúrbios fisiológicos (WEBER et al., 2017).

Embora tenham resultados saisfatórios a ACD-FC e ACD-QR tem um custo na implantação que esbarra no investimento desses sistemas. Assim, propôs-se a metodologia de

ACD monitorada pelo ponto de compensação anaeróbico (ACD-PCA) que monitora o LMO de maçãs, a qual utiliza do mesmo sistema de uma câmara de AC. O PCA é definido como sendo a concentração de O_2 na câmara na qual se alcança a produção de CO_2 mínima pelos frutos, quando ocorre o menor gasto energético do fruto para a manutenção do seu metabolismo (BOERSIG et al., 1988). A metodologia do ACD-PCA, ainda não citada em literatura, apresentou resultados positivos em testes preliminares no Núcleo de pesquisa em Pós-colheita da UFSM, sendo que nesta técnica a diminuição gradativa dos níveis de O_2 é baseada apenas na produção de CO_2 dos frutos, ou seja, a respiração dos frutos está próxima ao LMO, de forma semelhante ao método do QR.

A diminuição da concentração de O_2 em câmaras de armazenamento pelos métodos ACD-PCA e ACD-QR adaptam os frutos a tolerar, ao longo do tempo, uma gradativa diminuição do nível de oxigênio sem, no entanto, causar estresse em demasia. Com o passar do tempo, níveis extremamente baixos de oxigênio são alcançados (até inferiores a 0,08 kPa) o que dificulta um pouco a determinação precisa do nível deste gás na câmara, pois qualquer falta de calibração do analisador ou problemas na estanqueidade das câmaras poderá levar a erros na determinação no método do QR, resultando na estimação errônea do nível de O_2 na câmara. Por outro lado, o método do PCA baseia-se apenas na determinação do nível de CO_2, ou seja, a variação da produção de CO_2 na câmara entre duas determinações realizadas no intervalo de algumas horas irá indicar se a concentração de O_2 deve ser mantida, reduzida ou aumentada na câmara.

Comumente, a concentração de CO_2 utilizada depende da cultivar de maçã, varia de 0,8 a 2,0 kPa. Ao estudar o método do QR, diferentes autores têm utilizado a concentração de 1,2 kPa de CO_2 no armazenamento de maçãs mutantes da cultivar ‘Gala’ (BOTH et al., 2016, THEWES et al., 2017b; 2017c). O uso de pressões elevadas de CO_2 é desejável já que, ocorre a redução da entrada de O_2 durante a absorção de CO_2 acumulado pela respiração dos frutos e a inibição de fungos nas câmaras. Embora Brackmann; Weber; Both (2015) demonstraram que o armazenamento sob QR 1.5 com pressão parcial de 2,0 kPa CO_2 manteve menor número de frutos sadios pela maior incidência de polpa farinácea, demonstrando assim que há um nível ideal de CO_2 para cada método.

Em estudos preliminares foi observado que, à medida que se usam níveis mais elevados de CO_2 na câmara, o método do QR indica que o nível do O_2 deve ser reduzido, pois o alto CO_2 complementa o baixo O_2 na inibição do processo respiratório dos frutos, subestimando o método. Por outro lado, também em trabalhos preliminares, foi observado que em baixas concentrações de CO_2 o gradiente de concentração entre fruto e ambiente é maior,

o que tende a estimular a maior liberação de CO_2 pelo fruto, o que, consequentemente, aumenta o QR e, que por sua vez, sugere o aumento do nível do O_2 na câmara, quando na verdade este deveria ser reduzido. Com o método do PCA espera-se que este erro no estabelecimento correto do nível de O_2 em câmaras com alta concentração de CO_2 seja corrigido. Visto quê, o PCA acompanha a menor e maior liberação do produto da respiração em ambiente com alta e baixa pressão parcial de CO_2, sem no entanto subestimar o cálculo do *set point*.

Assim, buscou-se, por meio desse, avançar nas pesquisas com ACD, avaliando a possibilidade de utilizar apenas a produção de CO_2 pelos frutos, pelo método chamado de PCA, para estipular o limite mínimo de O_2, o que determinará o estabelecimento do nível de oxigênio (*set point*) da câmara por um período diário ou até semanal, este comparado à técnica do QR (BRACKMANN, 2015; WEBER et al., 2015). Erros nos analisadores de O_2 e CO_2 ainda podem estar presentes, mas como o estabelecimento do nível de O_2 da câmara será feito apenas com base na produção de CO_2, este será mais preciso, fato que poderá contribuir na aplicabilidade desta nova técnica em câmaras comerciais, bem como a menor sucetibilidade a erros no cálculo do QR pela entrada de O_2 em câmaas com menor estanqueidade. Além disso, pretende-se elucidar o efeito de diferentes níveis de CO_2 sobre a conservação da qualidade das maçãs na técnica de ACD monitorada pelo PCA, pois ainda não se conhece o efeito de diferentes níveis de CO_2 na câmara sobre a determinação do nível de O_2 nesta metodologia de monitoramento em câmaras de ACD. As maçãs serão avaliadas quanto a qualidade durante 21 dias de vida de prateleira após o armazenamento pelas técnicas de do QR e PCA, gerando importantes informações sobre situações que ocorrem na condição de comercialização da cadeia da maçã, uma vez que não há na literatura documentos que demonstrem estudos com período de vida de prateleira tão extenso após o armazenamento.

1.1 HIPÓTESES

O método do PCA mantém qualidade superior de maçãs ‘Maxi Gala’, quando comparado ao armazenamento pelo método de AC convencional sem e com a aplicação de 1-MCP, ACD-FC e ACD-QR 1,3, após o armazenamento e também após mais 21 dias de vida de prateleira.

O método do PCA permite armazenar maçãs da cultivar ‘Maxi Gala’ por longos períodos em alta concentração de CO_2 (2,0 kPa), o que otimiza o processo de adsorção deste, reduzindo a entrada de O_2 nas câmaras.

1.2 OBJETIVOS

a) Avaliar a eficiência do novo método de ACD monitorada pelo PCA na manutenção da qualidade de maçãs ‘Maxi Gala’ armazenadas por nove meses e mais 21 dias de vida de prateleira;

b) Avaliar a eficiência do novo método de ACD monitorada pelo ponto de compensação anaeróbico na manutenção do perfil de compostos voláteis;

c) Estabelecer o nível ideal de CO_2 para o armazenamento de maçãs ‘Maxi Gala’ pelo método do PCA;

2 REVISÃO DE LITERATURA

2.1 CARACTERIZAÇÃO DA PRODUÇÃO DE MAÇÃS

A maçã (*Malus domestica* Borkh.) é uma das frutas mais produzidas e consumidas no Brasil e no mundo (SANTOS et al., 2018), devido a características organolépticas que agradam ao consumidor (ALTISENT, 2008). A maçã é um fruto climatérico, que continua amadurecendo após a colheita (SEPPÄ et al., 2013), de ciclo sazonal, com curto período de colheita, exigindo alta tecnologia de armazenamento para que se possa garantir o prolongamento da oferta por todo o período de entressafra. Desta forma, a pesquisa em pós-colheita está focada no desenvolvimento de novas técnicas de armazenamento (THEWES et al., 2015). O Brasil ocupa o 11° lugar no ranking mundial de produção de maçã (FAO, 2017), com 1.094.116 toneladas produzidas no ano de 2018 (Kist et al., 2019). A alta produção garante ao país a autosufissiência para o consumo interno e, na entressafra europeia, parte é exportado (REENTZ et al., 2009).

Dentre as cultivares de maçãs mais produzidas no Brasil, destacam-se a ‘Gala’ e a ‘Fuji’ e suas mutantes, sendo que a ‘Maxi Gala’ juntamente com outras mutantes da ‘Gala’, tem uma participação crescente no mercado devido à renovação de pomares (THEWES,

2016; WEBER et al., 2013), já que é preferencialmente consumida no país devido à alta suculência e bom balanço entre ácidos e açúcares (THEWES et al., 2015), além do recobrimento e intensa coloração vermelha da epiderme (BOTH et al., 2018; BRACKMANN et al., 2009; WEBER et al., 2013). Porém, a alta produção de cultivares com ponto decolheita muito próximos, implica na necessidade de armazenamento para a conservação do remanescente que o mercado consumidor não consegue absorver na safra. Esses frutos são fornecidos na entressafra, que pode chegar a periodos superiores a nove meses.

2.2 CARACTERIZAÇÃO DO ARMAZENAMENTO DE MAÇÃS NO BRASIL E MUNDO

De acordo com Kist et al. (2016), os estados de Santa Catarina (SC) e Rio Grande do Sul (RS) que são os maiores produtores da fruta no Brasil, possuem a capacidade de armazenar 923.341 toneladas de maçãs destes, 30,2% são armazenados em câmaras de atmosfera refrigerada (AR) e 69,8% em câmaras de atmosfera controlada (AC). O armazenamento em atmosfera refrigerada, preconiza a redução da temperatura de 0,5 a 2,0 °C, além do aumento da umidade relativa do ar a 94 ±1%. Maçãs armazenadas em AR devem ser comercializadas em 3 até 4 meses após acolheita (BRACKMANN; CERRETA, 1999), sendo que após esse período perdas sinificativas na qualidade poderão ocorrer. Para suprir a demanda na entressafra, maçãs são armazenadas em atmosfera controlada (AC). No sistema de AC, além do controle de temperatura e umidade, pressão parcial de O_2 é reduzida e a concentração de CO_2 é aumentada nas câmaras, permanecendo em níveis estáticos durante todo o período de armazenamento. Para cultivares mutantes da Gala são recomendadas as pressões parciais de 1,2 kPa O_2 e 2,0 kPa CO_2 (BRACKMANN et al., 2008; BRACKMANN et al., 2009), a qual será referida no armazenamento em AC do presente trabalho.

Com a redução da concentração de O_2 nas câmaras de maçãs mantidas em AC, ocorre a diminuição da atividade metabólica do fruto pela restrição da taxa respiratória e o aumento da concentração de CO_2 que retarda o amadurecimento (VELTMAN et al., 2003). A redução da temperatura reduz o metabolismo dos frutos, concomitante a este, em AC a redução da conentração de O_2 restringe a respiração aeróbica uma vez que o O_2 é o aceptor final de elétrons da cadeia respiratória (TAIZ; ZEIGER, 2016). O oxigênio também é necessário para a conversão de ácido 1-aminociclopropano-1-carboxílico (ACC) em etileno (C_2H_4) (ASODA et al., 2009; YANG; HOFFMAN, 1984). O etileno por sua vez, é um fitormônio autocatalítico que atua na ativação de enzimas que degradam a parede celular (PAYASI et al., 2009), dentre

outros processos que desencadeiam a senescência da maçã. Quando comparado com AR, o armazenammento em AC ao reduzir a atividade metabólica dos frutos mantém maior acidez e firmeza de polpa, e reduz distúrbios fisiológicos, devido a redução da produção de etileno e da respiração dos frutos (BRACKMANN et al., 2008; LUMPKIN et al., 2015).

Porém, quando as maçãs são armazenadas em AC por período superior a oito meses ocorrem perdas significativas (BRACKMANN el al., 2008). Para minimizar este problema e prolongar o armazenamento, muitas emprezas combinam o armazenamento em AC com a aplicação de 1-metilciclopropeno (1-MCP), o qual inibe a ação de etileno, reduzindo perdas após longos períodos de armazenamento pelo retardo do amadurecimento dos frutos, mantendo maior da firmeza de polpa (THEWES et al., 2015; WATKINS; NOCK, 2012) e acidez titulável (LU et al., 2012).

2.3 1-METILCICLOPROPENO (1-MCP)

A aplicação de 1-metilciclopropeno no armazenamento de maçãs é implementado tanto no armazenamento em AR como em AC. O tratamento de maçãs com o fitorregulador 1-MCP, vem a ser uma tecnologia complementar ao armazenamento sob atmosfera controlada. O 1-MCP aplicado na forma de gás liga-se irreversivelmente aos receptores de etileno, impedindo assim a ligação do etileno e por consequência, os eventos desencadeados pelo mesmo (BLANKENSHIP; DOLE, 2003; SISLER; SEREK, 1997).

Embora mantenha as características de firmeza de polpa a acidez dos frutos, o 1-MCP possui desvantagens de reduzir a produção de compostos voláteis como ésteres e álcoois (THEWES et al., 2015), componentes fundamentais na formação do aroma da maçã (ESPINO-DIAZ et al., 2016). Além de não ser permitido na produção orgânica de maçãs (THEWES et al., 2017b; BOTH et al., 2014) e de apresentar maior incidência de contaminação por patulina, metabólico secundário carcinogênico liberado por fungos no armazenamento de maçãs (SANTOS et al, 2018), bem como apresenta ainda elevado custo no armazenamento de maçãs. Ainda assim, este produto é utilizado em um significativo volume de maçãs no Brasil e no mundo. O armazenamento por prolongado período sob baixo oxigênio pode substituir a aplicação de 1-MCP, porém possui suas particularidades.

2.4 ATMOSFERA CONTROLADA DINÂMICA (ACD)

Ao visar o armazenamento prolongado e manutenção da qualidade de frutos, foram desenvolvidos sistemas com base na AC, os quais monitoram o limite mínimo do oxigênio (LMO) tolerado pelos frutos, com pressões parciais abaixo daquela empregada em AC. O LMO tolerado pelos frutos é variável de acordo com a cultivar, ponto de colheita, ano de cultivo e condições climáticas, pressão parcial do CO_2 no armazenamento e também durante longos períodos de armazenamento. Por isso da necessidade de métodos que monitorem o LMO, visto que este consiste da concentração de oxigênnio onde as reações oxidativas e o processo da senescência são reduzidos (ZANELLA et al., 2008), estando abaixo do limite entre a respiração aeróbica e anaeróbica (THEWES, 2016), em contrapartida do que mencionou Wright et al. (2015), quando este referia-se ao ponto de compensação anaeróbico. Por se tratar de um limite próximo a respiração aeróbica e o metabolismo anaeróbico, o LMO deve ser monitorado em tempo real visto que determinações errôneas abaixo do LMO levam a danos irreversíveis pelo aumento demasiado da produção de compostos da fermentação como o acetaldeído, etanol e acetato de etila.

Assim denominada, atmosfera controlada dinâmica baseia-se em uma resposta fisiológica como emissão da fluorescência de clorofilas, pela emissão de produtos do metabolismo anaeróbico ou aumento da taxa respiratória (PRANGE, 2018). Em ACD corrige-se a concentração de O_2 de acordo com o metabolismo dos frutos (THEWES et al., 2017b), pela redução gradativa até atingir o LMO (THEWES et al., 2017a). Dentre as técnicas de ACD, as mais estudadas na atualidade são a ACD monitorada pela fluorescência da clorofila e ACD monitorada pelo quociente respiratório.

2.4.1 ACD monitorada pela fluorescência de clorofilas

Dentre as técnicas desenvolvidas para monitorar o LMO temos a ACD pelo método de emissão de fluorescência de clorofilas (ACD-FC) (PRANGE et al., 2007), no qual, para dectar o LMO, no início do armazenamento o oxigênio é reduzido até o fruto emitir fluorescência pelas clorofilas, como resposta a um estresse provocado pelo baixo O_2 na câmara. Continuamente, durate todo o período de armazenamento, uma luz de baixa intensidade que incide sobre a epiderme de uma amostra de frutos é emitida, e se novamente ocorrer estresse por baixo oxigênio na forma de emissão de um comprimento de onda menor do que aquele emitido pelo sistema, o oxigênio deve ser aumentado na câmara. A partir desse ponto, o nível de O_2 é aumentado em mais 0,20 kPa a 0,30 kPa, mas sempre ficando acima de

0,40 kPa ou 0,50 kPa adepender da cultivar, sendo monitorado constantemente durante todo o período de armazenamento a fim de verificar se não ocorre uma nova emissão da fluorescência da clorofila, necessitando elevação na pressão parcial de O_2 (GASSER et al., 2010; PRANGE et al., 2005). Este método é amplamente difundido na Europa e no mundo. Esse método é implementado no armazenamento de maçãs em 19,3 % das câmaras na região européia produtora de South Tyrol, no norte da Itália (correspondente a 743 câmaras), e 3 % no estado de Whashington, Estados Unidos e um montante de 1979 câmaras equipadas com essa tecnologia no mundo todo (Prange, 2018), com projeção de expansão nos próximos anos segundo este mesmo autor. Em suma, no método de ACD-CF o fruto é submetido a um estresse inicial por baixo oxigênio e após mantido a um nível estático até o final do armazenamento.

Porém, Se comparado à AC, a ACD-FC tem mostrado melhor manutenção da qualidade de frutos (), não diferindo de AC com aplicação de 1-MCP. Entretanto, devido ao elevado custo na aquisição do aparato para o monitoramento do LMO pela ACD-FC, o sistema pouco tem sido implantado em câmaras comerciais no Brasil. Além do mais, como prevê o método, amostras de 6 frutos são utilizadas como representativas para o monitoramento de emissão da clorofila para um elevado volume de frutos, o que pode induzir à leitura pouco representativa de grandes volumes de frutos armazenados.

2.4.2 ACD monitorada pelo quociente respiratório

O método do QR monitora o metabolismo anaeróbico dos frutos pelo quociente entre CO_2 produzido e O_2 consumido pelos frutos condicionados em uma câmara hermeticamente fechada, determinado num intervalo de 13 horas (THEWES et al., 2017a). Em ACD-QR o LMO é alcançado pela resposta do fruto, quando a produção de CO_2 excede o consumo de oxigênio, ou seja, QR maior que aquele pré definido significa que está ocorrendo o metabolismo anaeróbico, sendo então necessário aumentar a concentração de O_2 na câmara, do contrário diminui-se a concentração de O_2 em resposta a um QR inferior. Assim, gradativamente o fruto adaptar-se-á a hipóxia, podendo manter o nível de O_2 próximo ao LMO durante todo o período de armazenamento, oscilando os níveis de O_2 na câmara de acordo com o metabolismo avaliado pelo QR, podendo alcançar níveis próximos a 0,08 kPa de O_2 no final do período de armazenamento (BRACKMANN, 2015).

A detecção do LMO pelo QR demonstrou em maçãs ‘Fuji Suprema’ uma menor taxa respiratória e produção de etileno (WEBER et al., 2017) e menor perda de firmeza de polpa em QR 1,3 (THEWES et al., 2017a); em ‘Granny Smith’ uma menor incidência da escaldadura (BESSEMANS et al., 2016); e, em ‘Galaxy’, um maior percentual de frutos sadios e maior firmeza de polpa em QR 1,5 (BOTH et al., 2018) para maçãs armazenadas de 8 a 9 meses, quando comparado àquelas armazenados em AC convencional. Frutos armazenados em QR 1,3, quando comparados ao armazenamento em AC convencional, apresentaram menor produção de etileno e taxa respiratória (WEBER et al., 2017), menor incidência de distúrbios fisiológicos e redução da atividade da enzim ácido 1-carboxílico-1-aminociclopropano (ACC) oxidase (BOTH et al., 2017; WEBER et al., 2015), enzima responsável pela conversão de ácido 1-carboxílico-1-aminociclopropano a etileno. Além disso, comparando-se com AC mais aplicação de 1-MCP ou ACD-FC, frutos armazenados em ACD-QR apresentam maior concentração de compostos voláteis, tais como: ésteres, acetato de butila, acetato 2-metilbutila e acetato de hexila (BOTH et al., 2017), acetaldeído e acetato de etila (THEWES et al., 2017b), compostos estes responsáveis pelo aroma da maçã. O QR 1,3 demonstra ser eficiente no armazenamento de maçãs ‘Galaxy’ mesmo quando comparado a AC com aplicação de 1-MCP (THEWES et al., 2017b).

2.5 PONTO DE COMPENSAÇÃO ANAERÓBICA (PCA)

Como elucidado em outras metodologias, é possível diminuir ao longo do tempo de armazenamento a concentração a um nível ultrabaixo de oxigênio (do inglês *Ultralow Oxygen – ULO*). Para tanto, é necessário monitorar o limite mínimo de oxigênio (LMO), ou seja, o menor nível de oxigênio tolerado pelos frutos.

O PCA vem a ser o ponto crítico de pressão de O_2 onde a produção de CO_2 proveniente da respiração anaeróbica e aeróbica é mínima (BOERSIG; KADER; ROMANI, 1988; YEARSLEYA; BANKSB; GANESHC, 1997). Com o O_2 acima deste ponto, a respiração aeróbica tende a ser superior, ocasionando maior senescência (BESSEMANS et al., 2016) e, quando o O_2 estiver abaixo deste ponto, poderão ocorrer danos irreversíveis, dependendo do grau de fermentação.

No monitoramento pelo novo método em atmosfera controlada dinâmica pelo ponto de compensação anaeróbico (ACD-PCA), os níveis mínimos de O_2 também são alcançados de acordo com a respiração dos frutos, porém este baseado apenas na produção de CO_2, o qual

encontra-se próximo ao ponto de transição entre o metabolismo aeróbico e anaeróbico, onde qualquer mudança na respiração, para mais ou menos, é detectada e usada para a tomada de decisão na alteração do *set point* da câmara.

Pelo método de QR, são avaliados diferentes níveis de estresse, já que este preconiza trabalhar em níveis muito próximos do LMO, induzindo níveis seguros de fermentação, com os níveis de O_2 um pouco abaixo do estabelecido pelo método do PCA. Em outras palavras, o método do PCA não deve atingir níveis de estresse além daqueles alcançados pelo QR 1.3 e, segundo dados preliminares, mantém da mesma forma a qualidade de maçãs armazenadas por longos períodos. Porém, este método demonstra a vantagem de poder ser adotada em sistemas comerciais, já que apenas o CO_2 serve como base no controle dos níveis de O_2 nas câmaras, estando menos vulnerável a leituras errôneas pelos analisadores de gases e a problemas de estanqueidade das câmaras.

De um modo geral, o armazenamento de frutos e hortaliças evoluiu do frio do porão das antigas casas a sistemas especializados onde combinado ao aumento da concenração de CO_2, corrige-se a concentração do oxigênio de acordo com o metabolismo do próprio fruto. Porém, sistemas sofisticados tem esbarrado na determinação do LMO pela complexidade do sistema. Assim, visa-se nas seguintes páginas apresentar resultados promissores de uma nova metodologia de controle de O_2 pelo próprio metabolismo do fruto, porém sem a necessidade de incremento tecnológico além daquele utilizado em AC convencional.

2.6 EFEITOS DO CO_2 NO ARMAZENAMENTO

No armazenamento de frutos e hortaliças em atmosfera controlada, o aumento da concentração do gás cabônico (CO_2) dá-se como um complemento da redução da atividade metabólica e, indiretamente, na incidência de fungos. O CO_2 é produto da respiração, quando a pressão parcial é elevda, atua no processo auto inibitório pela inibição de enzimas da rota glicolítica e do ciclo dos ácidos tricarboxílicos, mais especificamente na fosfofrutoquinase, succinato desidrogenase e isocitrato desidrogenase (KE et al., 1995; LIU et al., 2004). O CO_2 também têm efeito na biossíntese de etileno, reduzindo a atividade da enzima ACC sintase e competindo com o etileno pelos receptores de etileno (MATHOOKO, 1996). Em morangos, a exposição dos frutos a 30 % de CO_2 por três horas reduziu a expressão de enzimas responsáveis pela degradação da parede celular (expansinas, pectinaesterase e β-xilosidase)

(BANG et al., 2019), possivelmente em detrimento a um efeito concomitante da inibição do efeito do etileno, uma vez que as referidas enzimas são dependentes de etileno.

Pressões elevadas de CO_2 em câmaras de AC comerciais são desejáveis, pois deste inibem o desenvolvimento de fungos (RIUDAVETS et al., 2018). Outro fator de suma importância é, a não entrada de O_2 nas câmaras durante a absorção do excedente de CO_2, uma vez que baixas pressões parciais de CO_2 oneram esse processo. Porém, o efeito de altas pressões parciais de CO_2 no armazenamento de maçãs em ACD não está bem elucidado, podendo ser positivo ao reduzir a respiração, manter a firmeza de polpa e acidez titulável (ARGENTA, 2000), ou negativo, ao causar desordens fisiológicas (MATHOOKO, 1996), dependendo das cultivares e das condições em que são armazenados os frutos (JAMES; JOBLING, 2009) e do ciclo de cultivo (SAQUET; STREIF; BANGERTH, 2000).

Em trabalho conduzido por DeEll (2005), a concentração de 2,5 kPa de O_2 com 2 kPa de CO_2, combinado à aplicação de 1-MCP ou não, manteve maior firmeza de polpa após armazenamento em AC por 240 dias, comparado a 0,0 Kpa de CO_2, com e sem a aplicação de 1-MCP. Isto demonstra que, dependendo das condições em que são armazenados os frutos, o alto CO_2 tem efeito positivo no armazenamento de maçãs. Brackmann et al. (2015), ao estudarem diferentes níveis de CO_2 em ACD-QR comparados a ACD-FC e AC (1,2 kPa de O_2 2,0 kPa de CO_2), também encontraram bons resultados em maçãs da cultivar ‘Galaxy’ armazenadas por nove meses, naqueles cuja pressão parcial de CO_2 foi de 1,2 e 1,6 kPa. Porém, não foram encontrados na literatura informações sobre o uso de baixa concentração de CO_2 no armazenamento em ACD.

Decorrente da pouca informação que se tem sobre o efeito do CO_2 no armazenamento em AC dinâmica monitorada pelo QR e nenhuma sobre ACD-PCA, serão comparadas diferentes concentrações de CO_2 quanto a influência destas na manutenção da qualidade de maçãs da cultivar ‘Maxi Gala’ armazenadas por nove meses e mais 21 dias de vida de prateleira.

2.7 EFEITO DAS CONDIÇÕES DE ARMAZENAMENTO DURANTE A VIDA DE PRATELEIRA

A perda de frutos na América Latina alcança as margens de 20% no processamento e desperdício de 12% nas prateleiras de supermercados (FAO, 2011). Em maçãs, muito disso é decorrente de longa exposição a altas temperaturas e concentração de gases ambiente. Após

passar por longo período de armazenamento, os frutos passam por processo de classificação e transporte até os pontos de comercialização e, ainda podem permanecer, durante a comercialização por até uma semana (*informação pessoal*), em condições com alta disponibilidade de O_2 e, muitas vezes, em temperaturas mais elevadas, o que acelera a amadurecimento e a senescência.

Comumente, trabalhos conduzidos para avaliar a qualidade de maçãs após o armazenamento, avaliam também a vida de prateleira por sete dias (THEWES et al., 2017a; 2017b; 2017c; WEBER et al., 2017; BRACKMANN; WEBER; BOTH, 2015), porém tem-se poucos dados sobre a qualidade além deste período. Desta maneira, há uma lacuna na avaliação da qualidade de maçãs após 14 e 21 dias de vida de prateleira, já que para reduzir o desperdício de alimentos seria de suma importância o desenvolvimento de tecnologias de armazenamento que pudessem prolongar a qualidade de frutas mesmo se mantidos em atmosfera e temperatura ambiente.

2.8 PERFIL VOLÁTIL DE MAÇÃS SOB PROLONGADO ARMAZENAMENTO

Atributos qualitativos que fazem da maçã um dos frutos mais consumidos no Brasil e no mundo, estes dizem respeito ao bom balanço entre a acidez, açúcares e firmeza, além de compostos voláteis que compõe o aroma (MEHINAGIC et al., 2006; SALAZAR; OROZCO, 2011). Dentre os compostos voláteis que dão o aroma característico da maçã pode-se citar os terpenos, cetonas, ácidos, aldeídos, alcoóis e ésteres, dentre os mais de 400 já identificados em maçã (SALAZAR; OROZCO, 2011). Porém, apenas 40 a 50 foram identificados como os de maior importância, principlmente os ésteres (ESPINO-DIAZ, et al., 2016; THEWES et al., 2017b), sendo responsáveis por 98% dos compostos voláteis em maçãs ‘Pink Lady’ (LÓPEZ et al., 2007). Dentre os ésteres característicos do aroma de maçãs ‘Gala’ estão o acetato de hexila, acetato de butila e acetato de 2-metilbutila (DUNEMANN et al., 2012; ESPINO-DIAZ et al., 2016; MEHINAGIC et al., 2006; SALAZAR; OROZCO, 2016; YOUNG et al., 1996). Por esse motivo, além da redução da incidência de distúrbios e podridões que causam perdas diretas aos armazenadores é desejável que maçãs armazenadas por longos períodos também mantenham compostos que caracterizam o aroma da maçã e a tornem atrativas ao consumidor e por consequência, incentivem a fidelidade deste (LU et al., 2018).

Ésteres são compostos formados na última reação de síntese de compostos voláteis pela ação da enzima álcool acil CoA transferase (AAT), responsável por combinar um ácido

acético do Acetil-CoA ao grupo hidroxila (OH) de um álcool (DEFILIPPI et al., 2005; St-PIERRE; De LUCA, 2000; YANG et al., 2016). Os alcoóis por sua vez, embora encontrados em menor concentração se comparados aos ésteres, são importantes na composição do aroma, sendo os mais abundantes o 2-metil-1-butanol, 1-butanol, 1-hexanol, 1-propanol e 2-metil-1-propanol (ECHEVERRÍA et al., 2004; Ló LÓPEZ, et al.1998; LÓPEZ et al., 1998), e sua importância é dada principalmente por se tratar de precursores de ésteres. Os alcoóis são formados pela ação da enzima álcool desidrogenase (ADH) na redução de um aldeído para seu respectivo álcool (BARTLEY; HINDLEY, 1980; DICKSON et al., 1997). A ADH é uma enzima dependente de etileno, onde de 10 genes identificados apenas a *ADH1* teve sua expressão reduzida pela presença do etileno (SCHAFFER et al., 2007). Por outro lado, a enzima AAT teve sua atividade inibida após 4 meses de armazenamento em maçãs ‘Gala’ (FELLMAN et al., 2000).

Sendo que um dos princípios do armazenamento tanto em AC quanto em ACD presa pela redução da atividade metabólica dos frutos pelo baixo oxigênio e, por consequência a redução da produção de etileno, estes negativamente resultam na redução da síntese de compostos voláteis na maçã.

Neste sentido, um dos atuais desafios do armazenamento de maçãs, especialmente por longos períodos, é além de manter os frutos com as características organolépticas atrativas ao consumidor, não prejudicar a emissão de compostos do aroma na maçã. Trabalhos conduzidos com ACD-QR 1,3 e ACD-QR 1,5 demonstraram que é possível armazenar frutos por longos períodos e manter maior quantidade de esteres de cadeia simples (THEWES e al., 2017b). O CO_2 em altas concentrações apresenta redução de compostos de cadeia ramificada BRACKMANN; STREIFF; BANGERTH, 1993).

3 ARTIGO 1

3.1 ANAEROBIC COMPENSATION POINT: NEW METHOD OF DYNAMIC CONTROLLED ATMOSPHERE

Abstract

The objective of this paper was to assess the efficacy of the new dynamic controlled atmosphere method based on the anaerobic compensation point to monitor the oxygen on 'Maxi Gala' apple during storage and the quality maintenance during prolonged shelf life at 20 °C. Thus, 'Maxi Gala' apples of a commercial orchard were stored for 9 months under 2 °C and evaluated after 7, 14, and 21 days of shelf life under 20 °C. The storage conditions were: static controlled atmosphere (CA) with 1.2 kPa O_2 + 2.0 kPa CO_2; CA with 1-methylcyclopropene treatment (0.625 µL L^{-1} of 14% active ingredient); dynamic CA (DCA) monitored by chlorophyll fluorescence (DCA-CF); DCA monitored by respiratory quotient 1.3 (DCA-RQ 1.3); and the new method of DCA monitored by the anaerobic compensation point (DCA-ACP). The level of 1.2 kPa CO_2 was adopted in all DCAstorage methods. All DCA conditions and CA+1-MCP suppressed ethylene production until 14 days of shelf life compared to CA. Hence, all DCA and CA+1-MCP conditions have lower IEC after 7 and 14 days of shelf life, lower ACC oxidase activity after 7 days of shelf life, and higher healthy fruit until 14 days of shelf life. After 21 days, DCA-RQ 1.3 and DCA-ACP maintained higher healthy fruit due lower mealiness and flesh breakdown incidence. DCA-ACP maintained higher hexyl acetate concentration compared to CA+1-MCP, DCA-CF, and DCA-RQ 1.3, but lower than CA. DCA-ACP also maintained higher thans-2-hexenal and DCA-RQ 1.3 and DCA-ACP maintained higher 2-methyl-butanal after 14 days of shelf life. DCA-ACP was efficient on apple quality maintenance, even after 21 days shelf life at 20 °C.

Key words: Apple, DCA-RQ 1.3, Shelf life, Volatile organic compounds.

[1]Artigo formatado segundo as normas da revista Scientia Horticulturae

3.1.1 Introduction

Maxi Gala is a strain Gala apple, which in turns belongs to the most cultivated apple in Brazilian orchards (Kist et al, 2016). Although Gala apples have a short harvest period that goes from the end of December until February, they can be found in the market during the entire year. Thus, CA is worldwide employed in most of the storage systems of the storage companies (Brackmann et al., 2009; Thewes et al., 2017a; Weber et al., 2012; Wright et al., 2015). Even though, there are losses of quality like flesh firmness loss (Lafer, 2008; Thewes et al., 2015b) and internal higher disorders occurrence (Thewes et al., 2015a; Weber et al., 2013a), agravated after prolongued storage. To diminish this problem, 1-methylcyclopropene (1-MCP) is applied before storage, which reduces ethylene action, hence avoids flesh firmness loss when compared to conventional CA (DeEll et al., 2007; Fawbush et al., 2009; Lafer, 2008; Lu et al., 2018; Schmidt et al., 2020; Thewes et al., 2017a). Although, 1-MCP negatively affects volatile compounds production, specially esters and alcohols (Lee et al., 2012; Thewes et al., 2015a; Yang et al., 2016).

Nowadays, aiming the apple supply on the off-season period, there is a trend to store apple under extreme low oxygen partial pressures (far under CA employed concentration), accompainish the lowest oxygen limit (LOL) tolerated by the apples, which is variable among prolongued storage. The low oxygen torage reduces apples metabolism, hence preservs fruit quality, with higher volatile compounds when compared to CA storage with 1-MCP application, but lower than conventional CA. Although, extreme low oxygen concentration damages may occure if subestimations on the ideal LOL are made, thus LOL has to be real time monitored to avoid losses by essessive anaerobic metabolism.

Methods to dynamically control the LOL tolerated by stored fruit are described in the literature (Bessemans et al., 2016; Prange et al., 2007; Veltman; Verschoor; Dugteren, Van, 2003; Weber et al., 2015; Wright et al., 2012). The most studied is the one based on chlorophyll fluorescence (DCA-CF) and the DCA based on respiratory quotient (DCA-RQ). In Brazil, DCA are less used because of the high costs and low quality of most of the old storage rooms, which still are of great number. DCA-CF is worldwide used by commercial storage companies, being responsible of 19.3% of the storage systems used in South Tyrol and 3% in the Washington State storage facilities (Prange, 2018). By DCA-CF, apples are initially exposed to declining levels of oxygen, until the stress induces a chlorophyll fluorescence spike. The oxygen in the storage atmosphere is then maintained in a static safety

level that varies from 0.20 to 0.30 kPa O_2 above the stressful oxygen concentration (Gasser et al., 2010; Prange et al., 2005; Weber et al., 2017). Basically, DCA-CF monitors the LOL through an initial low oxygen (ILO) stress and the oxygen is set at a safety concentration above the anaerobic compensation point (ACP).

More recently developed, DCA-RQ maintains higher apple quality when compared to DCA-CF and CA, and be adopted satisfactory without 1-MCP treatment (Thewes et al., 2017). In DCA-RQ the ratio between CO_2 production and the O_2 consumed by the fruit aerobic/anaerobic respiration is evaluated and, the ratio mostly employed according to research results are 1.3 and 1.5. DCA-RQ maintains higher flesh firmness, lower ethylene production, and lower ACC oxidase activity (Anese et al., 2019). DCA-RQ 1.3 and 1.5 monitor the LOL by the rate of consumed oxygen and produced carbon dioxide, maintaining the oxygen level under the ACP. This last one is supposed to have a RQ near 1.0. Although, DCA-RQ also requires an apparatus to be inserted in the chambers to determine the RQ of apples (Brackmann, 2015), due to its low tightness , which hinders the RQ calculation in the whole storage chambers.

Volatile compounds maintenance has an important weight on apple commerce with projection to rise, with an especial attention by consumers nowadays (Espino-Diaz et al., 2016a). The most apple aroma contributors are 2-methylbutyl acetate, butyl acetate, hexyl acetate and their respective alcohols precursors (Dunemann et al., 2012; Espino-Diaz et al., 2016a; Mehinagic et al., 2006; Salazar et al., 2016; Young et al., 1996).

Even that actual DCA storage methods maintain apple quality after long periods of storage when compared to conventional controlled atmosphere, especially over 8 – 9 months, the current methods available require the aquisition of apparatus to monitor the LOL. To solve this problem, we proposed a method that monitors the lowest oxygen limit tolerated by fruit through the anaerobic compensation point. The new DCA-ACP was previously tested by our research group and showed promissing results on apple quality maintenance, contrary to previous researches that found no possibility to use the ACP determination by CO_2 alone as a method to control the LOL of stored apples (Gasser et al., 2003; Prange, 2018).

After storage, apples take long periods until arriving to consumer's home, due to withdraw from chambers and afterwards, are submitted to long distances under transportation. Thus, prolonged shelf life until 21 days are supposed to represent the real conditions that apples are exposed during commercialization in the Brazilian market. The aim of this paper

was to compare the efficacy of the new storage method DCA-ACP in relation to CA, CA+1-MCP treatment, DCA-CF and DCA-RQ 1.3 on 'Maxi Gala' apple overall quality parameters and volatile compounds after 9 months of storage at 2 °C plus 7, 14, and 21 days of shelf life at 20 °C.

3.1.2 Materials and methods

3.1.2.1 Apple source and sampling

Apples of 'Maxi Gala' cultivar were harvested in a commercial orchard at Vacaria, RS, Brazil, in the 2017/2018 season and immediately transported to the Postharvest Research Center located at Santa Maria, RS, Brazil. Damaged and low size fruit were discarded. Each treatment was composed by four replicates of 25 fruit, in a completely randomized experimental design. Prior to the start of the storage, three replicates of 10 fruit were taken to characterize the initial quality and ripening stage at harvest. The iodine-starch index of the fruit was 6.88 (in a 0 to 10 scale) according to Streif (1984). The soluble solids content was 10.97 °Brix, titratable acidity 5.32 meq 100 mL^{-1}, respiration rate 3.12 µg kg^{-1} s^{-1}, ethylene production 0.21 ng kg^{-1} s^{-1}, flesh firmness 89.24 N, ACC oxidase enzyme activity 13.21 ng C_2H_4 kg^{-1} s^{-1}, and the internal ethylene concentration was 1.66 µg L^{-1}. Fruit were field treated with aminoethoxyvinylglycine (62.5 g ha^{-1} active ingredient) 30 days before harvest.

Fruit treated with 1-methylcyclopropene were placed in an 1800 L chamber at 2.0 ±0.1 °C and a solution of 1-MCP was prepared according to the manufacture directions to produce a concentration of 0.625 µL L^{-1} inside the experimental chamber. This solution was placed in Petri dishes located inside the chamber. The chamber was hermetically sealed, and the air inside the chamber was circulated with an aerator. After 24 h, the fruit were removed from the chamber and stored CA.

Afterwards, fruit of each treatment were allocated in their respective experimental chamber (230 L), and stored at 2.0 °C and 94 ±1 % relative humidity for 9 months. The following conditions were: [1] conventional controlled atmosphere (CA 1.2 kPa O_2 + 2.0 kPa CO_2); [2] CA+1-methylcyclopropene (CA+1-MCP); [3] dynamic CA (DCA) monitored by chlorophyll fluorescence (DCA-CF); [4] DCA monitored by respiratory quotient (DCARQ

1.3); and [5] DCA monitored by the new method named anaerobic compensation point. In all the DCA conditions, the CO_2 was set at 1.2 kPa.

3.1.2.2 Controlled atmosphere and dynamic CA establishment and monitoring

During the storage period, the O_2 and CO_2 partial pressure were monitored by an automatic control system (Valis®, Lajeado-RS, Brazil) and measured by a gas analyzer (Siemens®, model Ultramat, Germany). The O_2 partial pressure (pO_2) was reduced inside the experimental chambers by flushing them with N_2, until circa 5 kPa O_2 was reached. The pO_2 was decreased stepwise over five days, and then the carbon dioxide production was measured to determine the O_2 set point. The O_2 set point was determined two days per week, thus monitoring was break off two times per week during 13 hours, in order to recorder the CO_2 production and O_2 uptake, used to calculate the RQ and ACP.

Thereafter, when the pO_2 was below this set point, atmospheric air was injected into the chamber. The set point for CO_2 partial pressure (pCO_2) was obtained from apples respiration. When the level inside a chamber was above this set point, the CO_2 was removed by circulating the air of the chamber through a chamber containing calcium hydroxide. The relative humidity was maintained at 94 ± 1% during the entirely storage period by the addition of calcium chloride (7.5 g kg^{-1} of fruit) (Both et al., 2017) in the storage chamber. Figure 1 shows the oxygen set point behavior during the 9 months of storage under the different storage conditions.

Lowest oxygen limit (LOL) of dynamic controlled atmosphere chambers was monitored according to each storage system. DCA monitored by chlorophyll fluorescence was set according to manufacturer's method (Prange et al., 2007) where the chlorophyll spike was detected under 0.20 kPa O_2, thus set point was maintained at 0.40 kPa O_2. By DCA-RQ 1.3 and DCA-ACP, the O_2 set point was determined two times per week. DCA-RQ was set at 1.3 and O_2 was changed to keep this RQ constant. In such method the lowest oxygen limit was monitored through the RQ calculated, according to Brackmann (2015) and Weber, Brackmann, and Both (2015). To estimate the O_2 set point in the new method of DCA-ACP only the carbon dioxide production was mesured. The set point of each method among 9 months of storage is presented on figure 1.

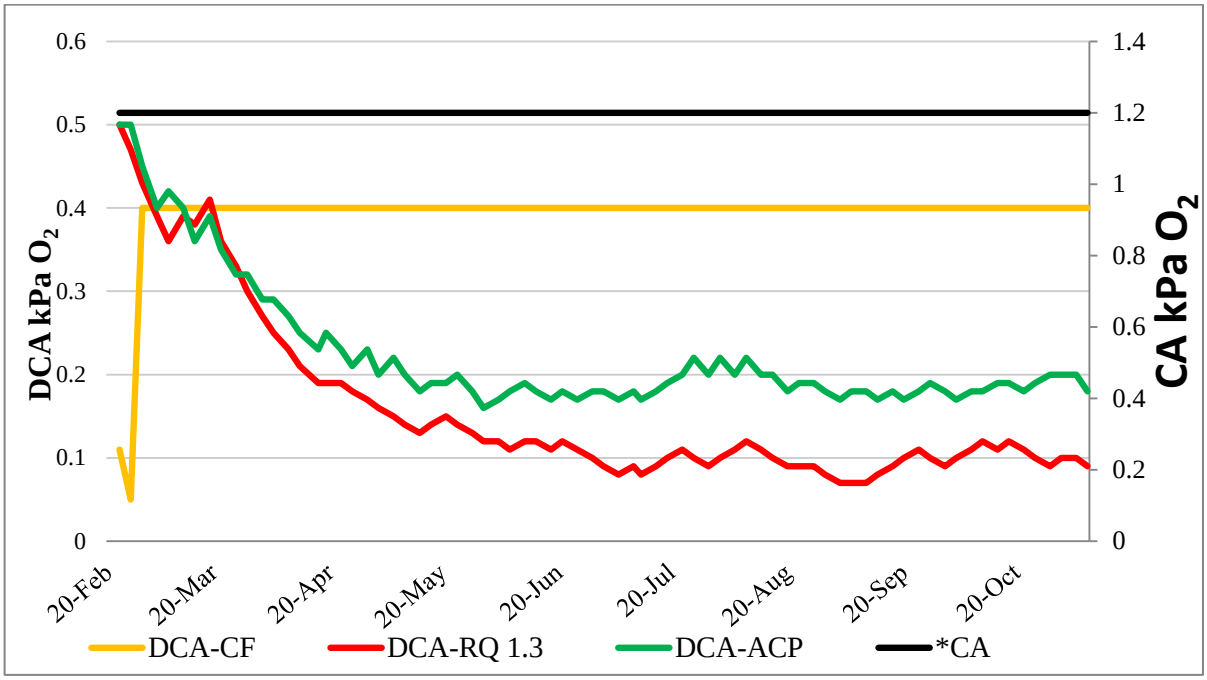


Figure 1 - Oxygen set point of 'Maxi Gala' apples during 9 months of storage under static *controlled atmosphere (CA) with 1.2 kPa O_2 + 2.0 kPa CO_2, dynamic CA (DCA) monitored by chlorophyll fluorescence (DCA-CF) with 1.2 kPa CO_2, DCA monitored by respiratory quotient (DCA-RQ 1.3) with 1.2 kPa CO_2, and the new method of DCA monitored by the anaerobic compensation point (DCA-ACP) with 1.2 kPa CO_2.

3.1.2.3 Quality analysis after 9 months of storage

After 9 months of storage, the fruit were withdrawn from the experimental chambers and held at 20 °C during 7, 14, and 21 days, to simulate the shelf life. After this period, the fruit quality was assessed as described below.

3.1.2.3.1 Ethylene production, and internal ethylene concentration (IEC)

Samples from each replication weighing approximately 1.5 kg, were placed in 5 L bottles and hermetically closed. After one hour, two samples of 1 mL of the headspace air were taken for ethylene determination, and injected into ainjector port of a Varian® gas chromatograph Star 3400CX model (Varian®, Palo Alto, CA, USA), equipped with a flame ionization detector (FID) and a Porapak N80/100 column. The injector, column and detector temperatures were 140, 90 and 200 °C, respectively, according to the method of Anese et al. (2016). Results were expressed in ng kg^{-1} s^{-1}.

Internal ethylene concentration (IEC) was determined according to methodology adapted from Mannapperuma et al. (1991). The internal air from the fruit was withdrawn using a vacuum pump at 565 mm Hg suction pressure and a closed container filled with water in which the fruit was submerged. An inverted funnel, with a septum in its thinner end, covered the fruit, allowing the air removed from it to be accumulated. Samples of this air were withdrawn using a syringe and injected into the same gas chromatograph used to determine ethylene production. Results were expressed in µg L^{-1}.

3.1.2.3.2 Aminocyclopropane-1-carboxylate (ACC) oxidase enzyme activity

ACC oxidase enzyme activity was determined based on the method proposed by Bufler (1986). A part of the skin of about 25 apples per replicate was removed (approximately 3 g), and immediately immersed for 30 min in a solution containing 0.1 mmol L^{-1}ACC and 10 mmol L^{-1} 2-(N-morpholin) ethanesulfonic acid (MES) buffer at pH 6.0. The peels were transferred into the barrel of a syringe and hermetically closed with 2 % CO_2. After further 30 min, two aliquots of 1 mL were taken from the vial of the syringe and the ethylene concentration was measured by a Varian® gas chromatograph (as described for ethylene production). Results were expressed in ng C_2H_4 kg^{-1} s^{-1}.

3.1.2.3.3 Soluble solids and titratable acidity

The juice of slices of apple flesh from the equatorial section of 25 fruits per replicate was obtained with the aid of a juice centrifuge (Philips®, Wallita). For titratable acidity, 10 mL of the juice was diluted in 100 mL distilled water and titrated with NaOH solution (0.1 N), up to pH 8.1 (Brackmann et al., 2008). The results of titratable acidity were expressed asmeq 100 mL. Soluble solids of each replicate were determined by refractometry, using 1 mL of juice, and expressed in °Brix (Brackmann et al., 2008).

3.1.2.3.4 Electrolyte leakage

Electrolyte leakage was measured according methodology proposed by Gago et al. (2015), with modifications. Then discs (5 mm of thickness and diameter) were taken from 10 diferent apples in each replicate. These discs were washed with distilled three times, to take the excessive juice and thereafter, immersed into a 30 mL distilled water for 1 h (20 ± 1 °C), afterward the conductivity of the suspension was measured with aid of a conductivity meter (ASKO® model AK51). The suspension was then placed for 30 min in a water bath at 100 °C and thereafter rapidly cooled down to 20 °C in afreezer, at -30 °C. Conductivity was measured again and taken as total leakage. Results were expressed in percentage.

3.1.2.3.5 Decay, mealiness and flesh breakdown incidence

Decay incidence was determined by counting each fruit that presented any sign of decay incidence, larger than 5 mm in diameter. The flesh breakdown was determined by slicing the 25 fruit of each replicate on the equatorial region and counting fruit that presented any sign of internal browning. As well, mealiness was determined after slicing the 25 apples of each replicate on the equatorial region and counting those that presented symptoms of meali pulp and low cell wall adherence. The number of affected fruit in each category was compared to the total number of fruit in each sample (25), and results were expressed in percentage (%).

3.1.2.3.6 Healthy fruit

The number of healthy fruit were determined by counting the fruits that did not present any apparent inner or outer physiological disorder and/or decay incidence. Results were expressed as a percentage (%).

3.1.2.3.7 Volatile compounds identification and quantification

The volatiles profile was determined after 9 months of storage following the methodology proposed by Both et al. (2014). Slices of the equatorial region of 25 apples were crushed and centrifuged at a juicer (Philips-Walitta®). Seed and endocarp were discharged, in

order to obtain the most representative juice of each sample. Immediately after extraction, the juice was put into 100 mL amber glasses and frozen at −30 °C, for later analysis. The semi-quantitative determination of volatile compounds was carried out relative to the concentration of the internal standard 3-octanol (82.2 μg mL^{-1}), as proposed by Both et al. (2014). An aliquot of 10 mL juice, 3 g sodium chloride and 10 μL of the internal standard were put inside a 20 mL vial and hermetically closed with a PTFE coated silicone lid and metal screw cap. The bottle was heated for 5 min with constant shaking in a water bath, at 35 °C. The volatiles were extracted by solid phase micro extraction (HS-SPME), by exposing a fiber (Supelco, 50/30 μm x 10 mm, covered with divinylbenzene/carboxen/polydimethylsiloxane polymers, previously preconditioned according to manufacturer's protocol), into the headspace of the vial for 60 min, while the vial remained in the water bath, at the same temperature. 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 volatile compounds were separated with a non-polar fused silica capillary column, DN-5 (30 m x 0.25 mm x 0.25 μm). Nitrogen gas was used as the carrier gas at a constant flow rate (1.0 mL min^{-1}). The initial oven temperature was 35 °C and was held for 3 min, an then the temperature gradient increased at 2 °C min^{-1} to 80 °C, and further increased at 5 °C min^{-1} to 230 °C, and remained at isothermal conditions for 5 min. The temperature of the detector was kept at 230 °C. To identify the volatile compounds, a Shimadzu QP2010 Plus gas chromatograph coupled to a mass spectrometer (GC/MS; Shimadzu Corporation, Kyoto, Japan) was used. 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 +70 eV, a scan range from m/z 35–350 and a temperature of 250 °C. Tentative identification of compounds was based on the National Institute of Standards and Technology library.

3.1.2.4 Statistical analysis

The study was carried out in a complete randomized design, in a uni factorial arrangement (storage condition). The results were submitted to analysis of variance (ANOVA). When the ANOVA result was significant ($p < 0.05$), the means were assessed using the Skott-Knott test at 5% probability error.

3.1.3 Results and discussion

3.1.3.1 Ethylene production, ACC oxidase activity and internal ethylene concentration (IEC)

ACC oxidase activity and IEC remained higher at CA stored apple after 7 days of shelf life, when compared to CA+1-MCP, DCA-CF, DCA-RQ 1.3 and DCA-ACP. Previous authors also found lower ACC oxidase activity on apples stored under DCA-CF (Both et al., 2017; Weber et al., 2015) DCA-RQ (Both et al., 2017; Thewes et al., 2017c) and 1-MCP treated apples (Schmidt et al., 2020; Thewes et al., 2018; Weber et al., 2017). The lower ACC oxidase activity of CA + 1-MCP may be related to the lower *MdACO*1 gene expression (Tsantili et al., 2007; Yang et al., 2013) and the lower oxygen availability of DCA condtions during storage, reflecting on low gene expression even if atmospheric oxygen concentration was re-stablished (Pesis, 2005). Curiously, after 14 days the ACC oxidase activity was higher at CA+1-MCP stored apple, although IEC and ethylene production remained at the lowest concentration. Probably the lower ethylene production and lower IEC of 1-MCP treated fruit is a result of the lower ACC concentration. Previous research found totally inhibition of the three identified ACC synthase genes expression of the system 2 by 1-MCP (Li et al., 2013), which could explain the lower ethylene production and lower IEC of 1-MCP treated fruit. Probably, as a result of the lower ACC concentration, even with higher ACC oxidase activity of this treatment. The raise on ACC oxidasey activity after 14 days of shelf life on CA + 1-MCP treated apple may indicate a loss of 1-MCP inhibition effect on ACC oxidase gene expression. An intermediate ACC oxidase activity was detected at CA and DCA-ACP, and lower activity was detected at DCA-CF and DCA-RQ 1.3 stored apple. After 21 days of shelf-life, CA, DCA-RQ 1.3, and DCA-ACP maintained the lowest ACC oxidase activity, but only DCA-ACP maintained the lower IEC, although no difference was found on ethylene production after 20 days of evaluation, only CA+1-MCP maitained lower production.

In an overview, DCA-ACP maintained an intermediate ACC oxidase activity during three weeks under shelf life and lower IEC. CA + 1-MCP did not inhibit ACC oxidase activity after 14 and 21 days of shelf-life, although suppressed IEC and ethylene production. Probably as an effect of the lower ACC synthase (*MdACS1*) genes expression (Tatsuki et al., 2007; Wakasa et al., 2006), which reduces the ACC availability, hence ethylene production was suppressed.

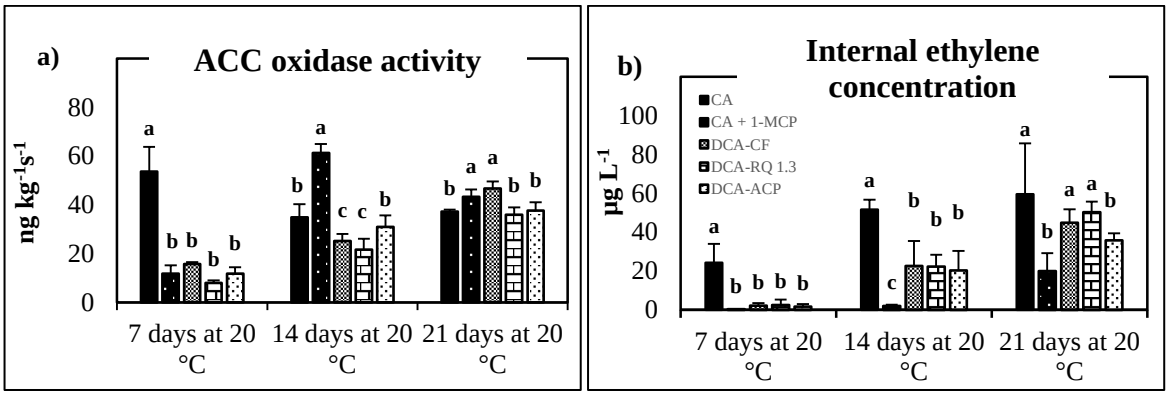


Figure 2 - ACC oxidase enzyme activity (a) and internal ethylene concentration (b) of 'Maxi Gala' apples after 9 months storage plus 7, 14 and 21 days of shelf-life at 20 °C. Means followed by the same letter at the same day, do not differ by the Scott-Knott test, at 5 % probability ($p>0.05$). Controlled atmosphere (CA) with 1.2 kPa O_2 + 2.0 kPa CO_2; 1-MCP: 1-methylcyclopropene; dynamic controlled atmosphere (DCA) monitored whether by chlorophyll fluorescence (CF); respiratory quotient (RQ); anaerobic compensation point (ACP). Initial ACC oxidase activity 13.21 ng C_2H_4 $kg^{-1}s^{-1}$ internal ethylene concentration 1.66 µg L^{-1}.

After the nine months of storage, apples were taken from the experimental chambers and placed in another room, at 20 °C and ambient O_2, during 20 days, in order to simulate prolongued shelf life. Every two days, the ethylene production was determined. Interesting fact is that CA+1-MCP and all DCAs conditions produced less ethylene than CA and did not statistically differ between them until the 14th day of measurement. Such result suggest that the lower oxygen partial pressure of this conditions and 1-MCP treatment have an effect on ACC oxidase (Pesis, 2005) and ACC synthase activity. Until 14 days of shelf life apples could be stored under any of this storage conditions. In DCA-ACP storage, it is not necessary the adoption of technical apparatus like in DCA-RQ 1.3 and DCA-CF and no high costs with 1-MCP treatment. CA+1MCP stored apple maintained lower ethylene production after 18 and 20 days of shelf life under 20 °C. This can be explain due to the 1-MCP irreversibly linkage on the ethylene receptors, and acts by blocking the ethylene perception (Sisler and Serek, 1997).

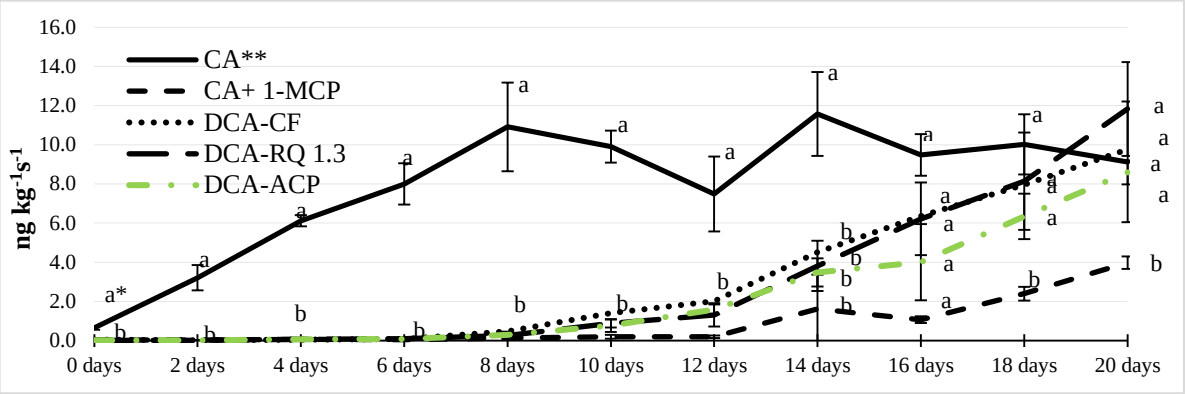


Figure 3 - Ethylene production of 'Maxi Gala' apples during 20 days at 20 °C after 9 months storage.*Means followed by the same letter at the same day, do not differ by the Scott-Knott test, at 5 % probability ($p>0.05$). **Controlled atmosphere (CA) with 1.2 kPa O_2 + 2.0 kPa CO_2; 1-MCP: 1-methylcyclopropene; dynamic controlled atmosphere (DCA) monitored whether by chlorophyll fluorescence (CF); respiratory quotient (RQ) with 1.2 kPa CO_2; anaerobic compensation point (ACP) with 1.2 kPa CO_2. Initial ethylene production 0.21 ng $kg^{-1}s^{-1}$.

3.1.3.2 Flesh firmness, soluble solids, titratable acidity and electrolyte leakage

Flesh firmness, soluble solids content and titratable acidity in harmony compose a part of the sensory attributes, which have an important role on the fruit acceptability (Roger Harker et al., 2002). At harvest, the 'Maxi Gala' apples have 89.24 N of flesh firmness. After 9 months of storage plus 7 days of shelf life, apples stored under CA + 1-MCP presented 74.09 N flesh firmness. Sudgesting that even the 1-MCP has no effect on apple flesh firmness maintenance at this year. At the first week of evaluation, when compared to CA+1-MCP, the DCA-RQ 1.3 maintained lower flesh firmness, but higher than CA, DCA-CF, and DCA-ACP. The DCA-ACP and DCA-CF condition did not differ between them, but maintained lower flesh firmness when compared with CA+1-MCP and DCA-RQ 1.3, and higher than CA. This result demonstrates that the lower oxygen partial pressure of DCA-RQ 1.3 has an effect on lower ethylene production (Fig. 3), thus may reduce cell wall enzyme activity reducing the cell wall degradation (Nishiyama et al., 2007), resulting in better flesh firmness maintenance.

Apples stored under CA+1-MCP also maintained higher flesh firmness after 14 and 21 days of shelf life, probably as a result of the lower cell wall degradation enzyme activity as an effect of the 1-MCP, although did not differ from DCA-RQ 1.3 after 14 days of shelf life. DCA-ACP maintained lower flesh firmness after 14 and 21 days of shelf life, when compared to CA+1MCP and DCA-RQ 1.3, but higher than DCA-CF and CA. CA without 1-MCP treatment maintained the lowest flesh firmness during the 21 days of evaluation, together with

DCA-CF on the 21th day of shelf life. Previous reports also found apple firmness loss avoidance when the fruit was stored under DCA-RQ compared to CA (Bessemans et al., 2016).

After 21 days of shelf life, CA+1-MCP maintained the higher flesh firmness. DCA-RQ lower but higher than DCA-ACP, DCA-CF and CA. The static and higher than the other DCA methods oxygen concentration of DCA-CF (Fig. 1) was not efficient to maintain flesh firmness after 21 days of shelf life when compared to DCA-ACP, DCA-RQ and CA with 1-MCP treatment (Fig. 3a).

Titratable acidity was higher on apples stored under CA, CA+1-MCP, and DCA-CF, while DCA-RQ and DCA-ACP maintained lower acidity after 7 days of shelf life (Fig.3b). Also, on electrolyte leakage, CA maintained the highest percentage, while CA+1-MCP, DCA-RQ, and DCA-ACP maintained an intermediate electrolyte leakage, and DCA-CF the lowest. Both titratable acidity and electrolyte leakage did not differ after 14 and 21 days of shelf life under 20 °C among all the storage conditions. Total soluble solids content did not differ among all the storage conditions between all the shelf life evaluations (Fig. 3c).

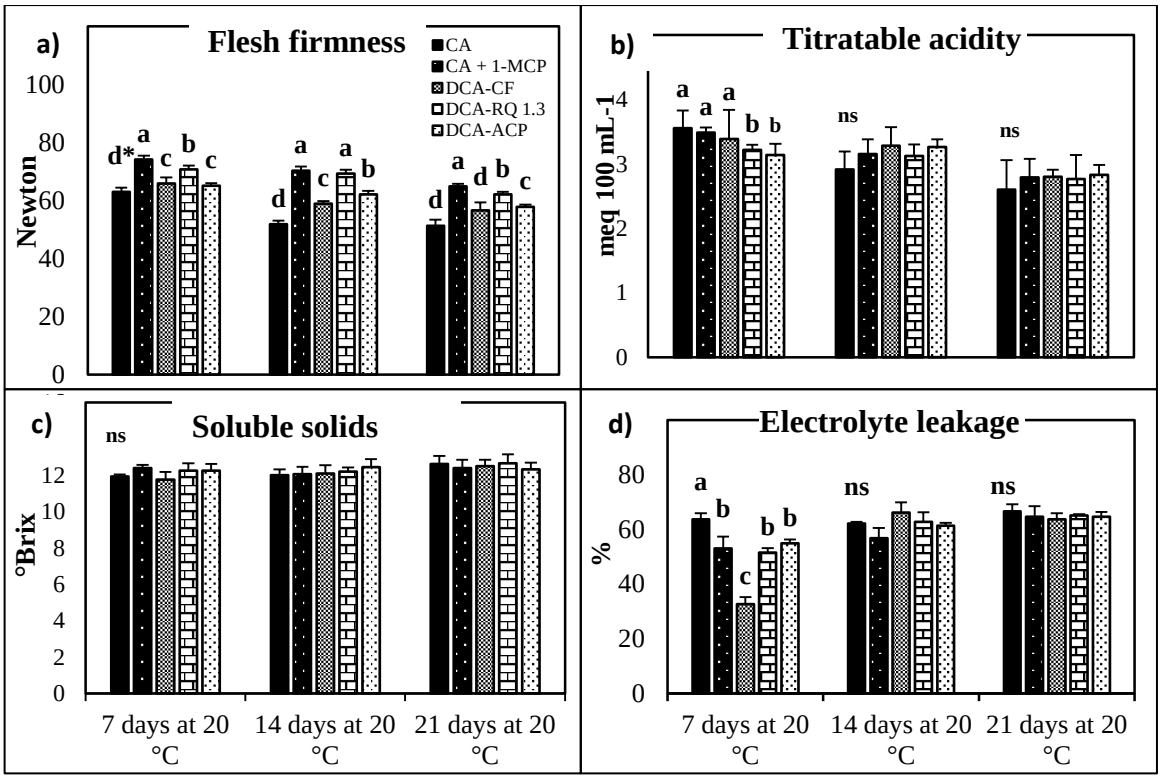


Figure 4 - Flesh firmness (a), soluble solids (b), titratable acidity (c) and electrolyte leakage (d) of 'Maxi Gala' apples after 9 months storage plus 7, 14 and 21 days of shelf-life at 20 °C. *Means followed by the same letter at the same day evaluation, do not differ by the Scott-Knott test, at 5 % probability ($p>0.05$). **Controlled atmosphere (CA) with 1.2 kPa O_2 + 2.0 kPa CO_2; 1-MCP: 1-methylcyclopropene; dynamic controlled

atmosphere (DCA) monitored whether by chlorophyll fluorescence (DCA-CF); respiratory quotient (RQ) with 1.2 kPa CO_2; anaerobic compensation point (ACP) with 1.2 kPa CO_2. Initial flesh firmness 89.24 N, soluble solids 10.97 %, titratable acidity 5.32 meq 100 mL^{-1} and electrolyte leakage 44.29 %. NS: not significant.

3.1.3.4 Healthy fruit, mealiness incidence, flesh breakdown, and decay incidence

Apples with mealiness incidence exhibit absence of juice, due middle layer adherence loss (Both et al., 2016). Mealiness incidence was low at the first week of evaluation, when CA, CA + 1-MCP, and DCA-RQ presented 1 % incidence, although there was no difference among all the storage conditions. However, after 14 days the static CA showed the highest mealiness incidence, reaching more than 31 % incidence in average, whilst DCA-RQ 1.3 have no mealiness incidence (Fig. 5a). The CA also showed higher mealiness incidence after 21 days of shelf life, CA+1-MCP and DCA-CF intermediate incidence, and DCA-RQ 1.3 and DCA-ACP the lowest incidence. Middle layer is mainly formed by pectin (Both et al., 2016). Pectin is degraded by ethylene dependent pectinmethylesterases (Nishiyama et al., 2007; Payasi et al., 2009; Prasanna et al., 2007). Curiously, the ethylene production of DCAs and CA+1-MCP differed only after 18 days of shelf life, although DCA-RQ presented lower mealiness incidence after 14 and 21 days of shelf life (Fig. 5a).

Another apple flesh disorder of great importance is flesh breakdown, which causes internal browning, because of low membrane integrity, and its loss. At this paper, no incidence was detected at the first week of evaluation, although after 14 of shelf life apples stored under CA presented the higher incidence (12%), compared with all the other storage conditions (Fig. 5b). The higher flesh breakdown may be related to the more advanced fruit ripening caused by prolonged CA storage (Castro et al., 2007; Winkinson and Fidler, 1973). After 21 days of shelf life, there was no difference among all the storage conditions. Also decay incidence did not differ among all the storage conditions and on all shelf life evaluations (Fig. 5c).

Total healthy fruit did not differ among all the storage conditions after 7 days of shelf life. However after 14 days, all DCA conditions and CA+1-MCP maintained higher amount of healthy fruit, demonstrating that lower oxygen partial pressure and 1-MCP treatment used during CA storage maintained higher healthy fruit (Fig. 5d). This may be a result of the lower ethylene production reported I these storage conditions (Fig. 3). Previous reports also found higher healthy fruit when storage was carried out in DCA-CF, DCA-RQ 1.5, and DCA-RQ

2.0 compared with CA of ‘Fuji Suprema’ apples (Thewes et al., 2017c). After 21 days of shelf life only DCA-RQ and DCA-ACP maintained higher healthy fruit and even 1-MCP treatment (in CA storage) was not effective in retain higher healphy fruit amount in prolonged shelf life (Fig. 5d). This is an important founding, once in Brazilian market it is reported that apples need around three weeks to arrive at some consumer’s home (*personal information*).

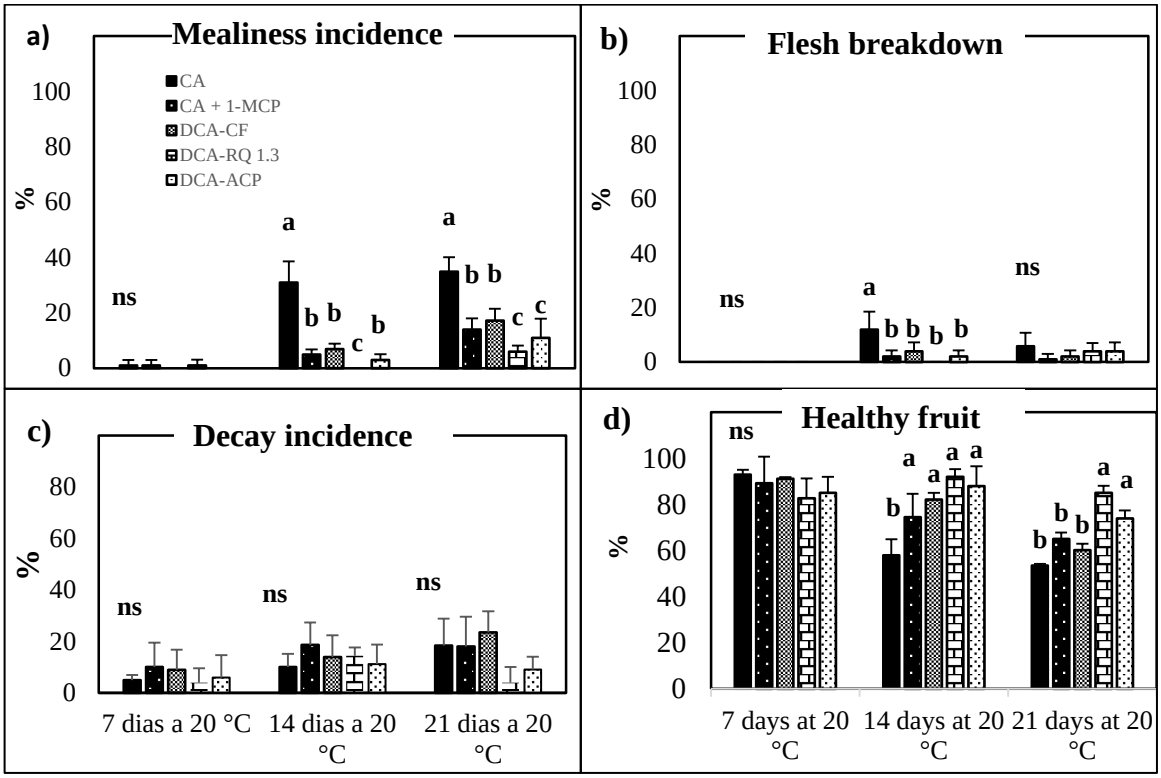


Figure 5 - Healthy fruit (a), mealiness incidence (b), flesh breakdown (c), and decay incidence (d) of 'Maxi Gala' apples after 9 months storage plus 7, 14 and 21 days of shelf-life at 20 °C. Means followed by the same letter at the same day evaluation, do not differ by the Scott-Knott test, at 5 % probability ($p>0.05$). Apples were stored under static controlled atmosphere (CA) with 1.2 kPa O_2 + 2.0 kPa CO_2; CA treated with 1-methylcyclopropene (1-MCP); dynamic controlled atmosphere (DCA) monitored whether by chlorophyll fluorescence (DCA-CF); respiratory quotient (RQ) with 1.2 kPa CO_2; Anaerobic compensation point (ACP) with 1.2 kPa CO_2. Apples assessment was conducted after 7, 14, and 21 days of shelf life. NS: not significant.

3.1.3.5 Volatile compounds analysis

3.1.3.5.1 Aldehydes

Aroma volatiles compounds contribute to the fruit flavor and are of great importance on fruit quality, with a special attention nowadays (Espino-Diaz et al., 2016b). Thus, volatile profile of the juice extracted from the fruit was evaluated after storage plus 7, 14, and 21 of shelf life. Total and main aldehyde, alcohols, and esters were determined for each storage condition and evaluation time. At the first week of evaluation, there was no difference among the storage conditions on total aldehydes concentration. After 14 days of shelf life, all DCAs conditions and CA+1-MCP maintained higher total aldehydes concentration compared to CA

(Fig. 6a). The higher total aldehydes concentration of DCAs conditions, at this evaluation period, is related to Z-2-hexenal which was in great amount and differed from CA and CA+1-MCP (Fig 6d). Z-2-hexenal has a ‘green’ odor (Lurie et al., 2002) and was related to ‘desirable essence’ in a previous sensorial analyze carried out with commercial apples (Petró-Turza et al., 1986). The higher total aldehydes of CA+1-MCP compared to CA stored apples, may be related to the sum of Z-2-hexenal and hexanal concentration (Fig. 6i). Apples stored under CA+1-MCP and DCA-CF have higher hexanal concentration after 14 days of shelf life. Hexanal have a ‘green apple’ descriptive odor (Mehinagic et al., 2004), and in greater concentration, both hexanal and Z-2-hexenal may remaind unripe apple (Flath et al., 1967; Rizzolo et al., 1989). Although in th present study, the rise of hexanal concentration with prolonging shelf life may be related to the membrane degradation, resulting in linoleic fatty acid degradation (Contreras et al., 2016). Probably because of the advance in fruit ripening which increases lipids degradation (Contreras et al., 2016). Thus, it is suggested that the higher Z-2-hexenal of DCA stored apple and higher hexanal of CA and CA+1-MCP stored apples concentrations have little relation to unripe apple, but to the onset of lipid fraction degradation, thus being concentration dependent to be meaning as green apple.

After 21 days of shelf life, CA have a pull in total aldehydes concentration probably because the higher hexanal concentration (Fig. 6i) corroborating with Contreras; Tjellström; Beaudry (2016), who linked the rise of hexanal to lipid degradation with advance in fruit ripening. However, CA+1-MCP also maintained similar levels of total aldehydes concentration which, in turn, resulted from the higher Z-2-hexenal concentration (Fig. 6d).

E-2-Hexenal is another important aroma volatile compound found in apple (Contreras and Beaudry, 2013; Espino-Diaz et al., 2016), which contributes to apple aroma intensity (Espino-Diaz et al., 2016), and as precursor of 1-hexanol and hexyl esters (Rowan et al., 1999). At the first week of evaluation, E-2-hexenal was found in higher concentration in DCA-ACP stored apple (Fig. 6c). Contrarily, BOTH et al (2017) assessing ‘Royal Gala’ apple volatile profile after 9 months of storage under 1.0 °C found higher E-2-hexenal concentration on apple stored under CA compared to DCA-RQ 1.5 and DCA-RQ 2.0, but did not differ from DCA-CF. Demonstrating that there may be a year or cultivar dependent response on E-2-hexenal concentration when comparing the CA with DCA technologies. After 14 days of shelf life E-2-hexenal concentration was reduced in all storage conditions, especially on CA stored apples which have the lowest concentration of E-2-hexenal (Fig.6c). The reduction of E-2-hexenal is related to the transition of pre-climacteric to ripening (Vallat et al., 2005) of

the apples assessed after 7 and 14 days of shelf life. E-2-hexenal concentration 21 days of shelf life did not differ among all the storage conditions.

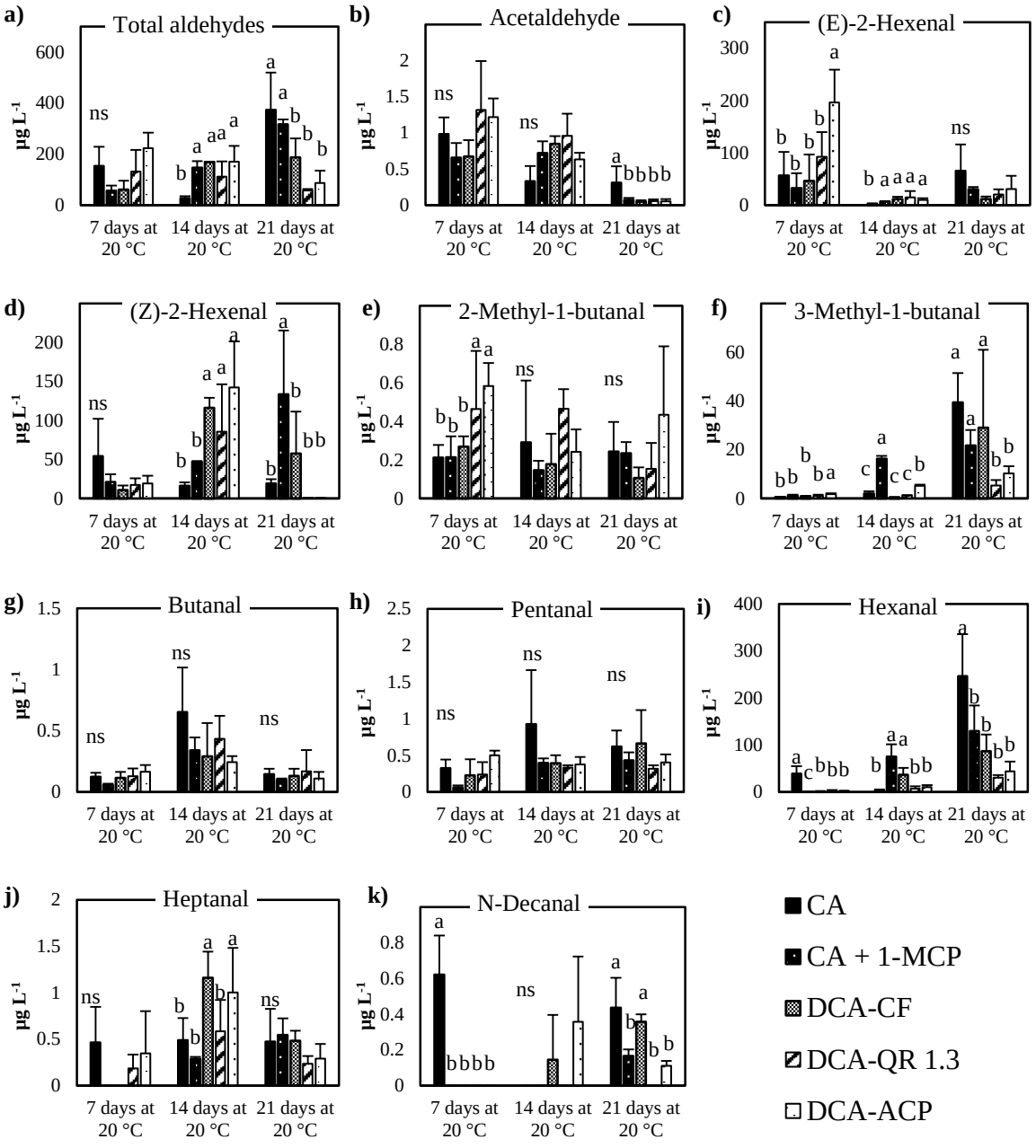


Figure 6 - Aldehydes of 'Maxi Gala' apples after 9 months storage plus 7, 14 and 21 days of shelf life at 20 °C, under five conditions: [1] static controlled atmosphere (CA); [2] CA with 1-methylcyclopropene treatment (CA+1-MCP); [3] dynamic CA (DCA) monitored by chlorophyll fluorescence (DCA-CF); [4] DCA monitored by respiratory quotient (DCA-RQ); and [5] DCA monitored by the new method monitored by the anaerobic compensation point (DCA-ACP). Mainly: total aldehydes (a), acetaldehyde (b), trans-2-hexanal (c), 2-hexanal (d), 2-methyl-1-butanal (e), 3-methyl-1-butanal (f), butanal (g), pentanal (h), hexanal (i), heptanal (j) and N-decanal (k).

3.1.3.5.2 Alcohols

Only four alcohols were identified by GC-MS: ethanol, 2-methyl-1-butanol, 1-hexanol and 6-methyl-5-hepten-2-ol. Total alcohols concentration was higher in CA, DCA-RQ 1.3, and DCA-ACP stored apple (Fig.7a), which may be related to the higher 2-methyl-1-butanol (Fig.7c) and 1-hexanol concentration (Fig.7d). The 2-methyl-1-butanol is a branched-chain alcohol (Espino-Diaz et al., 2016b) and, its precursor, the 2-methyl-1-butanal, derive from isoleucine (Hansen and Poll, 1993; Kochevenko et al., 2012). This last one, was found in higher concentration on DCA-RQ 1.3 and DCA-ACP (Fig. 6e) and lower in CA stored apples at the first week of evaluation.. Probably, the alcohol acyl transferase (AAT) enzyme activity, which catalyzes the reaction of esters formation, resulted in higher 2-methylbutyl acetate (Fig. 8f) on CA stored apple. Considering that at the first week of evaluation 2-methyl-1-butanol was found in higher concentration in CA, DCA-RQ 1.3, and DCA-ACP stored apple (Fig. 7c), and lower 2-mehylbuyl acetate in DCA-RQ 1.3 and DCA-ACP, there may be a low oxygen restriction on AAT activity. Previous authors found higher 2-methyl-1-butanol on ultra-low oxygen (ULO) storage with 0.70 kPa O_2 of 'Royal Gala' apples (Both et al., 2014), although 2-methyl-1-butanol was found in lower concentration on ULO with 0.50 kPa O_2. At the other hand, other authors studing 'Royal Gala' and 'Braeburn' did not found difference on 2-methyl-1-butanol concentration between apples stored under CA and DCAs conditions (Both et al., 2017; Schmidt et al., 2020).

After 14 days of shelf life, CA+1-MCP, DCA-CF, and DCA-ACP maintained higher 2-methyl-1-butanol concentration (Fig. 7c), but only DCA-CF and DCA-ACP maintained higher 2-methylbutyl acetate (Fig.8f), which may evidence the AAT activity restriction by 1-MCP treated fruit due low ethylene production (Thewes et al., 2015a). After 21 days of shelf life there was no difference among all the storage conditions in relation to 2-methyl-1-butanol concentration (Fig. 7c), nor on 2-methylbutyl acetate (Fig. 8f).

1-Hexanol was detected in higher concentration on CA, CA+1-MCP, DCA-RQ 1.3 and DCA-ACP stored apple after 7 days of shelf life (Fig.7d). The lower concentration of DCA-CF may be related to the lower precursor, the hexanal (Fig. 6i). 1-hexanol, is hexyl acetate precursor and both have a great impact in the apple aroma (Dunemann et al., 2012; Espino-Diaz et al., 2016a; Mehinagic et al., 2006). Thus, the lower 1-hexanol concentration would reflect in lower apple aroma perception of DCA-CF stored apples, although hexyl acetate concentration from fruit storesd under DCA-CF did not differed from CA+1-MCP and

DCA-RQ 1.3 (Fig. 8i). After 14 and 21 days of shelf life no difference was found among all the storage conditions on 1-hexanol concentration.

Ethanol is the ethyl acetate precursor, this last one is an important off-flavor marker of apple aroma composition (Wright et al., 2015). Thus, the higher ethanol concentration may result in higher ethyl acetate concentration. However, in this study the concentration of ethanol did not differe between all the storage conditions after 7 and 14 days of shelf life. After 21 days, lower concentration was found in DCA-RQ 1.3 stored apples. Although at all the storage conditions ethanol was found in low concentration, far bellow the OTH (100,000 µg L^{-1}) (Leffingwell and Leffingwell, 1991). Indicating that apples stored under DCA-ACP may not be subject to high ethanol accumulation.

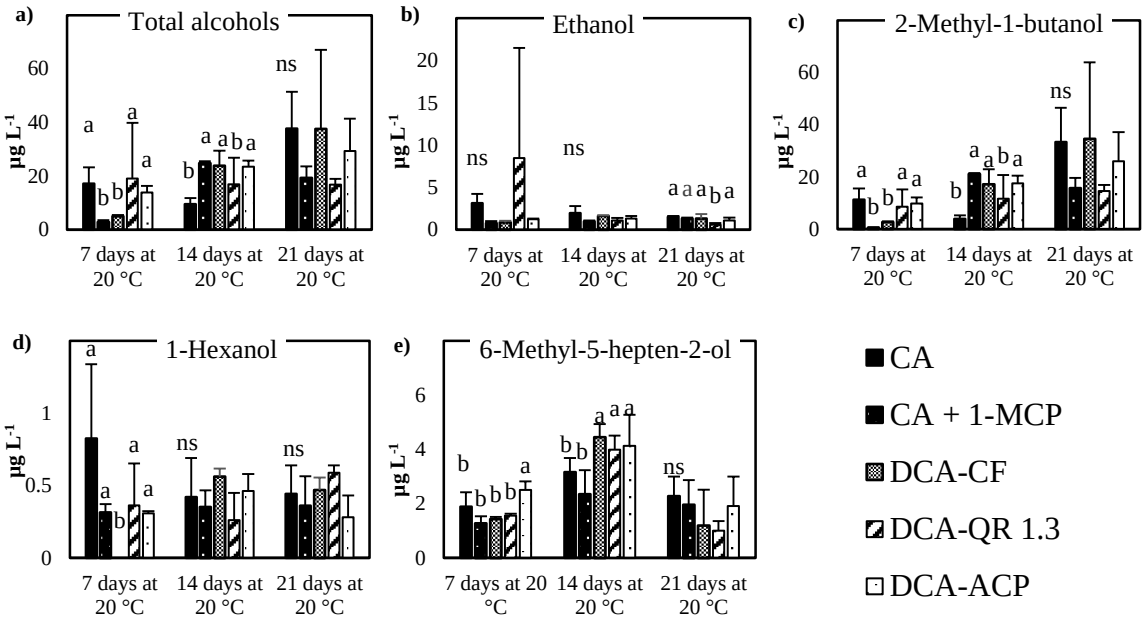


Figure 7 - Alcohols of 'Maxi Gala' apples after 9 months storage plus 7, 14 and 21 days of shelf life at 20 °C, under five conditions: [1] static controlled atmosphere (CA); [2] CA with 1-methylcyclopropene treatment (CA+1-MCP); [3] dynamic CA (DCA) monitored by chlorophyll fluorescence (DCA-CF); [4] DCA monitored by respiratory quotient (DCA-RQ); and [5] DCA monitored by the new method monitored by the anaerobic compensation point (DCA-ACP). Mainly: total alcohols (a), ethanol (b), 2-methyl-1-butanol (c), 1-hexanol (d) and 6-methyl-5-hepten-2-ol (e).

3.1.3.5.3 Esters

At this research, 13 esters were identified from 'Maxi Gala' apples at all the storage conditions. Total esters were found in higher concentration on apples stored under static CA after 7 days of shelf life, which was reduced with 1-MCP application or DCA storage.

Thewes *et al.* (2017) also found lower total ester concentration on apples stored under CA+1-MCP, but no difference between CA and DCA-RQ 1.3 of 'Galaxy' apple after 9 months of storage and 7 days shelf life of unripe and ripe stage harvested fruit. In that research, higher ester concentration on apple stored under DCA-RQ 1.3 was related to ethyl acetate, an anaerobic metabolism related volatile. At the present paper, the higher total ester concentration of apple in CA storage is related to 2-methylbutyl acetate (Fig. 8f) and hexyl acetate (Fig. 8i) esters, which are two of the three major apple aroma contributors (Salas et al., 2011; Young et al., 1996). Lower ethyl acetate was quantified at most of the storage conditions and over the three weeks of evaluation. After 14 days, total esters were found in higher concentrations in apple stored under CA+1-MCP, DCA-CF and DCA-ACP, which higher total esters concentration is related to 2-methylbutyl acetate, 3-methylbutyl acetate, and hexyl acetate, two branched chain esters and one straight chain esters respectively.

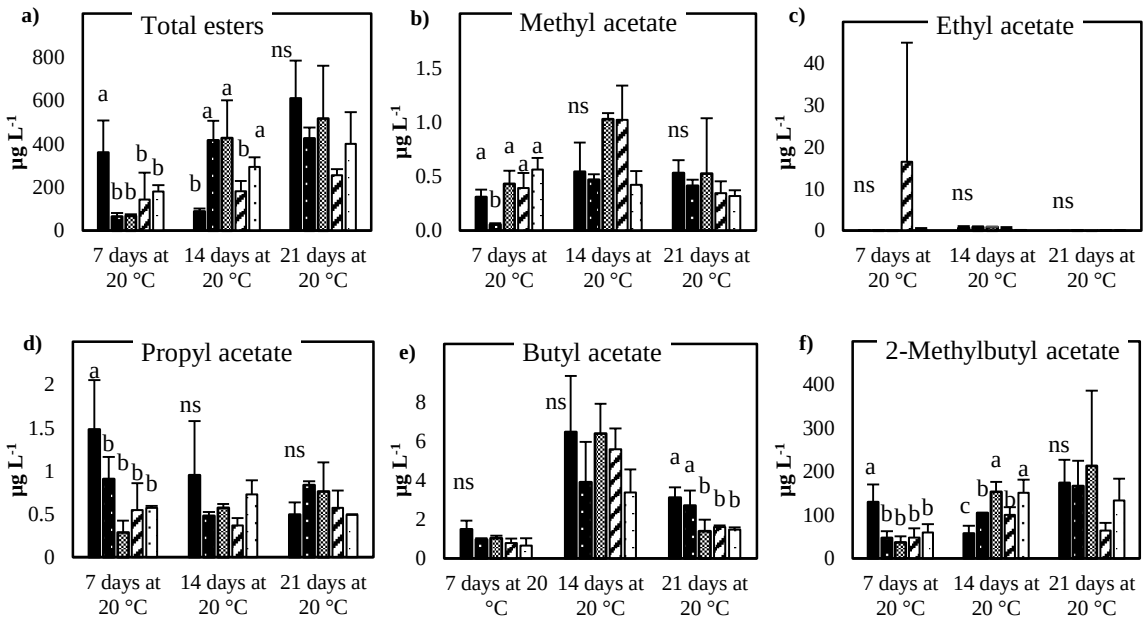
The most important esters of apple aroma are 2-methylbutyl acetate (11 μg kg^{-1} odor threshold (OTH)), hexyl acetate (2 μg kg^{-1} OTH), and butyl acetate (66 μg kg^{-1} OTH) (Dunemann et al., 2012; Espino-Diaz et al., 2016a; Mehinagic et al., 2006; Salazar et al., 2016; Young et al., 1996). Considering the OTH, 2-methylbutyl acetate and hexyl acetate were of great impact on aroma perception being far above the limit, spetially 2-methylbutyl acetate (Fig. 8f and 8i). At the first week, CA showed the highest 2-methylbutyl acetate concentration. Curiously, the precursor 2-methylbutanol was found in higher concentration in CA, DCA-RQ 1.3, and DCA-ACP stored apples. 2-methylbutanol's precursor, the 2-methylbutanal was found in higher concentration in DCA-RQ 1.3 and DCA-ACP stored apples. The result obtained with lower 2-methylbutyl acetate in DCA-RQ 1.3 and DCA-ACP stored apples suggest that the lower concentration may not be related to the precursor viability, but probably lower AAT activity, as proposed by a previous research (Both et al., 2017). AAT is ethylene dependent enzyme (Defilippi et al., 2005), which activity may be reduced in DCA-RQ 1.3 and DCA-ACP due lower ethylene production (Fig. 3). One week later, DCA-CF and DCA-ACP have the highest 2-methylbutyl acetate concentration after 14 days of shelf life, whereas, apples stored under CA showed the lowest concentration and CA+1-MCP and DCA-RQ 1.3 intermediate concentration. After 21 days of shelf life, no difference was found among the storage conditions on 2-methylbutyl acetate concentration (Fig. 8f).

After 7 days of shelf life, higher hexyl acetate concentration was found in CA stored apple, while DCA-ACP have an intermediate hexyl acetate concentration and CA+1-MCP,

DCA-CF and DCA-RQ 1.3 the lowest (Fig. 8i). Concerning to previous researches, that also found lower hexyl acetate concentration on apple stored under DCA-CF, DCA-RQ 1.5 and DCA-RQ 2.0 in ‘Royal Gala’ and ‘Fuji Suprema’ apples when compared to CA (Both et al., 2017; Thewes et al., 2017c). Both *et al.* (2017) correlated hexyl acetate lower concentration to hexanal once, the same was demonstrated by this research. Ester’s precursors are alcohol, which in turn derives from aldehydes (Espino-Diaz et al., 2016a).

Butyl acetate concentration did not differ among all the treatments at the first and second week of evaluation (Fig. 8e), probably as a response to the butanal (Fig. 6g), which also did not differed at the two first week of evaluation. As the butyl acetate concentration did not differ, there may be a precursor availability limiting effect, once neither the treatments influenced on butyl acetate concentration nor the precursor’s availability (Both et al., 2017; Schmidt et al., 2020). After 21 days of shelf life higher butyl acetate was identified on CA and CA+1-MCP stored apple.

Curiously, in this research the anaerobic volatile compounds markers responsible of the off-flavor development in higher concentrations, the ethyl acetate, ethanol, and acetaldehyde (Wright et al., 2015) were not found in great concentration, neither differed among the storage conditions at the two first weeks of evaluation (Fig. 6b, 7b, and 8c). Only after 21 days of shelf life the acetaldehyde was found in higher concentration in CA stored apple and DCA-RQ 1.3 maintained lower ethanol concentration.



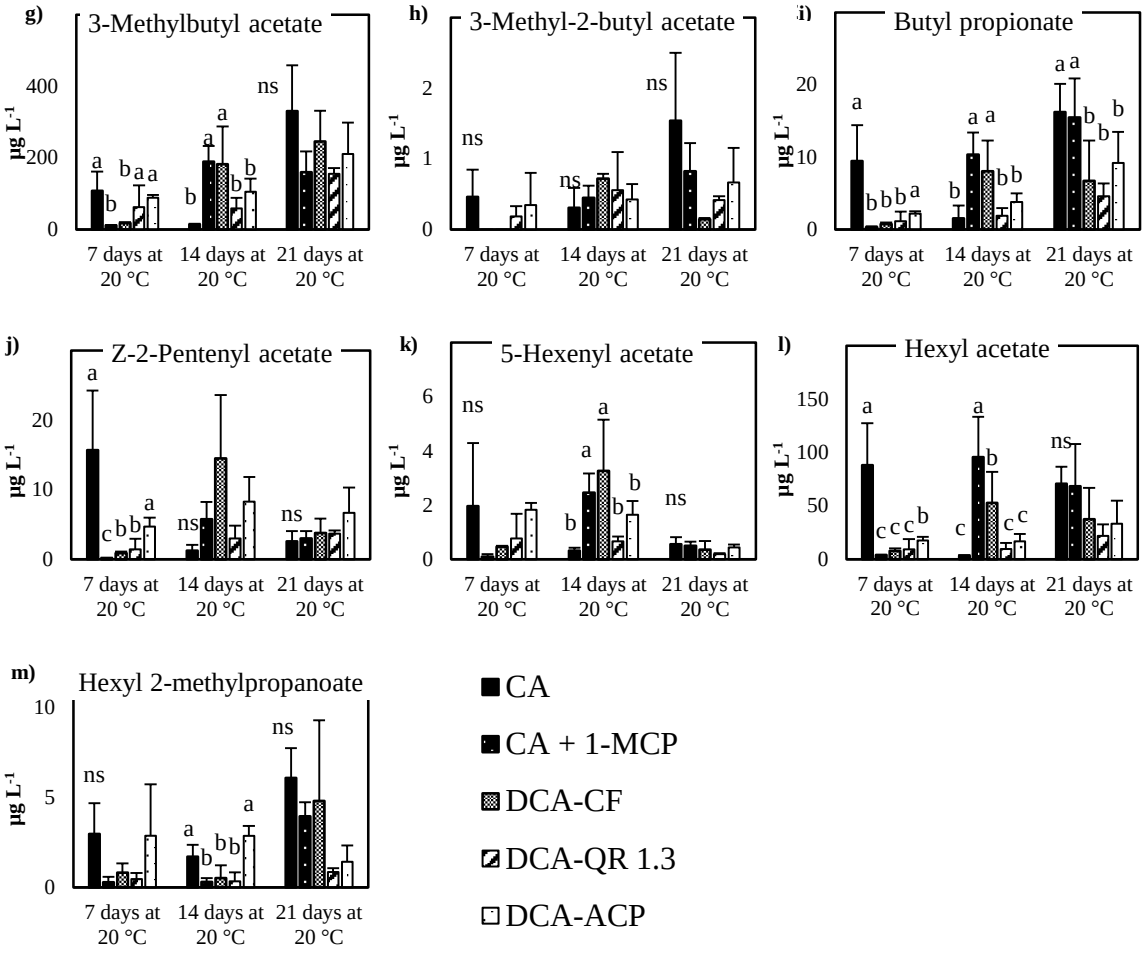


Figure 8 - Esters of 'Maxi Gala' apples after 9 months storage plus 7, 14 and 21 days of shelf life at 20 °C, under five conditions: [1] static controlled atmosphere (CA); [2] CA with 1-methylcyclopropene treatment (CA+1-MCP); [3] dynamic CA (DCA) monitored by chlorophyll fluorescence (DCA-CF); [4] DCA monitored by respiratory quotient (DCA-RQ); and [5] DCA monitored by the new method monitored by the anaerobic compensation point (DCA-ACP). Respectively: total esters (a), methyl acetate (b), ethyl acetate (c), propyl acetate (d), butyl acetate (e), 2-methyl-1-butyl acetate (f), 3-methyl-1-butyl acetate (g), 3-methyl-2-butyl acetate (h), butyl propionate (i), Z-2-pentenyl acetate (j), 5-hexenyl acetate (k), hexyl acetate (l) and hexyl 2-methylpropanoate (m).

3.1.4 Conclusion

All DCA conditions suppressed ethylene production until 14 days of shelf life compared to CA no differing from CA+1-MCP. Hence, CA+1-MCP and all DCA conditions have lower IEC after 7 and 14 days of shelf life, lower ACC oxidase activity after 7 days of shelf life, and higher healthy fruit until 14 days of shelf life. After 21 days, DCA-RQ 1.3 and

DCA-ACP maintained higher healthy fruit due lower mealiness and flesh breakdown incidence.

After 7 days of shelf life, CA stored apple maintained higher 2-methylbutyl acetate and hexyl acetate concentration. DCA-ACP maintained higher hexyl acetate concentration compared to CA+1-MCP, DCA-CF, and DCA-RQ 1.3, but lower than CA. DCA-ACP also maintained higher *thans*-2-hexenal, and DCA-RQ 1.3 and DCA-ACP maintained higher 2-methyl-butanal after 14 days of shelf life.

DCA-ACP was efficient in maintaining overall 'Maxi Gala' apple quality after 7 and 14 days, even after 21 days of shelf life. Thus, the new method of apple storage is recommended to monitor apple storage after long periods and prolonged shelf life.

3.1.5 References

Altisent, R., Graell, J., Lara, I., Lopez, L., Echeverría, G., 2008. Regeneration of volatile compounds in Fuji apples following ultra low oxygen atmosphere storage. J. Agric. Food Chem. 56, 8490–8497.

Anese, R. de O., Brackmann, A., Thewes, F.R., Schultz, E.E., De Gasperin, A.R., 2016. Mass loss by low relative humidity increases gas diffusion rates in apple flesh and allows the use of high CO_2 partial pressures during ultralow O_2 storage. Sci. Hortic. (Amsterdam). 198, 414–423. https://doi.org/10.1016/j.scienta.2015.12.015

Anese, R.D.O., Brackmann, A., Wendt, L.M., Rodrigo, F., Schultz, E.E., Ludwig, V., Berghetti, M.R.P., 2019. Interaction of 1-methylcyclopropene, temperature and dynamic controlled atmosphere by respiratory quotient on 'Galaxy' apples storage. Food Packag. Shelf Life 20, 100246. https://doi.org/10.1016/j.fpsl.2018.07.004

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.D.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., 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

Both, V., Thewes, F.R., Brackmann, A., Ferreira, D. de F., Pavanello, E.P., Wagner, R., 2016. Effect of low oxygen conditioning and ultralow oxygen storage on the volatile profile, ethylene production and respiration rate of ‘Royal Gala’ apples. Sci. Hortic. (Amsterdam). 209, 156–164. https://doi.org/10.1016/j.scienta.2016.06.028

Brackmann, A., 2015. Control apparatus for controlled atmosphere cells for storing perishable items. US2015/0257401A1.

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/https://doi.org/10.21273/JASHS.118.2.243

Brackmann, A., Weber, A., Both, V., 2015. CO_2 partial pressure for respiratory quotient and HarvestWatch™ dynamic controlled atmosphere for ‘Galaxy’ apples storage. Acta Hortic. 1079, 435–440.

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., Weber, A., Sestari, I., Peterle, M.E., Both, V., Pavanello, E.P., Vilela Pinto, J.A., 2009. Manejo do etileno e sua relação com a maturação de maçãs ‘Gala’ armazenadas em atmosfera controlada. Bragantia 68, 519–525. https://doi.org/10.1590/S0006-87052009000200027

Bufler, G., 1986. Ethylene-promoted conversion of 1-aminocyclopropane-1-carboxylic Acid 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

Castro, E. De, Barrett, D.M., Jobling, J., Mitcham, E.J., 2008. Biochemical factors associated with a CO_2-induced flesh browning disorder of Pink Lady apples 48, 182–191. https://doi.org/10.1016/j.postharvbio.2007.09.027

Castro, E. De, Biasi, B., Mitcham, E., Tustin, S., Tanner, D., Jobling, J., 2007. Carbon dioxide-induced flesh browning in pink lady apples. J. Am. Soc. Hortic. Sci. 132, 713–

719.

Contreras, C., Beaudry, R., 2013. Lipoxygenase-associated apple volatiles and their relationship with aroma perception during ripening. Postharvest Biol. Technol. 82, 28–38. https://doi.org/10.1016/j.postharvbio.2013.02.006

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

Corrêa, T.R., Steffens, C.A., do Amarante, C.V.T., Brackmann, A., Silveira, J.P.G., Tanaka, H., Both, V., 2010. Qualidade de maçãs "Fuji" armazenadas em atmosfera controlada e influência do clima na degenerescência da polpa. Pesqui. Agropecu. Bras. 45, 531–538. https://doi.org/10.1590/S0100-204X2010000600001

DeEll, J.R., Ayres, J.T., Murr, D.P., 2007. 1-Methylcyclopropene influences 'Empire' and 'Delicious' apple quality during long-term commercial storage. Hortic. Technol. 17, 46–51. https://doi.org/doi.org/10.21273/HORTTECH.17.1.46

Defilippi, B.G., Kader, A.A., Dandekar, A.M., 2005. Apple aroma : alcohol acyltransferase , a rate limiting step for ester biosynthesis , is regulated by ethylene. Plant Sci. 168, 1199–1210. https://doi.org/10.1016/j.plantsci.2004.12.018

Dunemann, F., Ulrich, D., Malysheva-Otto, L., Weber, W.E., Longhi, S., Velasco, R., Costa, F., 2012. Functional allelic diversity of the apple alcohol acyl-transferase gene MdAAT1 associated with fruit ester volatile contents in apple cultivars. Mol. Breed. 29, 609–625. https://doi.org/10.1007/s11032-011-9577-7

Espino-Diaz, M., Sepulveda, D.R., Gonzalez-Aguilar, G., Olivas, G.I., 2016a. Biochemistry of Apple Aroma. Food Technol. Biotechnol 54, 375–394. https://doi.org/10.17113/ftb.54.04.16.4248

Espino-Diaz, M., Sepulveda, D.R., Gonzalez-Aguilar, G., Olivas, G.I., 2016b. Biochemsitry of Apple Aroma. Food Technol. Biotechnol 54, 375–394. https://doi.org/10.17113/ftb.54.04.16.4248

Fawbush, F., Nock, J.F., Watkins, C.B., 2009. Antioxidant contents and activity of 1-methylcyclopropene (1-MCP)-treated "Empire" apples in air and controlled atmosphere storage. Postharvest Biol. Technol. 52, 30–37. https://doi.org/10.1016/j.postharvbio.2008.08.014

Flath, R.A., Black, D.R., Guadagni, D.G., Mcfadden, W.H., Schultz, T.H., 1967. Identification and Organoleptic Evaluation of Compounds in Delicious Apple Essence. J. Agric. Food Chem. 15, 29–35. https://doi.org/10.1021/jf60149a032

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., Dätwyler, D., Schneider, K., Naunheim, W., Hoehn, E., 2003. Effects of Decreasing Oxygen Levels in the Storage Atmosphere on the Respiration of Idared Apples. Acta Hortic. 600, 189–192.

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

Hansen, K., Poll, L., 1993. Conversion of L-isoleucine into 2-methyl-2-enyl esters in apples. LWT - Food Sci. Technol. 26, 178–180. https://doi.org/https://doi.org/10.1006/fstl.1993.1036

Herremans, E., Verboven, P., Bongaers, E., Estrade, P., Verlinden, B.E., Wevers, M., Hertog, M.L.A.T.M., Nicolai, B.M., 2013. Characterisation of 'Braeburn' browning disorder by means of X-ray micro-CT. Postharvest Biol. Technol. 75, 114–124. https://doi.org/10.1016/j.postharvbio.2012.08.008

Jelihovschi, E.G., Faria, J.C., Allaman, I.B., 2014. ScottKnott : a package for performing the Scott-Knott clustering algorithm in R. TEMA 1, 3–17. https://doi.org/10.5540/tema.2014.015.01.0003

Kist, B.B., Santos, C.E. dos, Carvalho, C. de, Beling, R.R., Jungblut, G., Helfer, L., Assmann, I., Ávila, S., Machado, M.O., Veiga, S.L., Montano, S., Langbecker, J., Moreira, B.G., 2019. Anuário Brasileiro da maçã 2019. Editora Gazeta Santa Cruz, Santa Cruz do Sul.

Kist et al, 2016. Anuário Brasileiro da Brazilian Apple Yearbook, 2016th ed, Editora Gazeta Santa Cruz. Editora Gazeta Santa Cruz, Santa Cruz do Sul.

Kochevenko, A., Araújo, W.L., Maloney, G.S., Tieman, D.M., Do, P.T., Taylor, M.G., Klee, H.J., Fernie, A.R., 2012. Catabolism of branched chain amino acids supports respiration

but not volatile synthesis in tomato fruits. Mol. Plant 5, 366–375. https://doi.org/10.1093/mp/ssr108

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. https://doi.org/10.17660/ActaHortic.2008.796.22

Larrigaudiere, C., Pintoó, E., Lentheric, S., Vendrell, M., 2001. Involvement of oxidative processes in the development of core browning in controlled-atmosphere stored pears. J. Hortic. Sci. Biotechnol. 76, 157–162. https://doi.org/10.1080/14620316.2001.11511343

Lee, J., Rudell, D.R., Peter, P.J., Watkins, C.B., 2012. Metabolic changes in 1-methylcyclopropene (1-MCP)-treated “Empire” apple at different storage temperatures. Acta Hortic. 1048, 113–120. https://doi.org/10.1007/s11306-011-0373-5

Leffingwell, J.C., Leffingwell, D., 1991. GRAS flavor chemicals detection thresholds. Perfum. Flavorist 16, 1–13.

Li, T., Tan, D., Yang, X., Wang, A., 2013. Exploring the apple genome reveals six ACC synthase genes expressed during fruit ripening. Sci. Hortic. (Amsterdam). 157, 119–123. https://doi.org/10.1016/j.scienta.2013.04.016

Lu, X., Meng, G., Jin, W., Gao, H., 2018. Effects of 1-MCP in combination with Ca application on aroma volatiles production and softening of ‘Fuji’ apple fruit. Sci. Hortic. (Amsterdam). 229, 91–98. https://doi.org/10.1016/j.scienta.2017.10.033

Lurie, S., Pre-Aymard, C., Ravid, U., Larkov, O., Fallik, E., 2002. Effect of 1-methylcyclopropene on volatile emission and aroma in cv. Anna apples. J. Agric. Food Chem. 50, 0–5. https://doi.org/10.1021/jf0200873

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.

Mehinagic, E., Prost, C., Demaimay, M., 2004. Optimization of extraction of apple aroma by dynamic headspace and influence of saliva on extraction of volatiles. J. Agric. Food Chem. 52, 5175–5182. https://doi.org/10.1021/jf049577g

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., Wangjin, L., Kato, K., Ushijima, K., Nakano, R., Inaba, A., Bouzayen, M., Latche, A., Pech, J.-C., Bennett, A.B., 2007a. 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

Nishiyama, K., Guis, M., Rose, J.K.C., Kubo, Y., Bennett, K.A., Wangjin, L., Kato, K., Ushijima, K., Nakano, R., Inaba, A., Bouzayen, M., Latche, A., Pech, J.C., Bennett, A.B., 2007b. 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., Lucia, A., Chaves, S., Singh, R., 2009. Biochemistry of fruit softening: an overview. Physiol. Mol. Biol. plants 15, 103–113.

Pesis, E., 2005. The role of the anaerobic metabolites, acetaldehyde and ethanol, in fruit ripening, enhancement of fruit quality and fruit deterioration. Postharvest Biol. Technol. 37, 1–19. https://doi.org/10.1016/j.postharvbio.2005.03.001

Prange, R., Delong, J., Harrison, P., Leyte, J., McLean, S.D., Scrutton, J.G.E., Cullen, J.J., 2007. Method and apparatus for monitoring a condition in chlorophyll containing matter. United States Pat. US 7,199,376 B2.

Prange, R., DeLong, J.M., Daniels-Lake, B., Harrison, P.A., 2005. Innovation in controlled atmosphere technology. Stewart Postharvest Rev. 3, 1–11. https://doi.org/10.2212/spr.2005.3.9

Prange, R.K., 2018. Dynamic controlled atmosphere (DCA) storage of fruits and vegetables, Reference Module in Food Science. Elsevier. https://doi.org/10.1016/B978-0-08-100596-5.21349-8

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/10.1080/10408390600976841

Rizzolo, A., Polesello, A., Teleky-Vàmossy, G., 1989. CGC/Sensory analysis of volatile compounds developed from ripening apple fruit. J. High Resolut. Chromatogr. 12, 824–827. https://doi.org/10.1002/jhrc.1240121214

Roger Harker, F., Anne Gunson, F., Brookfield, P.L., White, A., 2002. An apple a day: The influence of memory on consumer judgment of quality. Food Qual. Prefer. 13, 173–179. https://doi.org/10.1016/S0950-3293(02)00013-7

Rowan, D.D., Allen, J.M., Fielder, S., Hunt, M.B., 1999. Biosynthesis of straight-chain ester

volatiles in “Red Delicious” and “Granny Smith” apples using deuterium-labeled precursors. J. Food Chemmestry Nanotechnologie 47, 2553–2562. https://doi.org/htt p://dx.doi.org/10.1021/jf9809028

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. (Amsterdam). 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.

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. (Amsterdam). 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/10.1034/j.1399-3054.1997.1000320.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., Both, V., Brackmann, A., Ferreira, D. de F., Wagner, R., 2015a. 1-methylcyclopropene effects on volatile profile and quality of ‘Royal Gala’ apples produced in Southern Brazil and stored in controlled atmosphere. Ciência Rural 45, 2259–2266. https://doi.org/http://dx.doi.org/10.1590/0103-8478cr20141613

Thewes, F.R., Both, V., Brackmann, A., Weber, A., Anese, R.D.O., 2015b. Dynamic controlled atmosphere and ultralow oxygen storage on ‘Gala’ mutants quality maintenance. Food Chem. 188, 62–70. https://doi.org/10.1016/j.foodchem.2015.04.128

Thewes, F.R., Brackmann, A., Anese, R. de O., Bronzatto, E.S., Schultz, E.E., Wagner, R., 2017a. 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., Anese, R. de O., Ludwig, V., Schultz, E.E., dos Santos, L.F., Wendt, L.M., 2017b. 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., Brackmann, A., Both, V., Weber, A., Anese, R. de O., Ferrão, T. dos S., Wagner, R., 2017c. 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., 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. (Amsterdam). 227, 288–295. https://doi.org/10.1016/j.scienta.2017.09.028

Tsantili, E., Gapper, N.E., Arquiza, J.M.R.A., Whitaker, B.D., Watkins, C.B., 2007. Ethylene and r-farnesene metabolism in green and red skin of three apple cultivars in response to 1-methylcyclopropene (1-MCP) treatment. J. Agric. Food Chem. 55, 5267–5276. https://doi.org/10.1021/jf063775l

Vallat, A., Gu, H., Dorn, S., 2005. How rainfall, relative humidity and temperature influence volatile emissions from apple trees in situ. Phytochemistry 66, 1540–1550. https://doi.org/10.1016/j.phytochem.2005.04.038

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

Wakasa, Y., Kudo, H., Ishikawa, R., Akada, S., Senda, M., Niizeki, M., Harada, T., 2006. Low expression of an endopolygalacturonase gene in apple fruit with long-term storage potential. Postharvest Biol. Technol. 39, 193–198. https://doi.org/10.1016/j.postharvbio.2005.10.005

Weber, A., Brackmann, A., Anese, R. de O., Both, V., Pavanello, E.P., 2013a. Atmosfera controlada para o armazenamento da maçã 'Maxi Gala'. Rev. Cienc. Agron. 44, 294–

301.

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

Weber, A., Brackmann, A., Thewes, F.R., Both, V., Anese, R.D.O., Schorr, M.R.W., 2012. Relative humidity and its interaction with the storage temperature of “Gala” apples and mutants. Ciência Rural 42, 2159–2165. https://doi.org/10.1590/S0103-84782012001200009

Weber, A., Rodrigo, F., Sellwig, M., Brackmann, A., Wünsche, N.J., 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

Weber, A., Thewes, F.R., Anese, R. de O., Both, V., Pavanello, E.P., Brackmann, A., 2017. Dynamic controlled atmosphere (DCA): interaction between DCA methods and 1-methylcyclopropene on ‘Fuji Suprema’ apple quality. Food Chem. 235, 136–144. https://doi.org/10.1016/j.foodchem.2017.05.047

Weber, A., Thewes, F.R., Schorr, M.R.W., Gasperin, A.R. De, Venturini, T.L., Brackmann, A., 2013b. ‘Gala’, ‘Royal Gala’ and ‘Brookfield’ apples stored under ultra-low oxygen and two temperatures. Biosci. J. 29, 1887–1895.

Wild, H.P.J. De, 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

Yang, X., Song, J., Campbell-Palmer, L., Fillmore, S., Zhang, Z., 2013. Effect of ethylene and

1-MCP on expression of genes involved in ethylene biosynthesis and perception during ripening of apple fruit. Postharvest Biol. Technol. 78, 55–66. https://doi.org/10.1016/j.postharvbio.2012.11.012

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

Young, H., Gilbert, J.M., Murray, S.H., Ball, R.D., 1996. Causal effects of aroma compounds on Royal Gala apple flavours 18, 329–336.

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

4 ARTIGO 2

4.1 CO_2 CONCENTRATION IN STORAGE OF 'MAXI GALA' APPLES WITH THE NEW DCA-ACP METHOD

Abstract

Great amount of Brazilian apple are stored under refrigerated or static controlled atmosphere (CA), although it is know that losses after long periods still occur. Dynamic controlled atmosphere (DCA) monitored by chlorophyll fluorescence (DCA-CF) is less used because of its higher cost. Thus, there is a demand of new technologies to maintain apple quality after long periods of storage, to suppress off-season apple demand without quality losses and great investments on storage systems. Thus, we proposed the new method of DCA monitored by the anaerobic compensation point (DCA-ACP), which presented great results in previous tests, although there is a need to determine the ideal CO_2 concentration. 'Maxi Gala' apples were stored for 9 months at 2.0 °C and under: static CA [1] with 1.2 kPa O_2 and 2.0 kPa CO_2; [2] DCA-CF with 1.2 kPa CO_2; DCA-ACP with [3] 0.4, [4] 1.2, [5] 1.6 and [6] 2.0 kPa CO_2 plus 7, 14 or 21 days of shelf life under 20 °C. After 7 days of shelf life, all DCA-ACP with 1.6 kPa CO_2 maintained lower ACC oxidase activity, hence lower ethylene production, internal ethylene concentration (IEC), and lower electrolyte leakage. DCA-ACP with 1.6 and 2.0 kPa maintained lower internal ethylene concentration and higher soluble solids. After 14 days of shelf life all DCAs maintained higher healthy fruit, lower IEC, ethylene production and respiration rate, and lower mealiness incidence. Nevertheless, DCA-ACP with 1.2 and 1.6 kPa CO_2 maintained higher flesh firmness. After 21 days of shelf life DCA-ACP with 0.4, 1.2 and 1.6 kPa CO_2 maintained more healthy fruit, and DCA-ACP with 1.6 and 2.0 kPa CO_2 higher flesh firmness.

Keywords: Malus domestica, Physiological disorders, Flesh firmness.

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

4.1.1 Introduction

‘Maxi Gala’ apple is a spontaneous Gala mutant cultivar surged in the south Brazilian region of Vacaria, RS (Weber et al., 2013a). Gala mutants have an important role on the worldwide apples market. In Brazil, around 60% of total annual harvested apples are Gala srains (Kist et al., 2019; Kist et al, 2016). The Brazilian total apple production at 2018 season was of 1,069,126 tons of apple harvested in a short period (Kist et al., 2019). Thus, to maintain apple supply on the off-season, storage is required.

For the apple supply more than 4 months after harvest, the most used condition is the storage under controlled atmosphere (CA). CA consists in decrease of temperature, relative humidity control, oxygen partial pressure reduction and carbon dioxide increase until static concentrations during overall storage period (Brackmann et al., 2009, 2008; Corrêa et al., 2010).Compared with moderns dynamic CA (DCA), the storage in static CA resulted in fruit with higher physiological disorders incidence (Corrêa et al., 2010), lower flesh firmness and acidity (Brackmann et al., 2015; Lafer, 2008; Zanella et al., 2008), and higher ethylene production (Thewes et al., 2017b) on apples from different cultivars stored over 8 months.

One of the most worldwide used DCA, the DCA monitored by chlorophyll fluorescence (HarvestWatch^{TM}) (Prange et al., 2005; Prange, 2018; Weber et al., 2017) decreases the incidence of physiological disorders due to the low oxygen partial pressures used in this storage condition (Lafer, 2008). In DCA-CF, the oxygen partial pressure is gradually reduced, at the beginning of storage, until chlorophyll fluorescence is detected (Prange et al., 2005). After that, the O_2 partial pressure is set at 0.2 or 0.3 kPa above the oxygen set point where the chlorophyll fluorescence spikes (Prange et al., 2005; Zanella et al., 2008).However, chlorophyll fluorescence sensor have expensive costs, thus is less used by Brazilian storage companies compared to CA.

CO_2 at extreme high concentration of 20 kPa reduced ethylene biosynthesis by preventing 1-aminocyclopropane-1-carboxylate (ACC) accumulation, through ACC synthase inhibition and affected the expression of ACC oxidase gene families of tomatoes (*LE- ACO1*, *LE-ACO3*, and *LE-ACO4*) (Wild et al., 2005). Although higher levels of CO_2 have benefits on fungal control and lowering respiration, excessively higher CO_2 levels may cause damages on the membrane and occurrence of flesh brown and caves by increased accumulation of hydrogen peroxide in apples (Anese et al., 2016; Castro et al., 2008; Herremans et al., 2013; Larrigaudiere et al., 2001). Previous researches found 1.2 and 1.6 kPa CO_2 as the most

suitable for apples stored under DCA-CF contrarly to CA stored apples, where the 2.0 kPa CO_2 maintained higher 'Galaxy' apple quality (Brackmann et al., 2015), indicating that there may be a relation with the O_2 partial pressure on ideal CO_2 partial pressure maintenance. Thus, the ideal CO_2 concentration on apples storage is required to optimize storage and prevent damages caused by high levels.

At previous tests, the new DCA methodology (DCA-ACP) maintained the oxygen just above the lowest oxygen limit (LOL) tolerated by the fruits and near the anaerobic compensation point, it is supposed that this method adjust the lowest oxygen set point at different CO_2 concentrations and prevents apple disorders without quality losses. So we aimed to investigate the CO_2 concentration that better maintains overall 'Maxi Gala' apples quality characteristics after 9 months of storage and its effects on the extended shelf life until 21 days at 20 °C.

4.1.2 Materials and methods

4.1.2.1 Apple source and sampling

'Maxi Gala' apples were harvested in a commercial orchard at Vacaria, RS, Brazil, in the 2017/2018 season and immediately transported to the Postharvest Research Center located at Santa Maria, RS, Brazil. Damaged and low size fruit were discarded. Each treatment was composed by four replicates of 25 fruit, in a completely randomized experimental design. Prior to the start of the storage, three replicates of 10 fruit were taken to characterize the initial quality and maturity stage. The iodine-starch index of the fruit was 6.88 (in a 0 to 10 scale) according to Streif (1984). The soluble solids content was 10.97 °Brix, titratable acidity 0.36 g malic acid 100 mL^{-1}, respiration rate 3.12 µg CO_2 kg^{-1} s^{-1}, ethylene production 0.21 ng kg^{-1} s^{-1}, flesh firmness 89.24 N, ACC oxidase enzyme activity 13.21 ng C_2H_4 kg^{-1} s^{-1}, and the internal ethylene concentration was 1.66 µg C_2H_4 L^{-1}. The orchard was treated with aminoethoxyvinylglycine (62.5 g ha^{-1} active ingredient) 30 days before harvest.

After sampling, fruit of each treatment were allocated in their respective chamber with 230 L, and stored at 2.0 °C and 94 ±1 % relative humidity for 9 months. The treatments were composed by: [1] conventional controlled atmosphere (CA with 1.2 kPa O_2 + 2.0 kPa CO_2; [2] CA +1-methylcyclopropene (CA+1-MCP); [3] dynamic CA (DCA) monitored by chlorophyll fluorescence (DCA-CF with 1.2 kPa CO_2); and DCA monitored by the new

method named anaerobic compensation point with four CO_2 concentrations of [4] 0.4 kPa CO_2; [5] 1.2 kPa CO_2; [6] 1.6 kPa CO_2 and [7] 2.0 kPa CO_2.

4.1.2.2 Controlled atmosphere and dynamic CA establishment, monitoring, and controlling

During the 9 months of storage, temperature was daily monitored with the aid of a mercury thermometer. Minichambers of 230 L^{-1}, located inside of a refrigerated chamber were used to place the apples of each treatment individualy. The O_2 partial pressure (pO_2) of each minichamber was reduced inside the experimental chambers by flushing them with N_2, until circa 5 kPa O_2 was reached. The pO_2 was decreased stepwise over five days until reach 0.50 kPa O_2, and then the carbon dioxide production was measured to determine the O_2 set point.

During the storage period, the O_2 and CO_2 partial pressure were monitored by an automatic control system (Valis®, Lajeado-RS, Brazil) and measured by a gas analyzer (Siemens®, model Ultramat, Germany). When oxygen concentration was below the set point, atmospheric air was injected into the chamber atmosphere. The set point for CO_2 partial pressure (pCO_2) was obtained from apples respiration. When the level inside a chamber was above this set point, the CO_2 was removed by circulating the air of the chamber through a chamber containing calcium hydroxide. The relative humidity was maintained at 94 ± 1% during the entirely storage period by the addition of calcium chloride (7.5 g kg^{-1} of fruit) (BOTH et al., 2017) in the storage chamber.

Apples stored under conventional CA were maintained at 1.2 kPa O_2 and 2.0 kPa CO_2 during the 9 monthos of storage. Wile for DCA-CF stored apple, LOL was determined following manufactures instructions (Prange et al., 2005). The O_2 set point of DCA-ACP was determined two days per week, thus monitoring was break off two times per week during 13 hours, in order to recorder the CO_2 production, used to calculate the and ACP. The DCA-ACP LOL monitoring consists of the increase or decrease of CO_2 production compared to the prevew mesurament.

4.1.2.3 Quality analysis after 9 months of storage

After 9 months of storage, the fruit were withdrawn from the experimental chambers and held at 20 °C during 7, 14, and 21 days, to simulate shelf life periods. After this period, the fruit quality was evaluated as described below.

4.1.2.3.1 Determination of ethylene production, respiration rate, and internal ethylene concentration (IEC)

Samples from each replication weighing approximately 1.5 kg, were placed in 5 L bottles and hermetically closed. After one hour, two samples of 1 mL of the headspace air were taken for ethylene determination, and each was injected into a gas Varian® chromatograph Star 3400CX model (Varian®, Palo Alto, CA, USA), equipped with a flame ionization detector (FID) and a Porapak N80/100 column, with injector, column and detector temperatures were 140, 90 and 200 °C, respectively, according to the method of ANESE et al. (2016). Results were expressed in ng kg^{-1} s^{-1}. To measure respiration rate, the air of the same bottle was circulated through a gas analyzer (Isolcell®, Oxycarb 6, Italy), to determine the CO_2 production. Results were expressed in µg CO_2 kg^{-1} s^{-1}.

Internal ethylene concentration (IEC) was determined according to methodology adapted from Mannapperuma et al. (1991). The internal air from slices of 10 fruit was withdrawn using a vacuum pump at 565 mm Hg suction pressure. A closed container filled with water in which the fruit was submerged and an inverted funnel, with a septum in its thinner end, covered the fruit, allowing the air removed from it to be accumulated. Samples of this air were withdrawn through the septum, using a syringe, and injected into the same gas chromatograph used to determine ethylene production. Results were expressed in µg L^{-1}.

4.1.2.3.2 Aminocyclopropane-1-carboxylate (ACC) oxidase enzyme activity

ACC oxidase enzyme activity was determined based on the method proposed by Bufler (1986). A part of the skin of about 25 apples totaling approximately 3 g per replicate was removed, immediately immersed for 30 min in a solution containing 0.1 mol L^{-1}ACC and 10 mmol L^{-1} 2-(N-morpholin) ethanesulfonic acid (MES) buffer at pH 6.0. Thereafter these pieces of skin were transferred into the barrel of a syringe and hermetically closed with 2% CO_2. After further 30 min, two aliquots of 1 mL were taken from the vial of the syringe and

the ethylene concentration was measured by a Varian® gas chromatograph (as described for ethylene production). Results were expressed in ng C_2H_4 kg^{-1} s^{-1}.

4.1.2.3.3 Soluble solids and titratable acidity

The juice of slices of apple flesh from the equatorial section of 25 fruits per replicate was obtained with the aid of a juice centrifuge (Philips®, Wallita). For titratable acidity, 10 mL of the juice was diluted in 100 mL distilled water and titrated with NaOH solution (0.1 N), up to pH 8.1 (BRACKMANN et al., 2008). The results of titratable acidity were expressed as % malic acid. Soluble solids of each replicate were determined by refractometry, using 1 mL of juice, and expressed in °Brix (BRACKMANN et al., 2008).

4.1.2.3.4 Electrolyte leakage

Electrolyte leakage was measured according methodology proposed by Gago et al. (2015), with modifications.Ten discs pf the pulp (5 mm thickness and diameter) were taken from each replicate from 10 different apple. These discs were washed with distilled three times, to remove the excessive juice and thereafter, immersed into a 30 mL distilled water for 1 h (20 ± 1 °C), afterward the conductivity of the suspension was measured with aid of a conductivity meter (ASKO® model AK51). The suspension was then placed for 30 min in a water bath at 100 °C and thereafter rapidly cooled down to 20 °C in a freezer at -30 °C. Conductivity was measured again and taken as total leakage. Results were expressed in percentage.

4.1.3.5 Decay, mealiness and flesh breakdown incidence

Decay incidence was determined by counting each fruit that presented any sign of decay incidence, larger than 5 mm in diameter. The flesh breakdown was determined by slicing the 25 fruit of each replicate on the equatorial region and counting fruit that presented any sign of internal browning and melaliness by detection of mealy pulp. The number of affected fruit in each category was compared to the total number of fruit in each sample (25), and results were expressed as a percentage (%).

4.1.3.6 Healthy fruit

The number of healthy fruit were determined by counting the fruits that did not present any apparent physiological disorder and/or decay incidence. Results were expressed as a percentage (%).

4.1.3.7 Statistical analyses

The study was carried out in a complete randomized design, in a uni-factorial arrangement (storage condition). The results were submitted to analysis of variance (ANOVA). When the ANOVA result was significant ($p < 0.05$), the means were assessed using the Skott-Knott test (Jelihovschi et al., 2014) at 5 % probability error. Principal component analysis (PCA) was carried out for the volatile compounds, with the aid of The Unscrambler® X software (version 10.4, CAMO A/S, Trondheim, Norway). For the PCA, only those results with significant differences ($p < 0.05$) were assessed, and therefore the data matrix was auto scaled for each variable, in order to normalize the variance.

4.1.3 Results and discussion

4.1.3.1 ACC oxidase enzyme activity, electrolyte leakage, ethylene production, internal ethylene concentration (IEC) and respiration rate

The fruit assessment was carried out after 9 months of storage in CA or DCA, at 2.0 °C, plus 7, 14 and 21 days of shelf life. ACC oxidase enzyme activity is determined in view of its importance because it triggers the last step of ethylene biosynthesis, which has a direct relation on the ethylene production, hence on the ethylene dependent processes. After 7 days of shelf life, CA showed the higher, DCA-CF an intermediate, and all DCA-ACP conditions the lowest ACC oxidase activity (Figure 1a). The higher ACC oxidase activity on apples stored under CA condition may be directly related to the higher internal ethylene concentration (IEC) (Figure 1b), increase on ethylene production and respiration rate after 6 and 8 days of shelf life (Table 1 and 2). Previous researchs also reported higher ACC oxidase

enzyme activity on ‘Royal Gala’ and ‘Brookfield’ apples stored at CA plus 7 days of shelf life compared to lower oxygen storage conditions (BOTH et al., 2014; THEWES et al., 2015; WEBER et al., 2013b). Probably the higher O_2 partial pressure (1.2 kPa) employed at this condition stimulated the ethylene production and ACC oxidase during the storage period, once ACC oxidase activity requires oxygen to convert aminocyclopropane-1-carboxylic acid (ACC) in ethylene (Pesis, 2005). Thus, the storage under DCAs conditions have a positive effect on reduce ACC oxidase enzyme activity.

After 14 days, DCA-CF and DCA-ACP with 0.4 and 1.2 kPa CO_2 maintained lower ACC oxidase activity (Figure 1b), compared with CA and DCA-ACP with 1.6 and 2.0 kPa CO_2. DCA-ACP with 1.6 and 2.0 kPa O_2 maintained lower IEC when compared with CA, but higher when compared with DCA-CF and DCA-ACP with 0.4, at 14 days evaluation. This remains lower senescence when compared with the other storage conditions, even so apples of all storage conditions were in climacteric ripening stage (IEC > 0.6 µL C_2H_4 L^{-1}) (Brackmann et al., 1993), indicating the start in maturation. Apples maintained for 21 days at 20 °C to simulate prolonged commercial shelf life, at our research, did not statistically differ among all storage conditions on ACC oxidase activity, IEC, ethylene production after 16 days (Table 1), and respiration rate at 16 days of shelf life (Table 2). Probably, the ‘Maxi Gala’ apples in all conditions, at this point of shelf life were in advanced ripening stage, thus no statistically difference among the mentioned variables was observed.

Electrolyte leakage indicates membrane integrity (Gago et al., 2015) and high leakage may predict apples flesh disorders (Weber et al., 2019). Although CA storage condition maintained lower membrane integrity (higher electrolyte leakage) at the first evaluation week (Fig. 1c), there was no difference on flesh breakdown incidence among all the storage conditions on the three weeks of evaluation (Figure 3c). DCA-CF maintained the higher membrane integrity after 7 days of shelf life, followed by DCA-ACP with 0.4 kPa CO_2. DCA-ACP with 1.2, 1.6, and 2.0 kPa CO_2 maintained higher electrolyte leakage when compared to DCA-ACP with 0.4 kPa CO_2, but lower than CA after 7 days of shelf life. Thewes et al. (2017a) also found higher electrolyte leakage on ‘Galaxy’ apples stored under CA compared to low oxygen storage conditions (DCA-RQ 1.3 and DCA-RQ 1.5) of ‘Galaxy’ apples harvested at three maturity stages.

After 14 days of shelf life, DCA-ACP with 0.4 kPa CO_2 maintained lower electrolyte leakage, followed by CA, DCA-ACP with 1.2 kPa CO_2, and 1.6 kPa CO_2. Curiously, DCA-ACP with 2.0 kPa CO_2 maintained higher electrolyte leakage, suggesting that the higher CO_2 may have caused membrane damage after prolonged shelf life. DCA-CF maintained lower

electrolyte leakage compared to DCA-ACP with 2.0 kPa CO_2, but higher than the other condition after 14 days of shelf life at 20 °C.

After 21 days of shelf life only DCA-ACP with 1.2 kPa CO_2 maintained lower electrolyte leakage (Fig. 1c). As showed by the multivariate analisys of principal component analisys (PCA), at PC I electrolyte leakage after 21 days of shelf life was correlated to CA (Figure 4).

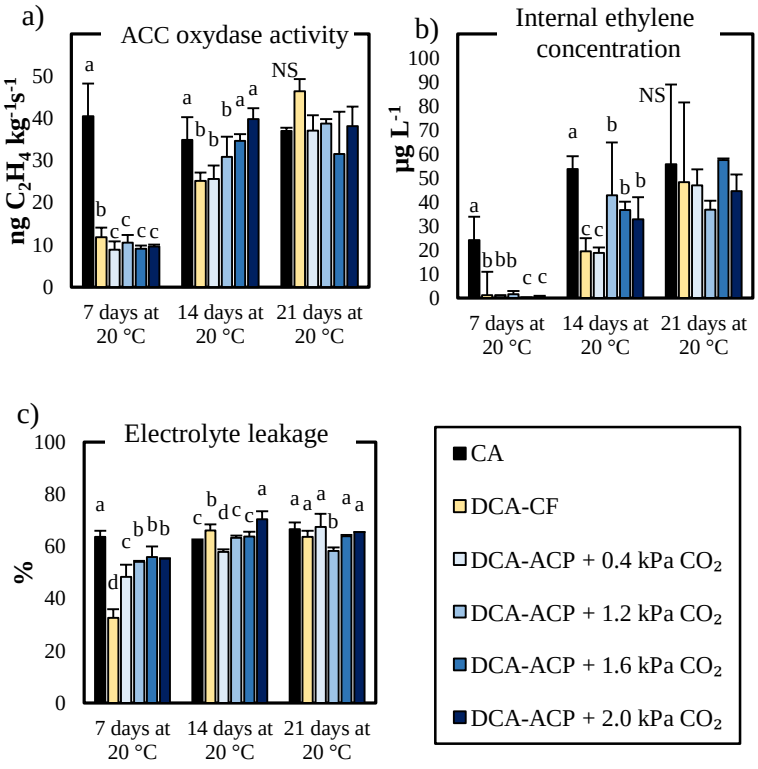


Figure 1 - ACC oxidase activity (a), internal ethylene concentration (b), and electrolyte leakage of 'Maxi Gala' apples after 9 months of storage plus 7, 14 and 21 days of shelf life at 20 °C. Bars with the same letter in the same shelf life assessment do not statistically differ by Skott-Knott test at 5 % probability ($p < 0.05$). NS: not significant.

To prolong apple storage period, metabolism has to be reduced as lower as possible without quality losses. In an overview, ethylene production and respiration rate were reduced by DCA storage conditions until 14 days of shelf life compared to CA (Table 1 and 2). At chamber opening, DCA-ACP with 0.4 and 1.6 kPa CO_2 maintained the lowest ethylene production and all DCA-ACP have lower respiration rate, while DCA-CF maintained higher ethylene production and respiration rate compared to DCA-ACP conditions, but lower than CA. According to Thewes et al. (2015b), DCA-CF maintained the lowest ethylene and

respiration rate right after chamber opening of 'Royal Gala' and 'Galaxy' apples after 9 months storage, compared with the static CA (with 1.2 kPa O_2 + 2.0 kPa CO_2) and ultra-low oxygen (ULO with static 0.4 kPa O_2 + 1.2 kPa CO_2) storage condition. Afterward, all DCAs conditions maintained lower ethylene and respiration rate at the 2th and 4th day of measurement, in exception of DCA-ACP with 1.6 and 2.0 kPa CO_2 but they did not differ from CA on the 2th day of respiration rate analysis.

At the 6th day of ethylene measurement, DCA-ACP with 2.0 kPa CO_2 has higher ethylene production when compared to the other DCA conditions, but lower than CA (Table 1). Although, by respiration rate did not differ from DCA-ACP conditions and was higher than DCA-CF (Table 2). All DCA-ACP conditions maintained lower respiration rate on the 8th day, when compared to DCA-CF and CA.

Table 1 - Ethylene production (ng C_2H_4 $kg^{-1}s^{-1}$) during 20 days of shelf life of 'Maxi Gala' apples stored for 9 months at 2.0 °C, and evaluated during 20 days while kept at 20 °C.

Day after chamber opening	CA*	DCA-CF	DCA-ACP			
			0.4 kPa CO_2	1.2 kPa CO_2	1.6 kPa CO_2	2.0 kPa CO_2
Initial	0.21 ng C_2H_4 $kg^{-1}s^{-1}$					
0 day	0.65a ±0.091	0.05 b ±0.008	0.01e ±0.002	0.02d ±0.003	0.01e ±0.002	0.03c ±0.003
2° day	3.21a ±0.064	0.02 b ±0.003	0.01b ±0.001	0.01b ±0.003	0.01b ±0.002	0.02b ±0.002
4° day	6.12a ±0.284	0.05 b ±0.005	0.03b ±0.006	0.06b ±0.06	0.03b ±0.007	0.05b ±0.038
6° day	8.00a ±1.054	0.07 c ±0.012	0.05c ±0.013	0.08c ±0.025	0.06c ±0.027	0.20b ±0.111
8° day	10.91a ±2.261	0.47 b ±0.240	0.16c ±0.054	0.31b ±0.161	0.15c ±0.083	0.53b ±0.216
10° day	9.90a ±0.812	1.41 b ±0.782	0.87b ±0.602	0.74b ±0.317	0.59b ±0.416	1.24b ±0.412
12° day	7.48a ±1.910	2.01 b ±1.292	1.08b ±0.314	1.59b ±0.250	2.01b ±1.396	2.70b ±0.974
14° day	11.57a ±2.142	4.51 b ±2.386	2.39b ±0.791	3.48b ±0.716	4.41b ±2.349	5.14b ±1.541
16° day	9.48^{ns} ±1.066	6.36 ±3.093	3.89 ±1.060	4.01 ±1.948	6.35 ±2.822	6.51 ±1.150
18° day	10.02^{ns} ±1.536	7.96 ±1.742	5.88 ±1.326	6.34 ±1.161	8.70 ±3.578	8.96 ±1.313
20° day	9.12^{ns} ±3.077	9.76 ±4.212	8.40 ±1.829	8.59 ±0.611	11.99 ±3.654	12.01 ±1.729

*Controlled atmosphere (1.2 kPa O_2 + 2.0 kPa CO_2); Dynamic CA (DCA) monitored by chlorophyll fluorescence (DCA-CF with 1.2 kPa CO2); DCA monitored by anaerobic compensation point (DCA-ACP) with four CO_2 concentrations: 0.4, 1.2, 1.6 and 2.0 kPa. **Means followed by the same letter in the row do not statistically differ by Skott-Knott test at 5% probability ($p < 0.05$). ns Not significant.

Table 2 - Respiration rate during 20 days of shelf life of 'Maxi Gala' apples stored for 9 months at 2.0 °C.

Days after chamber opening	CA*	DCA-CF	DCA-ACP			
			0.4 kPa CO_2	1.2 kPa CO_2	1.6 kPa CO_2	2.0 kPaCO_2
Initial	3.12 µg CO_2 kg^{-1} s^{-1}					

0 day	4.78a** ±0.338	3.60b ±0.443	2.14c ±0.109	2.60c ±0.242	2.7c ±0.082	2.62c ±0.190
2° day	3.28a ±0.222	2.17b ±0.089	2.09b ±0.132	2.23b ±0.332	3.77a ±0.317	3.35a ±1.115
4° day	4.06a ±0.063	2.40b ±0.208	2.34b ±0.186	2.51b ±0.477	2.37b ±0.079	2.24b ±0.133
6° day	4.67a ±0.103	2.25c ±0.330	4.03b ±0.365	3.74b ±0.304	3.66b ±0.198	3.70b ±0.166
8° day	5.36a ±0.193	2.59b ±0.409	2.04c ±0.083	2.07c ±0.080	1.97c ±0.078	2.12c ±0.065
10° day	4.65a ±0.185	2.61b ±0.593	2.06b ±0.058	2.06b ±0.105	2.27b ±0.182	2.13b ±0.231
12° day	3.78a ±0.152	2.16b ±0.516	2.15b ±0.219	2.26b ±0.154	2.68b ±0.484	2.29b ±0.461
14° day	4.29a ±0.442	2.84b ±0.595	2.22b ±0.141	2.32b ±0.088	2.72b ±0.531	2.06b ±0.553
16° day	4.59ns ±0.299	3.62 ±0.895	3.19 ±0.304	2.90 ±0.212	3.17b ±0.359	2.40b ±0.660
18° day	4.77a ±0.306	3.67b ±0.487	3.06b ±0.229	2.81b ±0.104	3.25b ±0.426	2.59b ±0.699
20° day	9.12a ±0.440	3.47a ±0.681	1.50b ±0.286	1.35b ±0.074	4.14a ±0.386	3.37a ±0.771

*Controlled atmosphere (1.2 kPa O_2 + 2.0 kPa CO_2); Dynamic CA (DCA) monitored by chlorophyll fluorescence (DCA- CF with 1.2 kPa CO_2); DCA monitored by anaerobic compensation point (DCA-ACP) with four CO_2 concentrations: 0.4, 1.2, 1.6 and 2.0 kPa. **Means followed by the same letter at the same day of measurement do not statistically differ by Skott-Knott test at 5% probability ($p < 0.05$). ns Not significant.

4.1.3.2 Flesh firmness, titratable acidity, and soluble solids

Flesh firmness is one of the most important apple quality indicators and appreciated by consumers, in combination of titratable acidity and soluble solids (Altisent et al., 2008). After 7 days of shelf life, no statistically difference on the flesh firmness and titratable acidity was observed between storage conditions, neither between the different CO_2 concentrations of DCA-ACP (Figure 2a). Brackmann et al. (2015) also did not found difference on flesh firmness between different CO_2 concentrations (1.2, 1.6 and 2.0 kPa) of 'Galaxy' apple stored under CA, DCA-CF, and DCA-RQ 1.5 after 9 months storage plus 7 days of shelf life (20 °C). Nevertheless, they found lower titratable acidity in CA with lower CO_2 partial pressure.

After 14 days of shelf life, it was possible to differentiate the best conditions that avoid firmness loss. DCA-ACP with 1.2 and 1.6 kPa CO_2 maintained higher flesh firmness, when compared with the other conditions assessed at the same day. Interesting fact is that the lowest and higher CO_2 concentrations, DCA-ACP with 0.4 and 2.0 kPa CO_2, were not as effective in retain flesh firmness compared to DCA-ACP 1.2 and DCA-CF 1.6, but higher than CA. There may be a relation of the higher cell wall damage at 2.0 kPa CO_2. CA had the lowest flesh firmness maintenance after 14 and 21 days shelf life 20 °C, probably as a result of the higher oxygen partial pressure used in this storage condition (1.2 kPa O_2), which also induces early during the shelf life the higher IEC (Figure 1b), ethylene production (Table 1), and respiration rate (Table 2). After 21 days, DCA-ACP with 1.6 and 2.0 kPa maintained higher flesh firmness. Evidencing that the higher CO_2 concentration has a benefitial effect to avoid flesh firmness loss. Titratable acidity was found in higher concentrations in DCA-CF and DCA-

ACP with 0.4 and 1.2 kPa after 14 days of shelf life, but no statistical difference was observed after 21 days of shelf life (Figure 2b).

Soluble solids content was higher in DCA-ACP with 1.6 and 2.0 kPa CO_2 after 7 days of shelf life. Previous researches did not find soluble solids difference in 'Royal Gala' and 'Galaxy' apples stored under CA or DCA-CF after 9 months plus 7 days of shelf life (Both et al., 2017; Thewes et al., 2017b, 2015b). This result indicates that there may be a CO_2 higher concentration effect during storage on soluble solids content, probably because of the lower respiration rate of these fruit. After 14 and 21 days of shelf life, no statistically difference was observed among all the storage conditions on soluble solids content (Figure 2c).

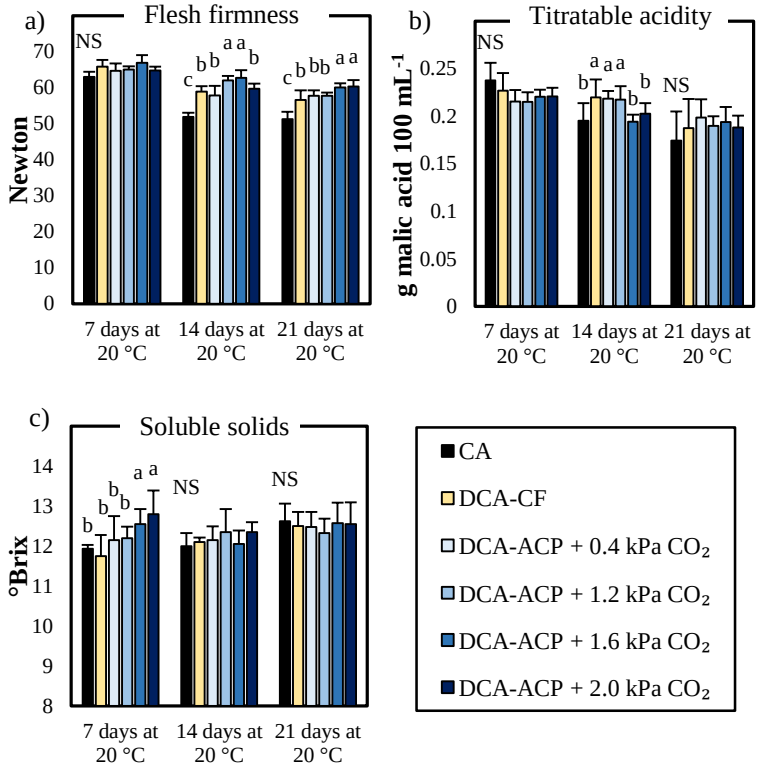


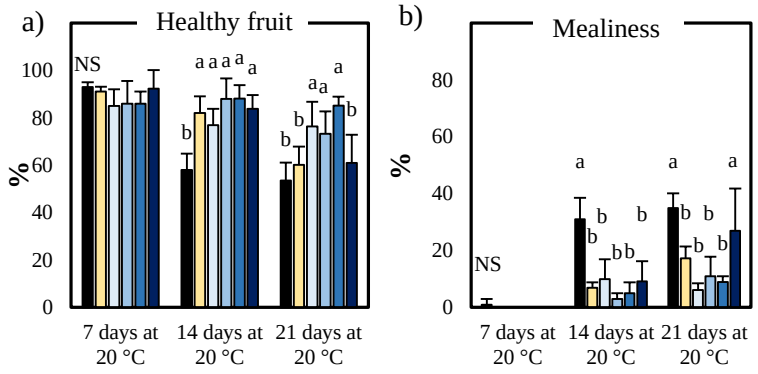
Figure 2 - Flesh firmness (a), titratable acidity (b), and soluble solids (c) after 9 months of storage plus 7, 14, and 21 days of shelf life at 20 °C. Apples were stored in these conditions: conventional controlled atmosphere (CA) with 1.2 kPa O_2 plus 2.0 kPa CO_2, dynamic CA (DCA) monitored by chlorophyll fluorescence (DCA-CF) with 1.2 kPa CO_2, DCA monitored by anaerobic compensation point (DCA-ACP) with 0.4, 1.2, 1.6 and 2.0 kPa CO_2. Means followed by the same letter at the same day of measurement do not statistically differ by Skott-Knott test at 5 % probability ($p < 0.05$). NS: Not significant.

4.1.3.3 Healthy fruit, mealiness incidence, flesh breakdown

At chambers opening after long term storage is desired to have a high amount of healthy fruit, because it has an impact on the amont of apple for marketing and for consumption at home of consomers. Healthy fruit are count as those that did not presented any internal physiologic disturbs that could be observed after cutting the apples at the equatorial region and without decay incidence. No statistical difference was observed after 7 days of shelf life (Figure 3a) and fruit stored in all conditions could be sold with low losses after 7 days of shelf life. However, after 14 days of shelf life all DCAs storage conditions maintained higher healthy fruit (Figure 3a). The lower healthy fruit in CA may be related to the higher mealiness incidence at this storage condition after 14 days of shelf life (Figure 3b).

DCA-ACP with 0.4, 1.2 and 1.6 kPa CO_2 maintained higher healthy fruit and lower mealiness incidence after the prolonged 21 days of shelf life under 20 °C, and DCA-CF also maintained lower mealiness incidence. Previous research also found lower mealiness incidence in 1.2 kPa and higher healthy fruit in 1.2 and 1.6 kPa storage under the DCA monitored by respiratory quotient (DCA-RQ) condition (Brackmann et al., 2015). It shows that the higher 2.0 kPa CO_2 concentration do not control mealiness and reduce healthy fruit.

There was no difference between the storage conditions for flesh breakdown and decay incidence, even after 21 days of shelf life (Figure 3c and 3d). By assessing 'Nicoter' apples after 8 months of storage, Weber et al. (2019) found flesh breakdown correlation to the lower storage temperature, suggesting that flesh breakdown may be a temperature dependent process, instead of CO_2 storage concentration, although new investigations are required.



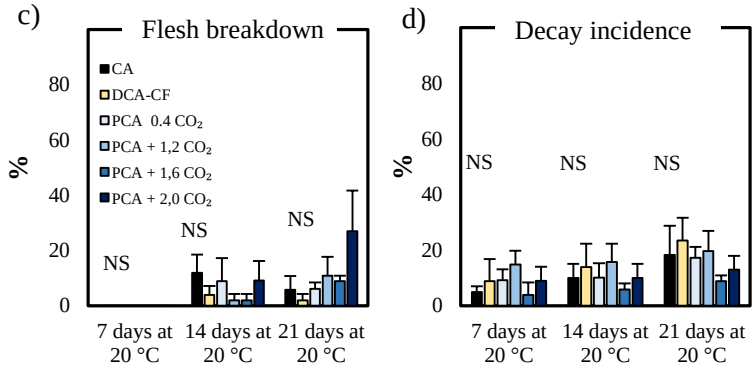


Figure 3 - Healthy fruit (a), mealiness incidence (b), flesh breakdown (c) and decay (d) incidence after 9 months of storage plus 7, 14, and 21 days of shelf life at 20 °C. Apples were stored at this conditions: conventional controlled atmosphere (CA) with 1.2 kPa O_2 plus 2.0 kPa CO_2, dynamic CA (DCA) monitored by chlorophyll fluorescence (DCA-CF) with 1.2 kPa CO_2, DCA monitored by anaerobic compensation point (DCA-ACP) with 0.4, 1.2, 1.6 and 2.0 kPa CO_2. Means followed by the same letter at the same day of measurement do not statistically differ by Skott-Knott test at 5 % probability ($p < 0.05$). NS: Not significant.

4.1.3.4 Principal component analysis (PCA)

To give a better overview, principal component analysis (PCA) was used to describe overall quality characteristics interaction. Together PC I and PC II explained 77% of the overall variation of the variables. Both et al. (2014) studding 'Royal Gala' apples explained 54.35% of overall variables with these two PCs. PCA I (53%) was important to discriminate the storage conditions in two groups, where all DCA storage conditions were separated from CA storage conditions$_2$ (Figure 4a). According to the PC I, soluble solids content after 7 days, healthy fruit after 14 and 21 days, flesh firmness after 14 and 21 days of shelf life were correlated with the higher CO_2 concentrations in DCA-ACP storage, mainly with 1.2, 1.6 and 2.0 kPa CO_2. On the other hand, CA was correlated with electrolyte leakage, higher ACC oxidase activity and internal ethylene concentration (IEC) after 7 days of shelf life, higher IEC, ACC oxidase activity, and mealiness after 14 days, also higher mealiness incidence after 21 days of shelf life. This emonstrates that most of the variables that indicate advanced ripening were correlated with the conventional static CA storage condition.

PCII (24%) discriminated the higher DCA-ACP CO_2 concentration from DCA-ACP with 0.4 kPa CO_2 and DCA-CF. Where DCA-CF and DCA-ACP with 0.4 kPa CO_2 were correlated with titratable acidity, while DCA-ACP conditions with higher CO_2 concentrations during storage were correlated with most of the quality parameters.

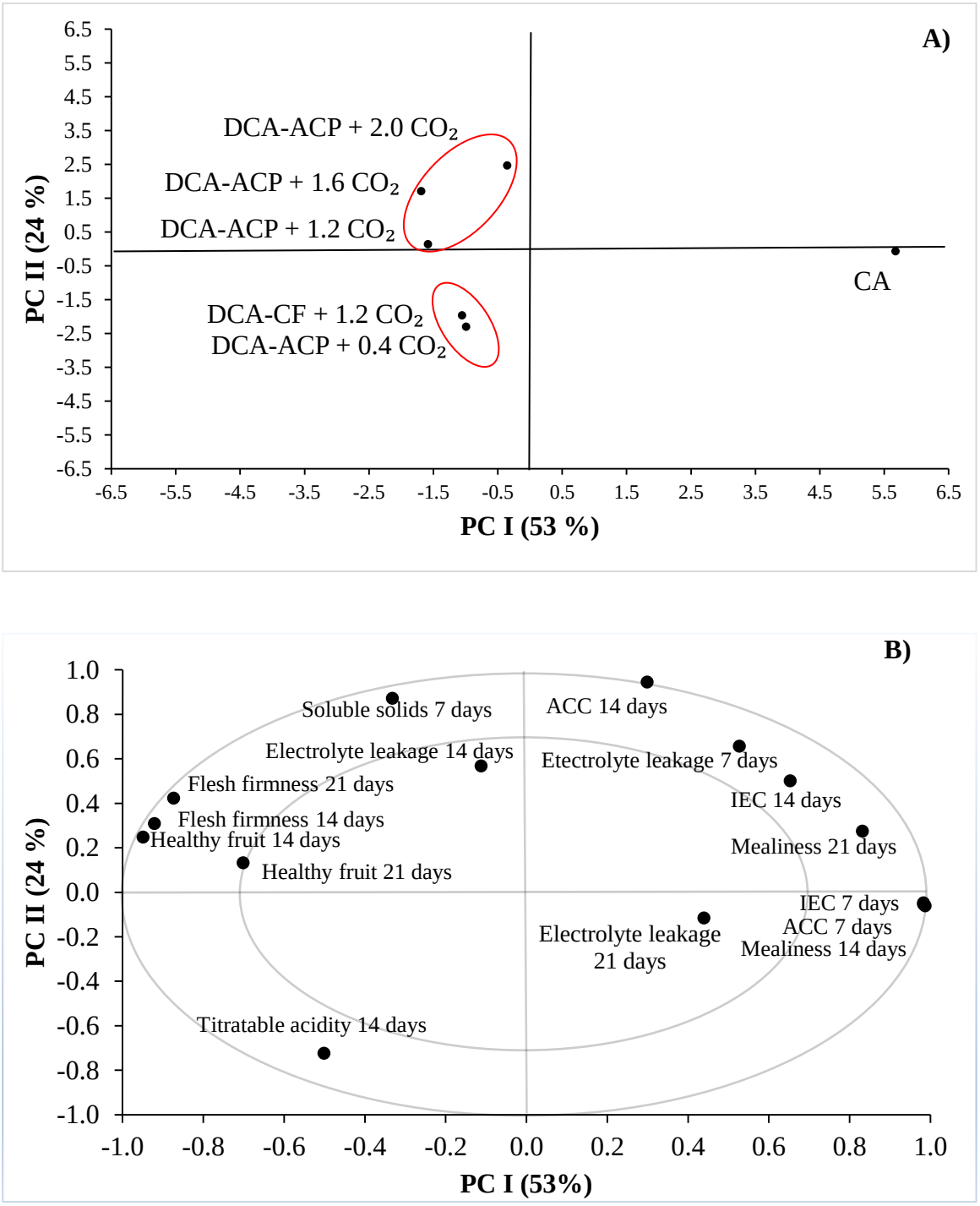


Figure 4 - Principal component analisys demonstrating the scores of treatments (A) and weights of the dependent variables of overall quality analysis (B) of 'Maxi Gala' apples after 9 months of storage plus 7, 14 and 21 days of shelf life at 20 °C. *CA: static controlled atmosphere with 1.2 kPa O_2 plus 2.0 kPa CO_2; DCA-CF: dynamic CA (DCA) monitored by chlorophyll fluorescence, with 1.2 kPa CO_2; DCA-ACP: DCA monitored by anaerobic compensation point, with 0.4; 1.2; 1.6 and 2.0 kPa CO_2; ACC:

ACC oxidase activity after 7 and 14 days; IEC: internal ethylene concentration after 7 and 14 days of shelf life.

4.1.4 Conclusion

DCA storage irrespective of CO_2 concentrations maintain lower fruit respiration rate and ethylene production untill 14 days of shelf life.

DCA-ACP with 1.6 and 2.0 kPa CO_2 maintained higher soluble solids content after 7 days of shelf life. DCA-ACP with 1.2 and 1.6 kPa CO_2 maintained higher flesh firmness, higher amount of healthy fruits, and lower mealiness incidence (after 14 and 21 days of shelf life), and IEC (after 7 days of shelf life).

DCA-CF did not avoid flesh firmness loss and maintained lower healphy fruit when compared to DCA-ACP with the same CO_2 partial pressure (1.2 kPa). Thus, DCA-ACP is recommended for apple storage istead DCA-CF.DCA-ACP with 1.2 or 1.6 kPa are the best conditions for store ‘Maxy Gala’ apple. DCA-ACP with 2.0 kPa CO_2 is not recomende due higher mealiness incidence after 21 days of shelf life.

4.1.5 References

Altisent, R., Graell, J., Lara, I., Lopez, L., Echeverría, G., 2008. Regeneration of volatile compounds in Fuji apples following ultra low oxygen atmosphere storage. J. Agric. Food Chem. 56, 8490–8497.

Anese, R. de O., Brackmann, A., Thewes, F.R., Schultz, E.E., De Gasperin, A.R., 2016. Mass loss by low relative humidity increases gas diffusion rates in apple flesh and allows the use of high CO_2 partial pressures during ultralow O_2 storage. Sci. Hortic. (Amsterdam). 198, 414–423. https://doi.org/10.1016/j.scienta.2015.12.015

Anese, R.D.O., Brackmann, A., Wendt, L.M., Rodrigo, F., Schultz, E.E., Ludwig, V., Berghetti, M.R.P., 2019. Interaction of 1-methylcyclopropene, temperature and dynamic controlled atmosphere by respiratory quotient on ‘Galaxy’ apples storage. Food Packag. Shelf Life 20, 100246. https://doi.org/10.1016/j.fpsl.2018.07.004

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.D.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., 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

Both, V., Thewes, F.R., Brackmann, A., Ferreira, D. de F., Pavanello, E.P., Wagner, R., 2016. Effect of low oxygen conditioning and ultralow oxygen storage on the volatile profile, ethylene production and respiration rate of 'Royal Gala' apples. Sci. Hortic. (Amsterdam). 209, 156–164. https://doi.org/10.1016/j.scienta.2016.06.028

Brackmann, A., 2015. Control apparatus for controlled atmosphere cells for storing perishable items. US2015/0257401A1.

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/https://doi.org/10.21273/JASHS.118.2.243

Brackmann, A., Weber, A., Both, V., 2015. CO_2 partial pressure for respiratory quotient and HarvestWatch^{TM} dynamic controlled atmosphere for 'Galaxy' apples storage. Acta Hortic. 1079, 435–440.

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., Weber, A., Sestari, I., Peterle, M.E., Both, V., Pavanello, E.P., Vilela Pinto, J.A., 2009. Manejo do etileno e sua relação com a maturação de maçãs 'Gala' armazenadas em atmosfera controlada. Bragantia 68, 519–525. https://doi.org/10.1590/S0006-87052009000200027

Bufler, G., 1986. Ethylene-promoted conversion of 1-aminocyclopropane-1-carboxylic Acid 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

Castro, E. De, Barrett, D.M., Jobling, J., Mitcham, E.J., 2008. Biochemical factors associated with a CO_2-induced flesh browning disorder of Pink Lady apples 48, 182–191. https://doi.org/10.1016/j.postharvbio.2007.09.027

Castro, E. De, Biasi, B., Mitcham, E., Tustin, S., Tanner, D., Jobling, J., 2007. Carbon dioxide-induced flesh browning in pink lady apples. J. Am. Soc. Hortic. Sci. 132, 713–719.

Contreras, C., Beaudry, R., 2013. Lipoxygenase-associated apple volatiles and their relationship with aroma perception during ripening. Postharvest Biol. Technol. 82, 28–38. https://doi.org/10.1016/j.postharvbio.2013.02.006

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

Corrêa, T.R., Steffens, C.A., do Amarante, C.V.T., Brackmann, A., Silveira, J.P.G., Tanaka, H., Both, V., 2010. Qualidade de maçãs "Fuji" armazenadas em atmosfera controlada e influência do clima na degenerescência da polpa. Pesqui. Agropecu. Bras. 45, 531–538. https://doi.org/10.1590/S0100-204X2010000600001

DeEll, J.R., Ayres, J.T., Murr, D.P., 2007. 1-Methylcyclopropene influences 'Empire' and 'Delicious' apple quality during long-term commercial storage. Hortic. Technol. 17, 46–51. https://doi.org/doi.org/10.21273/HORTTECH.17.1.46

Defilippi, B.G., Kader, A.A., Dandekar, A.M., 2005. Apple aroma : alcohol acyltransferase , a rate limiting step for ester biosynthesis , is regulated by ethylene. Plant Sci. 168, 1199–1210. https://doi.org/10.1016/j.plantsci.2004.12.018

Dunemann, F., Ulrich, D., Malysheva-Otto, L., Weber, W.E., Longhi, S., Velasco, R., Costa, F., 2012. Functional allelic diversity of the apple alcohol acyl-transferase gene MdAAT1 associated with fruit ester volatile contents in apple cultivars. Mol. Breed. 29, 609–625. https://doi.org/10.1007/s11032-011-9577-7

Espino-Diaz, M., Sepulveda, D.R., Gonzalez-Aguilar, G., Olivas, G.I., 2016a. Biochemistry of Apple Aroma. Food Technol. Biotechnol 54, 375–394. https://doi.org/10.17113/ftb.54.04.16.4248

Espino-Diaz, M., Sepulveda, D.R., Gonzalez-Aguilar, G., Olivas, G.I., 2016b. Biochemsitry of Apple Aroma. Food Technol. Biotechnol 54, 375–394. https://doi.org/10.17113/ftb.54.04.16.4248

Fawbush, F., Nock, J.F., Watkins, C.B., 2009. Antioxidant contents and activity of 1-methylcyclopropene (1-MCP)-treated "Empire" apples in air and controlled atmosphere storage. Postharvest Biol. Technol. 52, 30–37. https://doi.org/10.1016/j.postharvbio.2008.08.014

Flath, R.A., Black, D.R., Guadagni, D.G., Mcfadden, W.H., Schultz, T.H., 1967.

Identification and Organoleptic Evaluation of Compounds in Delicious Apple Essence. J. Agric. Food Chem. 15, 29–35. https://doi.org/10.1021/jf60149a032

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., Dätwyler, D., Schneider, K., Naunheim, W., Hoehn, E., 2003. Effects of Decreasing Oxygen Levels in the Storage Atmosphere on the Respiration of Idared Apples. Acta Hortic. 600, 189–192.

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

Hansen, K., Poll, L., 1993. Conversion of L-isoleucine into 2-methyl-2-enyl esters in apples. LWT - Food Sci. Technol. 26, 178–180. https://doi.org/https://doi.org/10.1006/fstl.1993.1036

Herremans, E., Verboven, P., Bongaers, E., Estrade, P., Verlinden, B.E., Wevers, M., Hertog, M.L.A.T.M., Nicolai, B.M., 2013. Characterisation of 'Braeburn' browning disorder by means of X-ray micro-CT. Postharvest Biol. Technol. 75, 114–124. https://doi.org/10.1016/j.postharvbio.2012.08.008

Jelihovschi, E.G., Faria, J.C., Allaman, I.B., 2014. ScottKnott : a package for performing the Scott-Knott clustering algorithm in R. TEMA 1, 3–17. https://doi.org/10.5540/tema.2014.015.01.0003

Kist, B.B., Santos, C.E. dos, Carvalho, C. de, Beling, R.R., Jungblut, G., Helfer, L., Assmann, I., Ávila, S., Machado, M.O., Veiga, S.L., Montano, S., Langbecker, J., Moreira, B.G., 2019. Anuário Brasileiro da maçã 2019. Editora Gazeta Santa Cruz, Santa Cruz do Sul.

Kist et al, 2016. Anuário Brasileiro da Brazilian Apple Yearbook, 2016th ed, Editora Gazeta Santa Cruz. Editora Gazeta Santa Cruz, Santa Cruz do Sul.

Kochevenko, A., Araújo, W.L., Maloney, G.S., Tieman, D.M., Do, P.T., Taylor, M.G., Klee, H.J., Fernie, A.R., 2012. Catabolism of branched chain amino acids supports respiration but not volatile synthesis in tomato fruits. Mol. Plant 5, 366–375. https://doi.org/10.1093/mp/ssr108

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.

https://doi.org/10.17660/ActaHortic.2008.796.22

Larrigaudiere, C., Pintoó, E., Lentheric, S., Vendrell, M., 2001. Involvement of oxidative processes in the development of core browning in controlled-atmosphere stored pears. J. Hortic. Sci. Biotechnol. 76, 157–162. https://doi.org/10.1080/14620316.2001.11511343

Lee, J., Rudell, D.R., Peter, P.J., Watkins, C.B., 2012. Metabolic changes in 1-methylcyclopropene (1-MCP)-treated "Empire" apple at different storage temperatures. Acta Hortic. 1048, 113–120. https://doi.org/10.1007/s11306-011-0373-5

Leffingwell, J.C., Leffingwell, D., 1991. GRAS flavor chemicals detection thresholds. Perfum. Flavorist 16, 1–13.

Li, T., Tan, D., Yang, X., Wang, A., 2013. Exploring the apple genome reveals six ACC synthase genes expressed during fruit ripening. Sci. Hortic. (Amsterdam). 157, 119–123. https://doi.org/10.1016/j.scienta.2013.04.016

Lu, X., Meng, G., Jin, W., Gao, H., 2018. Effects of 1-MCP in combination with Ca application on aroma volatiles production and softening of 'Fuji' apple fruit. Sci. Hortic. (Amsterdam). 229, 91–98. https://doi.org/10.1016/j.scienta.2017.10.033

Lurie, S., Pre-Aymard, C., Ravid, U., Larkov, O., Fallik, E., 2002. Effect of 1-methylcyclopropene on volatile emission and aroma in cv. Anna apples. J. Agric. Food Chem. 50, 0–5. https://doi.org/10.1021/jf0200873

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.

Mehinagic, E., Prost, C., Demaimay, M., 2004. Optimization of extraction of apple aroma by dynamic headspace and influence of saliva on extraction of volatiles. J. Agric. Food Chem. 52, 5175–5182. https://doi.org/10.1021/jf049577g

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., Wangjin, L., Kato, K., Ushijima, K., Nakano, R., Inaba, A., Bouzayen, M., Latche, A., Pech, J.-C., Bennett, A.B., 2007a. 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

Nishiyama, K., Guis, M., Rose, J.K.C., Kubo, Y., Bennett, K.A., Wangjin, L., Kato, K., Ushijima, K., Nakano, R., Inaba, A., Bouzayen, M., Latche, A., Pech, J.C., Bennett, A.B., 2007b. 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., Lucia, A., Chaves, S., Singh, R., 2009. Biochemistry of fruit softening: an overview. Physiol. Mol. Biol. plants 15, 103–113.

Pesis, E., 2005. The role of the anaerobic metabolites, acetaldehyde and ethanol, in fruit ripening, enhancement of fruit quality and fruit deterioration. Postharvest Biol. Technol. 37, 1–19. https://doi.org/10.1016/j.postharvbio.2005.03.001

Prange, R., Delong, J., Harrison, P., Leyte, J., McLean, S.D., Scrutton, J.G.E., Cullen, J.J., 2007. Method and apparatus for monitoring a condition in chlorophyll containing matter. United States Pat. US 7,199,376 B2.

Prange, R., DeLong, J.M., Daniels-Lake, B., Harrison, P.A., 2005. Innovation in controlled atmosphere technology. Stewart Postharvest Rev. 3, 1–11. https://doi.org/10.2212/spr.2005.3.9

Prange, R.K., 2018. Dynamic controlled atmosphere (DCA) storage of fruits and vegetables, Reference Module in Food Science. Elsevier. https://doi.org/10.1016/B978-0-08-100596-5.21349-8

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/10.1080/10408390600976841

Rizzolo, A., Polesello, A., Teleky-Vàmossy, G., 1989. CGC/Sensory analysis of volatile compounds developed from ripening apple fruit. J. High Resolut. Chromatogr. 12, 824–827. https://doi.org/10.1002/jhrc.1240121214

Roger Harker, F., Anne Gunson, F., Brookfield, P.L., White, A., 2002. An apple a day: The influence of memory on consumer judgment of quality. Food Qual. Prefer. 13, 173–179. https://doi.org/10.1016/S0950-3293(02)00013-7

Rowan, D.D., Allen, J.M., Fielder, S., Hunt, M.B., 1999. Biosynthesis of straight-chain ester volatiles in “Red Delicious” and “Granny Smith” apples using deuterium-labeled precursors. J. Food Chemmestry Nanotechnologie 47, 2553–2562. https://doi.org/http p://dx.doi.org/10.1021/jf9809028

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. (Amsterdam). 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.

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. (Amsterdam). 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/10.1034/j.1399-3054.1997.1000320.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., Both, V., Brackmann, A., Ferreira, D. de F., Wagner, R., 2015a. 1-methylcyclopropene effects on volatile profile and quality of 'Royal Gala' apples produced in Southern Brazil and stored in controlled atmosphere. Ciência Rural 45, 2259–2266. https://doi.org/http://dx.doi.org/10.1590/0103-8478cr20141613

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

Thewes, F.R., Brackmann, A., Anese, R. de O., Bronzatto, E.S., Schultz, E.E., Wagner, R., 2017a. 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., Anese, R. de O., Ludwig, V., Schultz, E.E., dos Santos, L.F., Wendt, L.M., 2017b. 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., Brackmann, A., Both, V., Weber, A., Anese, R. de O., Ferrão, T. dos S., Wagner, R., 2017c. 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., 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. (Amsterdam). 227, 288–295. https://doi.org/10.1016/j.scienta.2017.09.028

Tsantili, E., Gapper, N.E., Arquiza, J.M.R.A., Whitaker, B.D., Watkins, C.B., 2007. Ethylene and r-farnesene metabolism in green and red skin of three apple cultivars in response to 1-methylcyclopropene (1-MCP) treatment. J. Agric. Food Chem. 55, 5267–5276. https://doi.org/10.1021/jf063775l

Vallat, A., Gu, H., Dorn, S., 2005. How rainfall, relative humidity and temperature influence volatile emissions from apple trees in situ. Phytochemistry 66, 1540–1550. https://doi.org/10.1016/j.phytochem.2005.04.038

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

Wakasa, Y., Kudo, H., Ishikawa, R., Akada, S., Senda, M., Niizeki, M., Harada, T., 2006. Low expression of an endopolygalacturonase gene in apple fruit with long-term storage potential. Postharvest Biol. Technol. 39, 193–198. https://doi.org/10.1016/j.postharvbio.2005.10.005

Weber, A., Brackmann, A., Anese, R. de O., Both, V., Pavanello, E.P., 2013a. Atmosfera controlada para o armazenamento da maçã 'Maxi Gala'. Rev. Cienc. Agron. 44, 294–301.

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

Weber, A., Brackmann, A., Thewes, F.R., Both, V., Anese, R.D.O., Schorr, M.R.W., 2012. Relative humidity and its interaction with the storage temperature of "Gala" apples and mutants. Ciência Rural 42, 2159–2165. https://doi.org/10.1590/S0103-84782012001200009

Weber, A., Rodrigo, F., Sellwig, M., Brackmann, A., Wünsche, N.J., 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

Weber, A., Thewes, F.R., Anese, R. de O., Both, V., Pavanello, E.P., Brackmann, A., 2017. Dynamic controlled atmosphere (DCA): interaction between DCA methods and 1-methylcyclopropene on 'Fuji Suprema' apple quality. Food Chem. 235, 136–144. https://doi.org/10.1016/j.foodchem.2017.05.047

Weber, A., Thewes, F.R., Schorr, M.R.W., Gasperin, A.R. De, Venturini, T.L., Brackmann, A., 2013b. ‘Gala’, ‘Royal Gala’ and ‘Brookfield’ apples stored under ultra-low oxygen and two temperatures. Biosci. J. 29, 1887–1895.

Wild, H.P.J. De, 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

Yang, X., Song, J., Campbell-Palmer, L., Fillmore, S., Zhang, Z., 2013. Effect of ethylene and 1-MCP on expression of genes involved in ethylene biosynthesis and perception during ripening of apple fruit. Postharvest Biol. Technol. 78, 55–66. https://doi.org/10.1016/j.postharvbio.2012.11.012

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

Young, H., Gilbert, J.M., Murray, S.H., Ball, R.D., 1996. Causal effects of aroma compounds on Royal Gala apple flavours 18, 329–336.

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

5 DISCUSSÃO GERAL

De acordo com o artigo 1, se as maçãs fossem comercializadas em até 14 dias de vida de prateleira, todas as condições de ACD e AC +1-MCP não difeririam entre si na produção de etileno e frutos sadios. Se analizarmos pela praticidade, o método de monitoramento de O_2 pelo PCA seria o mais indicado, visto que vem a ser uma técnica de baixo custo de implantação, além de dispensar a necessidade de tratamento dos frutos com o fitorregulador 1-MCP que tem um alto custo anual adicional no armazenamento de maçãs (THEWES et al., 2017b). Porém, após 21 dias a 20 °C, apenas ACD-QR 1,3 e ACD-PCA mantiveram os maiores percentuais de frutos sadios. Este resultado é de suma importância, visto que confirma a eficiência de ACD-QR 1,3 mas também da nova tecnologia proposta no presente trabalho.

O armazenamento em AC sem aplicação de 1-MCP resultou em maior produção de etileno, o que refletiu em maior concentração de etileno interno, escurecimento de polpa, polpa farinácea e, consequentemente, menor número de frutos sadios após 14 dias de vida de prateleira. O que evidencia a menor eficácea do método de AC no armazenamento de maçãs da cultivar Maxi Gala após 9 meses mais até 21 dias de vida de prateleira, por tanto, não sendo indicado o uso desta técnica.

A maior incidência de polpa farinácea aos 21 dias de vida de prateleira, também influenciou em menor número de frutos sadios das condições de AC+1-MCP e ACD-FC. Em trabalhos anteriores, a aplicação de 1-MCP em maçãs Gala resultou na redução da incidência de polpa farinácea após 7 dias de vida de prateleira (BRCKMANN et al., 2014, WATKINS, 2006), porém, este trabalho evidenciou que o 1-MCP e o armazenamento em ACD-FC está relacionado a algum dano no caso do 1-MCP, e monitoramento errôneo do limite mínimo tolerado pelos frutos pela técnica de ACD-FC durante o armazenamento, o que resulta em menor eficácea de amabas as técnicas após 9 meses de armazenamento e prolongada vida de prateleira, ao contrário do efeito de concentrações extremamente baixas de oxigênio utilizadas na ACD-QR e ACD-PCA.

Quanto à produção de compostos voláteis, avaliados no primeio artigo, o armazenamento em AC sem 1-MCP manteve maior concentração de compostos que caracterizam o aroma da maçã (ESPINO-DIAS, 2016; YOUNG et al., 1996) como o acetato de 2-metilbutila e acetato de hexila em relação às demais condições de armazenamento,aos 7

dias de vida de prateleira. Embora com menor concentração de acetato de hexila se comparado a AC, a ACD monitorada pelo ponto de compensação anaeróbico manteve maior concentração se comparado às demais condições de armazenamento armazenamento neste mesmo período de avaliação. A ACD-PCA manteve maior concentração de *thans*-2-hexenal, e assim como a ACD-QR 1,3, mantiveram maior concentração de butanal 2-metila após 14 dias de vida de prateleira.

Se comparado à AC, AC+1-MCP e ACD-FC, a condição de ACD-PCA foi eficiente na manutenção da qualidade de maçãs da cultivar ‘Maxi Gala’ após 7, 14 e 21 dias de vidade prateleira, a qual juntamente com ACD-QR 1,3 mantiveram maior percentual de frutos sadios após 21 dias de vida de prateleira. Este último parâmetro esteve diretamente ligado à incidência de polpa farinácea. A incidência de polpa farinácea só é percebida após o corte de um fruto, o que pode desestimular o consumidor a adquirir novamente maçãs desta cultivar. Desta forma, acompanhando a ACDQR 1,3, a ACD monitorada pelo ponto de compensação anaeróbico demonstra sua eficîência no armazenamento de maçãs após longo período de armazenamento e até 21 dias de vida de prateleira a 20 °C. Assim, mesmo que as duas técnicas mantenham a mesma qualidade de maçãs ‘Maxi Gala’ após nove meses de armazenamento mais até os 21 dias de vida de prateleira, a ACD-PCA destaca-se pela possibilidade de ser implementada em câmaras comerciais onde a AC já é empregada, por ser menos sensível à estanqueidade de câmaras à entrada de O_2.

Assim, visando o aprimoramento da técnica, buscou-se determinar a melhor concentração de CO_2 para a ACD-PCA, onde objetivou-se determinar a pressão parcial de CO_2 que melhor mantém a qualidade maçãs ‘Maxi Gala’ monitradas pelo método do ACD-PCA comparados à AC e ADC-CF. Maçãs armazenadas em ACD mantiveram menor respiração e produção de etileno se comparados à AC, mesmo após mantidas por 14 dias a 20 °C, o que está relacionado à menor concentraão de O_2. Todas as condições de ACD-PCA mantiveram menor atividade da enzima ACC oxidase aos 7 dias de vidade prateleira, este efeito deve estar relacionado à menor pressão parcial de O_2, uma vez que a enzima ACC oxidase necessita de O_2 para conveter ACC em etileno (PESIS, 2005). Assim, percebe-se um efeito do baixo O_2 usado durante o armazenamento em ACD-PCA sobre a atividade da enzyma ACC oxidase mesmo após exposta a 20 °C por 7 dias.

Maçãs armazenadas em ACD-PCA com 1,2 e 1,6 kPa CO_2 mantiveram maior firmeza de polpa aos 14 dias de vida de prateleira, e 1,6 e 2,0 kPa CO_2 aos 21 dias de vida de prateleira o que pode estar relacionado à menor atividade de enzimas que degradam a parede

celular, provavelmente devido ao processo inibitório do alto CO_2 sobre espansinas, pectinametilesterases e ß-xilosidases (BANG et al., 2019), combinado ao efeito do baixo oxigêncio da condição de ACD-PCA. Entretanto, a maior concentração de CO_2 resultou em maior incidência de polpa farinácea, o que reduziu o percentual de frutos sadios enquanto que ACD-PCA com 0,4, 1,2 e 1,6 kPa de CO_2 mantiveram maior percentual. Assim, percebe-se que assim como resultados encontrados na literatura (BRACKMANN; WEBER; BOTH, 2015), a concentração de 2,0 kPa de CO_2 não é indicada para o armazenamento de maãs mutantes da cultivar Gala armazenadas nas condições de ACD-FC, ACD-QR 1,5 e ACD-PCA.

6 CONSIDERAÇÕES FINAIS

No armazenamento de maçãs ‘Maxi Gala’, a condição de AC+1-MCP e todas as condições de ACD mantiveram maior pecentual de frutos sadios até 14 dias de vida de prateleira. Após 21 dias de vida de prateleira, as duas condições de ACD-QR 1,3 e ACD-PCA mantiveram maior percentual de frutos sadios, em consequência da menor incidência de polpa farinácea e escurescimento de polpa.

O armazenamento em ACD manteve melhor qualidade de frutos, mesmo após prolongada vida de prateleira de 21 dias a 20 °C, em consequencia da baixa respiração e principalmente reduzida produção de etileno.

Após, 7 dias de vida de prateleira, maçãs armazenadas em AC mantiveram maior concentração de acetato de 2-metilbutla e acetato de hexila. A ACD-PCA manteve maior concentração de acetate de hexila quando comparado à AC+1-MCP, ACD-FC e ACD-QR 1,3, porém menor se comparado à AC. A ACD-PCA manteve também, maior concentraçã de *thans*-2-hexenal e, ACD-QR 1,3 e ACD-PCA mantiveram maior concentraçã de butanal 2-metil após 14 dias de vida de prateleira. Alternativamente, pode ser recomendado o armazenamento em ACD, sem a necessidade de aplicar o 1-MCP.

O armazenamento por 9 meses na condição de AC, manteve maior escurescimento de polpa após 14 dias de vida de prateleira e, maior incidência de polpa farinácea após 14 e 21 dias de vida de prateleira. Por consequência, menor número de frutos sadios. Por tanto, no armazenamento de maçãs por longos períodos de armazenamento e prolongada vida de prateleira, o que é comum nos mercados nacionais, não recomenda-se o uso da técnica de AC isolada, mas sim com a aplicação de 1-MCP ainda que perdas ocorram, pricipalmente nos compostos voláteis.

O amazenamento de maçãs ‘Maxi Gala’ em ACD-PCA nas concentrações de 1,2 e 1,6 kPa CO_2 mantiveram maior manutenção da firmeza de polpa, maior percentual de frutos sadios e menor incidência de polpa farinácea após 14 e 21 dias de vida de prateleira.

Portanto, tendo em vista que a maior concentração de CO_2 que não cause dano aos frutos é aquela que otimiza o processo de absorção deste nas câmaras, assim a concentração de CO_2 indicada para a metodologia de ACD-PCA é a de 1.6 kPa CO_2.

7 REFERÊNCIAS BIBLIOGRÁFICAS

ANTONIOLLI, L. R. et al. Qualidade de maçãs baseada nas condições nutricionais e meteorológica. NACHTIGALL, G. R.; GONÇALVES, B. (Ed.). **Inovações tecnológicas para o Set. da maçã** – Inova maçã relatório técnico. Bento Gonçalves Embrapa Uva e Vinho, p.275–310, 2011.

ARGENTA, L.; FAN, X.; MATTHEIS, J. Delaying establishment of controlled atmosphere or CO_2 exposure reduces 'Fuji' apple CO_2 injury without excessive fruit quality loss. **Postharvest Biology and Technology**, v.20, p.221-229, 2000.

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

BANG, J. et al. Integrated transcriptomic-metabolomic analysis reveals cellular responses of harvested strawberry fruit subjected to short-term exposure to high levels of carbon dioxide. **Postharvest Biology and Technology**, v.148, p.120–131, 2019.

BARTLEY IM, HINDLEY SJ. Alcohol dehydrogenase of apple. **Journal of Experimenal Botany**, v.31, p.449–459, 1980.

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

BLANKENSHIP, S.M., DOLE, J.M. 1-Methylcyclopropene: a review. **Postharvest Biology and Technology**, v.28, p.1-25, 2003.

BOERSIG, M.R. KADER, A.A. ROMANI, R.J. Aerobic–anaerobic respiratory transition in pear fruit and cultured pear fruit cells. **Journal of the American Society for Horticultural Science (USA)**, 113 (1988), pp. 869-873.

BOTH, V. et al. Effect of storage under extremely low oxygen on the volatile composition of 'Royal Gala' apples. **Food Chemistry**, 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,** v.209, p.156-164, 2016.

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**, v.16, p.168-177, 2018.

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 American Society Horticultural Science**, v.118, p.243–247, 1993.

BRACKMANN, A.; CERETTA, M. Efeito da redução nos níveis de etileno e da umidade relativa no armazenamento de maçã 'Gala' em atmosfera controlada. **Pesquisa Agropecuária Brasileira**, v. 34, p. 2169-2174, 1999.

BRACKMANN, A. et al. Armazenamento em atmosfera controlada de maçãs mutantes da cultivar 'Gala'. **Revista Brasileira de Armazenamento**, v.34, n.2, p.136-143, 2009.

BRACKMANN, A. et al. Effect of growth regulators on 'Brookfield' apple gas diffusion and metabolism under controlled atmosphere storage. **Pesquisa Agropecuária Brasileira**, v.49, p. 323-329, 2014.

BRACKMANN, A. Control apparatus for controlled atmosphere cells for perishable items. United States Patent. **Publication number US 2015/0257401A1**, 2015.

BRACKMANN, A.; WEBER, A.; BOTH, V. CO_2 partial pressure for respiratory quotient and harvestwatch^{tm} dynamic controlled atmosphere for 'Galaxy' apples storage. **Acta Horticulturae**, Leuven, v.1079, p.435-440, 2015.

BRIZOLA et al., A metabolomics approach to elucidate apple fruit responses to static and dynamic controlled atmosphere storage. **Postharvest Biology and Technology,** v.127, p.76–87, 2017.

BUFLER, G. Ethylene-promoted conversion of 1-aminocyclopropene-1-carboxylic acid to ethylene in peel of apple at various stages of fruit development. **Plant Physiology**, v.80, n.2, p.539-543, 1986.

CHERVIN, C. et al. Combining controlled atmosphere storage and ethanol vapors to control superficial scald of apple. **Horticulture Science**, v.36, p.951-952, 2001.

DEELL, J. R.; MURR, D. P.; MUELLER, R.; WILEY, L.; PORTEOUS, M. D. Influence of 1-methylcyclopropene (1-MCP), diphenylamine (DPA), and CO_2 concentration during storage on 'Empire' apple quality. **Postharvest Biology and Technology**, v.38, p.1-8, 2005.

Dickinson J. R. et al. A ^{13}C nuclear magnetic resonance investigation of the metabolism of leucine to isoamyl alcohol in Saccharomyces cerevisiae. **Journal of Biology and Chemistry**, v.272, p.26871–2678, 1997.

DUNEMANN, F. et al. Functional allelic diversity of the apple alcohol acyl-transferase gene MdAAT1 associated with fruit ester volatile contents in apple cultivars. **Molecular Breeding**, v.29, p.609–625, 2012.

ECHEVERRÍA, G. et al. Volatile produc- tion, quality and aroma-related enzyme activities during maturation of 'Fuji' apples. **Postharvest Biology and Technology**. v.31, p.217–227, 2004.

ESPINO-DIAZ, M.; SEPULVEDA, D.R.; GONZALEZ-AGUILAR, G.; OLIVAS, G.I. Biochemsitry of apple aroma. **Food Technology Biotechnology** v.54, p.375–394, 2016.

FAOSTAT, Food and Agriculture Organization of the United Nations Statistics. 2017. Disponível em: <http://www.fao.org/faostat/en/#rankings/countries_by_commodity>. Acesso em: 12 jan. 2018.

FELLMAN J. K. Factors that influence biosynthesis of volatile flavor compounds in apple fruits. Horticultural Science, v.35, p.1026–1033, 2000.

GAGO, C. M. L. et al. Effect of harvest date and 1-MCP (SmartFresh™) treatment on 'Golden Delicious' apple cold storage physiological disorders. **Postharvest Biology and Technology,** v.110, p.77-85, 2015.

GASSER, F., EPPLER, T., NAUNHEIM, W., GABIOUD, S., NISING, A.B. Dynamic CA storage of apples : monitoring of the critical oxygen concentration and adjustment of optimum conditions during oxygen reduction. **Acta Horticulturae**, v.876, p.39–46, 2010.

JAMES, H. J.; JOBLING, J. J. Contrasting the structure and morphology of the radial and diffuse flesh browning disorders and CO_2 injury of 'Cripps Pink' apples. **Postharvest Biology and Technology**, v.53, p.36-42, 2009.

KIST, B. B. et al. Anuário brasileiro da maçã 2016. **Santa Cruz do Sul: Editora Gazeta Santa Cruz**, v.56, 64 p.: il, 2016.

Ló LÓPEZ ML, et al. Influence of different oxygen and carbon dioxide concentrations during storage on production of volatile compounds by Starking Delicious apples. **Journal of Agricultural Food and Chemintry**. v.46, p.634–643, 1998.

LÓPEZ M. L. et al. Comparison of volatile compounds in two seasons in apples: Golden Deli- cious and Granny Smith. **Journal of Food Quality**. v.21, p.155–166, 1998.

LÓPEZ, M. L. Volatile compounds, quality parameters and consumer acceptance of 'Pink Lady®' apples stored in different conditions. **Postharvest Biology and Technology** 43, 55-66, 2007.

LU, X. et al. Effects of 1-MCP in combination with Ca application on aroma volatiles production and softening of 'Fuji' apple fruit. **Science Horticulturae** 229, 91–98, 2018.

LUMPKIN, C.; FELLMAN, J. K.; RUDELL, D. R.; MATTHEIS, J. P. 'Fuji' apple (*Malus domestica* Borkh.) volatile production during high pCO_2 controlled atmosphere storage. **Postharvest Biology and Technology**, v.100, p.234-243, 2015.

LUNARDI, R. et al. Suculência e solubilização de pectinas em maçãs 'Gala´, armazenadas em atmosfera controlada, em dois níveis de umidade relativa. **Ciência Rural**, v.34, n.3, p.743-747, 2004.

MANNAPPERUMA, J. D.; SINGH, R. P.; MONTERO, M. E. Simultaneous gas diffusion and chemical reaction in foods stored in modified atmosphere. **Journal of Food Engineering**, v.14, p.167-183, 1991.

MATHOOKO, F. M. Regulation of respiratory metabolism in fruits and vegetables by carbon dioxide. **Postharvest Biology and Technology**, v.9, p.247-264, 1996.

MEHINAGIC, E. et al. Characterisation of odor-active volatiles in apples: Influence of cultivars and maturity stage. **Journal of Agricultural Food and Chemistry**, v.54, p.2678–2687, 2006.

PAYASI, A. et al. Biochemistry of fruit softening: an overview. **Physiology and Molecular Biology of Plants**, v.15, p.103–113, 2009

PRANGE, R., DELONG, J.M., DANIELS-LAKE, B., HARRISON, P.A. Innovation in controlled atmosphere technology. **Stewart Postharvest Review,** v.3, p.1–11, 2005.

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

Prange, R.K. Dynamic controlled atmosphere (DCA) Storage of fruits and vegetables, **Reference Module in Food Science, v.1985, p.1-10,** 2018.

PESIS, E. The role of the anaerobic metabolites, acetaldehyde and ethanol, in fruit ripening, enhancement of fruit quality and fruit deterioration. **Postharvest Biology and Technology**, v.37, p.1–19, 2005.

REENTZ, E. R. et al. Anuário brasileiro da fruticultura. Santa Cruz do Sul: Gazeta Santa Cruz, 2009. 128 p.

RIUDAVETS, J.; PONS, M. J.; MESSEGUER, J.; GABARRA, R. Effect of CO_2 modified atmosphere packaging on aflatoxin production in maize infested with *Sitophilus zeamais*. **Journal of Stored Products Research**, v.77, p.89-91, 2018.

SALAZAR, N.O.; OROZCO, G.O. El aroma de la manzana. **Interciencia**, v.36, p.265-271, 2011.

SANTOS, I. D. et al. Patulin accumulation in apples under dynamic controlled atmosphere storage. **Food Chemintry**, v.255, p. 275-281, 2018.

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**, v.75:2, p.243-249, 2000.

Schaffer, R.J. et al. A genomics approach reveals that aroma pro- duction in apple is controlled by ethylene predominantly at the fi nal step in each biosynthetic pathway. Plant Physioly, v.144, p.1899–1912, 2007.

SEPPÄ, L. et al. Flavour and texture changes in apple cultivars during storage. **LWT - Food Science and Technology**, v.54, p.500-512, 2013.

St-PIERRE B, De LUCA V. Evolution of acyltransferase genes, origin and diversification of the BAHD superfamily of acyltransferases involved in secondary metabolism. In: Romeo JT, Ibrahim R, Varin L, De Luca V, editors. **Recent Advances in Phytochemistry**, vol. 34: Evolution of metabolic pathways. Oxford, UK: Elsevier Science Ltd; 2000. pp. 285–315.

Disponível em: htt p://dx.doi.org/10.1016/S0079-9920(00)80010-6

SISLER, E. C.; SEREK, M. Inhibitors of ethylene responses in plants at the receptor level: recent developments. **Physiologia Plantarum**, v.100, p.577-582, 1997.

Taiz, L. et al. **Fisiologia e desenvolvimento vegetal**. 6ed. Porto Alegre: Artmed, 2016. 858p.

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

THEWES, F. R. **Atmosfera controlada dinâmica monitorada pelo quociente respiratório e sua interação com estádios de maturação sobre a conservação da qualidade e o perfil volátil de maçãs 'Galaxy'.** 2016. 127 p. Dissertação (Mestrado em Agronomia) – Universidade Federal de Santa Maria, Santa Maria, RS, 2016.

THEWES, F. R. et al. The different impacts of dynamic controlled atmosphere and controlled atmosphere quality attributes of 'Fuji Suprema' apples. **Postharvest Biology and Technology**, v.130, p.2-20, 2017a.

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**, v.127, p.1-13, 2017b.

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**, v.222, p.84-93, 2017c.

.

VELTMAN, R. H. et al. Dynamic control system (DCS) for apples (*Malus domestica*, Borkh. cv. 'Elstar'): optimal quality through storage based on products response. **Postharvest Biology and Technology**, v.27, p.79-86, 2003.

WATKINS, C. B.; NOCK, J. F.; WHITAKER, B. D. Responses of early, mid and late season apple cultivars to postharvest application of 1 -methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. **Postharvest Biology and Technology**, v.19, p. 17-32, 2000.

WATKINS, C. B. The use of 1-metylcyclopropene (1-MCP) on fruit and vegetables. **Biotechnology Advances**, v.24, p.389-409, 2006.

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

WEBER, A. et al. Atmosfera controlada para o armazenamento da maçã 'Maxi Gala'. **Revista Ciência Agronômica**, v.44, n. 2, p.294-301, 2013.

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

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

YEARSLEY, C. W.; BANKS, N. H.; GANESH, S. Effect of carbon dioxide on the internal lower oxygen limits of apple fruit. **Postharvest Biology and Technology**, v.12, p.1-13, 1997.

YOUNG, H. et al. Causal effect of aroma compounds on Royal Gala apple flavours. **Journal of the Science of Food and Agriculture**, v.71, p.329-336, 1996.

YANG, S. F.; HOFFMAN, N. E. Ethylene biosynthesis and its regulation in higher plants. **Annual Review of Plant Physiology**, v. 35, p. 155-189, 1984.**)**