UNIVERSIDADE ESTADUAL PAULISTA – UNESP

CÂMPUS DE JABOTICABAL

ARMAZENAMENTO DE MANGA EM BAIXAS TEMPERATURAS USANDO POLIÓIS E ATMOSFERA CONTROLADA

Alex Guimarães Sanches

Engenheiro Agrônomo

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Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Doutor em Agronomia (Produção Vegetal).

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TÍTULO DA TESE: ARMAZENAMENTO DE MANGA EM BAIXAS TEMPERATURAS USANDO POLIÓIS E ATMOSFERA CONTROLADA

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DADOS CURRICULARES DO AUTOR

ALEX GUIMARÃES SANCHES, natural de Monte Alegre, estado do Pará, nasceu no dia 19 de fevereiro de 1991. Iniciou o curso de Engenharia Agronômica em março de 2010 pela Universidade Federal do Pará (UFPA), Campus Altamira – PA. Durante a sua graduação desenvolveu o Trabalho de Conclusão de Curso (TCC) intitulado “Avaliação da qualidade pós-colheita de cultivares de alface minimamente processadas oriundas de cultivo hidropônico’’ (março de 2014) e obteve o título de Engenheiro Agrônomo em novembro de 2014. Em agosto de 2016, ingressou no curso de Mestrado em Agronomia (Fitotecnia) da Universidade Federal do Ceará (UFC), Campus do Pici, Fortaleza, estado do Ceará, obtendo o título de Mestre em julho de 2018, com a dissertação intitulada “Influência da luz pulsada sobre a histologia, metabolismo de vitamina C e compostos fenólicos no armazenamento de acerolas’’. Em agosto de 2018, iniciou o curso de Doutorado no Programa de Pós-graduação em Agronomia (Produção Vegetal), da Universidade Estadual Paulista (UNESP), Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal (FCAV), Campus de Jaboticabal, integrando o grupo de pesquisa em Fruticultura e Pós-colheita (FrutUnesp), com bolsa inicial da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) código de financiamento 01 e posteriormente, do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). A trajetória acadêmica é dedicada ao estudo da Fisiologia Pós-colheita de frutas e hortaliças.

Acredite nos seus sonhos e trabalhe para realizá-lo. Um dia EU sonhei com isso.

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SUMÁRIO

ARMAZENAMENTO DE MANGA EM BAIXAS TEMPERATURAS USANDO POLIÓIS E ATMOSFERA CONTROLADA

RESUMO – Mangas (*Mangifera indica* L.) são frutas perecíveis e desenvolvem injúrias pelo frio (IF) quando armazenadas abaixo de 13 ºC, o que limita sua qualidade, capacidade de armazenamento e possibilidades de envio para mercados potenciais distantes. Para superar essas limitações, o objetivo deste estudo foi avaliar o uso de polióis (glicerol, propilenoglicol e sorbitol) em associação com atmosfera controlada (5 kPa O_2 + 5 kPa CO_2) para reduzir a susceptibilidade de mangas 'Palmer' a injúria pelo frio quando armazenadas a baixas temperaturas. As variáveis físico-químicas e bioquímicas, além da incidência da injúria pelo frio foram investigadas no epicarpo (casca) e mesocarpo (polpa). Em geral, as variáveis físico-químicas não foram comprometidas com os tratamentos. Durante o armazenamento a 8,0 ºC com diferentes concentrações de polióis (0,1, 0,5 e 2,5 % m/v) houve alívio dos sintomas da injúria pelo frio, especialmente em mangas tratadas com sorbitol 2,5 %, e isso foi associado com menor acúmulo de peróxido de hidrogênio (H_2O_2) e peroxidação lipídica (PL), preservação do conteúdo de ascorbato (AsA) e aumento nas atividades das enzimas antioxidantes superóxido dismutase (SOD), catalase (CAT) e ascorbato peroxidase (APX). Em temperaturas mais baixas (1,0 ºC), o efeito dos polióis (0,1 %) foi investigado, e a solução de sorbitol também aliviou os sintomas da IF durante 14 dias de armazenamento refrigerado e após 7 dias em ambiente. Além disso, baixas conteúdos de H_2O_2, PL e atividade da polifenoloxidase (PPO) e altas concentrações de AsA e de atividade das enzimas SOD, CAT e APX foram relacionados à tolerância à IF nessas frutas. Por fim, os frutos foram tratados com soluções de sorbitol (0,1 e 2,5 %) e armazenados sob AC (5 kPa O_2 + 5 kPa CO_2) a 8,0 ºC. Mangas tratadas com sorbitol 2,5 % + AC não desenvolveram sintomas de IF sob armazenamento refrigerado, enquanto no ambiente as lesões foram mínimas. Essa resposta foi associada a altas concentrações de AsA e polifenóis totais, aumento da atividade de SOD, CAT, APX, além de redução da atividade de PPO no epicarpo e mesocarpo. O desenvolvimento da injúria pelo frio foi associado à perda de massa fresca, cor do epicarpo (L *, hº e C *), aumento no conteúdo de H_2O_2, no vazamento de eletrólitos (EL), na PL e na atividade oxidativa da PPO. Esses resultados sugerem que o sorbitol (2,5 %) pode ser uma alternativa para o alívio da IF em mangas 'Palmer', especialmente quando associado a AC.

Palavras-chave: *Mangifera indica* L., antioxidantes enzimáticos e não enzimáticos, injúria pelo frio, polióis, atmosfera controlada.

MANGO STORAGE AT LOW TEMPERATURES USING OF POLYOLS AND CONTROLLED ATMOSPHERE

ABSTRACT - Mangoes (*Mangifera indica* L.) are perishable fruits and develop chilling injury (CI) when stored below 13 ºC, which limits their quality, storage capacity, and possibilities for shipping to distant potential markets. To overcome these limitations, the aim of this study was to evaluate the use of polyols (glycerol, propylene glycol, and sorbitol) in combination with a controlled atmosphere (5 kPa O_2 + 5 kPa CO_2) to reduce the susceptibility of 'Palmer' mangoes to chilling injury when stored at low temperatures. The physicochemical and biochemical variables, in addition to the incidence of CI, were investigated in the epicarp (peel) and mesocarp (pulp). In general, the physicochemical variables were not compromised by the treatments. During storage at 8.0 ºC with different concentrations of polyols (0.1, 0.5, and 2.5% m/v) there was relief from the symptoms of CI, especially in mangoes treated with 2.5% sorbitol, and this was associated with a lower accumulation of hydrogen peroxide (H_2O_2) and lipid peroxidation (LP), preservation of ascorbate (AsA) content, and increased activities of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). At lower temperatures (1.0 ºC), the effect of polyols (0.1%) was investigated, and the sorbitol solution also alleviated CI symptoms during 14 days of refrigerated storage and after 7 days in the room. Furthermore, low contents of H_2O_2, PL, and polyphenol oxidase (PPO) activity and high concentrations of AsA and activity of SOD, CAT, and APX enzymes were related to tolerance to CI in these fruits. Finally, the fruits were treated with sorbitol solutions (0.1 and 2.5%) and stored under AC (5 kPa O_2 + 5 kPa CO_2) at 8.0 ºC. Mangoes treated with 2.5% sorbitol + AC did not develop symptoms of CI under refrigerated storage, while in the environment the lesions were minimal. This response was associated with high concentrations of AsA and total polyphenols, increased SOD, CAT, APX activity, and reduced PPO activity in the epicarp and mesocarp. The development of cold injury was associated with loss of fresh weight, epicarp color (L *, hº and C *) increase in H_2O_2 content, electrolyte leakage (EL), LP, and PPO oxidative activity. These results suggest that sorbitol (2.5%) may be an alternative for the relief of CI in 'Palmer' mangoes, especially when associated with CA.

Keywords: *Mangifera indica* L., chilling injury, enzymatic and non-enzymatic antioxidants, polyols, controlled atmosphere.

CAPÍTULO 1 – Considerações gerais

1. Introdução

A mangueira (*Mangifera indica* L.) é uma árvore frutífera da família Anacardiaceae nativa do sul e sudeste da Ásia, sendo cultivada nas regiões tropicais e subtropicais ao redor do globo (Siddiq, Brecht e Sidhu, 2017). De acordo com a *Food and Agriculture Organization* (FAO), o Brasil ocupa o sétimo lugar no ranking dos maiores produtores de manga do mundo e o terceiro lugar na exportação desta fruta (FAOSTAT, 2019; Mango Crop Report, 2021). A produção nacional ocorre principalmente na região nordeste, que em 2020 contribuiu com mais de 87 % da produção dessa fruta. Nesse mesmo ano, a produção brasileira de mangas foi superior a 240 mil toneladas (t), o que classifica esta fruta como a primeira em geração de receita e volume exportado (EMBRAPA, 2020; ABRAFRUTAS, 2020). Dentre as cultivares produzidas, a Tommy Atkins representa 75 % da produção, porém a 'Palmer' vem ganhando a preferência dos consumidores em função de suas melhores características de qualidade, ou seja, frutos mais doces, aromáticos e com pouco ou nenhuma fibra (HORTIFRUTI BRASIL, 2018).

A manga é uma fruta climatérica, sendo altamente perecível e seu amadurecimento ocorre rapidamente, cerca de 7 dias, quando mantidas à temperatura ambiente (Eshetu et al., 2019; Ebrahimi e Rastegar, 2020). Esta característica limita a vida útil pós-colheita dessa fruta e reduz seu potencial de comercialização para mercados distantes. O uso da refrigeração vem sendo adotado visando aumentar a vida útil pós-colheita para 21 – 30 dias, dependendo da cultivar e do estádio de maturação (Miguel et al, 2011; Singh et al., 2013; Penchaiya et al., 2020). Todavia, em função da sensibilidade desta fruta à baixas temperaturas (< 13 ºC), o potencial de armazenamento também é limitado, pois nessa condição ocorre o desenvolvimento de um distúrbio fisiológico conhecido por dano por frio ou *chilling injury* (CI) (Zaharah e Singh, 2011; Zhang et al., 2017).

A injúria pelo frio (IF) é um dos principais problemas que ocorre durante o armazenamento de mangas, sendo economicamente importante, pois os sintomas da lesão, descoloração semelhante a queimaduras aprofundada, são mais evidentes no

epicarpo e dependendo dos padrões de qualidade estabelecidos pelo mercado consumidor, esses defeitos podem levar à desclassificação das mangas para o mercado de frutas frescas (Chaplin et al., 1991; Wang et al., 2008; Zhang et al., 2017). Soma-se a isso, é difícil prever um período seguro sob refrigeração, já que os sintomas são mais evidentes quando esses produtos são transferidos do resfriamento para a temperatura ambiente (Wang, 1989; Chidtragool et al., 2011).

Assim, o desenvolvimento ou a combinação de tecnologias de conservação para aumentar a tolerância ao frio e preservar a qualidade, poderia reduzir a incidência da IF possibilitar a comercialização de mangas para mercados potenciais. Nesse contexto, a atmosfera controlada (AC) é uma tecnologia que vem sendo utilizada em complementação à refrigeração durante o transporte dos frutos de mangueira visando retardar o processo de amadurecimento, preservando assim a qualidade (sabor, aroma, coloração) e aumentando a vida útil pós-colheita dependendo da concentração de oxigênio (O_2) e dióxido de carbono (CO_2) nas câmaras de armazenamento (Yahia et al., 2019; Brecht, 2020). Contudo, esta tecnologia também apresenta limitações, pois as mangas quando mantidas a temperaturas inferiores a 13 °C irão desenvolver danos por frio, o que reduz o potencial da AC em controlar os processos fisiológicos relacionados ao seu amadurecimento. Assim, uma possibilidade para maximizar a eficiência do uso da AC seria a sua associação a outras técnicas, e.g. o uso de aditivos alimentares comumente utilizados na indústria de alimentos.

Um grupo de aditivos que teria potencial em reduzir a injúria pelo frio seria o dos polióis. Esses compostos são substâncias que apresentam a capacidade de reduzir o ponto de congelamento e preservar a qualidade de alimentos a longo prazo (Azelee et al., 2019; Kalicka et al., 2019). No setor alimentício essas substâncias são classificadas como aditivos com função crioprotetora, umectante, edulcorante e texturizante (Pereira, 2013; Rice et al., 2020) e são considerados como produtos seguros, *Generally Recognized As Safe* (GRAS) (BRASIL, 1997; UE, 2004; FDA, 2016; CODEX ALIMENTARIUS, 2019).

Ante o exposto e considerando as limitações do armazenamento de mangas à baixas temperaturas (< 13 ºC), o objetivo geral deste trabalho é verificar a possibilidade do uso de polióis (glicerol, propileno glicol, sorbitol) e da associação com a atmosfera controlada (5 % O_2 e 5% CO_2) durante o armazenamento de mangas

‘Palmer’ sob baixas temperaturas (<13 ºC) visando o prolongamento da vida útil pós-colheita, sem o desenvolvimento de injúrias pelo frio. Os objetivos específicos foram: i) avaliar o efeito de polióis na redução da susceptibilidade de mangas a IF; ii) avaliar o efeito dos polióis e da combinação com AC no potencial de conservação e na preservação da qualidade físico-química e ii) investigar os processos bioquímicos relacionados ao metabolismo de defesa antioxidante em resposta a incidência de IF.

2. REVISÃO DE LITERATURA

2.1 Mangueira, aspectos botânicos e econômicos

A mangueira (*Mangifera indica* L.) é uma árvore frutífera originária da região Indo-Birmânia na Índia e vem sendo cultivada há pelo menos 4.000 anos (Mukherjee e Litz 2009). Essa espécie é considerada uma das mais importantes dentre às descritas na família Anacardiaceae e é cultivada comercialmente nas regiões tropicais e subtropicais do mundo.

O fruto da mangueira é denominado manga, sendo esse uma drupa carnosa indeiscente, com formato alongado, ovoide, oblongo ou arredondado, de aparência exótica que leva de três a seis meses para amadurecer. A manga é composta pelo exocarpo (casca), mesocarpo (polpa) e endocarpo (semente) (Litz e Lavi, 1997). O endocarpo contém um ou mais embriões, dependendo da cultivar ou raça. A coloração do exocarpo no fruto imaturo é verde e varia do amarelo ao roxo quando os frutos estão maduros. O mesocarpo é comestível, com sabor doce, semi-ácido e azedo, de espessura variável, suculento, geralmente fibroso com coloração que vai do amarelo ao alaranjado (Judd et al., 2009; Mukherjee e Litz, 2009).

Até o momento foram descritas mais de 1.000 cultivares de mangueiras ao redor do mundo, muitas das quais produzem frutos que amadurecem no verão, enquanto outras apresentam duas colheitas por ano (Solís-Fuentes e Durán-de-Bazúa, 2011).

De acordo com a *Food and Agriculture Organization* (FAO), o Brasil ocupa o sétimo lugar no *ranking* dos maiores produtores de manga do mundo (FAOSTAT, 2019). Em 2020, a produção brasileira de manga foi de 243,2 mil toneladas (t), oriundas de uma área cultivada de 76 mil hectares (ha), sendo que 87 % da produção foi proveniente da região Nordeste e 10 % da região Sudeste (EMBRAPA, 2020). Segundo o Mango Crop Report (2021), o Brasil foi o terceiro maior exportador de mangas em 2020. Nesse mesmo ano, esta atividade gerou US$ 246,9 milhões na balança comercial, com a exportação de 212 mil t de frutas, o que colocou a manga com a principal fruta em geração de receita e volume exportado (ABRAFRUTAS, 2020). Os maiores compradores da manga brasileira são a União Europeia (UE) e os

Estados Unidos da América (EUA), com destaque para as cultivares americanas, tais como: ‘Tommy Atkins’, ‘Palmer’, ‘Keitt’, ‘Kent’ e ‘Haden’ (Mango Crop Export, 2021). Nos últimos anos, as cultivares tardias como a ‘Palmer’ e ‘Keitt’ vem ganhando espaço no mercado europeu por conta da sazonalidade e devido às suas características de qualidade e preferência do consumidor (HORTIFRUTI BRASIL, 2018).

A cultivar Palmer é originária da Flórida, EUA, sendo caracterizada por apresentar frutos grandes (~400 a 600 g) e compridos. Quando imaturos os frutos apresentam coloração verde-arroxeada e, quando maduros, tornam-se vermelho-escuro, com polpa amarelada sem fibras (Fonseca et al., 2006). Em relação a cultivar mais plantada no Brasil, a ‘Tommy Atkins’, os frutos da ‘Palmer’ são mais doces, o que deve corresponder ao seu sabor superior, mais firmes favorecendo o manuseio e transporte, além de apresentarem maior relação polpa/fruto, teores de vitamina C, flavonoides, polifenóis e atividade antioxidante (Modesto et al., 2016; Costa et al., 2019).

2.2 Composição nutricional e fisiologia pós-colheita

A característica físico-química das mangas é influenciada pela variabilidade genética, regiões de cultivo, condições ambientais (precipitação, temperatura, altitude), práticas culturais (manejo do solo, adubação, irrigação, ocorrência de pragas e doenças) e tecnologias pós-colheita (Lobo e Sidhu, 2017; Ntsoane et al., 2019). Assim, considerando a composição química do fruto maduro, cada 100 gramas apresentam 60 – 250 kcal; 14,98 g de carboidratos; 0,38 g de lipídios; 0,82 g de proteínas e 1,6 g de fibras. Os minerais como potássio, K (168 mg), fósforo, P (14 mg), cálcio, Ca (11 mg) e magnésio, Mg (10 mg) aparecem como os mais abundantes, mas são encontrados em menor proporção ferro (Fe), sódio (Na), zinco (Zn), cobre (Cu), manganês (Mn) e selênio (Se) (USDA, 2016). Os principais açúcares são a sacarose (6,97 g), frutose (4,68 g) e glicose (2,01 g), enquanto na composição dos ácidos graxos destacam-se os ácidos graxos esteárico, oleico, palmítico e linoleico. As vitaminas mais abundantes são: vitamina C (6,56 – 60,1 mg), niacina (0,669 mg), riboflavina (0,038 mg) e tiamina (0,028 mg). Dentre os compostos bioativos a manga apresenta mangiferina (12,4 – 996 mg), β-caroteno (43,3 - 640 µg), α-caroteno (9,0

µg), β-criptoxantina (10 µg), licopeno (3,0 µg) α-tocopherol (0,90 mg), flavonoides (0,48 – 1,41 mg) e quercetina (0,15 – 4,52 mg) (Petry e Mercadante,2016; Alanón et al., 2019; Sousa et al., 2021).

Apesar do potencial nutricional da manga, esse é limitado pelos aspectos fisiológicos desta fruta, pois sua perecibilidade reduz o período de consumo deste alimento. A manga é classificada como um fruto climatérico que apresenta aumento da atividade respiratória (40 – 50 a 160 – 200 $mg.kg^{-1}.h^{-1}$) e da produção de etileno (0,1 – 0,2 a 1 – 3 $\mu L.kg^{-1}.h^{-1}$) quando mantida à 20 ºC (Rao e Rao, 2008). Além disso, ocorre a degradação da clorofila, biossíntese de carotenoides e antocianinas (Ma et al., 2018), redução da firmeza e aumento da suculência (Nambi et al., 2016), conversão de amido em açúcares (aumento dos sólidos solúveis), redução dos ácidos orgânicos (acidez titulável) e acúmulo de compostos voláteis (Thiruchelvam et al., 2020; Sousa et al., 2021). Essas mudanças fisiológicas, bioquímicas e organolépticas afetam a vida útil pós-colheita limitando-a aproximadamente sete dias sob condições de ambiente (Eshetu et al., 2019; Ebrahimi e Rastegar, 2020). Similarmente, o potencial de armazenamento, transporte e comercialização para mercados mais distantes também é limitado (Sivakumar et al., 2011).

2.3 Refrigeração e injúria pelo frio (IF)

O armazenamento refrigerado é a principal tecnologia pós-colheita utilizada para aumentar a vida útil da manga, uma vez que as baixas temperaturas diminuem sua atividade metabólica (Veja-Alvarez et al., 2020). Quando mantidas entre 8 – 13 ºC, a vida útil pós-colheita pode ser aumentada para até 21 – 30 dias, dependendo da cultivar e do estádio de maturação das frutas (Singh et al., 2013; Penchaiya et al., 2020). Porém, o armazenamento prolongado a temperaturas inferiores a 13 ºC pode levar ao desenvolvimento de um distúrbio fisiológico conhecido como injúria pelo frio (IF) ou *chilling injury* (CI), resultando em perdas quantitativas e qualitativas após a colheita (Zaharah e Singh, 2011; Zhang et al., 2017).

A IF ocorre quando produtos vegetais são armazenados a temperaturas abaixo de um valor crítico, mas acima do seu ponto de congelamento (Skog, 1998; Purvis, 2004). As baixas temperaturas induzem a mudanças estruturais nas células

resultando em vários processos metabólicos deletérios à qualidade dos frutos. As alterações induzidas pela IF podem ser consideradas como lesões primárias ou secundárias (Raison e Lyons, 1986). A lesão primária é a resposta rápida inicial às baixas temperaturas que são capazes de causar disfunções na célula vegetal e no processo metabólico podendo ser prontamente reversível se a temperatura for elevada a condições não injuriantes. Por outro lado, as lesões secundárias surgem em decorrência das lesões primárias, mas são irreversíveis levando a danos permanentes e morte celular independente do manuseio subsequente do produto (Rayson e Lyons, 1986; Wang, 1989; Shewfelt, 1992).

Os sintomas da IF em mangas são mais evidentes no epicarpo, porém o mesocarpo também é afetado. No epicarpo é comumente relatada a presença de descoloração semelhante a queimaduras aprofundadas (Figura 1) que prejudica a comercialização da fruta *in natura*, pois esse defeito pode tornar o produto inaceitável, dependendo dos padrões estabelecidos pelo mercado (Chaplin et al., 1991; Zhang et al., 2017).

Figura 1. Desenvolvimento de injúrias pelo frio em mangas ‘Palmer’.

No mesocarpo os sintomas mais comuns são o enrijecimento da polpa, ausência de sabor e aroma característico, amadurecimento desuniforme e susceptibilidade a podridões (Nair et al., 2003; Wang et al., 2008; Zaharah e Singh,

2011). Os sintomas se tornam mais evidentes quando as mangas são transferidas para a temperatura ambiente (Wang, 1989; Chidtragool et al., 2011).

A susceptibilidade a IF varia conforme a cultivar, estádio de maturação, condições ambientais de cultivo (temperatura, luminosidade) e de armazenamento (temperatura, tempo de exposição e umidade relativa). Por exemplo, mangas 'Rad' e 'Okrong' apresentaram menor incidência de IF ao longo de 25 dias de armazenamento (4, 8 e 12 ºC) em comparação às cultivares Kaew, Tongdum, Nam Dok Mai e Nungklangwun (Phakawatmongkol et al., 2004). Da mesma forma, o IF foi mais severo em mangas 'Sensation' em relação a 'Samar Bahisht' armazenadas a 6, 9 e 12 ºC por 16 dias (Farooqi et al., 1985).

Com relação ao estádio de maturação, mangas colhidas imaturas ou na maturidade fisiológica apresentaram-se mais sensíveis a IF do que as colhidas maduras (Mohammed e Brecht, 2002). Mangas 'Amelie', 'Tommy Atkins' e 'Keitt' armazenadas a 8, 10 e 12 ºC por 21 dias desenvolveram sintomas mais severos de CI quando colhidas antes da maturidade fisiológica em relação as frutas colhidas maduras (Medlicott, 1990). O índice de IF foi maior em mangas 'Zihua' colhidas 100 % verdes do que nas frutas colhidas com 20 e 50 % da casca amarela (Zhao et al., 2009). Uma possível explicação poderia ser inferida da análise de transcriptoma de fruto que foi armazenado à 5 ºC, pois nesta condição a manga aumentou a biossíntese e osmolaridade de etileno, ativando o metabolismo de açúcares, reduzindo assim o ponto de congelamento (Sivankalyani et al., 2016a). Neste sentido, as mangas maduras, em função da maior concentração de açúcares, são mais resistentes a injúria pelo frio (Patil et al., 2019).

Durante o cultivo, a temperatura e a luz (alta irradiância) promovem alterações estruturais em proteínas que ativam o metabolismo secundário (Mes et al., 2008). Nesse sentido, um estudo mostrou que mangas 'Shelly' acumularam mais flavonoides e antocianinas no epicarpo quando expostas a luz solar e isso foi correlacionado com a redução do dano pelo frio quando armazenadas a 10 ºC por 21 dias (Sivankalyani et al., 2016b). Esse resultado sugere que o acúmulo de compostos bioativos no epicarpo dos frutos da mangueira pode aumentar a resistência a IF e favorecer frutos mais atraentes.

O manejo da temperatura no ambiente de armazenamento é o principal responsável pelo desenvolvimento da injúria pelo frio nas células de vegetais, pois quanto mais baixa a temperatura de armazenamento, menor é o tempo de exposição necessário para desencadear o desenvolvimento dos sintomas de injúria pelo frio (Wang, 1989; Purvis, 2004). Mangas 'Palmer' mantidas por 7 dias a 2 e a 5 ºC precisaram de apenas 1 dia sob temperatura ambiente para desenvolver IF, por outro lado, quando armazenadas a 12 ºC não houve desenvolvimento destes sintomas (Miguel et al., 2013). Da mesma forma, o armazenamento na menor faixa de temperatura (0 – 10 ºC) provocou maior índice de injúria pelo frio em mangas 'Kensington Pride' (Nair e Singh, 2004), 'Kent' (5 – 12 ºC) (Dea et al., 2010), 'Tommy Atkins' (2 – 12 ºC) (Miguel et al., 2015), 'Carabao' (7 – 13 ºC) (Rodeo e Esguerra, 2013), 'Langra' (5 – 15 ºC) (Islam et al., 2018).

Apesar da injúria pelo frio ser muito estudada durante o armazenamento de mangas, ainda há uma grande dificuldade de se estabelecer os processos e mecanismos envolvidos na injúria pelo frio, assim como, a distinção entre lesões primárias e secundárias. Deste modo, algumas teorias foram propostas para explicar como as baixas temperaturas induzem às várias respostas que causam efeitos prejudiciais aos vegetais armazenados.

Nesse sentido, duas teorias principais têm sido discutidas como prováveis indicadores à resposta primária em vegetais. A teoria da ''fluidez da membrana'' proposta por Lyons (1973) sugere que o resfriamento induz a mudança na bicamada lipídica da membrana plasmática, ou seja, de um estado líquido flexível (móvel) para uma fase de gel mais sólida (imóvel). Essa rigidificação levaria a ruptura das membranas celulares e a morte celular (Lyons et al., 1979). Logo, a resistência ao resfriamento estaria associada a maiores concentrações de ácidos graxos insaturados nas membranas (Jin et al., 2014; Vazquez-Hernandez et al., 2020). Algumas evidências a favor da hipótese de rigidificação da membrana foram encontradas em mangas (Kane e Marcellin, 1978; Hidalgo et al., 1997; Agillon e Lizada, 2010; Kumpoun et al., 2017).

A segunda teoria propõe que o estresse pela baixa temperatura induz a produção de espécies reativas de oxigênio (ERO), tais como: peróxido de hidrogênio (H_2O_2) e radicais superóxido ($O_2^{\bullet-}$) e hidroxila ($^{\bullet}OH$) que reagem com várias moléculas,

incluindo o DNA e proteínas, resultando na peroxidação de lipídeos da membrana (Shewfelt e Rosario, 2000). Em mangas, o aumento no acúmulo de ERO tem sido correlacionado com a maior incidência de IF (Wang et al., 2008, Khaliq et al., 2016, Zhang et al., 2017 e Sanches et al., 2021) corroborando com a evidência da segunda teoria.

Após exposição prolongada ao resfriamento, essas respostas primárias levariam a uma série de eventos secundários que incluem perda de integridade da membrana, extravasamento de solutos, perda de compartimentação, diminuição da taxa de atividade oxidativa mitocondrial, aumento da energia de ativação das membranas, interrupção do fluxo protoplasmático, redução no fornecimento e utilização de energia, diminuição da taxa fotossintética, desorganização da estrutura celular, disfunção e desequilíbrio do metabolismo, acúmulo de substâncias tóxicas, estimulação da produção de etileno, aumento da taxa de respiração, alterações no metabolismo de poliaminas, além da manifestação de uma variedade de sintomas por frio no tecido injuriado (Lyons e Raison, 1970; Raison e Lyons, 1986; Raison e Orr 1990; Sevillano et al., 2009).

2.4 Metabolismo oxidativo e defesa antioxidante

O estresse oxidativo é o principal mecanismo de resposta das frutas e hortaliças ao estresse causado pela baixa temperatura e/ou pelos danos induzidos nessas condições, se caracterizando pelo aumento excessivo na produção de ERO, tais como o radical superóxido, peróxido de hidrogênio e radical hidroxila (Toivonen, 2004; Aghdam, 2013). O acúmulo dessas ERO leva à inativação de enzimas, degradação de proteínas, danos ao DNA, peroxidação lipídica (acúmulo de malondialdeído - MDA) e o extravazamento de eletrólitos como resultado do rompimento da membrana celular, com consequente aparição de sintomas visíveis de injúria pelo frio (Scandalios, 1993; Mitler, 2002; Del Río e López-Huertas, 2016).

Todavia, os efeitos nocivos da superprodução de ERO no metabolismo celular é contornado pelos metabólitos antioxidantes não enzimáticos, ou seja, por meio da presença de ascorbato, glutationa e polifenóis (α-tocoferol e β-caroteno). Similarmente, os mecanismos enzimáticos (Figura 2) envolvendo a atividade das

enzimas superóxido dismutase (SOD, EC1.15.1.1), catalase (CAT, EC 1.11.1.6), ascorbato peroxidase (APX, EC 1.11.1.11) e glutationa redutase (GR, EC1.8.1.7), também foram relacionados à minimização dos efeitos das ERO (Asada, 2006; Decros et al., 2019).

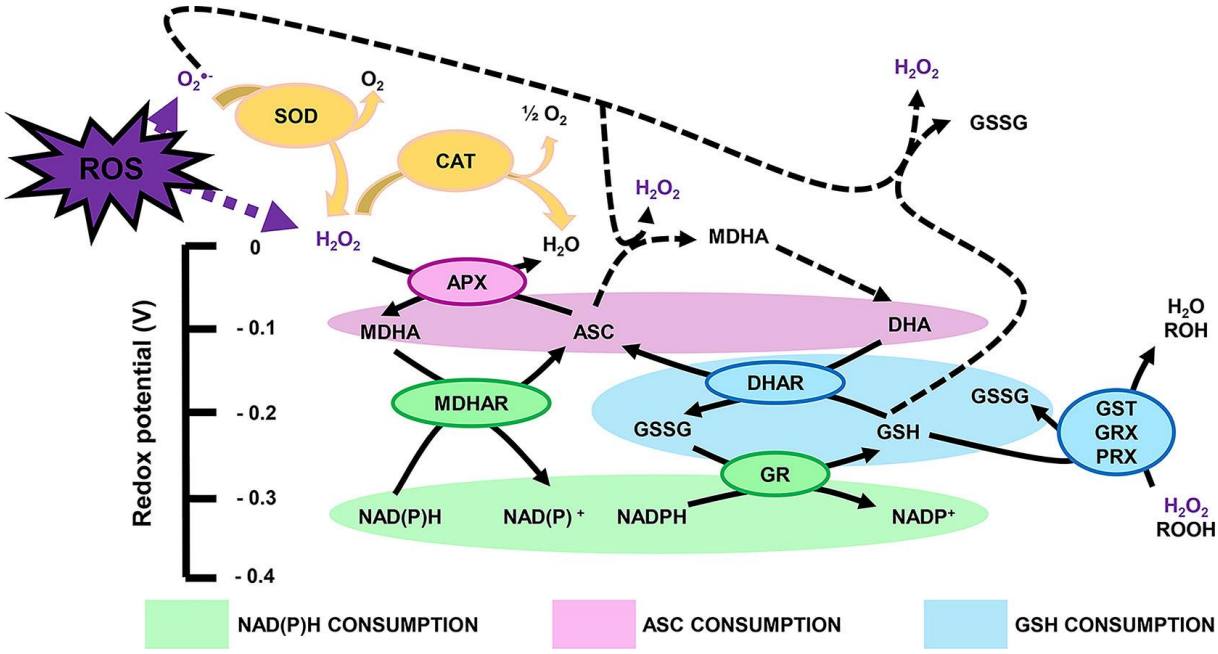

Figura 2. Principais compostos tampões (buffers) redox enzimáticos envolvidos na remoção de espécies reativas de oxigênio (ERO). Fonte: Decros et al. (2019). Setas simples e tracejadas representam reações enzimáticas e não enzimáticas, respectivamente. ASC, ascorbato reduzido; APX, ascorbato peroxidase; CAT, catalase; DHA, desidroascorbato; DHAR, desidroascorbato redutase; GSH, glutationa reduzida; GSSG, dissulfeto de glutationa; GR, glutationa redutase; GRX, glutaredoxina; GST, glutationa S-transferase; MDHA, monodeidroascorbato; MDHAR, monodeidroascorbato redutase; PRX, peroxirredoxina dependente de GRX; ROH, composto orgânico com grupo álcool; ROOH, composto orgânico com grupo peróxido; SOD, superóxido dismutase.

Estas enzimas parecem ter uma influência importante no grau de sensibilidade ou tolerância a injúria pelo frio (Sala e Lafuente, 2000; Sevillano et al., 2009). Sugere-se que a CAT seja a primeira enzima do sistema antioxidante a ser ativada em

resposta ao estresse oxidativo causado pela injúria pelo frio (Wang, 1994). O aumento na produção de ERO, juntamente com a redução da atividade enzimática da SOD, CAT e APX, e maior índice de IF, foram relatados em mangas 'Alphonso' (Niranjana et al., 2009) e 'Cogshall' (Rosalie et al., 2018) armazenadas a 5 e a 7 ºC, respectivamente. Mangas 'Nam Dok Mai No' armazenadas a 5 ºC por 42 dias, mostraram aumento na concentração de ERO ($O_2^{\bullet-}$, H_2O_2 e $^{\bullet}OH$) juntamente com diminuição no teor de glutationa total, ácido ascórbico e na atividade e expressão gênica das enzimas SOD, CAT, APX, resultando em aumento nos sintomas de injúria pelo frio (Junmatong et al., 2015). Por outro lado, a manutenção dos teores de vitamina C, compostos fenólicos totais e da atividade antioxidante foi correlacionada à redução da IF em mangas 'Choke Anan' (Khaliq et al., 2016). Da mesma forma, a maior atividade das enzimas do sistema antioxidante foi associada à tolerância natural a injúria pelo frio em várias cultivares de mangueira (Wang et al., 2008; Chidtragool et al., 2011; Chongchatuporn et al., 2013; Sanches et al., 2021). Assim, o desenvolvimento de abordagens para aumentar a tolerância ao resfriamento poderia reduzir as perdas pós-colheita de produtos hortícolas sensíveis à baixas temperaturas durante o armazenamento prolongado.

2.5 Polióis

Os polióis são carboidratos que têm sido utilizados como substitutos da sacarose (açúcar de mesa) por apresentarem baixo valor calórico. Parte de suas estruturas químicas é parecida com as estruturas de açúcares e álcoois e, por essa razão, os polióis também são conhecidos como álcoois de açúcar (SBD, 2019). Na indústria de alimentos os polióis são classificados como aditivos alimentares da classe dos edulcorantes, estabilizantes, antioxidantes e emulsificantes (Pereira, 2013; Rice et al., 2020) e considerados seguros pela *Food and Drug Administration* (FDA) recebendo o status de *Generally Recognized as Safe* (GRAS) FDA (2016). Também são considerados seguros na União Europeia (UE), tendo em vista sua aprovação pelo Parlamento Europeu de acordo com a Diretiva 94/35/CE (UE, 1994) e no Brasil através da Portaria nº 540, de 27 de outubro de 1997, do Ministério da Agricultura Pecuária e Abastecimento (MAPA) (BRASIL, 1997).

Dentre os polióis, o glicerol (propano-1,2,3-triol) é descrito como sendo um composto orgânico incolor, viscoso, higroscópico (absorve umidade), oleoso e com gosto adocicado (Zhang e Grinstaff, 2014). Trata-se de um poliálcool, com três hidroxilas em sua fórmula estrutural e que recebe outras denominações: glicerina, trihidroxipropano, glicil álcool, gliceril e 1,2,3-trihidroxipropano (Figura 3) podendo ser encontrado em azeites e óleos de coco, dendê, soja, algodão e oliva, ou mesmo em animais, na combinação de glicerina com ácido graxo (Beatriz et al., 2011).

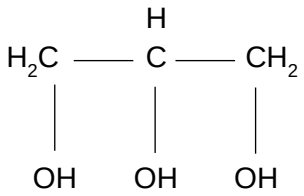

Figura 3. Fórmula estrutural do glicerol. Fonte: IUPAC (2013).

Como aditivo alimentar seguro, o glicerol foi avaliado pela primeira vez em 1976 pelo Comitê Conjunto FAO/OMS de Especialistas em Aditivos Alimentares (JECFA) e reavaliado em 2014. Com base nos estudos toxicológicos disponíveis e no fato de que o glicerol ocorre naturalmente nas gorduras e outras substâncias consumidas através dos alimentos, o JECFA alocou ao glicerol uma ingestão diária aceitável 'não especificado' para adultos e de 460 mg kg^{-1} para crianças (FDE, 2013; EFSA, 2017). Na indústria alimentícia o glicerol é amplamente utilizado em misturas à base de óleo e água, adoçando ou umedecendo o produto final, em alimentos congelados (carnes em geral, iogurtes, sorvetes, sobremesas) para evitar a formação de cristais de gelo, entre várias outras aplicações (Azelee et al., 2019). A incorporação de glicerol a 5 e 15 % em carnes processadas melhorou a umectância, preservou a qualidade sensorial, aumentou a atividade antioxidante e reduziu o dano oxidativo durante o processamento e o armazenamento (Jang et al., 2015; Sorapukdee et al., 2016).

Outro poliol comumente utilizado é o propilenoglicol (1,2-propanodiol ou metilglicol), que é um álcool poli-hídrico com fórmula química $C_3H_8O_2$ constituído por três carbonos e duas hidroxilas (Figura 4).

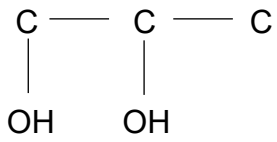

Figura 4. Fórmula estrutural do propilenoglicol. Fonte: IUPAC (2013).

O propilenoglicol é um líquido viscoso, muito higroscópico, incolor, solúvel em água e encontrado com os nomes 1,2-di-hidroxipropano, metil-etil-glicol e trimetil-glicol (Bischoff, 2013). Reconhecido como GRAS pelo FDA (FDA, 2016), a Organização Mundial da Saúde (OMS) recomenda uma ingestão máxima de 25 mg de propilenoglicol por kg de massa corporal por dia (EFSA, 2018). O uso principal do propileno glicol na indústria alimentar se dá na forma de aditivo (E-1520) em uma ampla variedade de alimentos e bebidas com as funções de agente crioproteor, umidificante, cristalizante, espessante e emulsionante melhorando a textura, sabor, aparência e o prazo de validade (Vulava, 2005; Sara et al., 2016).

O sorbitol ((2S,3R,4R,5R)-Hexane-1,2,3,4,5,6-hexol)) é um poliálcool constituído por seis moléculas de carbono e oxigênio e quatorze de hidrogênio (Figura 5).

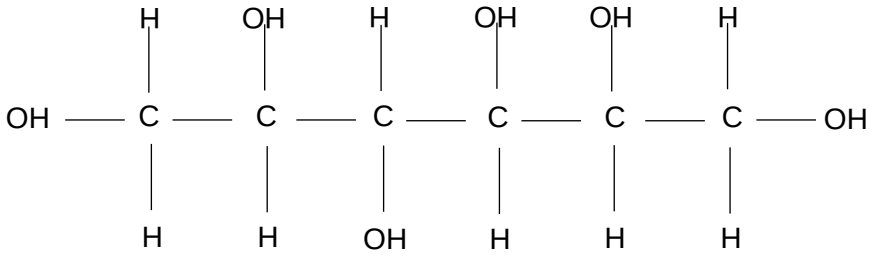

Figura 5. Fórmula estrutural do sorbitol. Fonte: IUPAC (2013).

Também chamado de D-glucitol, é um composto solúvel em água de ocorrência natural em frutas, tais como: maçã, framboesa, amora, morango, abacate, cereja, pêssego, entre outras (Rice et al., 2018; Fang et al., 2020). Por causa do seu baixo teor de calorias e sabor doce, o sorbitol é amplamente utilizado em adoçantes, doces, chocolate e bebidas sem açúcar como agente edulcorante, umectante, texturizante e estabilizante (Lee, 2015; Zhang et al., 2020). Além disso, o sorbitol tem ação

crioprotetora, pois diminui o ponto de congelamento, o que o torna adequado na produção de sorvetes (Ozdemir et al., 2003; Kalicka et al., 2019). No armazenamento do surimi e de carnes processadas, o sorbitol (2 – 5 %) costuma melhorar a qualidade, aumentar a vida de prateleira e reduzir o estresse oxidativo (Dey e Dora 2011; Liu et al., 2014; Fahrizal et al., 2018). Quanto à segurança ao uso do sorbitol, a JECFA categorizou a dose diária admissível do sorbitol como “não especificado,” que é a categoria mais segura para todo o ingrediente de alimento (Grembecka, 2015). O sorbitol foi classificado como GRAS pelo FDA e seu uso também é permitido como aditivo alimentar pela UE e em muitos outros países, além de ter a maior participação de mercado de todos os polióis no momento (Zhang et al., 2020).

Apesar do potencial dos polióis na conservação de alimentos e o uso seguro destes aditivos, não foram encontrados relatos quanto ao uso destes compostos em frutas *in natura*. Desta forma, os polióis podem ser uma alternativa para preservar a qualidade e reduzir o dano oxidativo causado pelo frio em mangas armazenadas à baixas temperaturas.

2.6 Atmosfera controlada (AC)

A atmosfera controlada (AC) é uma das tecnologias mais importantes em sistemas de armazenamento de frutas e hortaliças em associação à refrigeração, pois se relaciona ao controle das concentrações de oxigênio (O_2), dióxido de carbono (CO_2) e nitrogênio (N_2) na atmosfera (Bodbodak e Moshfeghifar, 2016; Yahia et al., 2019). Segundo Saltveit (2020), atmosferas com baixos teores O_2 reduzem a respiração (< 8,0 %), inibem a produção (6,0 %) e a ação do etileno (3,0 %). Da mesma forma, altos teores de dióxido de carbono (CO_2) reduzem a ação do etileno (5,0 %) e a respiração (Yahia et al., 2019). Todavia, as concentrações dos gases usados na AC variam conforme as diferenças físicas e químicas entre as cultivares, concentração inicial dos gases na câmara de armazenamento, temperatura, umidade, grau de maturação, concentração de etileno e as condições de pré e pós-colheita (Escobedo-Avellaneda e Welti-Chanes, 2016).

Na pós-colheita de mangas, o armazenamento sob AC tem sido utilizado para prolongar a vida útil e manter a qualidade de várias cultivares, tais como: Alphonso e

Banganapalli (Rao e Rao, 2008), Chaunsa (Ullah et al., 2010), Kensington Pride (Sumual et al., 2017), Kent (Sivakumar et al., 2012), Keitt (Hailu e Worku, 2017) e Shelly (Ntsoane et al., 2020).

De maneira geral, mangas podem ser armazenadas sob AC contendo 3-5 % O_2 e 5-8 % CO_2 a 13°C, o que resultará em uma vida útil pós-colheita de 21 a 42 dias, dependendo dos estádios de maturação (Brecht, 2020). Todavia, em concentrações mais baixos de oxigênio (1,0 % O_2) as mangas iniciam a produção de *off-flavors* (Nakasone and Paull, 1998; Ntsoane et al., 2019). Além disso, mangas 'Kensington Pride' armazenadas em atmosferas contendo 6,0 % de O_2 por 35 dias apresentaram redução na produção de compostos voláteis típicos do aroma de mangas (Lalel et al., 2003). Percentuais elevados de CO_2 (> 25 %) também afetam a qualidade das mangas, levando ao aumento na produção de etanol em mangas 'Tommy Atkins' armazenadas em atmosferas contendo 50 % CO_2 (Bender and Brecht, 2000).

Em relação a mangas 'Palmer', tem sido demostrado que o armazenamento sob AC contendo de 1 a 10% de O_2 foi capaz de reduzir a taxa respiratória, atrasar a maturação e manter a qualidade dos frutos ao longo de 28 dias de armazenamento a 12,8 ºC (Teixeira e Durigan, 2011). Por outro lado, o acréscimo de CO_2 (1 – 20 %) associado à baixo O_2 (5 %) não resultou em aumento da vida útil quando as mangas 'Palmer' foram armazenadas a 12,8 °C por 30 dias (Teixeira et al., 2018).

Além de retardar o processo de amadurecimento, foi relatado que mangas armazenadas sob AC apresentam sintomas menos severos de injúrias pelo frio. Por exemplo, O'Hare e Prasad (1992) relataram que AC contendo 5–10% de CO_2 aliviaram os sintomas de injúria pelo frio em mangas 'Kensington Pride' quando armazenadas abaixo de 10 °C. Similarmente, Pesis et al. (2000) relataram menor ocorrência de injúrias pelo frio em mangas 'Tommy Atkins' e 'Keitt' armazenadas por 3 semanas a 12 ºC em atmosferas contendo ~5 % de O_2 e ~10 % de CO_2. Apesar dos efeitos benéficos da AC em aliviar os sintomas de injúria pelo frio, esta tecnologia isoladamente não é suficiente para controlar o desenvolvimento deste distúrbio fisiológico. Desta forma, a associação com outras tecnologias de conservação como o uso dos polióis é necessário para reduzir os efeitos deletérios da baixa temperatura.

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CAPÍTULO 2 – Nota técnica: Estimativa da infiltração dos polióis em mangas ‘Palmer’

Resumo

Diferenciais de temperatura promovem a absorção da água para o interior dos produtos hortícolas através de um gradiente de pressão criado pela condensação do vapor quente da fruta aquecida com a água fria. Desta forma, foi testada a internalização do corante ácido azul 9 em mangas ‘Palmer’ visando simular como seria a absorção de soluções contendo polióis. Para isso, mangas fisiologicamente maduras foram aquecidas a 45 ºC em estufa de circulação de ar forçada e imediatamente imersas em solução contendo 1 % (m/v) de corante ácido azul 9 a 6 ºC por 0, 5, 15, 30, 45 e 60 minutos. Estas foram transferidas para uma câmara-fria à 8,0 ºC e avaliadas após 24 h quanto a taxa de infiltração. O aquecimento das mangas por 15 minutos foi suficiente para proporcionar a máxima absorção do corante, enquanto nas mangas sem aquecimento (~25 ºC) foram necessários 60 minutos. Independente do tratamento utilizado, a absorção do corante ocorreu de forma homogênea pelas lenticelas presentes no epicarpo e ocasionalmente pelo pedúnculo até o mesocarpo. Desta forma, considerando que a efetividade dos polióis no controle das injúrias pelo frio em mangas ‘Palmer’ depende da sua absorção e que o tratamento térmico pode levar a produção de proteínas de choque térmico, as mangas devem ser mantidas à 25 ºC e imersas nas soluções contendo os polióis por 60 minutos à 6 ºC.

Palavras-chave: *Mangifera indica* L., tratamento térmico, calor de campo, corante ácido azul 9, resfriamento.

1. Introdução

A manga ‘Palmer’ é uma cultivar de ciclo tardio que vem conquistando o mercado internacional por suas características de qualidade físico-químicas e sensoriais, pois são mais firmes, apresentam poucas fibras, são doces e aromáticos (HORTIFRUTI BRASIL, 2018).

Após a colheita, os frutos da mangueira são resfriados para reduzir vários processos metabólicos, tais como a transpiração, respiração, produção de etileno, entre outros (Duan et al., 2020). Uma técnica de pré-resfriamento bastante utilizada é feita com o uso de água fria (*hydrocooling*), que é o processo de se aplicar a água como meio de remoção do calor de campo.

Apesar da eficiência do uso do *hydrocooling*, a diferença de temperatura aumenta a absorção da água devido à expansão do volume do fruto com a queda de temperatura, que cria um vácuo e puxa a água fria por um gradiente de pressão para dentro dos produtos por meio de aberturas naturais (Bartz e Showalter, 1981; Warning et al., 2016). Em produtos frescos, as principais aberturas são as cicatrizes do pedúnculo, estômatos, lenticelas e ferimentos (Burnett et al., 2000). A absorção da água fria pode ser um meio para a entrada de organismos patogênicos (Bartz et al., 2015; Macarisin et al., 2017), porém pode ser utilizada para melhorar a penetração de aditivos visando aumentar a vida útil pós-colheita (Zheng et al., 2007; Lo'ay e Ameer, 2019).

Teoricamente, à medida que o diferencial de temperatura aumenta, maior será o volume de água que a fruta absorve (Bartz, 1999). Todavia, fatores como a profundidade e o tempo de imersão (Bartz, 1983; Smith et al., 2006), o tipo, tamanho e hidrofobicidade das aberturas (Burnett et al., 2000; Eblen et al., 2004; Bartz, 1983), lesões (Fatemi et al., 2006) e cultivares (Bartz, 1991; Smith et al., 2006) afetam a absorção de água nas diferentes temperaturas. Por exemplo, houve aumento da massa de morangos aquecidos a 45 ºC e posteriormente resfriados a 5 ºC em função da absorção de água (Ferreira et al., 1996). Da mesma forma, pimentas colhidas com temperaturas entre 22 – 25 ºC (calor de campo), ao serem resfriadas com água a 8 ºC também mostraram significativa absorção de água (Corey e Tan, 1990).

No epicarpo dos frutos da mangueira existe uma estrutura epidérmica chamada de lenticelas que se originam dos estômatos rompidos durante o crescimento e desenvolvimento dos frutos, atingindo seu tamanho máximo na maturação completa (Khader et al 1992; Bally, 1999; Du Plooy et al., 2009). As lenticelas são aberturas macroscópicas (poros) delimitados por células guarda não funcionais com cavidade subestomática revestida por células fracamente compactadas (Prinsloo et al., 2004; Du Plooy et al., 2006) e devido à sua posição superficial e integrada com camadas

subepidérmicas atuam como um canal para as trocas gasosas e a transpiração da fruta (Rymbai et al., 2012).

Considerando que a efetividade dos polióis no controle de injúrias pelo frio depende da sua absorção pela fruta, este estudo teve por objetivo testar a infiltração do corante ácido azul 9 em mangas ‘Palmer’ visando simular como seria a absorção de soluções contendo polióis.

2. Material e métodos

2.1 Material vegetal

Frutos de mangueira (*Mangifera indica* L.) da cultivar Palmer foram obtidos de um pomar comercial localizado em Cândido Rodrigues (21º 24' 23” South, 48º 30' 20” West, 579 m altitude), São Paulo, Brasil. Os frutos foram colhidos no estádio 3 de maturação com base na cartela de cores do epicarpo proposta por Trindade et al. (2015), que representa o estádio de maturidade fisiológica. Além disso, os frutos foram selecionados considerando tamanho uniforme, ausência de danos mecânicos ou fisiológicos e/ou ausência de pragas e doenças.

2.2 Simulação da infiltração dos polióis

Visando a simular a infiltração da solução contendo o corante ácido azul 9, que corresponderia as soluções de polióis, um lote de 50 frutas foi aquecido em estufas de circulação de ar forçado (Luca 82/250, Lucadema, Brasil) até que a polpa atingisse à 45 ºC (Jacobi et al., 2001). Em seguida, as mangas foram imersas em água fria (6 ºC) contendo o corante azul ácido 9 (Chem-Impex Int’l Inc., Wood Dale, IL) a 1% (m/v) por 5, 15, 30, 45 e 60 minutos (Macarisin et al., 2017). Da mesma forma, um outro lote de 50 frutas com calor de campo (~25 ºC) foram imersas na solução corada por igual período. A estimativa da temperatura (calor de campo) foi realizada em amostras de 10 frutas com auxílio de um termômetro. Após a imersão nas soluções contendo o corante, as frutas foram lavadas em água corrente e armazenadas em câmara fria a 8,0 ± 1,0 ºC por 24 horas.

A estimativa da infiltração da solução contendo o corante azul ácido 9 foi realizada cortando-se longitudinalmente os frutos. As duas metades foram escaneadas e o percentual de infiltração do corante na polpa foi determinado usando o programa ImageJ (Rueden et al., 2017).

O experimento foi instalado utilizando um delineamento inteiramente casualizado (DIC) em esquema fatorial 2 (tratamentos: aquecimento e sem aquecimento) x 6 (tempos de imersão: 0, 5, 15, 30, 45 e 60 minutos) com 10 repetições de um fruto, totalizando 120 frutos.

2.3 Análise estatística

Os dados referentes ao percentual de infiltração foram submetidos à análise de variância (ANOVA) utilizando o software R (R Core Team, 2020) e as médias comparadas por meio do teste de Tukey ao nível de significância de 0.05%.

3. Resultados

O aquecimento dos frutos (45 ºC) promoveu maior infiltração do corante azul ácido 9 (média 2,27 %) em relação àqueles sob temperatura ambiente, calor de campo (média 0,68 %).

Este processo ocorreu rapidamente e após 15 minutos de imersão em água fria foi possível observar maior infiltração nos frutos aquecidos (2,97 %) em relação aos 60 minutos necessários para ocorrer 1,84 % nas frutas não aquecidas (Tabela 1). Independentemente do tratamento utilizado, o epicarpo foi o tecido mais corado em relação ao mesocarpo (Figura 1).

4. Discussão

A diferença de temperatura favoreceu uma maior e mais rápida infiltração do corante nas frutas aquecidas (45 ºC) em relação as frutas na temperatura ambiente, calor de campo (~25 ºC). Possivelmente, esse diferencial de temperatura estimulou a

absorção de água pela expansão do volume do fruto criando um gradiente de pressão para o interior das frutas (Warning et al., 2016) (Figura 2).

Da mesma forma, um estudo com absorção de corante (ácido azul 9) em melões ‘Cantaloupe’ mostrou maior infiltração quando as frutas foram aquecidas a 42 ºC e resfriadas a 6 ºC do que aquelas aquecidas a 18 ºC (Mararisin et al., 2017). Mangas ‘Tommy Atkins’ apresentaram maior taxa de infiltração de corante quando foram aquecidas a 47 ºC e resfriadas em água a 24 ºC mais fria (Penteado et al., 2004). Também, uma diferença de temperatura de 17 °C entre laranjas e uma solução de corante mais fria levou à maior infiltração do corante na fruta (Eblen et al., 2004). Todavia, assim como existe uma quantidade finita de espaço livre interno, já que estes não são contínuos, há um limite para a entrada de água nesses espaços, isso justifica a saturação na absorção do corante após 15 minutos de imersão das frutas aquecidas em água fria. Provavelmente, a velocidade de infiltração da água resultou na expansão de gases internos aumentando a sua densidade e reduzindo a absorção de água (Bartz e Showalter, 1981).

De modo geral, a infiltração do corante no epicarpo ocorreu de forma homogênea, provavelmente por conta dos poros (lenticelas) que compõem esse tecido (Paul et al., 2007) e promoveram o influxo da água corada. Essa é uma característica interessante do ponto de vista de preservação do epicarpo já que distúrbios fisiológicos como a injúria pelo frio acometem esse tecido, principalmente. Assim, a maior concentração do corante no epicarpo sugere maior proteção deste tecido em relação aos efeitos da baixa temperatura. No mesocarpo a intensidade de infiltração foi menor, porém, quando houve ocorreu principalmente pela cicatriz do pedúnculo e se espalhou ocasionalmente por feixes vasculares (Figura 1). Além disso, em mangas, a distribuição das lenticelas é 2 – 3 vezes maior na região apical do fruto do que na porção do meio ou do ombro (Khader, 1992).

Apesar do aquecimento do fruto ter resultado em maior infiltração do corante azul ácido 9, este procedimento não foi adotado para a aplicação dos polióis. A justificativa para não ser utilizado se relaciona ao fato que o aquecimento pode levar à síntese de proteínas de choque térmico e mascarar a ação dos polióis, pois estas proteínas diminuem os sintomas de injúrias pelo frio em mangas (Dautt-Castro et al., 2018). Além disso, do ponto de vista comercial o aquecimento das frutas requer um

manejo adicional e maior gasto energético para ser realizado em relação ao controle (calor de campo). Assim, optou-se por não aquecer os frutos e imergi-los por 60 minutos nas soluções de glicerol, propileno glicol e sorbitol em função da maior taxa de infiltração (Figura 1).

5. Conclusão

A imersão de mangas 'Palmer' na temperatura de 25 ºC por 60 minutos em soluções contendo corante azul ácido 9 a 6,0 ºC resultou em maior taxa de infiltração desse corante, o que seria um indicativo da possível infiltração dos polióis.

6. Agradecimentos

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Tabela

Tabela 1. Percentual de infiltração do corante azul ácido 9 em mangas 'Palmer' tratadas termicamente (45 ºC) ou não sob diferentes tempos de imersão em água à 6,0 ºC através de estimativa pelo programa ImageJ.

Tempo de imersão (minutos)	Tratamentos	
	Aquecimento 45 ºC	Temperatura ambiente (~25 ºC)
0	0,00 ± 0,00 cA	0,00 ± 0,00 eA
5	1,76 ± 0,18 bA	0,00 ± 0,00 eB
15	2,97 ± 0,14 aA	0,31 ± 0,08 dB
30	2,91 ± 0,21 aA	0,76 ± 0,15 cB
45	3,02 ± 0,19 aA	1,42 ± 0,11 bB
60	2,90 ± 0,16 aA	1,84 ± 0,15 aB
CV (%)	2,15	1,91

Letras minúsculas nas linhas (tempos de imersão) e maiúsculas na coluna (com e sem aquecimento) não diferem entre si pelo teste de Tukey ($p<0,05$).

Figuras

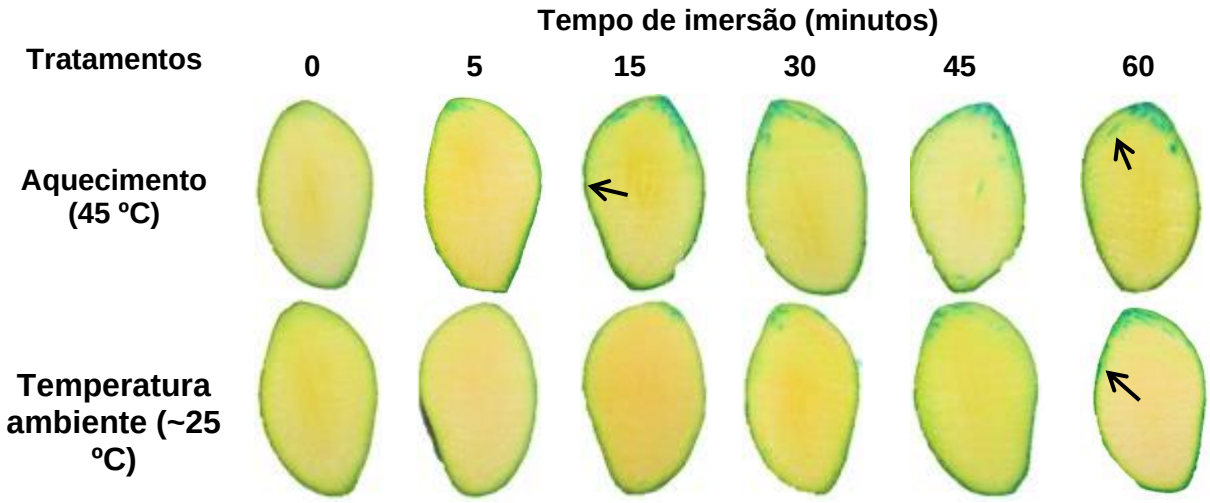

Figura 1. Infiltração do corante azul ácido 9 em mangas 'Palmer' tratadas termicamente (45 ºC) ou não sob diferentes tempos de imersão em água à 6,0 ºC. (→ detalhe da infiltração do corante entre o epicarpo e o mesocarpo).

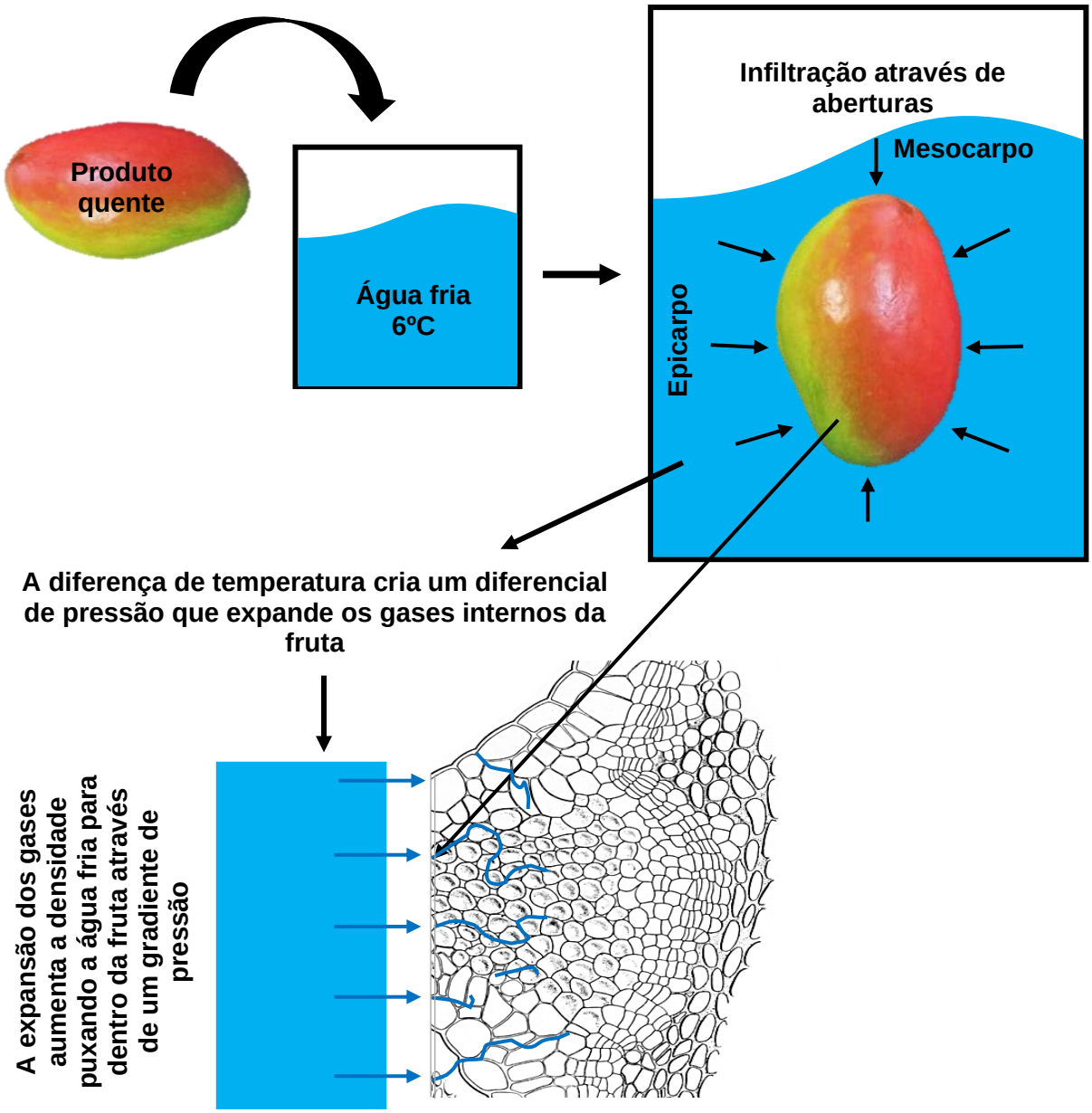

Figura 2. Um esquema da física do resfriamento por água. A manga aquecida (45 ºC) ou a temperatura ambiente (~25 ºC) é colocada em água fria 6,0 ºC. A diferença de temperatura promove a expansão de gases internos que aumenta a densidade da fruta e estimula a infiltração da água com o corante ácido azul 9 através de um gradiente de pressão pelas lenticelas (epicarpo) e pedúnculo (mesocarpo).

CAPÍTULO 3 – Polyols can alleviate chilling injury in ‘Palmer’ mangoes during cold storage[1]

Abstract

Considering the susceptibility of mangoes to chilling injury (CI) during storage at low temperatures (< 13ºC), the objective of this study was to evaluate the use of polyols to alleviate the damage caused by CI, and to verify the effect of these additives on the quality and oxidative metabolism of ‘Palmer’ mangoes stored at 8.0ºC for 28 days with subsequent transfer to ambient conditions (23ºC). Therefore, physiologically mature ‘Palmer’ mangoes were harvested and immersed in solutions containing propylene glycol, sorbitol, and glycerol (concentrations, 0.1, 0.5, and 2.5 %, respectively for 1 h). The fruits were stored at 8 ± 1.0°C for 28 days and transferred to ambient temperature (23 ± 2.0°C) every 7 days to evaluate the development of CI, modification of physicochemical parameters, and oxidative metabolism. The symptoms of CI were minimized during cold storage, mainly when the mangoes were treated with sorbitol solutions. Fruits treated with polyols were firmer and showed less fresh weight loss and browning (luminosity), higher titratable acidity, and soluble solids content than the controls. The epicarp (peel) and mesocarp (pulp) fruits treated with 2.5% sorbitol exhibited lower accumulation of H_2O_2 and polyphenol oxidase (PPO) activity, higher membrane integrity (malondialdehyde – MDA), and higher activities of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX); further, sorbitol treatment preserved the ascorbate content in these tissues compared to other treatments. Thus, immersion of mangoes in sorbitol can serve as an alternate strategy to alleviate CI during cold storage of ‘Palmer’ mangoes.

Keywords: *Mangifera indica* L., glycerol, propylene glycol, sorbitol, oxidative metabolism.

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[1]Sanches AG, Pedrosa VMD, Checchio MV, Fernandes TFS, Guevara JEM, Gratão, PL, Teixeira GHA (2021) Polyols alleviate chilling injury in ‘Palmer’ mangoes during cold storage. **Food Control** 129: 108248. https://doi.org/10.1016/j.foodcont.2021.108248

1. Introduction

Chilling injury (CI) is a physiological disorder that affects fruits and vegetables stored below a critical threshold temperature and above the freezing point (Purvis, 2004). Mangoes (*Mangifera indica* L.) are economically important fruits, but their sensitivity to CI when stored at temperatures below 13ºC (Mitra and Baldwin, 1997; Ayele et al., 2017) limits their storage potential for long periods because of the development of black spots, pitting, and sunken lesions on the epicarp (Nair et al., 2003; Sivankalyani et al., 2016), abnormal ripening (peel and pulp), and increased susceptibility to post-harvest decay, especially when transferred from low temperatures to ambient conditions (Nair and Singh, 2009).

Natural products such as methyl jasmonate (Gonzalez-Aguilar et al., 2000), and salicylic acid (Ding et al., 2007) have been reported to alleviate the development of CI in mangoes during cold storage (< 13ºC). However, the results only showed retardation in the onset of CI symptoms. Thus, a possible alternative to prevent or alleviate this physiological disorder would be the use of polyols, which are commonly used additives in the food industry (Alvarez et al., 2011; Liu et al., 2014; Murthy et al., 2017).

Polyols or polyhydric alcohols are used for controlling viscosity and texture, adding bulk, retaining moisture, reducing water activity, and as humectant, emulsifier, and osmoprotective agents (Linday, 2008). Polyols also have the ability to penetrate cells, leading to a reduction in the freezing temperature inside them and preventing the formation of ice crystals (MacDonald and Lanier, 1997; Elliott et al., 2017). Polyols such as glycerol, propylene glycol, and sorbitol are classified as generally recognized as safe (GRAS) additives (FDA, 2016; Codex Alimentarius, 2019) and have been used in many frozen products (Chikalikar et al., 2000; Velickova et al., 2013; Bikaki et al., 2013; Temkov et al., 2015). However, to our knowledge, no studies have been conducted regarding the use of these compounds during the cold storage of fresh fruits to control CI.

Polyols can bind water and decrease the dielectric constant even at temperatures above freezing (Shirgire et al., 2012). Therefore, these additives are osmoprotective agents that have been related to CI tolerance (Bustamante et al., 2016), contributing to membrane stabilization and structural damage avoidance

(Thomashow, 1999). It is important to state the relationship between molecular mobility and stability of foods. As food is cooled, molecular mobility decreases, and at some point, the mobility of large molecules is so constrained that their diffusion is highly restricted and the processes depending on their mobility slow down markedly (Reid & Fennema, 2008). This phenomenon increases the production of reactive oxygen species (ROS) and causes membrane damage, and is particularly important in mangoes, as it is a tropical fruit, and cold-stress-induced ROS ultimately react with the monounsaturated and polyunsaturated fatty acids present in the cell membrane, leading to lipid peroxidation (Vega-Alvarez et al., 2020).

Thus, considering the CI susceptibility of mangoes during storage at low temperatures (< 13ºC), the objective of this study was to evaluate the use of polyols (glycerol, propylene glycol, and sorbitol) to reduce fruit susceptibility to CI, and to verify the effect of these additives on the quality and oxidative metabolism of 'Palmer' mangoes stored at 8.0ºC for 28 days, with subsequent transfer to ambient conditions (23ºC).

2. Material and methods

2.1 Plant material

Palmer mangoes (*Mangifera indica* L.) were obtained from a commercial orchard located Taquaritinga (21º 24' 23" South, 48º 30' 20" West, 579 m altitude), São Paulo, Brazil. Fruits were harvested at maturity stage 2 based on the epicarp color chart proposed by Trindade et al. (2015), which represents the physiological maturity stage. Further, the fruits were selected considering uniform size absence of mechanical or physiological damage, and/or absence of pests and diseases. Dry matter content of 15 ± 1% was determined in a batch of 20 fruits.

2.2 Polyol treatments

Mangoes were washed with neutral detergent (Ypê Clear, São Paulo, Brazil) and rinsed in running water prior to treatment with polyols. The fruits were immersed

in distilled water (control) and in three different polyol solutions containing glycerol (Mendel, São Paulo, Brazil), propylene glycol (Mendel, São Paulo, Brazil), and sorbitol (Sigma-Aldrich, St. Louis, USA), respectively at 5°C for 60 min at three concentrations, i.e., 0.1, 0.5, and 2.5 % (w/v). These concentrations were chosen based on the Codex Alimentarius recommendation for fresh fruit consumption (Codex Alimentarius, 2019). Notably, Codex Alimentarius recommends a maximum dose of 0.1% of these products for the consumption of fresh fruits (Codex Alimentarius, 2019). After immersion in these solutions, the fruits were transferred to a cold room at 8.0 ± 1.0°C and 75 ± 3.0% RH and stored for 28 days. At 7-day intervals, the fruits were transferred to the ambient environment (23 ± 2.0°C and 65 ± 4.0% RH) to determine the development of injuries caused by low-temperature storage (CI), in addition to the physicochemical and oxidative metabolism parameters.

The experiment was set with a completely randomized design (DIC) in a factorial arrangement of 3 (polyols: glycerol, propylene glycol, and sorbitol) × 3 (concentrations: 0.1, 0.5, and 2.5%) × 5 (storage period: 0, 7, 14, 21, and 28 days) + 1 (control), with five repetitions of one fruit for each condition. A total of 500 fruits were used in this study.

2.3 Fruit quality evaluations

Fruit quality was assessed by determining the following parameters:

2.3.1 Chilling injury severity - CI: The CI symptoms (black spots, depressions, and sunken lesions in the epicarp) were recorded visually using the scale proposed by Miguel et al. (2011), with some modifications as follows: 1 = no visible symptoms (CI = 0 %), 2 = mild symptoms (CI < 25 %), 3 = moderate symptoms (CI = 25–50 %), and 4 = severe symptoms (CI > 50 %).

2.3.2 Fresh weight loss (FWL): This was calculated according to the variation of fruit mass at different storage periods by weighing the mangoes on a semi-analytical balance with an accuracy of 0.01 g (Mars, model AS 2000, São Paulo, Brazil). The results were expressed as percentages (%).

2.3.3 Color: The color of the epicarp (peel) was determined using a colorimeter (CR-400, Minolta, Osaka, Japan), and was recorded as L*, a*, and b*. These values were transformed into chromaticity and hue angles, as described by McGuire (1992).

2.3.4 Firmness: For this evaluation, the epicarp was removed and fruit firmness was measured on two opposite sides of five replicate fruits using a texturometer (Effegi Fruit Tester, Italy) equipped with an 8 mm tip. The results were expressed in Newtons (N) as described by Watkins and Harman (1981).

2.3.5 Physicochemical analysis: The soluble solid content (SSC) of the liquid obtained by pressing 10 g of pulp was determined using a digital refractometer (Alpha, Atago Co., Ltd, Japan) and the results expressed as °Brix. Titratable acidity (TA) was determined by titrating 10 g of pulp with 0.1 N NaOH, using 0.1% phenolphthalein as an indicator. The results were expressed as equivalent grams of citric acid in 100 g of pulp. The ratio was calculated using the SSC/TA. The pH of the samples was measured using a pHmeter (Thermo Scientific, Orion 3 Star, USA) directly on the fruit pulp. The ascorbic acid (AsA) content was determined by titrating a 10 mL aliquot of the extract with 2,6-dichlorophenolindophenol. The results were expressed as mg 100 g^{-1} (AOAC, 2016).

2.4 Oxidative metabolism determinations

2.4.1 Sample preparation: From each of the 500 fruits, 10 g of epicarp (peel) and mesocarp (pulp) were frozen using liquid nitrogen and stored at -20 ºC. The epicarp was removed using a potato peeler, and the mesocarp was immediately (2 cm) was cut with a stainless steel. These samples were ground using a ceramic mortar and pestle using liquid nitrogen to obtain a fine powder for oxidative metabolism analysis. The samples were prepared in triplicate.

2.4.2 Lipid peroxidation: Peel (0.3 g) and pulp (0.4 g) samples were homogenized with 2 mL of 0.1% (w/v) trichloroacetic acid (TCA) and 20%

polyvinylpyrrolidone (PVPP) and were centrifuged (Thermo Scientific, ST16-R, USA) at 15,000 × *g* for 15 min at 4°C. To the supernatant (250 µL), 1 mL of a solution containing 20% TCA (w/v) and 0.5% thiobarbituric acid (TBA) (w/v) was added, followed by incubation in a water bath at 95°C for 30 min. The readings were obtained using a spectrophotometer (Shimadzu, UV-1280, Japan) at 535 and 600 nm. The malondialdehyde concentration equivalent (MDA) was calculated using the molar extinction coefficient of 155 $M^{-1}.cm^{-1}$, and the results were expressed as nmol of MDA g^{-1} of fresh weight mass (Gratão et al., 2012).

2.4.3 Hydrogen peroxide (H_2O_2): H_2O_2 content was determined as described by Alexieva et al. (2001). Peel (0.5 g) and pulp (1.0 g) samples were homogenized in 0.1% trichloroacetic acid at 4°C and centrifuged at 10,000 × *g* for 15 min. Then, 200 µL of the supernatant was mixed with 200 µL of 100 mM potassium phosphate buffer (pH 7.5) and 800 µL of 1 M^{-1} potassium iodide (KI). H_2O_2 content was determined using a standard curve at 390 nm, and the results were expressed as µmol H_2O_2 mg^{-1} of fresh weight mass.

2.4.4 Enzyme activities: The enzymes superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), and ascorbate peroxidase (APX, EC 1.11.1.1) were extracted as described by Boaretto et al. (2014), using 3.0 g of peel and 1.0 g of pulp. These samples were macerated in 100 mM potassium phosphate buffer (pH 7.5) containing 1 mM ethylenediaminetetraacetic acid (EDTA), 3 mM DL-dithiothreitol, and 5% polyvinylpolypyrrolidone (w/v). The homogenate was filtered through a fine nylon mesh and centrifuged at 10,000 × *g* for 30 min. The supernatant was used as an extract to determine the following enzymes:

2.4.5 Superoxide dismutase (SOD): The activity of this enzyme was determined according to the method described by Rendón et al. (2013). The reaction mixture (1.0 mL) contained 50 mM sodium phosphate (pH 7.8), methionine (13 mM), NBT (75 mM), EDTA (0.1 mM), and riboflavin (2 µM), and 50 µL of the extract. After 15 min of reaction in a chamber under light, absorbance was measured at 560 nm using a

spectrophotometer (Shimadzu, UV-1280, Japan), and the results were expressed as UAE SOD mg^{-1} of protein.

2.4.6 Catalase (CAT): CAT activity was measured as per the methodology described by Nogueirol et al. (2015). The reaction medium comprised 1.0 mL of 100 mM potassium phosphate buffer (pH 7.5), 25 µL of 1 mM H_2O_2, and 25 µL of the extract. The degradation of H_2O_2 was monitored and quantified using the molar extinction coefficient (36 $M^{-1}.cm^{-1}$) at 420 nm. The results were expressed as µmol H_2O_2 min^{-1} mg^{-1} of protein.

2.4.7 Ascorbate peroxidase (APS): APX activity was determined by reacting 750 µL of 80 mM potassium phosphate buffer (pH 7.0) containing EDTA (1 mM), 100 µL of ascorbic acid (5 mM), 100 µL of 1 mM H_2O_2, and 20 µL of the extract. Enzyme activity was determined by monitoring ascorbate oxidation at 290 nm. The activity results were expressed as µmol H_2O_2 mg^{-1} min^{-1} of protein using the molar extinction coefficient of ascorbate (2.8 $M^{-1}.cm^{-1}$) (Nakano and Asada, 1981).

2.4.8 Polyphenoloxidase (PPO): For determining PPO (EC1. 14.18.1) activity, it was necessary to carry out a different extraction following the method described by Sojo et al. (1998). Briefly, 0.5 g of peel and 1.0 g of pulp were homogenized with 0.1 M potassium phosphate buffer (pH 6.1) containing 4% (v/v) Triton X-100, and 0.02 g of polyvinylpolypyrrolidone (PVPP). The reaction medium comprised 1.85 mL of 100 mM potassium phosphate buffer (pH 6.0), 30 µL of 0.1 M pyrocatechol, and 300 µL of the extract. After incubation at 30°C for 30 min, absorbance was measured at 395 nm and the results were expressed as UEA min^{-1} mg^{-1} of protein (Robinson, 1987).

2.4.9 Protein determination: The total soluble protein content in the peel and pulp was determined following the method described by Bradford (1976) using bovine serum albumin as the standard.

2.5 Statistical analysis

The data were subjected to analysis of variance (ANOVA) using R software, and the means were compared using Tukey's test at a significance level of 0.05%. The effect of polyol concentration, when significant ($P < 0.05$), was subjected to polynomial regression analysis (R Core Team, 2020).

3. Results

3.1 Polyol treatments

With respect to chilling injury (CI) symptoms, no significant effect ($P > 0.05$) of polyol concentration (0.1, 0.5, and 2.5%) was observed during cold storage; however, there was a significant interaction ($P < 0.05$) between polyols and the storage period (Figure 1).

During cold storage, fruits treated with polyols showed less severity of the damage caused by CI compared to the control (Figure 1A). The CI lesions became visible only after 14 days of cold storage and were less severe in fruits treated with sorbitol compared to other polyols (Figure 1A and 2S). After 21 days, this treatment still showed less severe lesions, but without significant differences from the fruit treated with glycerol. Propylene glycol alleviated the CI symptoms in relation to the control fruit, which were the most affected (Figure 1A). However, on the last day of cold storage (28 days), no differences were observed between the polyols, though they reduced the severity of CI symptoms in relation to the control (Figure 1A).

When the fruit were transferred to ambient temperature (23°C), CI damage began to be observed after 7 days of storage at 8.0°C, but no difference was observed between the treatments (Figure 1B and 2S). However, after 14 and 21 days of cold storage and subsequent transfer to ambient conditions, the fruit treated with polyols showed less severity of CI damage compared to the control (Figure 1B). In the last transfer (28 + 7 days), no further effect of polyols was observed compared to the control fruit (Figure 1B).

CI damage affected the color of the fruit, mainly the luminosity (L*), with a significant interaction ($P < 0.05$) between polyols and the storage period, both when refrigerated (Table 1S) and after transfer to ambient conditions (Table 2S). During cold

storage, reductions in L* values were observed, which were associated with epicarp browning, especially in the final third of the storage period (21 and 28 days), when the control fruit showed lower L* values compared to those treated with polyols (Figure 2A). Similar results were found when the fruits were transferred to ambient conditions (Figure 2B). Chromaticity and hue angle (°h) were not affected by any factor when the fruits were subjected to cold storage (Table 1S); however, after transfer to ambient conditions, a significant effect of the storage period was observed, along with a consequent increase in the values of these two parameters (Table 2S).

Regarding changes in other quality parameters during cold storage, no significant effect ($P > 0.05$) of the polyol levels was observed (Table 1S). However, there was a significant interaction ($P < 0.05$) between polyols and storage period for fresh weight loss (Figure 3), firmness (Figure 4), titratable acidity (Figure 5), and the ratio of SSC to TA (Figure 6). SSC was affected by polyol treatments, as well as by the storage period, which resulted in increased SSC during cold storage (Table 1S). pH was not affected by any of these factors (Table 1S). After transfer to ambient conditions, it was possible to observe the effect of polyols on SSC, with the control fruit showing lower SSC in relation to those treated with propylene glycol and sorbitol (Table 2S). Likewise, SSC was increased in different samples and reached an average value of 14.96 ± 0.37°Brix (Table 2S). The pH values were also affected by the storage period and increased over time (Table 2S).

3.2 Oxidative metabolism

Significant increases ($P < 0.05$) in malondialdehyde (MDA) were observed in the epicarp (peel) and mesocarp (pulp) of the fruit during cold storage and after transfer to ambient conditions. However, sorbitol treatment significantly decreased ($P < 0.05$) the degree of lipid peroxidation of the membrane in the peel of fruit stored at 8.0ºC for 28 days (3.66 ± 0.11 nmol MDA g^{-1}) and until the fourth transfer to 23ºC (21 + 7 days) (4.85 ± 0.25 nmol MDA g^{-1}) (Figures 7 and 8A). Similarly, sorbitol reduced the MDA in pulp during cold storage (3.01 ± 0.13 nmol MDA g^{-1}) and after 14 +7 days at ambient conditions (2.61 ± 0.21 nmol MDA g^{-1}) in relation to the other treatments (Figures 9 and 10A). The sorbitol concentration of 2.5% resulted in peel (Figure 9) and pulp

(Figure 10A) with lower lipid peroxidation ($P < 0.05$), regardless of storage conditions (Table 4S).

Immersion of mangoes in sorbitol resulted in lower levels ($P < 0.05$) of hydrogen peroxide (H_2O_2) in the peel (72.51 ± 2.18 µmol H_2O_2 mg^{-1}) and pulp (17.23 ± 0.27 µmol H_2O_2 mg^{-1}) during cold storage in relation to the other treatments, Tables 3S and 5S. A similar result was observed after transferring the fruit to ambient conditions. The H_2O_2 levels were lower until the fourth (21 + 7 days) and third transfer (14 + 7 days) in the peel (288.95 ± 31.80 µmol H_2O_2 mg^{-1}) and pulp (49.98 ± 3.19 µmol H_2O_2 mg^{-1}) (Figures 8B and 10C), respectively. Regarding the peel, the concentration of 2.5% for both sorbitol (62.84 ± 3.81 µmol H_2O_2 mg^{-1}) and propylene glycol (67.07 ± 2.06 µmol H_2O_2 mg^{-1}) resulted in less accumulation of H_2O_2 ($P < 0.01$) (Figure 7B) under refrigeration. However, when the fruit were transferred to ambient conditions (23ºC) the H_2O_2 level was lower ($P < 0.05$) only in mangoes treated with sorbitol (197.52 ± 3.69 µmol H_2O_2 mg^{-1}) (Table 4S). In the pulp, there was no significant effect ($P > 0.05$) of polyol concentration on the H_2O_2 content, regardless of storage temperature (Tables 5S and 6S).

Despite the reduction in AsA content during storage, the levels remained significantly higher ($P < 0.05$) in the peel of the fruit treated with sorbitol (31.03 ± 1.27 mg 100 g^{-1}) and propylene glycol (30.43 ± 1.81 mg 100 g^{-1}) during cold storage (Table 3S) and after transfer to ambient conditions, but here, only sorbitol maintained the highest content of AsA (19.02 ± 1.68 mg 100 g^{-1}) in relation to the other treatments (Table 4S). In the ambient condition, the highest levels of AsA ($P < 0.05$) were observed in the pulp of fruit treated with sorbitol (43.34 ± 1.69 mg 100 g^{-1}) and glycerol (41.24 ± 1.17 mg 100 g^{-1}) (Table 6S). There was a significant difference in the AsA concentrations ($P < 0.05$) only in the pulp of fruit under refrigeration and in response to treatment with 0.5% and 2.5% polyols (Table 5S).

During cold storage, superoxide dismutase (SOD) activity increased from day zero (72.86 ± 3.35 UAE SOD mg^{-1}) to day 14 (85.72 ± 2.86 UAE SOD mg^{-1}), with a subsequent reduction in activity ($P < 0.01$). Further, greater SOD activity ($p < 0.01$) was observed in the peel of the fruit treated with sorbitol (83.07 ± 2.64 UAE SOD mg^{-1}) and glycerol (81.25 ± 3.35 UAE SOD mg^{-1}) in relation to propylene glycol and the control treatments, (79.87 ± 2.06 and 78.44 ± 2.23 UAE SOD mg^{-1}, respectively) (Table

3S). Further, a significant effect ($P<0.05$) of the treatment with sorbitol (31.10 ± 1.08 UAE SOD mg^{-1}) was observed compared to the other treatments (mean 27.79 ± 0.98 UAE SOD mg^{-1}) in the pulp (Table 5S). After transfer to ambient conditions, SOD activity remained higher ($P < 0.05$) in the peel (14 + 7 days) and pulp (21 + 7 days) when mangoes were treated with sorbitol (129.19 ± 5.84 and 49.23 ± 1.23 UAE SOD mg^{-1}, respectively) (Figures 8C and 10D). The concentration of the polyol also increased the SOD activity in the pulp (Tables 5S and 6S) and the peel (Table 3S), mainly in mangoes treated with 2.5% sorbitol solution (122.34 ± 2.95 UAE SOD mg^{-1}) and propylene glycol (117.46 ± 2.83 UAE SOD mg^{-1}) (Figure 8D).

The catalase (CAT) activity in the peel of fruit treated with polyols was not significantly affected ($P > 0.05$) during cold storage, but significant increase ($P < 0.05$) was observed between day 0 (327.77 ± 13.08 µmol H_2O_2 min^{-1} mg^{-1}) and day 14 (406.32 ± 12.19 µmol H_2O_2 min^{-1} mg^{-1}) (Table 3). After the increase in CAT activity up to 14 + 7 days in the ambient environment, the activity of this enzyme remained significantly higher ($P < 0.05$) in fruits treated with sorbitol (990.29 ± 39.18 µmol H_2O_2 min^{-1} mg^{-1}) until the fourth transfer to ambient conditions (21 + 7 days) in relation to the other polyols (average of 824.89 ± 38.09 µmol H_2O_2 min^{-1} mg^{-1}) (Figure 8E). During cold storage, the pulp of fruit treated with sorbitol had a higher CAT activity (253.77 ± 7.28 µmol H_2O_2 min^{-1} mg^{-1}) (Figure 9C) and the same trend was observed for up to the 21 + 7 days of transfer to ambient conditions (182.98 ± 6.23 µmol H_2O_2 min^{-1} mg^{-1}) (Figure 10E). Regarding the peel, the concentrations of 0.5% and 2.5% resulted in higher CAT activities, especially when transferred to ambient conditions (Table 3S). Polyol solutions containing 0.5% glycerol (901.58 ± 12.19 µmol H_2O_2 min^{-1} mg^{-1}) and propylene glycol (912.80 ± 35.13 µmol H_2O_2 min^{-1} mg^{-1}), and 2.5% sorbitol (947.17 ± 32.45 µmol H_2O_2 min^{-1} mg^{-1}) showed greater CAT activities (Figure 8F). However, a significant effect ($P < 0.05$) was observed only after the fruit was transferred to ambient conditions, and the highest CAT activity was observed in the pulp of fruit treated with 2.5% sorbitol (176.90 ± 11.95 µmol H_2O_2 min^{-1} mg^{-1}) (Figure 10F).

During cold storage, ascorbate peroxidase (APX) activity in the peel remained significantly high ($P < 0.05$) when mangoes were treated with sorbitol (31.82 ± 0.51 µmol H_2O_2 min^{-1} mg^{-1}) (Figure 7C). Similarly, a higher APX activity (36.98 ± 1.23 µmol H_2O_2 min^{-1} mg^{-1}) was observed after transfer to ambient condition (14 + 7 days) (Figure

8G), and the concentration of 2.5% resulted in increased APX activity (Table 3S and Figure 8H). Regarding the pulp, the greatest APX activity was observed when the fruit were treated with sorbitol (12.48 ± 0.46 µmol H_2O_2 min^{-1} mg^{-1}) and glycerol (10.23 ± 0.32 µmol H_2O_2 min^{-1} mg^{-1}) under refrigeration (Figure 9D) and after transfer to ambient condition (8.79 ± 0.87 and 9.01 ± 0.58 µmol H_2O_2 min^{-1} mg^{-1}) (Table 6S).

Despite the increase ($P < 0.05$) in polyphenoloxidase (PPO) activity during cold storage and after transfer to ambient condition, the PPO activity was significantly lower ($P < 0.01$) in the peel of fruit treated with sorbitol for up to 21 days under refrigeration (309.27 ± 18.69 UAE min^{-1} mg^{-1}) (Figure 7D) and, when transferred to ambient conditions (502.51 ± 13.51 UAE min^{-1} mg^{-1}) in relation to the other treatments (Table 4S). In contrast, the PPO activity in the pulp was higher (163.89 ± 4.07 UAE min^{-1} mg^{-1}) when the fruit were treated with propylene glycol (Table 5S). At ambient temperature, there was no effect on PPO activity ($P > 0.05$) between the polyol solutions and control treatment (Table 6S). Regardless of the storage condition, the concentrations of 0.5% (299.00 ± 10.27 UAE min^{-1} mg^{-1}) and 2.5% (510.62 ± 11.18 UAE min^{-1} mg^{-1}) resulted in lower PPO activity in the peel compared to 0.1%, with 306.41 ± 10.05 and 527.90 ± 15.68 UAE min^{-1} mg^{-1}, respectively (Tables 3S and 4S). Regarding the pulp, the lowest PPO activity was observed at a concentration of 2.5% during cold storage (Table 5S) and when fruits were transferred to ambient conditions (Table 6S).

4. Discussion

4.1 Polyol treatment

Chilling injury (CI) lesions develop in ‘Palmer’ mangoes when the fruits are stored at temperatures below 13°C (Chaplin, 1991; Miguel et al., 2013). Thus, during storage at 8.0 ± 1.0ºC the fruit were subjected to temperatures that led to CI development after 7 days (Figure 1A). However, the CI symptoms were less intense in fruits treated with solutions containing polyols (Figures 1A and 1B). The alleviation of CI by polyols might be due to their osmoprotective effect (Linday, 2008; Bustamante et al., 2016), which contributes to membrane stabilization and prevents structural damage (Thomashow, 1999). Likewise, polyols maintain cytoplasmic movement

(Fuller, 2004), which reduces the accumulation of compounds that can lead to CI development.

One of the main symptoms of CI is epicarp browning with the development of black spots, pitting, and sunken lesions (Nair et al., 2003). These symptoms are associated with a decrease in the luminosity (L*) of the epidermis during the cold storage of ‘Palmer’ mangoes (Miguel et al., 2013) and ‘Tommy Atkins’ (Miguel et al., 2016). Therefore, maintenance of higher L* values in fruit treated with polyols (with respect to the control fruit) indicated the protective action of these compounds in alleviating the cumulative effect of CI, both during cold storage and at ambient conditions (Figure 2A and 2B). However, the mangoes did not develop the typical yellow-reddish color of ripe ‘Palmer’ mangoes (Table 1S), possibly due to the combined effect of maturity stage (stage 2) and low storage temperature (8°C). These effects may have affected the activity and expression of enzymes involved in chlorophyll degradation and carotenoid synthesis. Abnormal ripening has also been reported by Ambuko et al. (2018) in ‘Apple’ mangoes and by Wannabussapawich and Seraypheap (2018) in ‘Nam Dok Mai No.4’ mangoes. Further, when fruits were transferred to ambient temperature (23°C), some changes in the color parameters were observed after 14 days of storage, but without significant differences (Table 2S). These results might be related to the period of exposure to low temperature and the development of CI, even in fruits treated with polyols.

Another positive effect of polyols in controlling CI may be related to the decrease in fresh weight loss during cold storage, especially after 14 days (Figure 3). According to MacDonald and Lanier (1997), food preservation is related to the stability of cellular structure and control of water movement to avoid collapse at low temperatures, which may justify these results. Likewise, firmer fruit when treated with solutions containing polyols during cold storage (Figures 4A and 4B) demonstrated that these compounds also delayed secondary responses such as the loss of membrane integrity, which is a characteristic symptom of cold stress (Lyons and Raison, 1970; Raison and Orr, 1990) and is associated with loss of firmness. Similarly, higher SSC was observed in fruits treated with polyol solutions (Table 1S), which can be related to their role in reducing the freezing temperature (MacDonald and Lanier, 1997), thus allowing ethylene biosynthesis and activation of sugar metabolism (Sivankalyani et al., 2016), making

them more tolerant to CI as ripe mangoes are more resistant to CI due to their higher sugar concentration (Patil et al., 2019) compared to immature fruit (Medlicott, 1990; Zhao et al., 2009).

Polyols also affected the titratable acidity (TA), especially after 14 days of cold storage (Figure 5A) and up to the fourth transfer to ambient conditions (21 + 7) (Figure 5B), when an increase in TA was observed in relation to the control fruit. This suggests that fruit treated with polyols have less irregular and slow ripening as TA normally reduces during mango ripening (Mitra and Baldwin, 1997). In this respect, reduction in the SSC/TA ratio from 21 days of cold storage (Figure 6A and 6B) was related to the increase in SSC (Tables 1s and 2s) and maintenance of TA, even though mangoes treated with polyols presented higher SSC/TA ratios (Figure 6A and 6B).

4.2 Oxidative metabolism and antioxidant defense

The main effect of polyols in alleviating the development of CI symptoms is related to oxidative metabolism. During cold storage, a 3.5- and 2.5-fold increase was observed in the malondialdehyde (MDA) content of the peel and pulp, respectively. These increases indicated that lipid peroxidation occurred during storage, which was associated with increasing levels of hydrogen peroxide (H_2O_2) in the peel (59.17 - 414.44 µmol H_2O_2 mg^{-1}) and pulp (19.30 - 94.33 µmol H_2O_2 mg^{-1}), irrespective of the storage temperature (Tables 3S, 4S, 5S, and 6S). MDA is a product of lipid peroxidation and an indicator of the structural integrity of cell membranes (Khaliq et al., 2016). Therefore, accumulation of reactive oxygen species (ROS) during storage promoted lipid degradation in the membranes and consequently increased the MDA levels.

As CI developed only after 7 days of storage (Figures 1A and 1B), oxidative stress caused by H_2O_2 accumulation may be an early response of ‘Palmer’ mangoes to this physiological disorder, as low ROS concentrations act as signaling molecules (Halliwel, 2006). A correlation between the increase in the MDA content and H_2O_2 was also associated with CI development during the cold storage of ‘Guifei’ (Zhang et al., 2017), ‘Cogshall’ (Rosalie et al., 2018), and ‘Keitt’ mangoes (Patil et al., 2019). Therefore, lower accumulation of MDA and H_2O_2 in the peel and pulp of fruit treated

with sorbitol suggests a reduction in oxidative damage and thus, better integrity of the cell membrane during the storage period (Tables 3S, 4S, 5S, and 6S).

Polyols are also involved in non-enzymatic antioxidants (ascorbate content) and enzymatic (SOD, CAT, and APX) defense systems, which mainly act by eliminating ROS accumulation in the cellular environment (Halliwel, 2006). Reports involving CI in mangoes have demonstrated a positive relationship between antioxidant defense mechanisms and cold tolerance (Jiang et al., 2015; Ren et al., 2017; Sudheeran et al., 2018; Tarabih, 2020). Regarding the non-enzymatic antioxidant defense system, an association between increased oxidative stress (MDA and H_2O_2) and reduction in AsA content was observed during storage (Tables 3S, 4S, 5S, and 6S). This demonstrates the role of AsA in ROS detoxification, either through electron donation or as a cofactor for the activity of peroxidase enzymes, especially APXs (Akran et al., 2017). Overall, the greatest decline in AsA content of the peel and pulp occurred when the fruits were transferred to ambient conditions (63.76 and 45.56%) in relation to cold storage (40.75% and 25.40%), respectively. These results occurred due to the intensification of CI lesions when the fruits were exposed to ambient temperature (Figure 1B) and to the ripening process itself, resulting in greater oxidative stress where AsA is required more. Thus, the highest levels of AsA observed in the peel (Tables 3S and 4S) and pulp (Tables 5S and 6S) of mangoes treated with sorbitol demonstrate that this polyol alleviates the stress associated with low temperatures.

Regarding the enzymatic defense system, Wannabussapawich and Seraypheap (2018) reported that oxidative stress reduces the activity of enzymes associated with ROS accumulation. This justifies the increase and reduction in SOD, CAT, and APX activities due to the lesser/greater accumulation of H_2O_2 at the beginning and end of the storage period, respectively (Tables 3S, 4S, 5S, and 6S).

SOD is primarily involved in scavenging ROS by catalyzing the dismutation of superoxide radicals ($O2^-$) into H_2O_2 and molecular oxygen (Mittler, 2002). Increased SOD activity in the peel and pulp during cold storage indicates that the enzymatic mechanism of superoxide radical catalysis remained active over 28 days, corroborating the low MDA accumulation in the fruit (Halliwell, 2006). In contrast, the decreased SOD activity after the second transfer to ambient conditions (7 + 7 days) can be attributed to the increase in oxidative stress characterized by the intensification

of CI (Figure 1B) and the accumulation of MDA itself, associated with low dismutation of the $O2^{-}$ anion (Tables 4S and 6S).

In turn, CAT and APX act in the conversion of H_2O_2 into water; CAT activity was 35- and 15-fold higher in the peel (Tables 3S and 4S) and 17-fold higher in the pulp (Tables 5S and 6S) in relation to APX, suggesting that CAT acts directly in the removal of excess H_2O_2, whereas APX plays a more refined role in modulating ROS, especially at the beginning of the storage period, when H_2O_2 accumulation was lower and AsA was higher. Further, APX uses AsA as a substrate to remove H_2O_2, and a decrease in the content of this antioxidant during storage contributed to the low expression of this enzyme. Chongchatuporn et al. (2013) reported that the increases in SOD and CAT activities in 'Nam Dok Mai' mangoes and of APX in 'Choke Anan' mangoes served as a mechanism for neutralizing ROS and enhancing CI tolerance. Further, the overexpression of SOD, CAT, and APX genes in 'Nam Dok Mai No. 4' mangoes was correlated with increased activity of these enzymes and resistance to CI over 6 weeks of storage at 5°C (Junmatong et al., 2015). Therefore, the higher activities of SOD, CAT, and APX in the peel and pulp for up to 21 days of cold storage and the third transfer to ambient conditions (14 + 7 days), when mangoes were treated with 2.5% sorbitol, suggests alleviation of oxidative stress by eliminating ROS and improvement of CI tolerance in these fruits.

Polyphenoloxidase (PPO) catalyzes the conversion of polyphenols to polymeric quinones via enzymatic oxidation, resulting in tissue browning (Yoruk and Marshall, 2003). The loss of membrane structural integrity is the main factor for the solubilization of phenolic compounds in cellular medium with consequent browning due to their oxidation by PPO (Vela et al., 2003). In this regard, the stress caused by low temperatures to induce lipid peroxidation is in agreement with the increments in PPO activity observed during the storage period (Tables 3S, 4S, 5S, and 6S). This was more evident in the peel, where the activity was 3-fold greater than the pulp, as the CI (browning) was more evident in the peel, especially when fruits were transferred to ambient conditions (Table 4S). In 'Tainong' mangoes stored at 20ºC for 20 days, the increase in PPO activity was also related to the intensification of CI (Ren et al., 2017). Likewise, the effect of polyols was observed only in the peel (Tables 3S and 4S), and

mangoes treated with sorbitol shoed less PPO activity, which might be associated with low lipid peroxidation (MDA) and CI intensity in these fruits (Figure 3).

Low temperature stress triggers the accumulation of sugars that provide cryoprotection as part of a cold acclimatization mechanism contributing to the osmotic stabilization of phospholipid membranes (Thomashow, 1999; Wang et al., 2013; Wang et al., 2020). In 'Keitt' mangoes stored at 5ºC, transcriptome analysis showed that CI tolerance was related to the activation of genes involved in sugar metabolism (sucrose, trehalose, raffinose, and stachyose), possibly to maintain osmotic balance and deal with the stress caused by low temperature (Sivankalyani et al., 2016). Sorbitol is a six-carbon sugar belonging to the group of alcohols and is found naturally in fruit such as cherry (19.13 mg g^{-1}), peach (1.2 - 19.4 mg g^{-1}), pear (35 - 70.2 mg g^{-1}), and apple (0.31 - 72.15 mg g^{-1}) (Ma et al., 2014; Wang et al., 2018; Yu et al., 2019; Fang et al., 2019). In this context, absorption of sorbitol solution during the immersion of 'Palmer' mangoes may have allowed improved adaptation of the fruit to cold stress at a cellular level based on the status of sugar, conferring increased resistance to the CI (Figure 3) and homeostasis of oxidative metabolism.

5. Conclusions

The use of solutions containing glycerol, propylene glycol, and sorbitol alleviated CI development caused by cold storage in 'Palmer' mangoes, with sorbitol being the most efficient in controlling the development of this physiological disorder. These additives did not compromise the physicochemical quality of fruit.

Regarding polyol doses, chilling injury symptoms were not fully evident at the dose recommended by Codex Alimentarius (0.1%), and this was related to lower levels of lipid peroxidation (MDA) and H_2O_2 accumulation. The antioxidant defense system was associated with the maintenance of AsA, reduction in MDA and H_2O_2 levels content and increased activities of SOD, CAT, and APX enzymes, mainly in mangoes treated with solutions containing 2.5% sorbitol.

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Figures

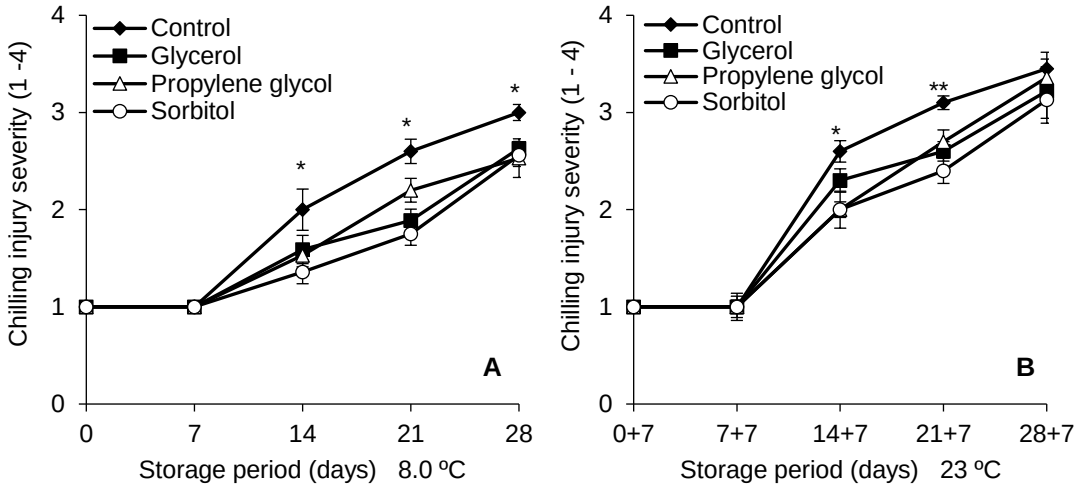


Figure 1. Chilling injury development in 'Palmer' mangoes treated with polyols and stored at 8.0 ± 2 ºC for up to 28 days (A) and transfer to ambient (23 ± 2 ºC) for 7 more days (B). Statistical significance at P<0.05 (**) and P<0.01 (*) for the treatments within each storage period. The bars represent the standard deviation of 5 repetitions.

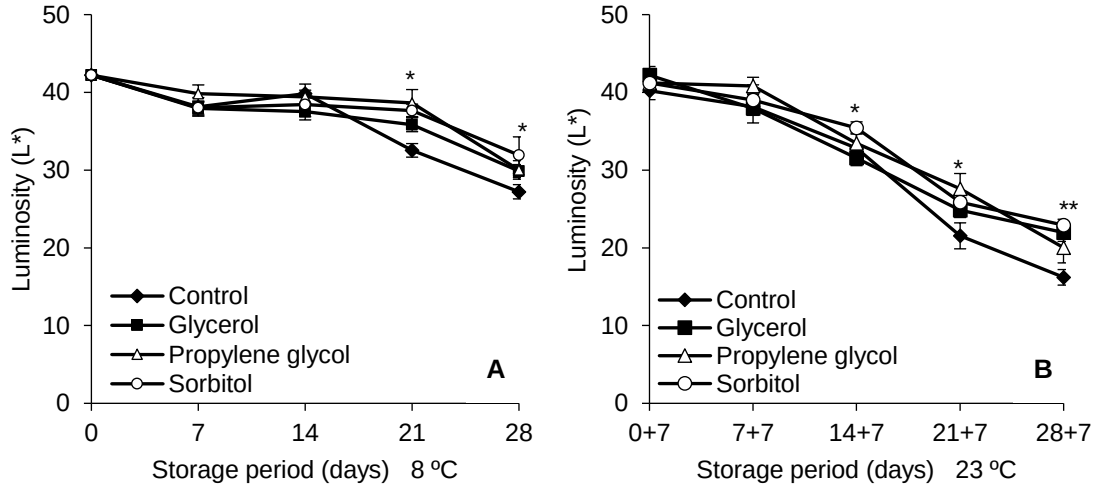


Figure 2. Luminosity (L*) of 'Palmer' mangoes epicarp (peel) treated with polyols and stored at 8.0 ± 2 ºC for up to 28 days (A) and transfer to ambient (23 ± 2 ºC) for 7 more days (B). Statistical significance at P<0.05 (**) and P<0.01 (*) for the treatments within each storage period. The bars represent the standard deviation of 5 repetitions.

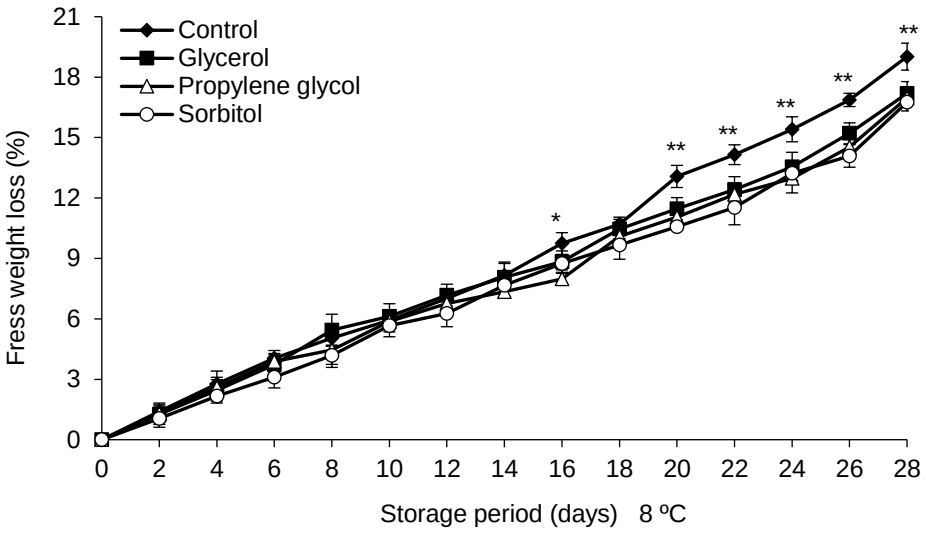


Figure 3. Fresh weight loss of 'Palmer' mangoes treated with polyols and stored at 8.0 ± 2 ºC for up to 28 days. Statistical significance at P<0.05 (**) and P<0.01 (*) for the treatments within each storage period. The bars represent the standard deviation of 5 repetitions.

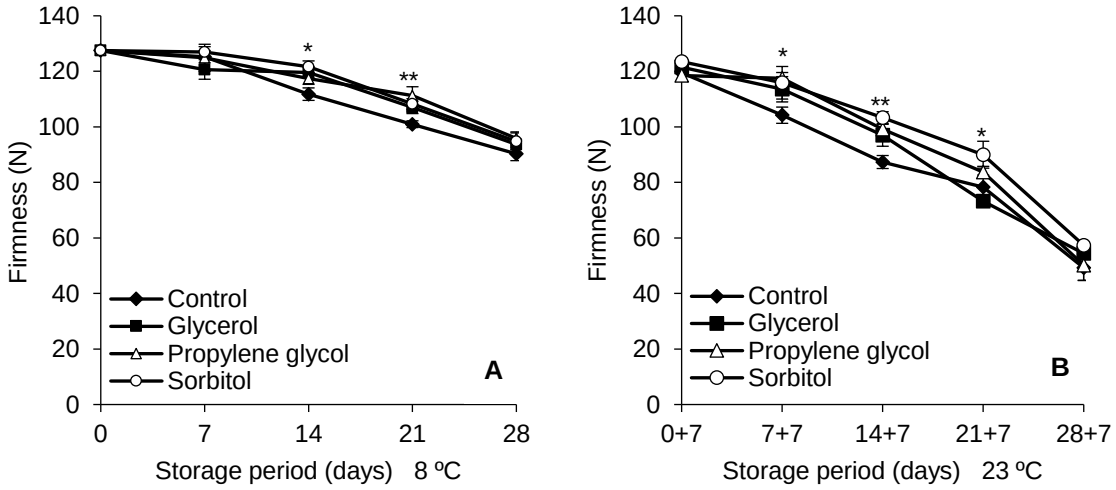


Figure 4. Firmness (N) of 'Palmer' mangoes treated with polyols and stored at 8.0 ± 2 ºC for up to 28 days (A) and transfer to ambient (23 ± 2 ºC) for 7 days (B). Statistical significance at P<0.05 (**) and P<0.01 (*) for the treatments within each storage period. The bars represent the standard deviation of 5 repetitions.

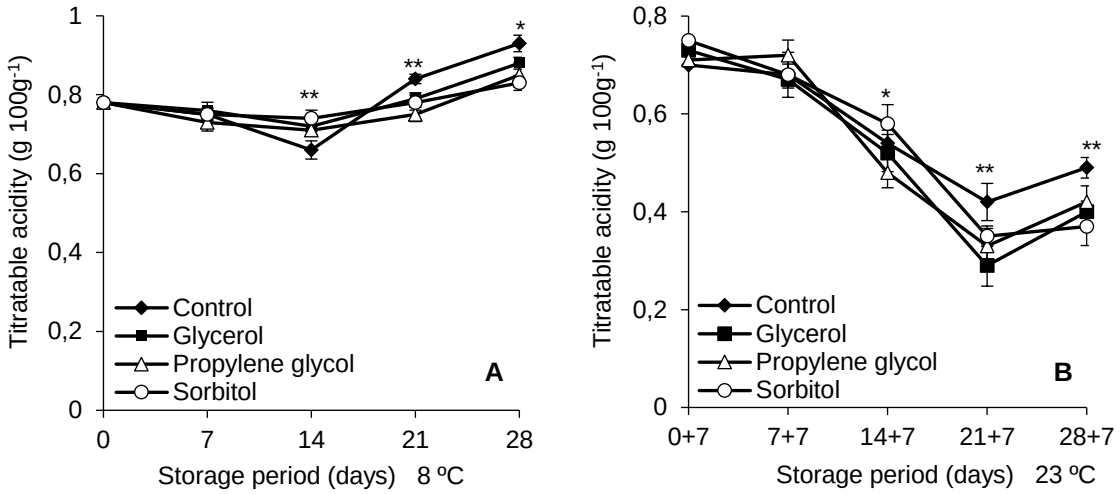


Figure 5. Titratable acidity of 'Palmer' mangoes treated with polyols and stored at 8.0 ± 2 ºC for up to 28 days (A) and transfer to ambient (23 ± 2 ºC) for 7 days (B). Statistical significance at P<0.05 (**) and P<0.01 (*) for the treatments within each storage period. The bars represent the standard deviation of 5 repetitions.

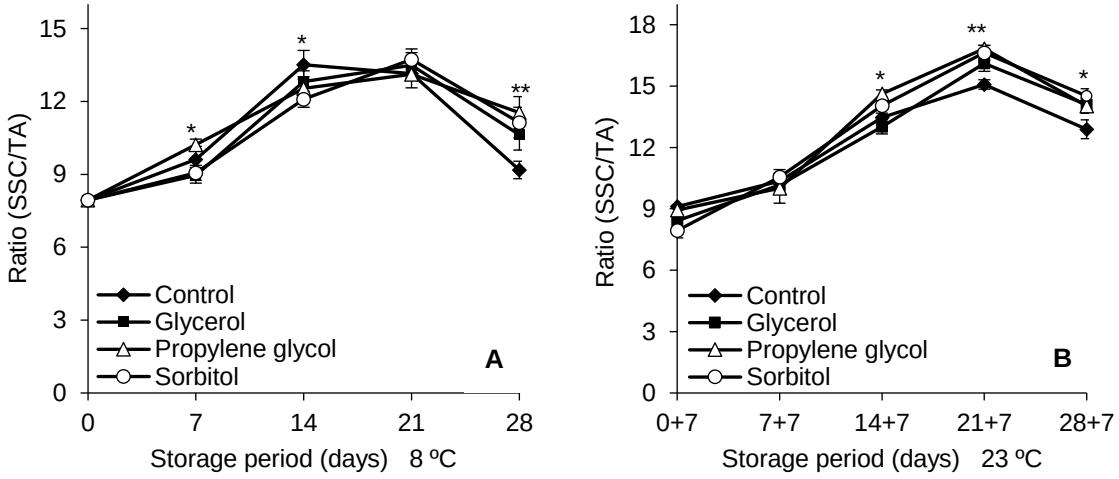


Figure 6. Ration (SSC/TA) of 'Palmer' mangoes treated with polyols and stored at 8.0 ± 2 ºC for up to 28 days (A) and transfer to ambient (23 ± 2 ºC) for 7 days (B). Statistical significance at P<0.05 (**) and P<0.01 (*) for the treatments within each storage period. The bars represent the standard deviation of 5 repetitions.

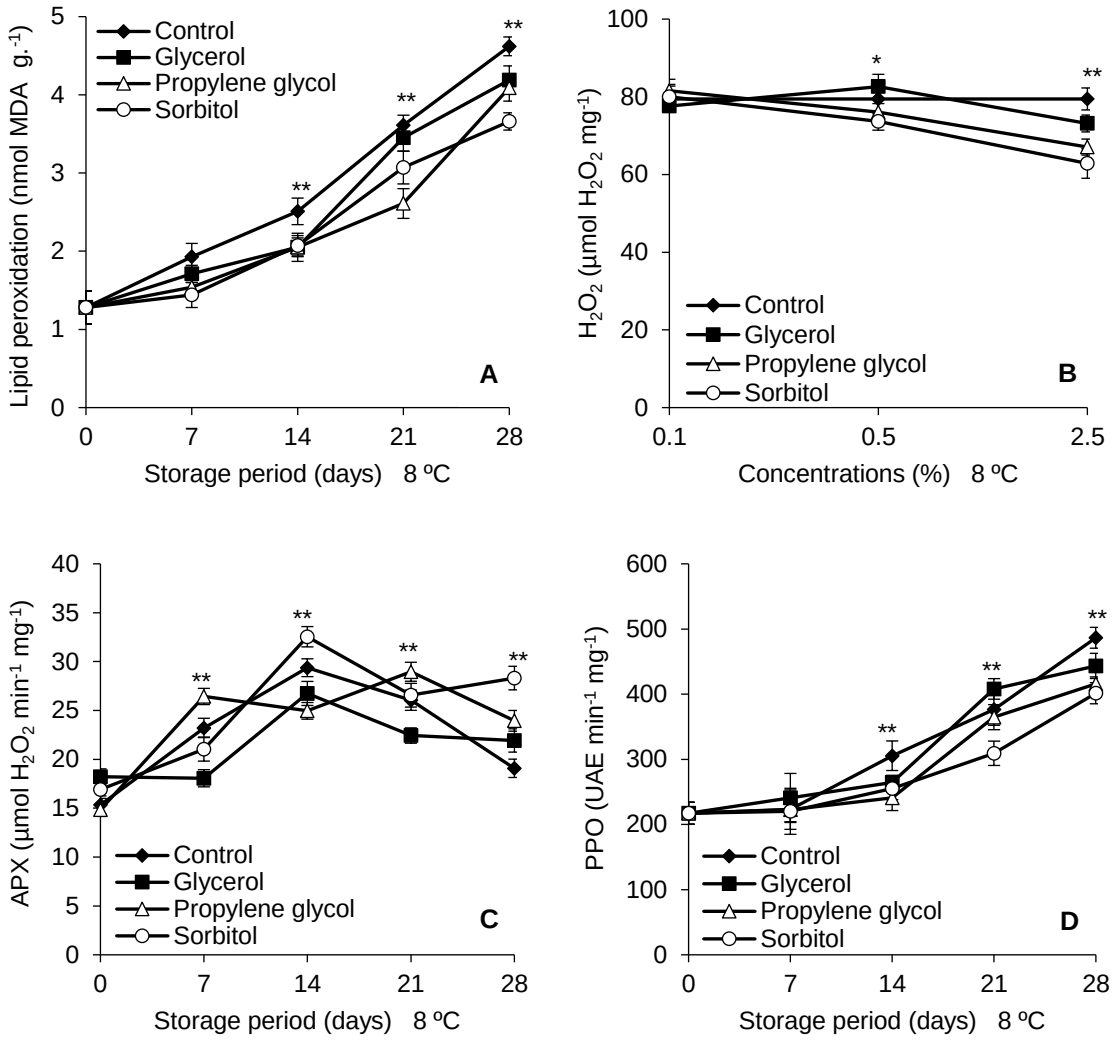
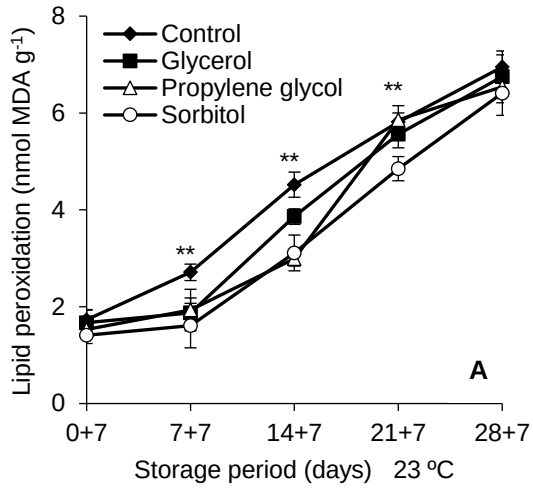
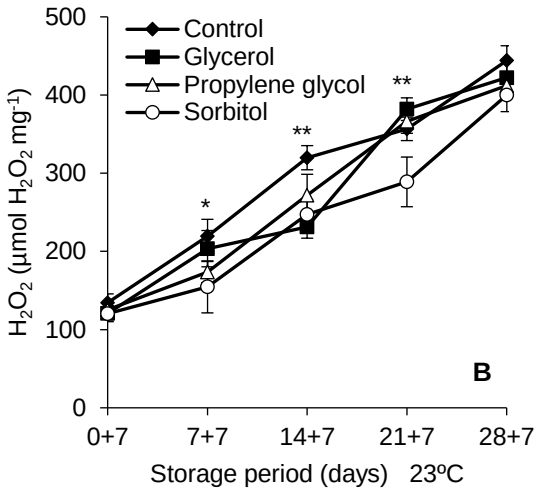
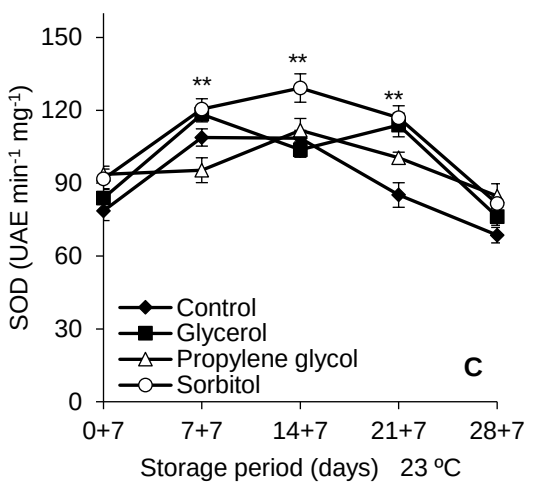
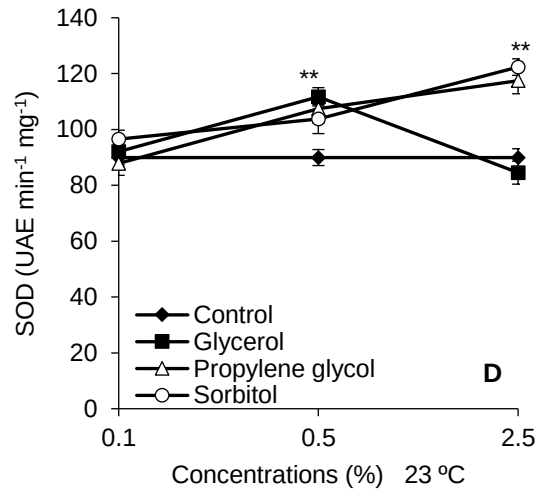


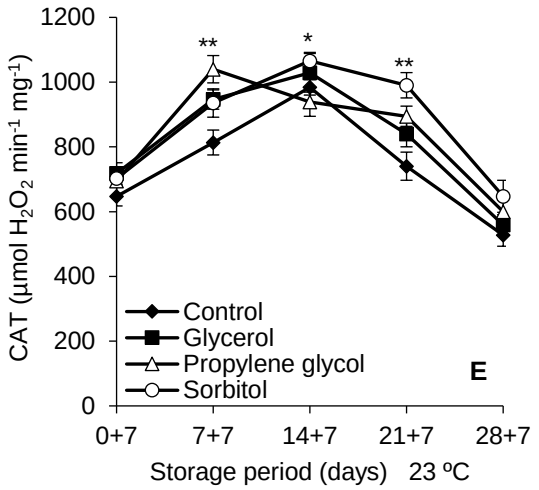
Figure 7. Lipid peroxidation (A), hydrogen peroxide content (B), ascorbate peroxidase (C) and polyphenoloxidase (D) activities of 'Palmer' mangoes epicarp (peel) treated with polyols and stored at 8.0 ± 2 ºC for up to 28 days. Statistical significance at $P<0.05$ (**) and $P<0.01$ (*) for the treatments within each storage period. The bars represent the standard deviation of 5 repetitions.

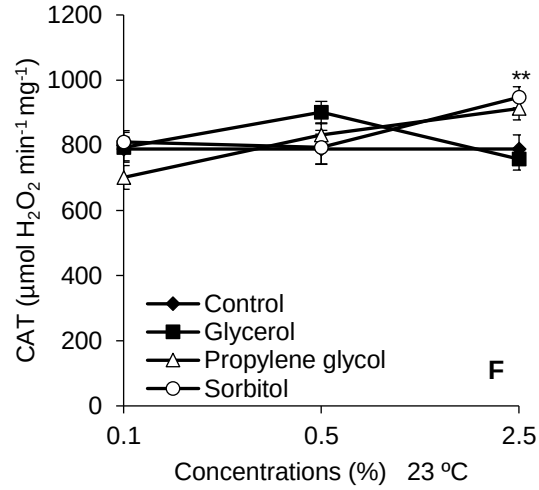

Lipid peroxidation (nmol MDA g-1)
Control
Glycerol
Propylene glycol
Sorbitol
A
0+7
7+7
14+7
21+7
28+7
Storage period (days) 23 ºC


H2O2 (µmol H2O2 mg-1)
Control
Glycerol
Propylene glycol
Sorbitol
B
Storage period (days) 23ºC


SOD (UAE min-1 mg-1)
Control
Glycerol
Propylene glycol
Sorbitol
C
Storage period (days) 23 ºC


SOD (UAE min-1 mg-1)
Control
Glycerol
Propylene glycol
Sorbitol
D
Concentrations (%) 23 ºC


CAT (µmol H2O2 min-1 mg-1)
Control
Glycerol
Propylene glycol
Sorbitol
E
Storage period (days) 23 ºC


CAT (µmol H2O2 min-1 mg-1)
Control
Glycerol
Propylene glycol
Sorbitol
F
Concentrations (%) 23 ºC

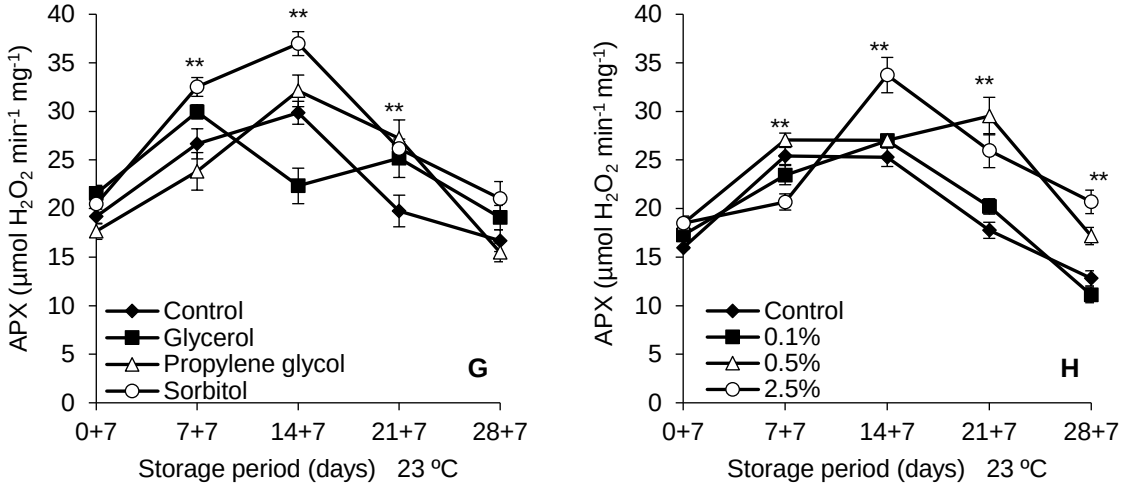
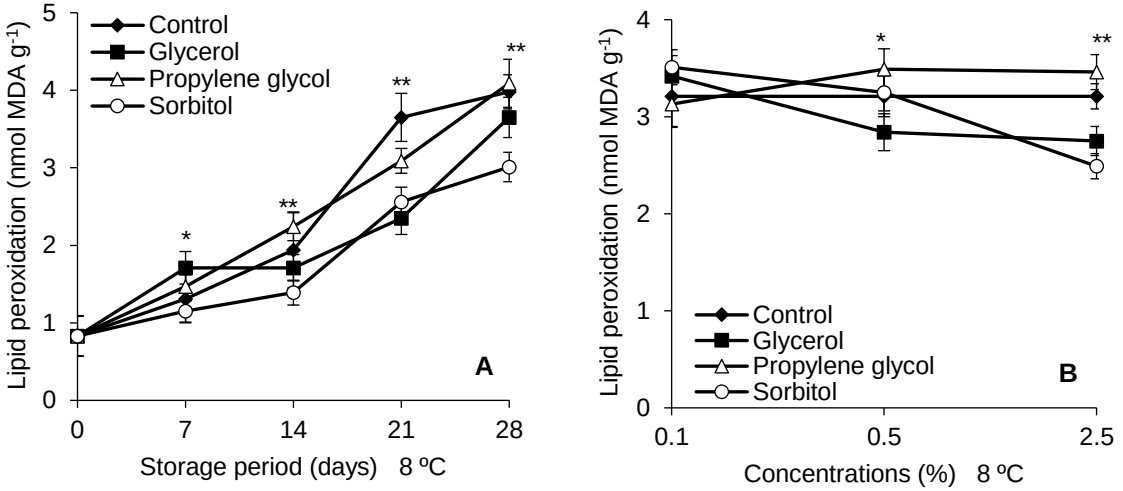


Figure 8. Lipid peroxidation (A), hydrogen peroxide content (B), superoxide dismutase (C - D), catalase (E - F) and ascorbate peroxidase (G - F) activities of 'Palmer' mangoes epicarp (peel) treated with polyols and stored at 8.0 ± 2 ºC for up to 28 days and transfer to ambient (23 ± 2 ºC) for 7 days. Statistical significance at P<0.05 (**) and P<0.01 (*) for the treatments within each storage period. The bars represent the standard deviation of 5 repetitions**.**



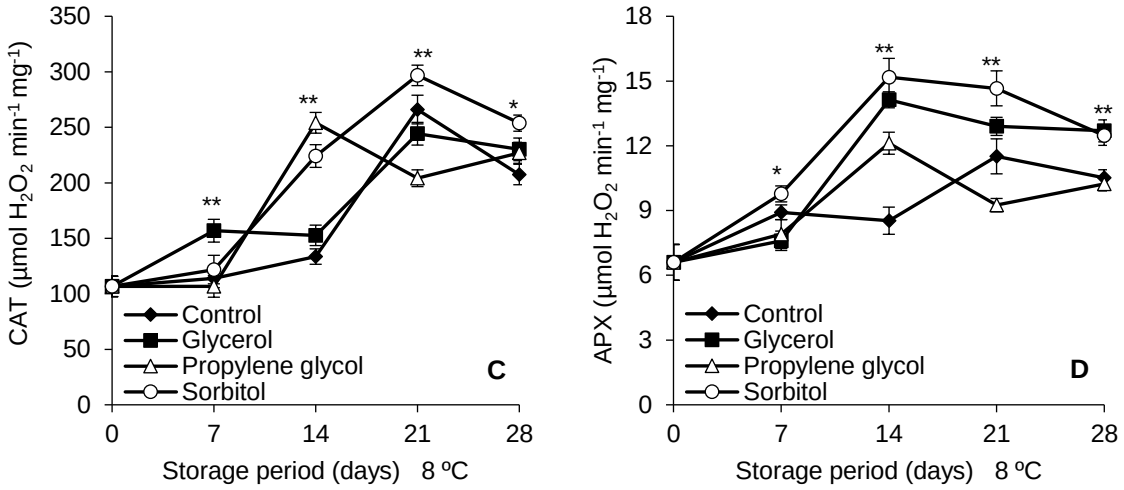
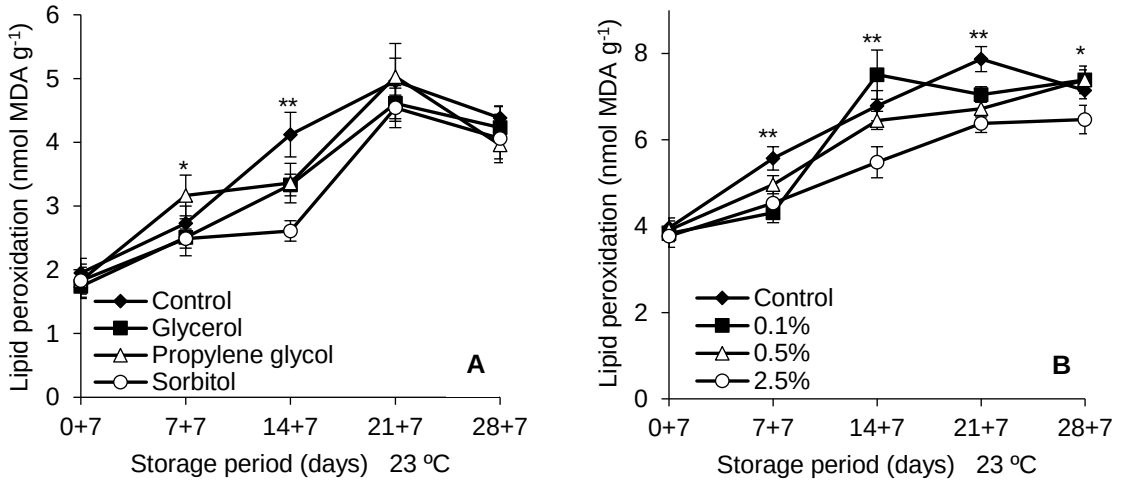


Figure 9. Lipid peroxidation (A - B), catalase (C) and ascorbate peroxidase (D) activities of 'Palmer' mangoes mesocarp (pulp) treated with polyols and stored at 8.0 ± 2 ºC for up to 28. Statistical significance at P<0.05 (**) and P<0.01 (*) for the treatments within each storage period. The bars represent the standard deviation of 5 repetitions.



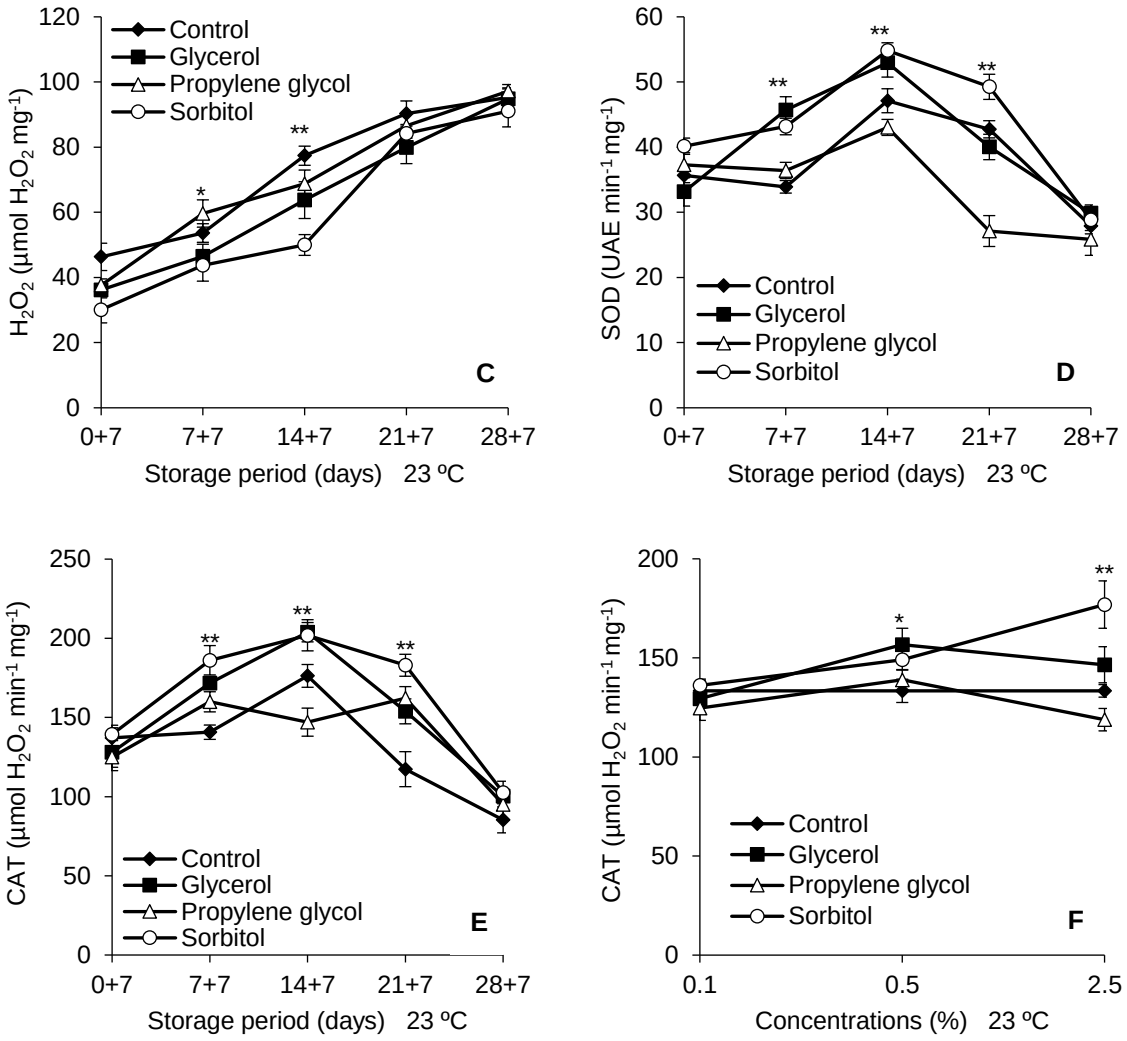


Figure 10. Lipid peroxidation (A - B), hydrogen peroxide content (C), superoxide dismutase (D), catalase (E - F) activities of 'Palmer' mangoes mesocarp (pulp) treated with polyols and stored at 8.0 ± 2 ºC for up to 28 days and transfer to ambient (23 ± 2 ºC) for 7 days. Statistical significance at P<0.05 (**) and P<0.01 (*) for the treatments within each storage period. The bars represent the standard deviation of 5 repetitions.

Supplementary material

Table 1S. Simple effects and interactions of the polyol solutions on physicochemical parameters of 'Palmer' mangoes stored at low temperature (8 ± 2 ºC) for up to 28 days.

Principal effects	Firmness (N)	Color			SSC[a] (ºBrix)	TA[b] (g 100g^{-1})	SSC/TA	pH
		Luminosity	Angle hue	Chromaticity				
Treatments (A)								
Control	104.03 ± 3.01 b	37.10 ± 1.38 b	86.58 ± 0.78 a	15.88 ± 1.19 a	8.02 ± 0.20 b	0.79 ± 0.13 a	10.49 ± 1.25 b	3.56 ± 0.17 a
Glycerol	108.59 ± 3.12 a	38.21 ± 1.56 ab	86.48 ± 1.12 a	15.71 ± 1.12 a	8.42 ± 0.18 a	0.74 ± 0.19 ab	12.33 ± 1.23 a	3.51 ± 0.11 a
Propylene glycol	110.71 ± 2.89 a	39.51 ± 1.93 a	88.89 ± 0.93 a	17.02 ± 1.03 a	8.58 ± 0.28 a	0.68 ± 0.09 b	11.96 ± 1.73 a	3.52 ± 0.21 a
Sorbitol	110.25 ± 3.03 a	38.07 ± 1.21 ab	87.73 ± 0.67 a	16.60 ± 1.17 a	8.64 ± 0.32 a	0.70 ± 0.13 b	12.08 ± 1.02 a	3.49 ± 0.12 a
Concentrations (B)								
0.1 %	104.60 ± 2.56 a	37.93 ± 1.87 a	88.00 ± 1.02 a	16.27 ± 0,89 a	8.16 ± 0.14 a	0.76 ± 0.16 a	11.37 ± 0.96 a	3.53 ± 0.12 a
0.5 %	103.84 ± 3.04 a	38.57 ± 0.93 a	87.40 ± 0.91 a	16.59 ± 1.04 a	8.14 ± 0.23 a	0.69 ± 0.31 a	11.23 ± 1.11 a	3.53 ± 0.18 a
2.5 %	104.79 ± 2.88 a	38.31 ± 1.34 a	87.91 ± 0.87 a	16.22 ± 0.91 a	8.20 ± 0.21 a	0.73 ± 0.21 a	11.88 ± 1.07 a	3.55 ± 0.13 a
Storage (C)								
0	127.49 ± 2.92 a	42.21 ± 0.83 a	86.62 ± 1.56 a	14.15 ± 0.77 a	6.22 ± 0.19 a	0.76 ± 0.14 c	7.94 ± 0.89 c	3.52 ± 0.10 a
7	125.76 ± 3.06 a	37.87 ± 1.27 b	85.79 ± 0.77 a	14.26 ± 1.96 a	7.33 ± 0.26 b	0.72 ± 0.12 c	10.83 ± 1.29 b	3.47 ± 0.26 a
14	116.60 ± 2.77 b	39.04 ± 1.65 ab	86.25 ± 0.80 a	15.53 ± 0.62 a	7.88 ± 0.25 c	0.69 ± 0.11 c	12.47 ± 0.96 a	3.51 ± 0.17 a
21	107.41 ± 3.13 c	34.13 ± 0.71 c	86.91 ± 1.11 a	14.20 ± 1.12 a	9.03 ± 0.29 c	0.82 ± 0.17 b	12.87 ± 1.18 a	3.45 ± 0.20 a
28	93.47 ± 2.81 d	31.45 ± 0.77 d	87.89 ± 1.61 a	16.09 ± 1.88 a	10.67 ± 0.21 d	0.88 ± 0.21 a	12.59 ±057 a	3.53 ± 0.31 a
Interactions								
AxB	NS	NS	NS	NS	NS	NS	NS	NS
AxC	4.19**	2.66*	NS	NS	NS	6.48**	5.09**	NS
BxC	NS	NS	NS	NS	NS	NS	NS	NS
AxBxC	NS	NS	NS	NS	NS	NS	NS	NS

[a]Soluble solids content (SSC), [b]titratable acidity (TA). Means followed by the same letter within each column do not differ statistically from each other by the Tukey test (P<0.05). Non-significant interaction (NS), significant interaction at P<0.05 (**) and significant interaction at P<0.01 (*).

Table 2S. Simple effects and interactions of the polyol solutions on physicochemical parameters of 'Palmer' mangoes stored at low temperature (8 ± 2 ºC) for up to 28 days plus 7 more days at ambient (23 ± 2 ºC).

Principal effects	Firmness (N)	Color			SSC[a] (ºBrix)	TA[b] (g 100g^{-1})	SSC/TA	pH
		Luminosity	Angle hue	Chromaticity				
Treatments (A)								
Control	87.71 ± 3.04 b	31.18 ± 1.09 b	90.65 ± 1.06 a	18.11 ± 1.32 a	10.82 ± 1.33 b	0.57 ± 0.09 b	13.15 ± 1.21 b	4.23 ± 0.47 a
Glycerol	91.32 ± 2.09 ab	32.33 ± 1.21 ab	89.71 ± 0.99 a	18.21 ± 1.11 a	11.78 ± 1.88 ab	0.51 ± 0.13 ab	13.94 ± 0.98 a	4.27 ± 0.51 a
Propylene glycol	97.21 ± 1.51 a	33.42 ± 0.96 a	90.21 ± 1.15 a	19.52 ± 1.26 a	12.18 ± 0.52 a	0.49 ± 0.18 a	14.12 ± 1.12 a	4.18 ± 0.43 a
Sorbitol	95.60 ± 1.98 a	33.34 ± 1.52 a	89.33 ± 1.20 a	18.76 ± 1.05 a	12.23 ± 0.75 a	0.46 ± 0.21 a	14.26 ± 1.26 a	4.14 ± 0.56 a
Concentrations (B)								
0.1 %	90.45 ± 1.21 a	34.52 ± 0.89 a	88.03 ± 1.12 a	18.70 ± 1.09 a	12.17 ± 0.29 a	0.49 ± 0.22 a	13.95 ± 0.91 a	4.04 ± 0.34 a
0.5 %	88.92 ± 2.94 a	35.84 ± 1.76 a	89.00 ± 1.03 a	19.22 ± 0.87 a	12.24 ± 0.38 a	0.46 ± 0.11 a	14.03 + 1.17 a	4.11 ± 0.45 a
2.5 %	91.06 ± 1.55 a	35.19 ± 1.03 a	88.91 ± 0.97 a	18.81 ± 1.01 a	12.13 ± 0.21 a	0.44 ± 0.19 a	13.91 ± 1.32 a	4.09 ± 0.28 a
Storage (C)								
0+7	119.71 ± 2.92 a	41.21 ± 0.83 a	87.62 ± 0.56 b	14.85 ± 0.77 b	8.12 ± 0.19 d	0.71 ± 0.14 d	8.60 ± 0.89 d	3.81 ± 0.17 c
7+7	109.52 ± 1.86 b	38.97 ± 1.23 b	86.89 ± 1.11 b	15.31 ± 0.86 b	9.33 ± 0.26 c	0.64 ± 0.27 c	11.93 ± 1.05 c	4.11 ± 0.31 b
14+7	96.22 ± 3.20 c	32.81 ± 1.05 c	89.56 ± 0.82 ab	17.95 ± 0.67 a	11.68 ± 0.45 b	0.53 ± 0.28 b	14.56 ± 1.19 b	3.89 ± 0.26 bc
21+7	80.79 ± 2.18 d	25.98 ± 0.99 d	91.67 ± 0.44 a	18.58 ± 1.04 a	12.31 ± 0.26 b	0.31 ± 0.45 a	16.12 ± 0.93 a	4.36 ± 0.19 a
28+7	52.82 ± 1.63 e	23.01 ± 1.22 d	93.75 ± 0.91 a	19.92 ± 0.81 a	14.96 ± 0.37 a	0.42 0.19 b	14.22 ± 1.22 b	4.41 ± 0.32 a
Interactions								
AxB	NS	NS	NS	NS	NS	NS	NS	NS
AxC	5.31**	4.78**	NS	2.91*	NS	5.93**	6.21**	NS
BxC	NS	NS	NS	NS	NS	NS	NS	NS
AxBxC	NS	NS	NS	NS	NS	NS	NS	NS

[a]Soluble solids content (SSC), [b]titratable acidity (TA). Means followed by the same letter within each column do not differ statistically from each other by the Tukey test (P<0.05). Non-significant interaction (NS), significant interaction at P<0.05 (**) and significant interaction at P<0.01 (*).

Table 3S. Simple effects and interactions of the polyol solutions on oxidative metabolism parameters of 'Palmer' mangoes epidermis (peel) stored at low temperature (8 ± 2 ºC) for up to 28 days.

Principal effects	LP[a] (nmol MDA g^{-1})	H_2O_2[b] (μmol H_2O_2 mg^{-1})	AsA[c] (mg $100g^{-1}$)	SOD[d] (UAE min^{-1} mg^{-1})	CAT[e] (μmol H_2O_2 min^{-1} mg^{-1})	APX[f] (μmol H_2O_2 min^{-1} mg^{-1})	PPO[g] (UAE min^{-1} mg^{-1})
Treatments (A)							
Control	2.69 ± 0.65 a	79.44 ± 2.37 a	27.65 ± 1.26 b	78.44 ± 2.23 b	361.65 ± 16.06 a	17.78 ± 0.66 ab	311.77 ± 7.92 a
Glycerol	2.57 ± 0.34 ab	77.13 ± 2.05 a	26.41 ± 0.92 b	81.25 ± 1.92 a	363.85 ± 20.36 a	17.77 ± 0.79 b	302.52 ± 11.65 ab
Propylene glycol	2.46 ± 0.96 b	74.87 ± 1.76 ab	30.43 ± 1.81 a	79.87 ± 2.06 b	366.75 ± 19.21 a	17.81 ± 0.47 ab	298.24 ± 7.48 b
Sorbitol	2.39 ± 0.63 b	72.51 ± 2.18 b	31.03 ± 1.27 a	83.07 ± 2.64 a	369.81 ± 22.07 a	19.82 ± 0.51 a	293.02 ± 13.51 b
Concentrations (B)							
0.1 %	2.56 ± 0.56 a	80.16 ± 3.17 a	26.41 ± 1.93 a	78.80 ± 2.45 c	356.51 ± 16.12 b	17.73 ± 0.75 b	306.41 ± 10.05 a
0.5 %	2.58 ± 0.29 a	75.33 ± 2.08 b	26.93 ± 2.06 a	81.13 ± 3.18 b	368.97 ± 10.38 a	18.06 ± 0.81 b	301.19 ± 9.33 b
2.5 %	2.50 ± 0.33 a	71.72 ± 3.01 c	27.65 ± 1.72 a	83.53 ± 2.83 a	371.06 ± 13.77 a	19.83 ± 0.49 a	296.81 ± 11.21 b
Storage (C)							
0	1.28 ± 0.52 e	59.17 ± 1.92 c	34.86 ± 2.13 b	72.86 ± 3.35 c	327.77 ± 13.08 c	17.52 ± 0.83 c	217.21 ± 7.47 d
7	1.70 ± 1.06 d	52.89 ± 2.03 d	38.19 ± 1.10 a	79.18 ± 1.97 b	375.74 ± 17.94 b	19.56 ± 0.98 c	226.72 ± 10.61 d
14	2.10 ± 0.17 c	61.20 ± 2.27 c	33.56 ± 0.96 b	85.72 ± 2.86 a	406.32 ± 12.19 a	26.68 ± 0.51 b	258.86 ± 9.07 c
21	3.11 ± 0.80 b	96.03 ± 3.55 b	26.07 ± 1.07 c	82.91 ± 2.18 ab	393.03 ± 15.91 ab	29.02 ± 0.21 a	363.11 ± 12.19 b
28	4.45 ± 0.26 a	112.40 ± 3.28 a	16.11 ± 0.89 d	83.11 ± 3.91 a	324.71 ± 12.63 c	21.09 ± 0.79 c	444.78 ± 13.62 a
Interactions							
AxB	NS	1.80*	NS	NS	NS	NS	NS
AxC	2.56**	NS	NS	NS	NS	NS	2.09*
BxC	NS	NS	NS	NS	NS	2.31*	NS
AxBxC	NS	NS	NS	NS	NS	NS	NS

[a]Lipid peroxidation - malondialdehyde (MDA), [b]hydrogen peroxide, [c]ascorbate, [d]superoxide dismutase (SOD), [e]catalase (CAT), [f]ascorbate peroxidase (APX). Means followed by the same letter within each column do not differ statistically from each other by the Tukey test (P<0.05). Non-significant interaction (NS), significant interaction at P<0.05 (**) and significant interaction at P<0.01 (*).

Table 4S. Simple effects and interactions of the polyol solutions on oxidative metabolism parameters of 'Palmer' mangoes epidermis (peel) stored at low temperature (8 ± 2 ºC) for up to 28 days plus 7 more days in the ambient (23 ± 2 ºC).

Principal effects	LP[a] (nmol MDA g^{-1})	H_2O_2[b] (µmol H_2O_2 mg^{-1})	AsA[c] (mg $100g^{-1}$)	SOD[d] (UAE min^{-1} mg^{-1})	CAT[e] (µmol H_2O_2 min^{-1} mg^{-1})	APX[f] (µmol H_2O_2 min^{-1} mg^{-1})	PPO[g] (UAE min^{-1} mg^{-1})
Treatments (A)							
Control	3.37 ± 1.28 a	211.01 ± 7.87 a	15.81 ± 1.09 b	89.94 ± 1.13 c	788.56 ± 21.44 b	20.41 ± 0.34 c	524.77 ± 11.34 a
Glycerol	3.15 ± 2.34 b	204.94 ± 6.61 b	16.56 ± 1.65 b	96.44 ± 2.26 b	821.00 ± 15.21 ab	22.62 ± 0.13 b	524.02 ± 9.89 a
Propylene glycol	3.11 ± 1.03 bc	205.24 ± 7.18 b	16.13 ± 0.95 b	102.59 ± 3.35 a	827.51 ± 13.36 ab	22.68 ± 0.22 b	518.81 ± 8.76 ab
Sorbitol	3.05 ± 0.83 c	197.52 ± 3.69 c	19.02 ± 1.68 a	105.20 ± 218 a	850.28 ± 24.19 a	24.77 ± 0.19 a	502.51 ± 13.51 b
Concentrations (B)							
0.1 %	3.20 ± 0.16 a	212.30 ± 5.89 a	16.53 ± 0.84 a	91.60 ± 1.06 c	777.71 ± 24.83 b	23.43 ± 0.21 c	527.90 ± 15.68 a
0.5 %	3.26 ± 0.29 a	205.87 ± 7.21 b	17.01 ± 1.43 a	98.45 ± 1.11 b	828.68 ± 13.05 a	25.62 ± 0.89 b	513.86 ± 12.67 b
2.5 %	3.05 ± 0.23 b	195.46 ± 6.22 c	17.65 ± 2.18 a	103.57 ± 0.37 a	859.02 ± 21.11 a	26.81 ± 0.23 a	507.38 ± 9.70 b
Storage (C)							
0+7	1.92 ± 0.21 e	125.20 ± 8.23 e	23.07 ± 1.83 a	85.71 ± 2.19 d	702.92 ± 22.05 d	23.41 ± 0.55 e	260.56 ± 9.83 e
7+7	3.24 ± 0.53 d	173.74 ± 6.34 d	19.56 ± 2.51 b	115.25 ± 3.17 a	928.81 ± 14.19 b	33.59 ± 0.78 d	375.87 ± 17.67 d
14+7	4.68 ± 1.67 c	280.22 ± 5.76 c	17.13 ± 2.71 b	109.84 ± 2.41 b	1015.07 ± 25.25 a	24.49 ± 0.21 c	461.71 ± 12.30 c
21+7	5.52 ± 1.20 b	329.80 ± 12.11 b	14.53 ± 1.68 c	104.13 ± 3.67 c	871.31 ± 23.97 c	21.53 ± 0.54 b	642.90 ± 16.45 b
28+7	6.83 ± 1.38 a	414.44 ± 10.93 a	8.36 ± 2.05 d	77.78 ± 2.02 e	591.06 ± 17.85 e	16.93 ± 0.43 a	854.10 ± 11.78 a
Interactions							
AxB	NS	NS	NS	4.35**	6.38**	NS	NS
AxC	5.68**	3.56**	NS	7.01**	3.96**	4.69**	NS
BxC	NS	NS	NS	NS	NS	3.11**	NS
AxBXC	NS	NS	NS	NS	NS	NS	NS

[a]Lipid peroxidation - malondialdehyde (MDA), [b]hydrogen peroxide, [c]ascorbate, [d]superoxide dismutase (SOD), [e]catalase (CAT), [f]ascorbate peroxidase (APX). Means followed by the same letter within each column do not differ statistically from each other by the Tukey test (P<0.05). Non-significant interaction (NS), significant interaction at P<0.05 (**) and significant interaction at P<0.01 (*).

Table 5S. Simple effects and interactions of the polyol solutions on oxidative metabolism parameters of 'Palmer' mangoes pulp stored at low temperature (8 ± 2 ºC) for up to 28 days.

Principal effects	LP[a] (nmol MDA g^{-1})	H_2O_2[b] (µmol H_2O_2 mg^{-1})	AsA[c] (mg $100g^{-1}$)	SOD[d] (UAE min^{-1} mg^{-1})	CAT[e] (µmol H_2O_2 min^{-1} mg^{-1})	APX[f] (µmol H_2O_2 min^{-1} mg^{-1})	PPO[g] (UAE min^{-1} mg^{-1})
Treatments (A)							
Control	3.21 ± 0.85 b	18.72 ± 0.18 ab	53.02 ± 0.94 b	27.91 ± 1.03 b	165.49 ± 8.43 b	9.39 ± 1.27 bc	150.34 ± 3.29 b
Glycerol	3.06 ± 1.03 c	18.15 ± 0.31 bc	54.07 ± 1.19 b	28.79 ± 1.69 b	168.91 ± 11.23 b	9.57 ± 1.04 b	148.14 ± 5.21 b
Propylene glycol	3.41 ± 0.75 a	18.92 ± 0.22 a	51.92 ± 2.01 c	26.68 ± 1.08 c	158.77 ± 7.66 b	8.82 ± 1.34 c	163.89 ± 4.07 a
Sorbitol	3.12 ± 0.91 c	17.23 ± 0.27 c	56.91 ± 1.23 a	31.10 ± 1.08 a	183.93 ± 10.04 a	11.32 ± 0.91 a	146.32 ± 7.18 b
Concentrations (B)							
0.1 %	3.22 ± 0.79 a	18.67 ± 0.09 a	53.16 ± 1.17 b	28.22 ± 0.95 b	176.65 ± 8.12 a	9.13 ± 0.63 b	157.15 ± 4.20 a
0.5 %	3.17 ± 1.16 b	18.56 ± 0.20 a	56.06 ± 2.06 a	26.93 ± 1.81 b	178.76 ± 9.17 a	10.15 ± 0.81 a	153.32 ± 2.06 ab
2.5 %	3.00 ± 0.84 c	18.51 ± 0.17 a	57.11 ± 1.93 a	30.10 ± 1.06 a	182.44 ± 10.18 a	10.04 ± 1.06 a	148.04 ± 2.34 b
Storage (C)							
0	1.54 ± 0.93 e	19.30 ± 0.34 c	59.25 ± 2.61 b	19.05 ± 1.11 e	106.69 ± 9.02 d	6.60 ± 1.76 d	67.47 ± 3.91 d
7	2.58 ± 0.87 d	18.28 ± 0.27 c	63.21 ± 1.84 b	23.12 ± 0.76 d	115.80 ± 12.43 d	7.42 ± 0.94 d	86.03 ± 1.18 c
14	3.38 ± 1.06 c	24.13 ± 0.37 d	69.67 ± 1.16 a	25.76 ± 1.93 c	136.54 ± 6.21 c	9.67 ± 1.31 c	112.65 ± 6.21 b
21	4.02 ± 1.13 b	29.00 ± 0.26 b	53.21 ± 1.93 c	39.14 ± 1.67 a	256.26 ± 7.11 a	14.25 ± 0.97 a	243.83 ± 4.11 a
28	4.14 ± 0.91 a	33.19 ± 0.30 a	44.20 ± 2.06 d	36.02 ± 0.89 b	231.10 ± 11.08 b	10.93 ± 1.09 b	250.88 ± 7.12 a
Interactions							
AxB	7.07**	NS	NS	NS	NS	NS	NS
AxC	5.09**	NS	NS	NS	4.01**	4.61**	NS
BxC	NS	NS	NS	NS	NS	NS	NS
AxBxC	NS	NS	NS	NS	NS	NS	NS

[a]Lipid peroxidation - malondialdehyde (MDA), [b]hydrogen peroxide, [c]ascorbate, [d]superoxide dismutase (SOD), [e]catalase (CAT), [f]ascorbate peroxidase (APX). Means followed by the same letter within each column do not differ statistically from each other by the Tukey test (P<0.05). Non-significant interaction (NS), significant interaction at P<0.05 (**) and significant interaction at P<0.01 (*).

Table 6S. Simple effects and interactions of the polyol solutions on oxidative metabolism parameters of 'Palmer' mangoes pulp stored at low temperature (8 ± 2 ºC) for up to 28 days plus 7 more days in the ambient (23 ± 2 ºC).

Principal effects	LP[a] (nmol MDA g^{-1})	H_2O_2[b] (µmol H_2O_2 mg^{-1})	AsA[c] (mg $100g^{-1}$)	SOD[d] (UAE min^{-1} mg^{-1})	CAT[e] (µmol H_2O_2 min^{-1} mg^{-1})	APX[f] (µmol H_2O_2 min^{-1} mg^{-1})	PPO[g] (UAE min^{-1} mg^{-1})
Treatments (A)							
Control	5.93 ± 1.82 b	64.19 ± 2.11 a	38.42 ± 1.92 b	37.07 ± 0.49 c	133.36 ± 8.18 b	7.99 ± 1.46 b	125.20 ± 3.50 a
Glycerol	5.89 ± 1.16 b	61.42 ± 1.96 b	41.24 ± 1.17 a	39.33 ± 1.02 b	146.55 ± 6.31 a	8.79 ± 0.87 a	124.17 ± 2.88 a
Propylene glycol	6.78 ± 0.79 a	64.74 ± 0.78 b	37.21 ± 2.13 b	35.15 ± 0.58 d	129.83 ± 11.26 b	7.77 ± 0.93 b	127.26 ± 4.01 a
Sorbitol	5.38 ± 1.21 c	59.62 ± 0.93 c	42.34 ± 1.69 a	41.06 ± 0.96 a	151.01 ± 6.08 a	9.01 ± 0.58 a	123.40 ± 3.16 a
Concentrations (B)							
0.1 %	6.13 ± 1.34 a	62.84 ± 2.16 a	37.61 ± 2.01 a	36.08 ± 0.87 b	133.40 ± 7.19 b	7.99 ± 1.06 b	132.59 ± 4.19 a
0.5 %	6.06 ± 1.67 a	62.59 ± 1.74 a	39.10 ± 1.05 a	39.63 ± 1.12 a	142.26 ± 11.07 a	8.47 ± 0.95 a	121.70 ± 3.61 b
2.5 %	5.81 ± 0.72 b	61.05 ± 2.02 a	39.93 ± 2.26 a	38.74 ± 1.06 a	145.65 ± 5.12 a	8.71 ± 0.61 a	120.74 ± 2.83 b
Storage (C)							
0+7	3.83 ± 0.86 e	37.56 ± 0.91 e	52.36 ± 1.67 a	36.56 ± 2.21 d	132.39 ± 11.04 c	7.92 ± 0.45 c	93.36 ± 2.19 d
7+7	5.90 ± 1.27 d	52.12 ± 3.18 d	50.41 ± 1.18 a	37.80 ± 1.73 c	162.09 ± 4.18 b	11.32 ± 1.11 a	117.27 ± 4.82 c
14+7	6.58± 0.89 c	57.21 ± 2.82 c	42.08 ± 1.82 b	48.23 ± 1.42 a	183.28 ± 7.56 a	9.29 ± 0.87 b	171.92 ± 1.83 a
21+7	6.72 ± 0.94 b	71.25 ± 1.19 b	37.11 ± 1.07 c	39.54 ± 1.12 b	128.59 ± 9.01 c	7.69 ± 0.55 c	155.12 ± 5.06 b
28+7	6.96 ± 1.11 a	94.33 ± 1.06 a	28.50 ± 1.73 d	28.62 ± 2.39 e	95.83 ± 6.67 d	5.73 ± 0.81 d	87.38 ± 3.44 d
Interactions							
AxB	NS	NS	NS	NS	2.46*	NS	NS
AxC	4.33**	4.71**	NS	6.75**	5.81**	NS	NS
BxC	3.19**	NS	NS	NS	NS	NS	NS
AxBXC	NS	NS	NS	NS	NS	NS	NS

[a]Lipid peroxidation - malondialdehyde (MDA), [b]hydrogen peroxide, [c]ascorbate, [d]superoxide dismutase (SOD), [e]catalase (CAT), [f]ascorbate peroxidase (APX). Means followed by the same letter within each column do not differ statistically from each other by the Tukey test (P<0.05). Non-significant interaction (NS), significant interaction at P<0.05 (**) and significant interaction at P<0.01 (*).

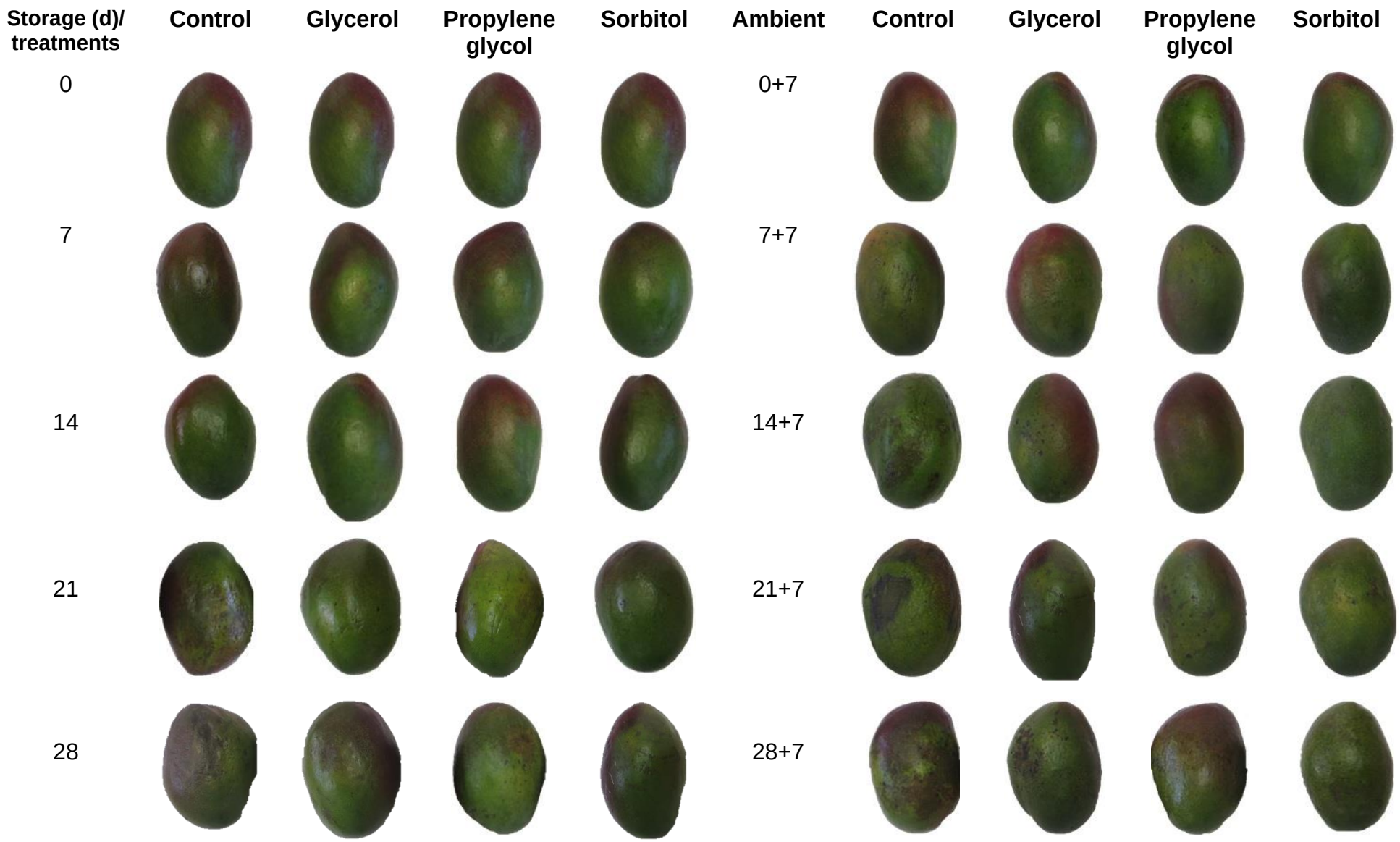


Figure 2S. Chilling injury symptoms in ‘Palmer’ mangoes treated with polyols and stored at 8.0 ± 2 ºC for up to 28 days and transfer to ambient (23 ± 2 ºC) for 7 more days.

CAPÍTULO 4 - Polyols alleviate chilling injury symptoms in 'Palmer' mangoes subjected to quarantine cold treatment[2]

Abstract

Treatment at low temperatures (1 °C for 14 d) can be used to quarantine mango fruit against fruit fly infestation. However, mangoes can develop chilling injury (CI) when stored at temperatures below 13 °C. We have demonstrated that the use of polyols (glycerol, propylene glycol, and sorbitol) can alleviate CI symptoms in 'Palmer' mangoes stored at 8 °C. These results suggest that these products can be used to reduce CI in mangoes during quarantine at low temperatures. Thus, we investigated the efficacy of applying 0.1 % (v/v) glycerol, propylene glycol, or sorbitol to 'Palmer' mangoes subjected to cold treatment (1.0 ºC) for 28 d at mitigating CI. Mangoes were then ripened at 23 ºC for 7 d. Among these polyols, sorbitol was most effective in alleviating CI symptoms during 14 d of cold storage, but could not protect the fruit for longer than 14 d. Sorbitol maintained oxidative metabolism, maintaining low levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), elevated levels of ascorbate (AsA), especially in the epicarp of the fruit, and increased superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) activities, resulting in reduced polyphenol oxidase (PPO) activity. These results suggest that 0.1 % sorbitol can be applied to mangoes during low temperature quarantines of up to 14 d to reduce CI and oxidative damage by improving antioxidant defense systems.

Keywords: *Mangifera indica* L., low temperature, glycerol, propylene glycol, sorbitol, fruit fly.

1. Introduction

Temperature is the most important factor governing the post-harvest maintenance of fruit and vegetable quality (Wills et al., 1998). During storage, low temperatures (0 – 10 °C) are used to reduce respiration rates, ethylene production,

[2]Este capítulo corresponde ao artigo científico submetido à revista Journal of Food Processing and Preservation e encontra-se em avaliação para publicação.

and nutrient degradation, and maintain texture, minimize membrane peroxidation, and inhibit the growth of microorganisms (Fan et al., 2019; Liu et al., 2020) and insect pests (Patil et al., 2019).

Low temperatures (1-2 °C for 12-15 d) have also been used to quarantine fruit to control fruit fly species, including *Ceratitis capitata*, *Bactrocera sp*., and *Anastrepha sp*. (USDA, 2002). However, cold treatments cannot be employed to their full potential with many tropical and subtropical fruit because of the susceptibility of these products to chilling injury (CI), especially when they are exposed to temperatures below 10-13 °C (Chaplin et al., 1991; Zhang et al., 2012).

Chilling injury (CI) is a physiological disorder associated with damage to cell membranes and increased oxidative reactions resulting from overproduction of reactive oxygen species (ROS) (Siboza et al., 2017). Cold stress decreases the ratio of unsaturated to saturated fatty acids in membranes, making them rigid and susceptible to lipid peroxidation by ROS. Under prolonged stress conditions, membrane damage becomes irreversible, and the fruit develops CI symptoms (Sevillano et al., 2009). In mangoes these symptoms manifest as epicarp discoloration (browning), development of sunken lesions, irregular ripening (pulp discoloration, reduced aromas and flavors), and increased susceptibility to decay (Zaharah & Singh, 2011; López-López et al., 2018).

Therefore, it is necessary to develop methods for minimizing CI symptoms when applying cold quarantines. Zhao et al. (2006) reported that CI symptoms were inhibited in ‘Wacheng’ mangoes when fruit were subjected to cold shock at 0 ºC for 4 h prior to quarantine at 2 ºC for 12 d. Patil et al. (2019) reported that application of ethylene (150 ppm for 24 hours), use of modified atmosphere (low density polyethylene plastic bags with 30 0.5 mm holes), and gradual temperature reduction (1 d at 12 °C with 75 % RH followed by 1 d at 5 °C with 87 % RH followed by 1 d at 2 °C with 92 % RH) minimized development of CI symptoms in both ‘Keitt’ and ‘Shelly’ mangoes subjected to quarantine treatment at 2 °C for 19 d. Recently, we showed that glycerol, propylene glycol, and sorbitol could be effectively used to alleviate CI symptoms and activate oxidative metabolism in ‘Palmer’ mangoes stored at 8.0 °C for 28 d (Sanches et al., 2021). Thus, these compounds could be used to prepare mangoes for cold quarantine.

Polyols are sugar alcohols formed by catalytic hydrogenation of carbohydrates. In addition to occurring naturally in fruit and vegetables, they are commonly added to various fresh and processed foods as sweeteners, humectants, and cryoprotectants (Lenhart and Chey, 2017), and are “generally recognized as safe” (GRAS) by the FDA (2016). Thus, the aim of this study was to investigate the effect of applying 0.1% (v/v) glycerol, propylene glycol and sorbitol on the incidence of IC, quality and oxidative metabolism of 'Palmer' mangoes stored at 1 ºC for 28 d, then matured at 23 ºC for 7 d.

2. Material and methods

2.1 Plant material

Palmer mangoes (*Mangifera indica* L.) were obtained harvested in the morning at a commercial orchard located in Cândido Rodrigues (21º 24' 23” South, 48º 30' 20” West, 579 m altitude), São Paulo, Brazil. The fruit were harvested at stage 3 based on the epicarp color chart proposed by Trindade et al. (2015). Fruit was selected by size, absence of mechanical and physiological damage, and absence of pests and diseases. Dry matter content was determined to be 15 ± 7 % in a batch of 20 fruit.

2.2 Polyol treatment

Mangoes were washed with neutral detergent (Ypê Clear, São Paulo, Brazil) and rinsed under running water before treatment with polyols. Mangoes were immersed in distilled water (control), or one of the following three solutions: 0.1 % (v/v) glycerol (Mendel, São Paulo, Brazil), propylene glycol (Mendel, São Paulo, Brazil), or sorbitol (Sigma-Aldrich, St. Louis, USA) all at 5 °C for 60 min. The 0.1 % concentration was chosen based on the Codex Alimentarius recommendation (2019) and because it did not differ from 2.5 % in alleviating CI in ‘Palmer’ mangoes (Sanches et al., 2021). Mangoes were then transferred to a cold room at 1.0 ± 0.5 °C and 75 ± 3.5 % RH for a period of 28 d. At 7-d intervals, fruit were transferred to ambient conditions (~23 ±

2.0 °C and 75 ± 3.0 % RH) to check for development of CI and assess quality parameters and oxidative metabolism.

The experiment was carried out according to a completely randomized design (CRD) with a factorial arrangement of 4 treatments: (control, glycerol, propylene glycol, and sorbitol) × 5 storage periods: (0, 7, 14, 21, and 28 d) with five replicate fruits for each condition at each time point, totaling 100 fruits. The same was done for fruit transferred to an ambient environment (n = 100 fruit).

2.3 Fruit quality evaluations

Fruit quality was assessed by determining the following parameters:

2.3.1 IC incidence: IC symptoms were recorded visually using the scale proposed by Miguel et al. (2011), with some modifications, as follows: 1 = no visible symptoms (CI = 0 %), 2 = mild symptoms (CI < 25 %), 3 = moderate symptoms (CI = 25–50 %), and 4 = severe symptoms (CI > 50 %).

2.3.2 Fresh weight loss (FWL): FWL was calculated based on change in fruit mass after different storage periods by weighing the mangoes on a semi-analytical balance with an accuracy of 0.01 g (Mars, model AS 2000, São Paulo, Brazil). The results are expressed as percentages (%).

2.3.3 Color: Epicarp (peel) color was determined using a colorimeter (CR-400, Minolta, Osaka, Japan) using parameters L*, a*, and b* proposed by the Commission Internationale de l' Éclairage (CIE). These values were transformed into chromaticity and hue angles, as described by McGuire (1992).

2.3.4 Firmness: Mesocarp (pulp) firmness was evaluated after removing two regions of epicarp on opposite sides of five replicate fruit, using a texturometer (Effegi Fruit Tester, Italy) equipped with an 8 mm tip. The results are expressed in Newtons (N) as described by Watkins & Harman (1981).

2.3.5 Physicochemical analysis: Soluble solid content (SSC) was quantitated using liquid samples obtained by pressing 10 g of pulp with a digital refractometer (Alpha, Atago Co., Ltd, Japan). Results were expressed as percentages (%). Titratable acidity (TA) was determined by titrating 10 g of pulp with 0.1 N NaOH, using 0.1 % phenolphthalein as an indicator. The results are expressed as gram equivalents of citric acid per kilogram of pulp (g kg-1). These ratios were calculated using the SSC/TA. Sample pHs were measured directly in fruit pulp using a pH meter (Thermo Scientific, Orion 3 Star, USA). AsA content was determined by titrating a 10 mL aliquot of extract with 2,6-dichlorophenolindophenol. Results are expressed as g kg^{-1} (AOAC, 2016).

2.4. Assessments of oxidative metabolism

Sample preparation: A total of 200 epicarp (peel) and 200 mesocarp (pulp) samples were frozen in liquid nitrogen and stored at -20 °C. Epicarp samples were removed using a potato peeler, and mesocarp samples (2 cm) were immediately cut with a sharp knife. Samples were frozen in liquid nitrogen, and ground using a ceramic mortar and pestle to obtain a fine powder for oxidative metabolism analysis. Analyzes were performed in triplicate for each repetition.

2.4.1 Lipid peroxidation (PL): Peel and pulp samples (0.5 g) were homogenized with 2.5 mL of 0.1 % (w/v) trichloroacetic acid (TCA) and 20 % polyvinylpyrrolidone (PVPP), and were centrifuged (Thermo Scientific, ST16-R, USA) at 12.298 x g for 15 min at 4 °C. Supernatants (250 µL) were removed and mixed with 1 mL of a solution containing 20 % TCA (w/v) and 0.5 % thiobarbituric acid (TBA) (w/v), followed by incubation in a water bath at 95 °C for 30 min. Tubes were then cooled in an ice bath for 10 min and centrifuged at 12.298 x g for 15 min at 4 °C. Absorbances were read using a spectrophotometer (Shimadzu, UV-1280, Japan) at 535 and 600 nm. Thiobarbituric acid reactive species (TBARS) were calculated using the molar extinction coefficient 155 mM cm^{-1}, and results were expressed as µmol of MDA kg^{-1} of fresh mass (Gratão et al., 2012).

2.4.2 Hydrogen peroxide (H_2O_2): Peel and pulp samples (1.0 g) were homogenized in 0.1 % trichloroacetic acid at 4 °C, and centrifuged at 12.298 x g for 20 min at 4 °C. Then, 200 µL of supernatant was mixed with 200 µL of 100 mM potassium phosphate buffer (pH 7.5) and 800 µL of 1 M potassium iodide (KI). Tubes were maintained in an ice bath for 1 h, protected from external light. H_2O_2 content was determined using a standard curve produced at 390 nm. Results are expressed as mol H_2O_2 kg^{-1} fresh mass (Alexieva et al., 2001).

2.5. Enzyme extraction and protein quantification

Enzymatic activity was determined in control fruit and in those treated with 0.1 % sorbitol, as this treatment most strongly alleviated CI and reduced oxidative stress (lipid peroxidation and H_2O_2 content) among the tested polyols. Superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), and ascorbate peroxidase (APX, EC 1.11.1.1) assays were performed as described by Yang et al. (2009), using 1.0 g of peel or pulp. These samples were macerated in 100 mM potassium phosphate buffer (pH 7.0) containing 1 mM ethylenediaminetetraacetic acid (EDTA) and 20 % polyvinylpolypyrrolidone (PVPP). Homogenates were filtered through a fine nylon mesh and centrifuged at 12.298 x g for 25 min. Supernatants were stored at -80 °C for later determination of SOD, CAT, APX, and PPO activities. Protein concentrations were determined by the Bradford method (1976) using bovine serum albumin as a standard.

2.5.1 Superoxide dismutase (SOD) assay: The activity of this enzyme was determined as described by Giannopolitis and Ries (1977). Reaction mixtures contained 50 µL of enzymatic extract, 1.0 mL of 50 mM sodium phosphate buffer (pH 7.8) containing 19.5 mM methionine, 150 µL of NBT, and 300 µL of riboflavin. After 15 min of reaction in a chamber under light, absorbance was measured at 560 nm using a spectrophotometer (Shimadzu, UV-1280, Japan). One unit of enzyme activity (U) was defined as the amount of enzyme required to cause a 50 % reduction in the NBT photoreduction rate. Results were expressed in U min^{-1} kg^{-1} 10^6 of protein (Beauchamp and Fridovich, 1971).

2.5.2 Catalase (CAT) assay: CAT activity was measured as described by Beers & Sizer (1952). Decrease in H_2O_2 was monitored and quantified using the molar extinction coefficient (36 mM-1.cm-1). Reactions contained 50 µL of enzymatic extract, 900 µL of 0.1 M potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA, and 50 µL H2O2 (0.5 M). Measurements were taken every 15 s at 240 nm spanning an interval of 1 min with the aid of a spectrophotometer (Shimadzu, UV-1280, Japan). Results are expressed in mol H_2O_2 min^{-1} kg^{-1} of protein (P).

2.5.3 Ascorbate peroxidase (APX) assay: APX activity was measured as described by Nakano and Asada (1981) by monitoring ascorbic acid oxidation at 290 nm. Reactions contained 50 µL of enzymatic extract, 800 µL of 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA, 100 µL of H2O2 (0.03 M), and 50 µL of ascorbic acid (0.015 M). Measurements were taken every 20 s for 1 min using a spectrophotometer (Shimadzu, UV-1280, Japan). Results are expressed in mol H_2O_2 min^{-1} kg^{-1} of protein (P) using the molar extinction coefficient of ascorbate (2.8 mM^{-1} cm^{-1}).

2.5.4 Polyphenoloxidase (PPO) assay: PPO containing extracts (EC 1.14.18.1) were obtained as described by Sojo et al. (1998). Peel and pulp (1.0 g) were homogenized with cold 50 mM potassium phosphate buffer (pH 7.0), containing 1 % polyvinylpyrrolidone (PVP), and centrifuged at 12.298 x g for 10 min at 4 °C. Supernatants were collected and used as enzyme extracts. PPO activity was quantitated as described by Wissemann and Lee (1980) using 100 µL of enzymatic extract and 1.85 mL of 100 mM potassium phosphate buffer (pH 6.0) containing 100 mM catechol as the reaction medium. After incubation in a water bath at 30 °C for 30 min, reactions were stopped by addition of 800 µL of 2 N perchloric acid. Absorbances were recorded at 395 nm, and the results are expressed as U min^{-1} kg^{-1} 10^6 of protein (P).

2.6 Statistical analysis

Data were subjected to analysis of variance (ANOVA) using R software (R Core Team, 2020), and means were compared using Tukey's test, with a significance threshold of 0.05 %.

3. Results

3.1 Chilling injury (CI)

During quarantine in the cold (1.0 ± 0.5 °C and 75 ± 3 % RH), an interaction between treatments and storage period was observed (Figure 1A and Figure 1S). Under these conditions, fruit treated with 0.1 % sorbitol displayed reduced CI symptoms until 14 d. Beyond this period, CI symptoms were similar to those of the other polyol treated groups and the control group (Figures 1A and 1S). Under ambient conditions (~23 ± 2.0 °C and 75 ± 3.0 % RH), mangoes treated with 0.1 % sorbitol displayed milder CI symptoms after 7 d (7 + 7); however, at 14 d, all treatments displayed the same CI incidence (Figures 1B and 1S).

3.2 Fruit quality

During quarantine under cold conditions, fruit treated with 0.1 % sorbitol underwent less fresh weight loss (4.1 %) up to d 16 compared to the other polyols (average of 6.4 %) (Figure 2). Firmness was not affected by polyol treatments, but varied during cold treatment, with a reduction of 11.2 % over 28 d (Table 1). A reduction in luminosity (L*) and an increase in hue angle (ºh) values were observed until d 21 of cold treatment. Fruit treated with 0.1 % sorbitol displayed higher luminosity (L*=45.53 ± 1.60) and hue angle (ºh =76.62 ± 2.01) compared with the control group and groups treated with the other polyols (Table 1). By contrast, chromaticity values (Chroma) did not vary between treatments (Table 1). During cold treatment, soluble solids content (SSC) and titratable acidity (TA) increased, and the SSC/TA ratio and pH values decreased (Table 1). SSC and SSC/TA values were higher in fruit treated with 0.1 % sorbitol, 9.09 ± 0.3 %, and 26.56 ± 2.01 %, respectively. Titratable acidity (TA) was 23.5 % lower in fruit treated with 0.1 % sorbitol and propylene glycol, compared to the 0.1 % glycerol and control groups. pH did not differ between treatments (Table 1).

During ripening under ambient conditions, loss of firmness was greater than 60 %. However, treatment with 0.1 % sorbitol (61.38 ± 2.65 N) and propylene glycol (59.73 ± 3.17 N) maintained fruit texture relative to that of control mangoes (52.27 ± 1.95 N), and mangoes treated with 0.1 % glycerol (50.06 ± 2.83 N) (Table 2). In the last transfer to ambient temperature (28 + 7 d), firmness was not assessed due to incidence of CI (Table 2 and Figure 1S).

Regarding color, L* values decreased by 53.7 %. Fruit treated with 0.1 % sorbitol or propylene glycol showed less variation in this parameter. Hue angle (ºh) remained higher in fruit treated with 0.1 % sorbitol (76.30 ± 2.34) compared to fruit treated with the other polyols (mean 69.62 ± 2.46), without a defined variation during storage (Table 2). Chromaticity did not vary between polyol treatments, and values decreased significantly after 14 and 7 d (Table 2).

During ripening, mangoes treated with 0.1 % sorbitol exhibited higher SSC (15.96 ± 0.41 %) (Figure 3) and SSC/TA (69.13 ± 3.02) than mangoes treated with the other polyols (Table 2). Lower TA levels and higher pH values were observed in fruit treated with 0.1 % sorbitol and propylene glycol (Table 2).

3.3 Oxidative metabolism

During quarantine in cold conditions, only hydrogen peroxide (H_2O_2) content was detected in the peel (Figure 4). Lipid peroxidation (LP) in the peel, H_2O_2 content in the pulp, and ascorbic acid (AsA) content in the peel and pulp were affected, either by polyol treatment or by cold treatment (Table 3). By contrast, LP in the pulp was not affected by the polyols, but was influenced by cold treatment (Table 3).

Lipid peroxidation (LP), expressed as malondialdehyde (MDA) content, increased in the peel (62.4 %) and pulp (59.7 %) during cold treatment. In the peel, an effect of polyol treatment was observed, especially in mangoes treated with 0.1 % sorbitol, whose MDA content was 12.3 % lower than those of the other groups (Table 3). Mangoes treated with 0.1 % sorbitol accumulated about 1.6 times more H_2O_2 in the peel than mangoes treated with the other polyols after 7 d of cold treatment. However, this content decreased after 14 d, and remained lower until d 21 (131.19 ± 3.66 mol H_2O_2 kg^{-1}) relative to the other polyol treated groups (mean 159.45 ± 3.44 mol H_2O_2

kg^{-1}) (Figure 4). H_2O_2 content in the pulp increased by 67.6 % during cold treatment, and was 11.3 % lower in fruit treated with 0.1 % sorbitol or propylene glycol relative to control and 0.1 % glycerol treated groups (Table 3).

Vitamin C (AsA) content increased in peel and pulp at d 7. Even with the reductions seen in subsequent evaluations, mango peels treated with 0.1 % sorbitol showed higher AsA content (0.29 ± 0.016 g kg^{-1}) than the other treatment groups (0.23 ± 0.023 g kg^{-1}). In addition, in the pulp of fruit treated with 0.1 % sorbitol or propylene glycol, AsA content was 21.9 % higher than that in control fruit or fruit treated with 0.1 % glycerol (Table 3).

During ripening under ambient conditions, an interactive effect of polyol addition and point of transfer to ambient conditions was observed for LP and H_2O_2 content in peel and pulp. AsA content in the peel was not influenced by treatments, but varied over the period spent in ambient temperature. Polyol treatments and the period spent at ambient temperature both influenced AsA content in the pulp (Table 4).

In the ambient, increases of 151.8 % and 332.4 % were observed for MDA and H_2O_2 content in the peel (21 + 7 d). However, treatment with 0.1 % sorbitol reduced accumulation of these compounds to 14.7 and 12.9 %, respectively, when compared to the other polyol treated groups (Table 4). In the pulp, MDA and H_2O_2 levels reached 167.8 % and 258.3 %, respectively, and treatment with 0.1 % sorbitol or propylene glycol resulted in 12.2 % and 15.1 % reduced MDA and H_2O_2, respectively (Table 4). After the transfer to ambient temperature at 21 + 7 d, AsA content decreased by 64 % in the peel, and 60 % in the pulp. Only the pulp of the fruit treated with polyols showed high levels of AsA (0.11 ± 0.015 g kg^{-1}), more noticeably in mangoes treated with 0.1 % sorbitol or propylene glycol than in the control or 0.1 % glycerol treated groups (Table 4).

3.4 Enzymatic activity

During quarantine cold treatment, an interaction was observed between superoxide dismutase (SOD) activity in the pulp (Figure 6A) and ascorbate peroxidase (APX) activity in peel and pulp (Figures 5A and 6B). In the peel, SOD and CAT activities varied between polyol treatments, and varied with length of cold treatment (Table 4).

No difference in CAT (pulp) or PPO (peel and pulp) activity was observed between groups treated with different polyols. However, activity levels of these enzymes did vary with length of cold exposure (Table 4).

SOD activity increased by 36.8 % (peel) and 28 % (pulp) by d 14. This increase was higher in the peel (11.3 %) and pulp (12.5 %) of fruit treated with 0.1 % sorbitol compared to control fruit (Table 5 and Figure 6A). In the peel, CAT activity was 12.4 % higher in fruit treated with 0.1 % sorbitol compared to control fruit (Table 5), whereas in the pulp, no difference was observed between these two treatments. CAT activity increased by 97.3 % (peel) and 90.3 % (pulp) over 14 d of cold exposure (Table 5), then tended to decrease after this period (Table 5). Treatment with 0.1 % sorbitol enhanced APX activity after 14 d of cold exposure (56.19 ± 3.34 and 49.38 ± 3.01 mol H_2O_2 min^{-1} kg^{-1}) relative to control fruit (49.11 ± 1.33 and 37.81 ± 2.45 mol H_2O_2 min^{-1} kg^{-1}) in peel and pulp (Figures 5A and 6B), respectively. PPO activity was lower (229.86 ± 6.18 10^6 U min^{-1} kg^{-1}) in fruit treated with 0.1 % sorbitol compared to that in control fruit (255 ± 5.83 10^6 U min^{-1} kg^{-1}). A significant increase of 77.7 % (peel) and 48.3 % (pulp) was observed after 28 d of cold exposure (Table 5).

At ambient temperature, an effect of polyol treatment on CAT activity was observed only in the peel (Figure 5B). In the pulp, an isolated correlation was observed between polyol treatment and length of cold exposure (Table 6). In the peel, SOD, APX, and PPO activities differed among polyol treatments, and with duration of cold exposure. However, duration of cold exposure only affected enzyme activity levels in the pulp (Table 6).

In general, SOD and APX activities were 28.1 % and 40.3 % higher in peels treated with 0.1 % sorbitol, respectively. During cold exposure, SOD and APX activities increased by 17.9 % and 20 % until the second transfer to ambient temperature (7 + 7 d), respectively. In the pulp, SOD increased by 13.6 % up to 7 + 7 d, while APX activity decreased during the transfer to ambient temperature (Table 6). CAT activity increased by 63.3 % (peel) and 78.9 % (pulp) over 14 + 7 d, then subsequently decreased (Table 6). Fruit treated with 0.1 % sorbitol showed greater CAT activity in the peel (26.4 %) and pulp (17.9 %) than that of the control fruit (Figure 5B and Table 6). PPO activity increased until the third transfer (14 + 7 d) to ambient temperature, independently of the evaluated tissue, but in the peel of fruit treated with 0.1 % sorbitol there was less

PPO activity (191.09 ± 4.45 10^6 U min^{-1} kg^{-1}) relative to the control (218.20 ± 5.71 10^6 U min^{-1} kg^{-1}), while in the pulp, no effect of treatment was observed (Table 6).

4. Discussion

4.1 Cold treatment and chilling injury (CI)

For cold treatment to effectively control fruit flies in mangoes, the fruit must remain at low temperatures (1-2 °C) for specific periods (12-15 d) to expose larvae, eggs, and pupae to these conditions long enough to eliminate these stages (Richardson, 1958). Unfortunately, when mangoes are exposed to temperatures below 10-13 °C, even for short periods (Zhang et al., 2012), they develop CI symptoms (Chaplin et al., 1991).

Therefore, when ‘Palmer’ mangoes were subjected to cold treatment at 1 °C, CI symptoms were observed after 14 d under these conditions. However, treatment with 0.1 % sorbitol minimized symptoms of this injury more effectively than the other polyols. These results are in agreement with Sanches et al. (2021), who reported that polyols such as glycerol, propylene glycol, and sorbitol were effective in alleviating CI symptoms in ‘Palmer’ mangoes stored at 8.0 °C for 28 d. Thus, 0.1 % sorbitol treatment can be used to better preserve mangoes subjected to quarantine cold treatment. It is worth mentioning that sorbitol protected mangoes; however, this protection was temporary, since CI symptoms intensified with prolonged exposure of the fruit to low temperature (1 °C).

As the efficacy of 0.1 % sorbitol treatment lasted for only 14 d, it could be applied together with other treatments, such as cold shock (Zhao et al., 2006), application of ethylene, or modified atmosphere packaging (Patil et al., 2019) in combination with other treatments.

4.2 Fruit quality

Alleviation of CI symptoms in fruit treated with 0.1 % sorbitol might also be related to less fresh weight loss. Bower et al. (2003) and Zaharah & Singh (2011)

reported a relationship between FWL and development of CI in mangoes. As sorbitol can bind to water and decrease the dielectric constant, even at temperatures above zero (Shirgire et al., 2012), the fruit treated with this polyol showed lower FWL.

Reductions in L* values are related to epicarp browning (Miguel et al., 2013), thus the higher L* values in fruit treated with 0.1 % sorbitol may be related to less severe CI symptoms. At ambient temperature, fruit treated with 0.1 % sorbitol and propylene glycol also showed higher L* values, indicating less severe CI symptoms. During cold exposure, increased CI incidence was observed, resulting in a reduction in L* values, especially after transfer to ambient temperature. Likewise, uneven maturation was observed, possibly due to the low quarantine temperature, which may have inhibited carotenoid synthesis (Medilicott et al., 1990; Rosalie et al., 2018). The higher ºh value observed in mangoes treated with 0.1 % sorbitol indicates that these fruit acquired an intense red color, indicating that the ripening process of these fruit was less affected than that of other treatment groups.

During ripening, mangoes catalyze starch hydrolysis (Mitcham & McDonald, 1992), with a consequent increase in SSC and a reduction in TA, resulting in an increased SSC/TA (Perumal et al., 2021). However, low SSC concentrations and increased TA were observed during cold treatment (1°C), which led to a low SSC/TA ratio. This ratio persisted after transfer to ambient conditions. Even though ripening was partially inhibited by low temperature (1°C), mangoes treated with 0.1 % sorbitol ripened normally until 14 + 7 d in ambient conditions compared to the other treatments, suggesting that this polyol aided in preserving mango quality even under extreme low temperature conditions.

4.3 Oxidative metabolism

In agreement with our observed effects on CI development, fruit treated with 0.1 % sorbitol displayed less lipid peroxidation (LP), which can be associated with reduced MDA, especially in the epicarp (peel) where CI symptoms are most evident. Thus, the improved tolerance to CI in mangoes treated with 0.1 % sorbitol may have resulted in less accumulation MDA and H_2O_2 content, and increased antioxidant enzyme activities, especially in the epicarp. This might have resulted in better membrane

integrity, since increased MDA content positively correlates with CI development in ‘Nam Dok Mai No. 4’ mango epicarp and mesocarp (Junmatong et al., 2012).

H_2O_2 is a primary environmental stress response signaling molecule (Apel & Hirt, 2004). This could explain the high H_2O_2 content (39.2 %) in the epicarp of fruit treated with 0.1 % sorbitol after 7 d of cold treatment. This concentration suggests that ROS signal the activation of low temperature (1 °C) protection and acclimatization mechanisms. Thus, both non-enzymatic systems (ascorbate-AsA) and enzymatic systems (SOD, CAT, and APX) acted to detoxify accumulating H_2O_2, decreasing the content of this molecule in subsequent periods of quarantine cold treatment correlating with low H_2O_2 accumulation in peel and pulp, and corroborating the reduced MDA content.

Mangoes treated with 0.1 % sorbitol had 24.9 % more AsA in their epicarp than the other polyol treated groups. Therefore, sorbitol might have been able to prevent H_2O_2 accumulation, as AsA plays an important role in controlling oxidative stress through detoxification of different ROS (Foyer & Noctor, 2011). In this respect, ‘Choke Anan’ and ‘Cogshall’ mangoes with higher AsA content have shown greater repression of oxidative stress (Khaliq et al., 2016; Rosalie et al., 2018). In addition, the 17.2 % increase in AsA in the epicarp of mangoes until d 7 may be related to a response to accumulating H_2O_2 which signals a response to the stress caused by low temperature (1 °C), since cool conditions induce overproduction of ROS (Hodges et al., 2004).

4.4 Enzymatic activity

Treatment with 0.1 % sorbitol also affected enzymatic defense systems, permitting greater antioxidant enzyme activity (SOD, CAT, and APX) during quarantine cold treatment (up to 14 d) and after transfer to ambient temperature (7 + 7 d) relative to the control. Increased SOD activity in fruit treated with 0.1 % sorbitol, especially under low temperature, may have contributed to increased H_2O_2 accumulation and reduced MDA content in the epicarp, because the superoxide anion (O_2^-) is the main ROS associated with membrane degradation (Shi et al., 2020). In addition, SOD plays a fundamental role in H_2O_2 biosynthesis by converting it to O_2^- (Mittler, 2002).

In turn, CAT and APX eliminate toxic H_2O_2 from the cell, with APX being dependent on AsA concentration (Mittler, 2002). Thus, greater CAT and APX activities at the beginning of the cold treatment (14 and 7 + 7 d) corresponded with reduced H_2O_2 accumulation in fruit treated with 0.1 % sorbitol, minimizing oxidative stress, and consequently minimizing CI symptoms. In addition, CAT more potently modulated H_2O_2 elimination responses in epicarp and mesocarp during cold treatment and ambient conditions than APX activity. This is explained by the specificity of CAT for H_2O_2, especially at high concentrations, and H_2O_2 is the main substrate for CAT activation (Halliwell, 2006). APX uses AsA as an electron donor to eliminate H_2O_2 (Foyer & Noctor, 2011); thus, AsA content was reduced when the fruit were transferred to ambient conditions.

Finally, the incidence of CI may correlate with oxidation of phenolic compounds present in plant tissues by the enzyme PPO, which converts them into dark colored quinones (Stewart et al., 2001). Thus, during cold treatment and after transfer to ambient conditions, increased PPO activity was associated with more severe CI symptoms and a reduction in L* values. These modifications are related to pericarp browning. However, reduced PPO activity in the epicarp of mangoes treated with 0.1 % sorbitol clearly indicates that the use of this polyol prevented the formation of quinones that caused browning, thereby reducing CI symptoms and maintaining L* of the fruit in relation to the control fruit. In addition, the improved membrane stability (MDA) in 0.1% sorbitol treated fruit restricted the interaction between the phenolic compounds and PPO, alleviating CI symptoms.

5. Conclusions

Polyol treatment alleviated development of chilling injury (IC) in ‘Palmer’ mangoes subjected to quarantine cold treatment (1 ºC). Immersion in solutions containing 0.1 % sorbitol was the most effective treatment, reducing CI symptoms for up to 14 d. However, cold treatment should be reduced to 7 d, as CI symptoms were observed after 7 d when fruit were transferred to ambient temperature (23 ºC).

CI alleviation by 0.1 % sorbitol correlated with reduced fresh weight loss, better preservation of membrane integrity (H_2O_2 and MDA), and activation of non-enzymatic and enzymatic antioxidant defense systems.

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Figures

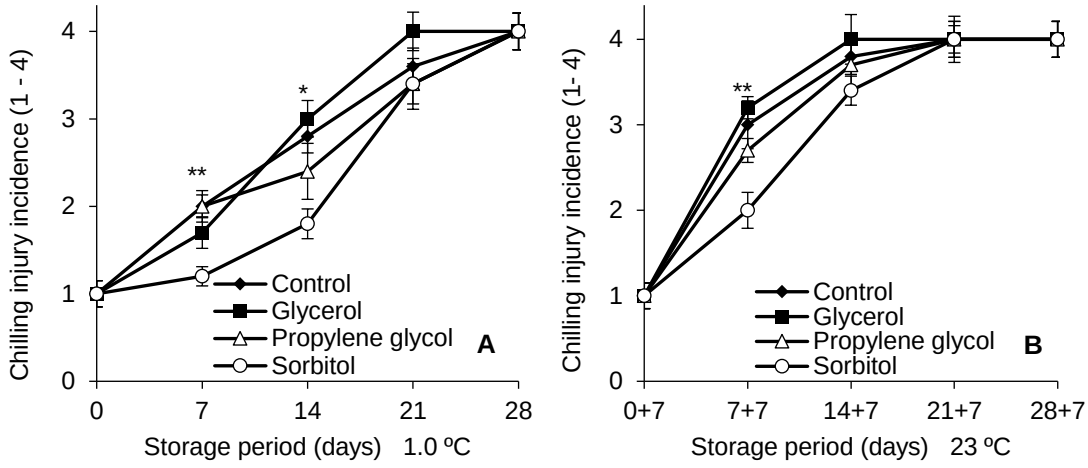

Figure 1. Interaction between polyols (0.1 %) and cold treatment period (1.0 ± 0.5 ºC) for 28 d (A) on chilling injury (CI) incidence in 'Palmer' mangoes followed by transfer to ambient (23 ± 2 ºC) for 7 d (B). Significant difference at P<0.05 (**) and P<0.01 (*) for treatments within each storage period. The bars represent the standard deviation of 5 repetitions.

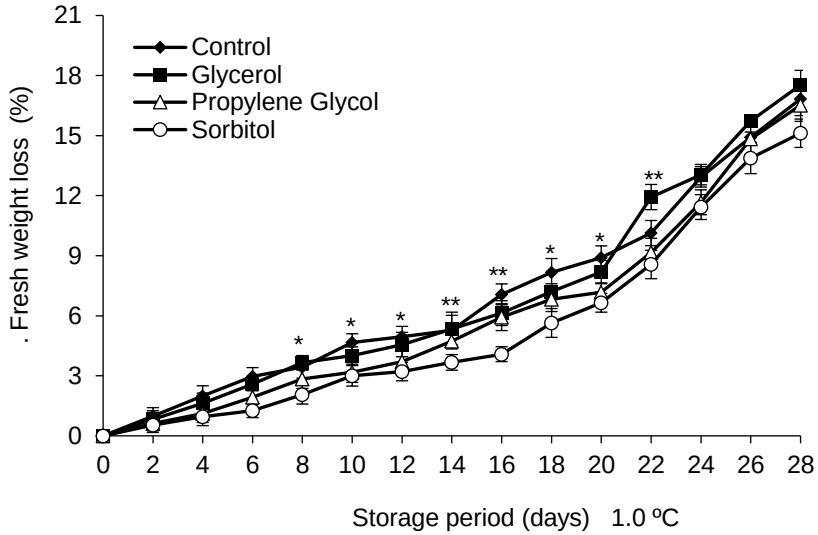

Figure 2. Fresh weight loss (%) of 'Palmer' treated with polyols (0.1 %) and subjected to quarantine cold treatment (1.0 ± 0.5 ºC) for 28 d (A). Significant difference at P<0.05 (**) and P<0.01 (*) for treatments within each storage period. The bars represent the standard deviation of 5 repetitions.

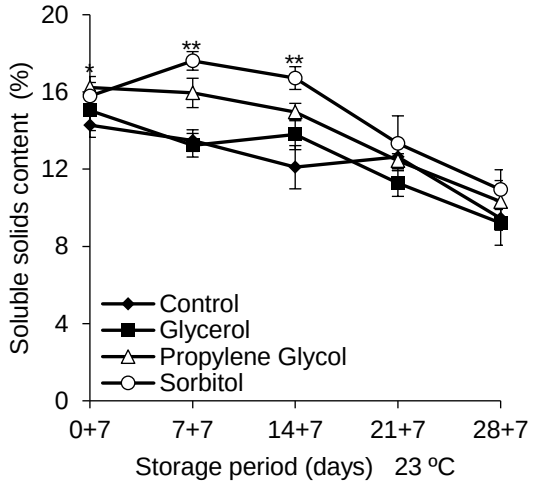

Figure 3. Interaction between polyols (0.1 %) and cold treatment period (1.0 ± 0.5 ºC) for 28 d on soluble solids content (SSC - %) in 'Palmer' mangoes followed by transfer to ambient (23 ± 2 ºC) for 7 d. Significant difference at P<0.05 (**) and P<0.01 (*) for treatments within each storage period. The bars represent the standard deviation of 5 repetitions.

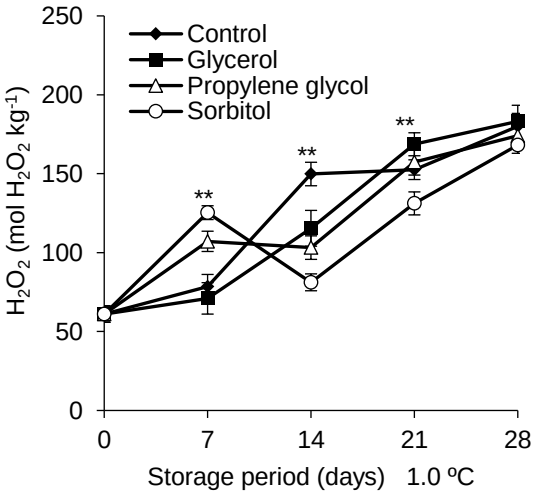

Figure 4. Interaction between polyols (0.1 %) and cold treatment period (1.0 ± 0.5 ºC) for 28 d on hydrogen peroxide (H_2O_2) in 'Palmer' mangoes epicarp (peel). Significant difference at P<0.05 (**) and P<0.01 (*) for treatments within each storage period. The bars represent the standard deviation of 5 repetitions.

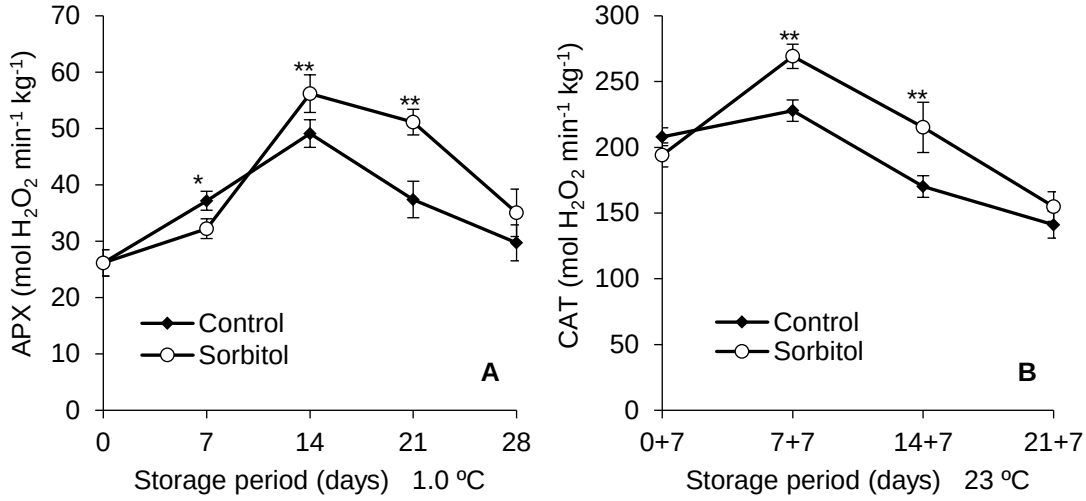

Figure 5. Interaction between polyols (0.1 %) and cold treatment period (1.0 ± 0.5 ºC) for 28 d on the activity of ascorbate peroxidase (A) and catalase (B) in 'Palmer' mangoes epicarp (peel) followed by transfer to ambient (23 ± 2 ºC) for 7 d. Significant difference at P<0.05 (**) and P<0.01 (*) for treatments within each storage period. The bars represent the standard deviation of 5 repetitions.

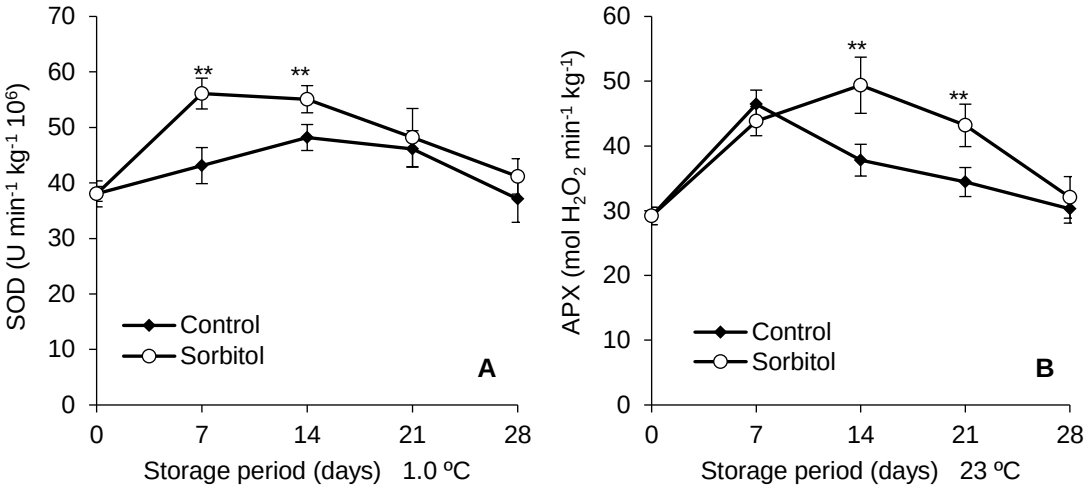

Figure 6. Interaction between polyols (0.1 %) and cold treatment period (1.0 ± 0.5 ºC) for 28 d on the activity of superoxide dismutase (A) and ascorbate peroxidase (B) in 'Palmer' mangoes mesocarp (pulp) followed by transfer to ambient (23 ± 2 ºC) for 7 d. Significant difference at P<0.05 (**) and P<0.01 (*) for treatments within each storage period. The bars represent the standard deviation of 5 repetitions.

Table 1. Effect of solutions containing 0.1 % of glycerol, propylene glycol, and sorbitol on firmness, color parameters (luminosity – L*, hue angle, and chromaticity, soluble solids content (SSC), titratable acidity (TA), ratio (SSC/TA), and pH of 'Palmer' mangoes submitted to cold treatment (1.0 ± 0.5 ºC) for up to 28 d.

Principal effects	Firmness (N)	Color			SSC (%)	TA (g kg^{-1})	SSC/TA	pH
		Luminosity	Hue angle	Chromaticity				
Treatments (A)								
Control	124.10 ± 1.46 a	40.68 ± 1.07 b	68.56 ± 2.21 c	24.03 ± 1.82 a	7.61 ± 0.44 b	0.0044 ± 0.0002 b	19.23 ± 3.81 b	3.29 ± 0.31 a
Glycerol	123.95 ± 2.04 a	39.16 ± 1.12 b	69.03 ± 1.94 c	26.31 ± 2.03 a	7.82 ± 0.31 b	0.0041 ± 0.0004 b	20.67 ± 2.64 b	3.17 ± 0.51 a
Propylene Glycol	124.21 ± 1.63 a	41.94 ± 1.87 b	73.07 ± 1.43 b	24.94 ± 1.16 a	8.11 ± 0.52 b	0.0034 ± 0.0002 a	23.08 ± 3.15 b	3.61 ± 0.36 a
Sorbitol	125.40 ± 2.11 a	45.53 ± 1.60 a	76.62 ± 2.01 a	25.85 ± 1.87 a	9.09 ± 0.27 a	0.0031 ± 0.0003 a	26.56 ± 2.01 a	3.72 ± 0.57 a
Storage – days (B)								
0	127.48 ± 0.13 a	51.97 ± 1.21 a	65.45 ± 3.11 c	23.34 ± 1.68 b	7.47 ± 0.43 c	0.0017 ± 0.0003 d	38.09 ± 2.33 a	4.15 ± 0.16 a
7	127.40 ± 0.13 a	43.13 ± 2.06 b	73.16 ± 2.33 b	26.99 ± 2.47 a	8.11 ± 0.41 b	0.0034 ± 0.0002 c	22.86 ± 2.19 b	3.97 ± 0.12 b
14	127.13 ± 1.12 a	39.73 ± 2.18 c	84.27 ± 3.01 a	28.26 ± 2.18 a	8.50 ± 0.37 b	0.0047 ± 0.0002 a	18.47 ± 1.95 b	3.69 ± 0.15 c
21	126.01 ± 1.64 a	41.85 ± 3.62 bc	85.49 ± 2.55 a	28.81 ± 3.02 a	9.31 ± 0.39 a	0.0051 ± 0.0004 a	19.56 ± 2.06 b	3.74 ± 0.19 c
28	113.23 ± 2.02 b	37.20 ± 2.73 c	62.93 ± 3.16 c	24.91 ± 2.56 b	9.23 ± 0.61 a	0.0042 ± 0.0003 b	20.74 ± 3.03 b	3.62 ± 0.21 c
Interaction								
AxB	NS	NS	NS	NS	NS	NS	NS	NS

Means followed by the same letter within each column do not differ statistically from each other by the Tukey test (P<0.05). Non-significant interaction (NS), significant interaction at P<0.05 (**) and significant interaction at P<0.01 (*).

Table 2. Effect of solutions containing 0.1 % of glycerol, propylene glycol, and sorbitol on firmness, color parameters (luminosity – L*, hue angle, and chromaticity, soluble solids content (SSC), titratable acidity (TA), ratio (SSC/TA), and pH of 'Palmer' mangoes submitted to cold treatment (1.0 ± 0.5 ºC) for up to 28 d followed by transfer to ambient (23 ± 2 ºC) for 7 d.

Principal effects	Firmness (N)	Color			SSC (%)	TA (g kg^{-1})	SSC/TA	pH
		Luminosity	Hue angle	Chromaticity				
Treatments (A)								
Control	52.27 ± 1.95 b	36.78 ± 1.21 b	69.75 ± 2.06 b	21.05 ± 1.90 a	11.39 ± 1.21 c	0.0029 ± 0.0002 a	52.74 ± 2.36 c	4.21 ± 0.16 b
Glycerol	50.06 ± 2.83 b	36.41 ± 1.08 b	68.43 ± 3.14 b	20.84 ± 2.17 a	11.42 ± 1.06 c	0.0031 ± 0.0005 a	50.21 ± 3.18 c	4.18 ± 0.20 b
Propylene Glycol	59.73 ± 3.17 a	39.23 ± 2.32 a	70.69 ± 2.19 b	21.97 ± 3.01 a	12.54 ± 0.91 b	0.0023 ± 0.0003 b	64.19 ± 3.11 b	4.42 ± 0.19 a
Sorbitol	61.38 ± 2.65 a	41.70 ± 1.74 a	76.30 ± 2.34 a	23.51 ± 2.72 a	14.18 ± 0.93 a	0.0021 ± 0.0002 b	69.13 ± 3.02 a	4.45 ± 0.23 a
Storage – days (B)								
0+7	99.46 ± 2.21 a	46.13 ± 1.87 a	69.47 ± 5.12 c	28.43 ± 2.05 a	13.54 ± 1.36 b	0.0021 ± 0.0002 c	74.56 ± 3.18 b	4.38 ± 0.25 a
7+7	75.82 ± 1.89 b	42.07 ± 2.18 b	75.76 ± 1.13 b	30.97 ± 1.72 a	16.81 ± 0.92 a	0.0019 ± 0.0003 d	85.03 ± 4.01 a	4.46 ± 0.21 a
14+7	61.47 ± 2.04 c	40.43 ± 1.94 b	79.36 ± 2.01 a	31.16 ± 2.94 a	15.70 ± 1.14 b	0.0023 ± 0.0002 c	71.47 ± 5.12 c	4.20 ± 0.27 a
21+7	36.12 ± 2.11 d	33.10 ± 2.32 c	72.48 ± 4.08 b	17.32 ± 2.12 b	11.84 ± 1.72 c	0.0029 ± 0.0003 b	49.18 ± 4.11 d	3.71 ± 0.19 b
28+7	WE[a]	21.35 ± 2.18 d	51.27 ± 6.14 d	11.75 ± 2.91 c	9.13 ± 1.26 d	0.0034 ± 0.0002 a	23.19 ± 4.29 e	3.63 ± 0.26 b
Interaction								
AxB	NS	NS	NS	NS	4.83**	NS	NS	NS

Means followed by the same letter within each column do not differ statistically from each other by the Tukey test (P<0.05). [a]WE = without evaluation, Non-significant interaction (NS), significant interaction at P<0.05 (**) and significant interaction at P<0.01 (*).

Table 3. Effect of solutions containing 0.1 % of glycerol, propylene glycol, and sorbitol on lipid peroxidation (LP), hydrogen peroxide (H_2O_2), ascorbic acid (AsA) of 'Palmer' mangoes epicarp (peel) and mesocarp (pulp) submitted to cold treatment (1.0 ± 0.5 ºC) for up to 28 d.

Principal effects	LP – peel (µmol MDA kg^{-1})	LP – pulp (µmol MDA kg^{-1})	H_2O_2 – peel (mol H_2O_2 kg^{-1})	H_2O_2 – pulp (mol H_2O_2 kg^{-1})	AsA - peel (g kg^{-1})	AsA - pulp (g kg^{-1})
Treatments (A)						
Control	2.18 ± 0.13 a	1.24 ± 0.13 a	90.61 ± 4.37 a	61.80 ± 3.11 a	0.23 ± 0.017 c	0.13 ± 0.019 b
Glycerol	2.33 ± 0.15 a	1.29 ± 0.14 a	94.04 ± 4.21 a	63.01 ± 2.98 a	0.21 ± 0.028 c	0.11 ± 0.014 b
Propylene glycol	2.09 ± 0.07 b	1.21 ± 0.18 a	77.01 ± 3.63 b	56.62 ± 2.74 b	0.26 ± 0.026 b	0.16 ± 0.019 a
Sorbitol	1.93 ± 0.09 c	1.15 ± 0.20 a	69.13 ± 4.05 c	54.09 ± 3.50 b	0.29 ± 0.016 a	0.16 ± 0.017 a
Storage – days (B)						
0	1.16 ± 0.23 d	0.81 ± 0.19 c	65.05 ± 5.07 d	34.12 ± 5.16 d	0.34 ± 0.022 b	0.18 ± 0.017 a
7	1.29 ± 0.21 d	0.76 ± 0.21 c	59.34 ± 8.19 d	30.04 ± 3.08 d	0.40 ± 0.021 a	0.21 ± 0.023 a
14	2.01 ± 0.19 c	0.97 ± 0.26 c	81.82 ± 4.38 c	59.17 ± 3.65 c	0.32 ± 0.014 b	0.19 ± 0.018 a
21	2.61 ± 0.12 b	1.49 ± 0.18 b	134.43 ± 6.92 b	71.03 ± 4.00 b	0.18 ± 0.016 c	0.15 ± 0.015 b
28	3.09 ± 0.11 a	2.01 ± 0.19 a	196.01 ± 5.81 a	105.45 ± 3.12 a	0.13 ± 0.020 d	0.10 ± 0.018 c
Interactions						
AxB	NS	NS	3.09**	NS	NS	NS

Means followed by the same letter within each column do not differ statistically from each other by the Tukey test (P<0.05). Non-significant interaction (NS), significant interaction at P<0.05 (**) and significant interaction at P <0.01 (*).

Table 4. Effect of solutions containing 0.1 % of glycerol, propylene glycol, and sorbitol on lipid peroxidation (LP), hydrogen peroxide (H_2O_2), ascorbic acid (AsA) of 'Palmer' mangoes epicarp (peel) and mesocarp (pulp) submitted to cold treatment (1.0 ± 0.5 ºC) for up to 28 d followed by transfer to ambient (23 ± 2 ºC) for 7 d.

Principal effects	LP – peel (µmol MDA kg^{-1})	LP – pulp (µmol MDA kg^{-1})	H_2O_2 – peel (mol H_2O_2 kg^{-1})	H_2O_2 – pulp (mol H_2O_2 kg^{-1})	AsA - peel (g kg^{-1})	AsA - pulp (g kg^{-1})
Treatments (A)						
Control	3.47 ± 0.11 a	1.93 ± 0.13 a	179.81 ± 7.16 a	70.03 ± 4.18 a	0.18 ± 0.017 a	0.08 ± 0.014 b
Glycerol	3.51 ± 0.09 a	2.01 ± 0.16 a	184.20 ± 4.34 a	73.81 ± 2.77 a	0.16 ± 0.021 a	0.07 ± 0.018 b
Propylene glycol	3.43 ± 0.10 a	1.79 ± 0.14 b	158.32 ± 5.11 b	63.11 ± 5.09 b	0.19 ± 0.018 a	0.11 ± 0.016 a
Sorbitol	3.02 ± 0.13 b	1.67 ± 0.17 b	146.03 ± 4.52 c	59.50 ± 3.56 b	0.20 ± 0.016 a	0.12 ± 0.017 a
Storage – days (B)						
0+7	1.95 ± 0.16 e	1.18 ± 0.21 c	79.34 ± 9.34 d	32.67 ± 3.17 d	0.30 ± 0.025 a	0.14 ± 0.016 a
7+7	2.16 ± 0.15 d	1.32 ± 0.18 c	93.03 ± 11.05 d	41.17 ± 5.06 c	0.26 ± 0.017 a	0.13 ± 0.011 a
14+7	3.32 ± 0.22 c	2.09 ± 0.29 b	169.71 ± 9.87 c	89.30 ± 3.18 b	0.21 ± 0.018 b	0.09 ± 0.016 b
21+7	4.91 ± 0.43 b	3.16 ± 0.32 a	343.07 ± 11.53 a	117.06 ± 4.94 a	0.10 ± 0.021 c	0.05 ± 0.019 c
28+7	WE[a]	WE[a]	WE[a]	WE[a]	WE[a]	WE[a]
Interactions						
AxB	NS	NS	NS	NS	NS	NS

[a]WE = without evaluation. Means followed by the same letter within each column do not differ statistically from each other by the Tukey test (P<0.05). Non-significant interaction (NS), significant interaction at P<0.05 (**) and significant interaction at P <0.01 (*).

Table 5. Effect of solutions containing 0.1 % sorbitol on superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and polyphenol oxidase (PPO) of 'Palmer' mangoes epicarp (peel) and mesocarp (pulp) submitted to cold treatment (1.0 ± 0.5 ºC) for up to 28 d.

Principal effects	**SOD- peel (U min^{-1} kg^{-1} 10^6)**	**SOD – pulp (U min^{-1} kg^{-1} 10^6)**	**CAT – peel (mol H_2O_2 min^{-1} kg^{-1})**	**CAT – pulp (mol H_2O_2 min^{-1} kg^{-1})**	**APX – peel (mol H_2O_2 min^{-1} kg^{-1})**	**APX – pulp (mol H_2O_2 min^{-1} kg^{-1})**	**PPO – peel (U min^{-1} kg^{-1} 10^6)**	**PPO – pulp (U min^{-1} kg^{-1} 10^6)**
Treatments (A)								
Control	59.02 ± 1.19 b	36.07 ± 1.85 b	175.01 ± 7.21 b	109.62 ± 3.88 a	30.06 ± 3.03 a	24.03 ± 1.84 b	255.74 ± 5.83 a	100.14 ± 3.01 a
Sorbitol	68.77 ± 2.11 a	41.81 ± 2.01 a	200.12 ± 8.04 a	113.17 ± 5.51 a	39.83 ± 2.11 b	34.98 ± 2.11 a	229.86 ± 6.18 b	95.18 ± 6.27 a
Storage – days (B)								
0	61.19 ± 1.08 c	38.05 ± 3.18 c	103.06 ± 6.81 d	55.21 ± 5.98 c	26.17 ± 3.44 c	31.03 ± 1.18 c	83.19 ± 8.21 d	67.12 ± 3.13 cd
7	72.19 ± 3.17 b	45.10 ± 2.74 b	142.19 ± 8.13 c	78.70 ± 7.36 b	42.28 ± 2.97 b	39.29 ± 2.76 b	101.19 ± 15.17 d	62.90 ± 4.67 d
14	96.13 ± 2.01 a	52.87 ± 2.96 a	203.35 ± 6.10 a	105.09 ± 6.12 a	53.13 ± 3.34 a	48.27 ± 1.25 a	243.72 ± 13.31 c	71.56 ± 6.10 c
21	71.18 ± 3.78 b	49.29 ± 1.19 a	179.80 ± 7.63 b	90.27 ± 5.02 a	37.19 ± 4.19 b	41.72 ± 1.94 b	306.17 ± 8.63 b	102.36 ± 5.18 b
28	63.20 ± 2.10 c	41.73 ± 2.07 c	137.92 ± 9.06 c	69.83 ± 8.03 b	29.00 ± 5.06 c	32.03 ± 3.06 c	375.03 ± 9.19 a	129.74 ± 4.06 a
Interactions								
AxB	NS	3.81**	NS	NS	5.27**	1.19*	NS	NS

Means followed by the same letter within each column do not differ statistically from each other by the Tukey test (P<0.05). Non-significant interaction (NS), significant interaction at P<0.05 (**) and significant interaction at P<0.01 (*).

Table 6. Effect of solutions containing 0.1 % sorbitol on superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and polyphenol oxidase (PPO) of 'Palmer' mangoes epicarp (peel) and mesocarp (pulp) submitted to cold treatment (1.0 ± 0.5 ºC) for up to 28 d followed by transfer to ambient (23 ± 2 ºC) for 7 d.

Principal effects	SOD- peel (U min^{-1} kg^{-1} 10^6)	SOD – pulp (U min^{-1} kg^{-1} 10^6)	CAT – peel (mol H_2O_2 min^{-1} kg^{-1})	CAT – pulp (mol H_2O_2 min^{-1} kg^{-1})	APX – peel (mol H_2O_2 min^{-1} kg^{-1})	APX – pulp (mol H_2O_2 min^{-1} kg^{-1})	PPO – peel (U min^{-1} kg^{-1} 10^6)	PPO – pulp (U min^{-1} kg^{-1} 10^6)
Treatments (A)								
Control	30.14 ± 2.17 b	21.74 ± 2.38 a	137.12 ± 11.36 b	121.62 ± 5.18 b	19.39 ± 2.84 b	24.91 ± 3.55 a	218.20 ± 5.71 a	147.72 ± 6.90 a
Sorbitol	38.60 ± 1.05 a	23.20 ± 1.17 a	159.20 ± 9.64 a	143.41 ± 6.70 a	27.20 ± 1.76 a	26.72 ± 4.10 a	191.09 ± 4.45 b	142.66 ± 4.06 a
Storage – days (B)								
0+7	44.12 ± 0.95 b	29.12 ± 1.18 b	128.05 ± 7.19 c	89.34 ± 3.11 d	31.18 ± 2.11 b	41.13 ± 5.85 a	105.10 ± 6.21 d	86.81 ± 5.10 d
7+7	52.01 ± 1.11 a	33.07 ± 2.94 a	137.92 ± 9.13 c	106.27 ± 4.85 c	37.43 ± 3.28 a	33.71 ± 3.74 b	178.47 ± 9.93 c	121.72 ± 7.36 c
14+7	46.74 ± 2.17 b	27.51 ± 2.53 b	209.18 ± 6.18 a	159.83 ± 9.92 a	33.65 ± 4.74 a	29.54 ± 5.19 bc	341.95 ± 11.18 a	218.39 ± 4.07 a
21+7	31.82 ± 1.85 c	19.05 ± 2.10 c	164.11 ± 6.77 b	121.01 ± 7.19 b	21.94 ± 1.81 c	19.18 ± 9.94 c	266.03 ± 7.02 b	182.45 ± 5.91 b
28+7	WE[a]	WE[a]	WE[a]	WE[a]	WE[a]	WE[a]	WE[a]	WE[a]
Interactions								
AxB	NS	NS	4.02**	NS	NS	NS	NS	NS

[a]WE = without evaluation. Means followed by the same letter within each column do not differ statistically from each other by the Tukey test (P<0.05). Non-significant interaction (NS), significant interaction at P<0.05 (**) and significant interaction at P<0.01 (*).

Supplementary material

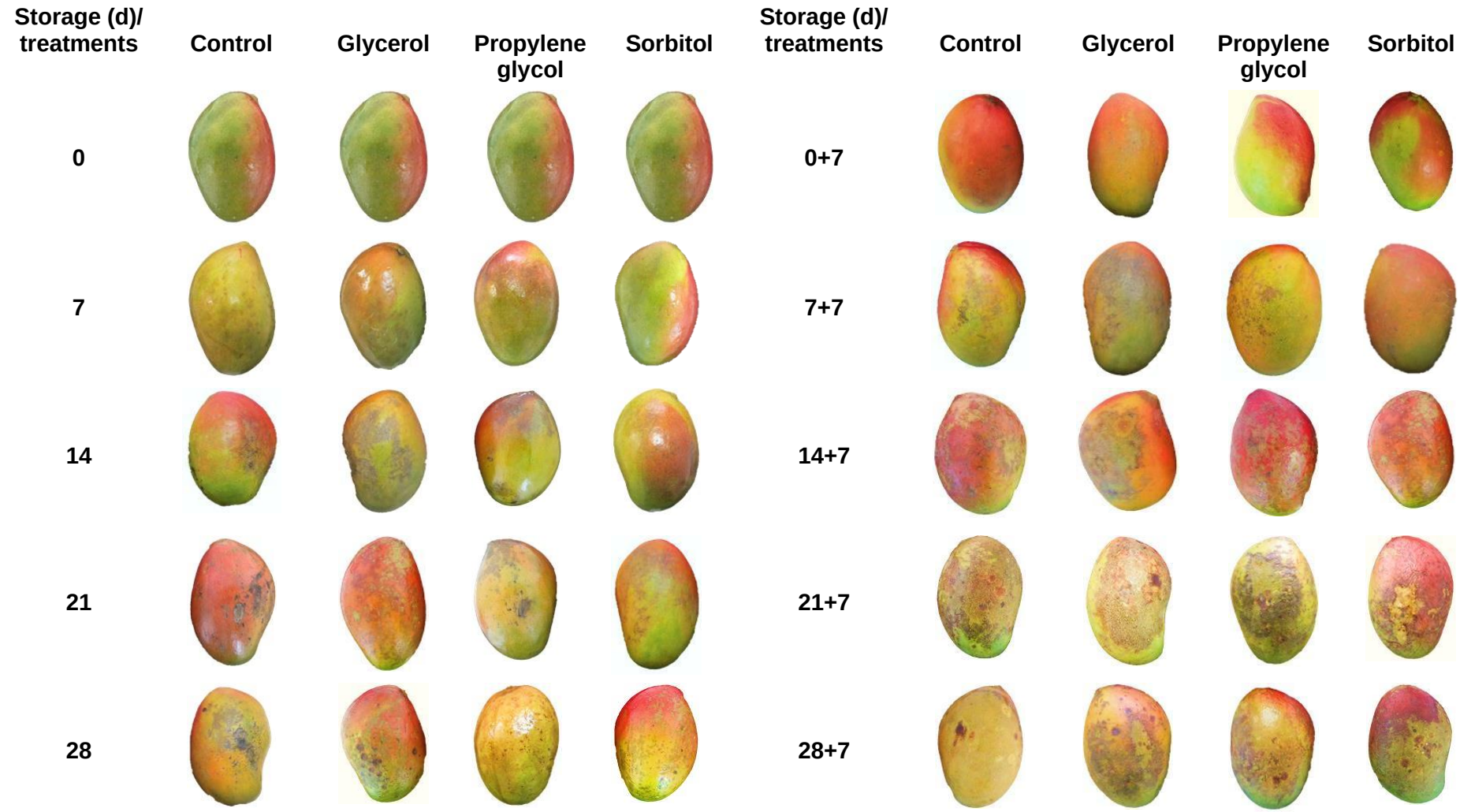

Figure 1S. Development of chilling injury (CI) in 'Palmer' mangoes treated with polyols (0.1 %) and submitted to cold treatment (1.0 ± 0.5 ºC) for up to 28 d followed by transfer to ambient (23 ± 2 ºC) for 7 d.

CAPÍTULO 5 - Sorbitol immersion controls chilling injury in CA stored ‘Palmer’ mangoes[3]

Abstract

The objective of this study was to evaluate the effectiveness of ‘Palmer’ mangoes were immersed in 0.1 and 2.5 % (w/v) sorbitol solutions and stored under CA 5 % O_2 + 5 % CO_2 at 8 ºC for 30 d. The evaluations were performed 0, 10, 20, and 30 d, after which the fruit were transferred to ambient temperature environment ~23 °C for 7 days. CI was in mangoes treated with 2.5 % sorbitol and stored in CA. The soluble solids content, titratable acidity, SSC/TA ratio, and pH, firmness, and mesocarp color (L*, hº, and C*) were not affected. CI development was associated with increased fresh weight loss and epicarp color (L*, hº, and C*). Hydrogen peroxide (H_2O_2) levels were related to CI symptom development, which intensified with the transfer to an ambient temperature, mainly due to increased electrolyte leakage (EL), lipid peroxidation (LP), and polyphenol oxidase (PPO) activity. CI inhibition in mangoes treated with 2.5 % sorbitol under a CA was related to the non-enzymatic (vitamin C and total polyphenols) and enzymatic (superoxide dismutase – SOD, catalase – CAT, and ascorbate peroxidase – APX) defense metabolisms, allowing for the quality of the fruit to be maintained for up to 30 d at 8 °C.

Keywords: *Mangifera indica* L.*,* enzymatic metabolism, non-enzymatic metabolism, principal component analysis, physiological disorder.

1. Introduction

Controlled atmosphere (CA) storage is used in association with low temperatures to extend the shelf life of many horticultural products (Kader, 2003). According to Saltveit (2020), atmospheres with low oxygen (O_2) reduce respiration,

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[3]Sanches AG, Silva MB, Wong MCC, Oliveira ARG, Pedrosa VMD, Fernandes TFS, Gratão PL, Teixeira GHA (2022) Sorbitol immersion controls chilling injury in CA stored ‘Palmer’ mangoes. **Postharvest Biology and Technology**, 185, 111800. https://doi.org/10.1016/j.postharvbio.2021.111800

and inhibit the production and action of ethylene, while high levels of carbon dioxide (CO_2) also reduce ethylene action and respiration (Yahia et al., 2019).

Mangoes can be stored in atmospheres containing 3–5 % O_2 and 5–8 % CO_2 at 13 °C to extend their shelf life to 21 to 42 d, depending on the cultivars and maturity stage (Brecht, 2020); however, lower levels of oxygen (1.0 % O_2) initiate the production of off-flavors (Nakasone and Paull, 1998; Ntsoane et al., 2019). In addition, 'Kensington Pride' mangoes stored in atmospheres containing 6.0 % O_2 for 35 d showed reduced production of the typical volatile compounds (Lalel et al., 2003). Elevated levels of CO_2 (> 25 %) can also affect the quality of mangoes, such as by increasing ethanol production in 'Tommy Atkins' mangoes stored in atmospheres with 50 % CO_2 (Bender and Brecht, 2000).

As tropical fruits, mangoes must be stored at temperatures above 13 °C (Chaplin et al., 1991), which limits the efficiency of a CA, because lower temperatures are required to extend the shelf life. However, CA conditions could reduce in chilling injury (CI) development, which is characterized by epicarp discoloration, sunken dark lesions (Wang et al., 2008), irregular ripening (Zhao et al., 2009), and other damage (Nair et al., 2003; Zaharah and Singh, 2011; Sivankalyani et al., 2017). O'Hare and Prasad (1992) reported that a CA with 5–10% CO_2 alleviated the symptoms of CI in 'Kensington Pride' mangoes stored below 10 °C. Similarly, Pesis et al. (2000) reported a lower incidence of CI in 'Tommy Atkins' and 'Keitt' mangoes stored at 12 °C under a CA with 5 % O_2 and 10 % CO_2 for 3 weeks.

It has been recently demonstrated that immersion of 'Palmer' mangoes in sorbitol (0.1 and 2.5%) solutions alleviated the development of CI when stored at 8 °C for 28 d, particularly through the activation of the antioxidant metabolism (Sanches et al., 2021; Sanches et al., in press). Sorbitol (D-glucitol) is a carbohydrate known as sugar alcohol or polyol, that is naturally synthesized in fruit and it is broadly used by the food industry as a sweetening, wetting, and texturizing agent (Lee, 2015) to improve quality and extend shelf-life (Liu et al., 2014; Sorapukdee et al., 2016; Fahrizal et al., 2018). The Joint Expert Committee on Food Additives (JECFA) categorized the allowable daily intake of sorbitol as "unspecified," which is the safest category for any food ingredient (Grembecka, 2018).

Therefore, the objective of this study was to evaluate the effectiveness of combining sorbitol immersion (0.1 and 2.5 %) with CA storage (5 kPa O_2 + 5 kPa CO_2) to control the development of CI in ‘Palmer’ mangoes stored at 8 ºC for 30 d.

2. Material and methods

2.1 Plant material

Palmer mangoes (*Mangifera indica* L.) were obtained from a commercial orchard located in Cândido Rodrigues (21º24'23" S, 48º30'20" W; 579 m altitude), São Paulo, Brazil. The fruits were harvested at physiological maturity, stage 3, based on the epicarp color scale proposed by Trindade et al. (2015), and sorted based on size, presence of mechanical injury, and pest and disease lesions. A batch of 20 fruits was used to determine the dry matter (DM) content (15 ± 7 %).

2.2 Sorbitol treatment and controlled atmosphere (CA) storage

Mangoes were washed with neutral soap (Ypê Clear, São Paulo, Brazil) and rinsed with tap water before sorbitol treatment. The fruits were immersed in distilled water (control) and solutions containing 0.1 and 2.5% (w/v) food-grade sorbitol (Sigma-Aldrich, St. Louis, USA) at 5 °C for 60 min (Sanches et al., 2021). Subsequently, the mangoes were stored in a cold room at 8.0 ± 1.0 °C and 95 ± 0.5 % RH for 30 d in CA cabinets (Fruit Control Equipment, model Venezia PCM 1.000, Milan, Italy). The treatments involved fruits subjected to the following: i. distilled water immersion and CA storage with 21 % O_2 + 0.03 % CO_2 (negative control), ii. distilled water and CA storage with 5 % O_2 + 5 % CO_2, as recommended by Santos Neto et al. (2019), iii. 0.1 % sorbitol immersion and CA storage with 5 % O_2 + 5 % CO_2, and iv. 2.5 % sorbitol and CA storage with 5 % O_2 + 5 % CO_2. The O_2, CO_2, and ethylene (C_2H_4) levels were determined and adjusted using the SWINGLOS ® software (Fruit Control Equipment, Milan, Italy).

Mangoes were evaluated every 10 d (0, 10, 20, and 30 d) and one batch was transferred to ambient conditions (~23 ± 2.0 °C and 75 ± 2.0% RH) for a further 7 d to

evaluate for CI development and to analyze the quality variables and oxidative metabolism responses. A completely randomized design (CRD) was used in a 4 factorial arrangement (treatments: control without Ca, control with CA, 0.1% sorbitol with CA, and 2.5% sorbitol with CA) × 4 (storage period: 0, 10, 20, and 30 d) factorial arrangement with 5 repetitions, totaling 80 fruits. The same procedure was used to evaluate fruit transferred to the ambient environment (n=80 fruit).

2.3 Chilling injury (CI) incidence and physicochemical variables

2.3.1 Chilling injury (CI): CI symptoms were evaluated visually using a scale from 1 to 4, as proposed by Miguel et al. (2011), with slight modifications: 1 = without visible symptoms; 2 = slight symptoms (CI = 0–25%); 3 = moderate symptoms (CI = 25–50% CI); and 4 = severe symptoms (CI = > 50%).

2.3.2 Fresh weight loss (FWL): FWL was determined by weighing the fruit on an analytical scale (Mars, model AS 2000, São Paulo, Brazil) at the beginning of the experiment (initial weight) and again every evaluation day (final weight). The results were expressed as a percentage (%) based on the following equation: FWL (%) = WL initial - WL final / WL initial × 100.

2.3.3 Color: Epicarp (peel) and mesocarp (pulp) color were determined on the central position of the opposite sides of the fruit, and the average value was used. A colorimeter (Minolta, CR-400, Osaka, Japan) was used to obtain the variables L*, a*, and b*, which were then used to calculate the chromaticity and hue angle (McGuire, 1992).

2.3.4 Firmness: This evaluation was conducted on the opposite sides of each fruit after removing the peel. A penetrometer (Effegi Fruit Tester, Rome, Italy) equipped with an 8 mm tip was used to determine fruit firmness, which was expressed in Newtons (N), as stated by Watkins and Harman (1981).

2.3.5 Soluble solids content (SSC): The SSC was determined using a digital refractometer (Alpha, Atago Co., Ltd, Japan), and the results were expressed as percentages (AOAC, 2016).

2.3.6 Titratable acidity (TA): TA was determined via titration, and the results were expressed as g kg^{-1} of citric acid (AOAC, 2016).

2.3.7 Ratio (SSC/TA): This ratio was calculated by dividing the results of SSC by the TA (AOAC, 2016).

2.3.8 pH: The pH of the fruit pulp was determined using a pH-meter (Orion 3 Star, Thermo Scientific, USA) (AOAC, 2016).

2.4 Oxidative metabolism variables

2.4.1 Electrolyte leakage (EL): EL was determined according to Khaliq et al. (2016) with slight modifications as follows: 10 discs (0.5 cm^2) of peel and pulp were collected using a stain steel tool. The discs were washed three times and incubated in 25 mL of distilled water at 25 °C for 30 min. The initial values of the electrolytes in the solution were determined using a conductivity meter (DiST®3 HI98303, Hanna Instruments, Limena, Italy). After incubation, the solution was boiled at 95 °C for 15 min, cooled, and the electrolyte value was re-measured. The relative electrolyte rate was expressed as a percentage (%) of the value obtained after 30 min of incubation in relation to the total electrolyte value after boiling.

2.4.2 Lipid peroxidation (LP): LP was quantified based on the malondialdehyde (MDA) content in the peel and pulp following the method described by Sanches et al. (2021) and Gratão et al. (2012). The results were expressed in mol MDA kg^{-1}.

2.4.3 Hydrogen peroxide (H_2O_2): The H_2O_2 content in the peel and pulp was determined according to Sanches et al. (2021) following the modification described by Alexieva et al. (2001).

2.5 Non-enzymatic and enzymatic metabolism

2.5.1 Vitamin C (AsA): AsA determination was performed according to AOAC (2016), and the results were expressed in g kg^{-1}.

2.5.2 Total polyphenols: The total polyphenol content was determined via colorimetry using the Folin-Ciocalteu method described by Obanda et al. (1997). The results were expressed as gallic acid equivalents of (GAE) kg^{-1} of fresh weight.

2.5.3 Enzyme extraction: The enzymes superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), and ascorbate peroxidase (APX, EC 1.11.1.1) were extracted following the method described by Yang et al. (2009). The protein content was determined using the Bradford (1976) method with bovine serum albumin as the standard.

2.5.4 Superoxide dismutase (SOD): SOD activity was determined as described by Sanches et al. (2021), following the method of Giannopolitis and Ries (1977). The results were expressed in U min^{-1} kg^{-1} 10^6 of protein.

2.5.5 Catalase (CAT): CAT activity was determined according to Sanches et al. (2021), using the method reported by Beers and Sizer (1952). The results were expressed in mol H_2O_2 min^{-1} kg^{-1} of protein.

2.5.6 Ascorbate peroxidase (APX): The APX activity was determined according to Sanches et al. (2021), following the method described by Nakano and Asada (1981). The results were expressed in mol H_2O_2 min^{-1} kg^{-1} of protein using the ascorbate molar extinction coefficient of 2.8 mM^{-1} cm^{-1}.

2.5.7 Polyphenol oxidase (PPO): The enzymatic extract to determine this enzyme was obtained following the method proposed by Sojo et al. (1998). The PPO (EC1.14.18.1) activity was determined according to Sanches et al. (2021), using the

method described by Wissemann and Lee (1980). The results were expressed in U min^{-1} kg^{-1} 10^6 of protein.

2.6 Statistical analysis

2.6.1 Multivariate analysis

Numerical matrices were constructed using the CI symptoms, quality variables, and oxidative metabolism for the different periods of CA (0, 10, 20, and 30 days) and ambient storage (0+10, 10+7, 20+7, and 30+7 days), in both the peel and pulp. Prior to the principal component analysis (PCA), the variable data were self-scaled (centered mean with subsequent scale for variance) and the relevant information was obtained using the CANOCO software (Ter Braak and Smilauer, 1998) using the first two principal components (PC1 and PC2), with a 95% confidence level.

2.6.2 Univariate analysis

The data was subjected to variance analysis (ANOVA) using the software R (R Core Team - 2020, Auckland, New Zealand). The means were compared using Tukey's test at a 0.05% probability level (supplementary material).

3. Results

3.1 CI and physicochemical variables

PC1 and PC2 represented 84.9 % of the data variance related to CI and the physicochemical variables during CA storage (Figure 1A). The CI vector was located in the upper right quadrant of PC1, clustered with the peel color parameters (L*, hº, and C*) and fresh weight loss (FWL). The negative control, control + CA, and 0.1 % sorbitol + CA treatments were closely related to CI, especially at the end of the storage period (Figure 1A). However, CI development was not associated with firmness, titratable acidity (TA), or pulp color (hº), particularly at the beginning of storage (0 and 10 d) (Figure 1A).

When the mangoes were transferred to ambient conditions, PC1 and PC2 represented 68.5 % 19.1 % of the variance, respectively totaling 87.6 % (Figure 1B). The CI vector remained in the upper right quadrant of PC1, clustering with FWL, and L*, and hº of the peel. CI was associated with mangoes in the negative control and control + CA treatments, mainly from the 20+7 d forward (Figure 1B). CI was not related to SSC, SSC/TA, pH, TA, firmness, and pulp color (L*, hº, and C*), especially for the fruit treated with 2.5 % sorbitol + CA (Figure 1B).

3.2 CI and oxidative metabolism

The PCA explained a large amount of the variation (73.7%), and the oxidative metabolites of the peel provided a more comprehensive CI development (Figure 2A). The CI vector was located in the upper right quadrant of PC1, forming a cluster with H_2O_2 content and PPO activity. This vector was in the opposite direction of SOD, APX, and CAT activities (Figure 2A). CI was more severe at 30 d of storage, particularly with mangoes in the negative control, control + CA, and 0.1 % sorbitol + CA treatments (Figure 2A). In contrast, CI was inhibited by 2.5 % sorbitol + CA, which contained less MDA and consequently revealed less lipid peroxidation (LP) activity. As opposed to LP, electrolyte leakage (EL), vitamin C (AsA), and total phenol (TP) contents were clustered in the negative axis of PC2 (Figure 2A).

Regarding the metabolites in the pulp of the fruit maintained under a CA, the total variance explained by the PCA was 90.5 % (78.6 % from PC1 and 11.9 % from PC2; Figure 2B). The CI vector was also located in the upper right quadrant of the PC1 and clustered with H_2O_2 content and PPO activity, but showed less interaction with these metabolites (Figure 2B). The non-enzymatic (AsA and TP) and enzymatic (SOD and CAT) antioxidant defenses were associated with mangoes treated with 2.5 % sorbitol + CA (Figure 2B). In PC2, LP was negatively correlated with EL (Figure 2B).

The PCA of the metabolites present in the peel of the fruit transferred to ambient conditions explained 76.2 % of the variation (Figure 3A). CI symptoms were more severe in ambient conditions and when clustered with H_2O_2 content, PPO activity, EL, and LP, while AsA and TP were located on the opposite side of PC1 in relation to CI at the end of the storage period (20+7 and 30+7 d) (Figure 3A). The SOD, CAT, and

APX activities clustered on PC2, and the vectors of these variables showed a strong correlation (Figure 3A).

The pulp of the fruit transferred to the ambient environment also presented an association between CI and H_2O_2, EL, LP, and PPO activity, but the CI vector was located in the upper left quadrant of PC1 (Figure 3B). The variation explained by PCA was high (83.6 %). CAT, SOD, and APX activities were opposite to the CI vector and the mangoes treated with 2.5 % sorbitol + CA clustered with these enzymes and showed inhibition of CI development (supplementary material). CI was related to the negative control and the control + CA treatments, especially at the end of the storage period (20+7 and 30+7 d) (Figure 3B).

4. Discussion

4.1 CI and physicochemical variables

During CA storage, peel discoloration was observed mainly in mangoes from the negative control and control + CA treatments, while sunken dark lesions were only present after transfer of the fruit to ambient conditions (Figure 1S). The intensification of CI symptoms corroborated Sivakumar et al. (2011), who reported more severe CI symptoms when mangoes were withdrawn from cold storage and subjected to ambient temperatures. According to Miguel et al. (2016) and Rosalie et al. (2018), color parameters are affected by CI due to tissue browning and the inhibition of carotenoid synthesis. As CI is an accumulative injury and mango epicarp is the most affected tissue of the fruit (Wang et al., 2008), symptoms are exacerbated at the end of the storage period, both in mangoes kept under CA and those transferred to ambient conditions. In contrast, mangoes treated with 2.5 % sorbitol + CA did not show peel color modifications, suggesting a control and/or alleviation of CI symptoms.

The intensification of CI symptoms was related to increasing FWL, which was positively correlated with CI development in mangoes that presented dehydration (Pesis et al., 2000; Zaharah and Singh, 2011). However, as the FWL during CA storage was very low (2.07 %) due to the high relative humidity (RH) inside the CA chambers (95 ± 2.0 %), CI development was minimized, particularly on the fruit treated with 2.5

% sorbitol + CA. However, when mangoes were transferred to ambient conditions with a lower RH (75 ± 2.0 %), the FWL was higher (14.19 %) and more severe CI symptoms were observed (Figure 1S).

During CA storage, very few modifications were observed in the physicochemical variables (supplementary material), and these modifications were not related to CI. Overall, the maintenance of fruit firmness, peel and pulp color, SSC, TA, and pH were related to the low storage temperature (8.0 ºC), high RH (95 ± 2.0 %), and the beneficial effects of the storage atmosphere (5 % O_2 + 5 % CO_2), which reduced the metabolic activity of the fruit and controlled the ripening process. The effectiveness of this storage atmosphere was reported by Santos Neto et al. (2019) with 'Palmer' mangoes at 13 °C under a CA (5 % O_2 + 5 % CO_2) for 28 d.

The development of CI in mangoes during cold storage is associated with a reduction in fruit firmness due to the loss of membrane integrity, as well as the presence of whitish spots in the pulp and irregular ripening that affects SSC, TA, and the SSC/TA ratio (González-Aguilar et al., 2001; Cantre et al., 2017). However, in the current study, this was not observed during CA storage, while in the ambient environment, a reduction in fruit firmness, L*, and hº, and an increase in C* and FWL was observed because of the higher temperature (23 ± 2.0 °C) and lower RH (75 ± 2.0 %). However, mangoes treated with 2.5 % sorbitol + CA had higher values for fruit firmness and the color parameters (L*, hº, and C*) up to 20+7 d, and since these modifications were not related to CI development, ripening was deferred in this treatment.

4.2 CI and oxidative metabolism

CI was related to H_2O_2 content irrespective of the fruit tissue (peel or pulp) and storage condition (CA or ambient environment), which can explain the accumulation of reactive oxygen species (ROS) as an early response to CI in 'Palmer' mangoes, which corroborates previous studies (Sanches et al., 2021, Sanches et al., in press). Shewfelt and Rosario (2000) reported that cooling induces ROS production, and these compounds react with various molecules, including DNA and proteins, resulting in membrane lipid peroxidation. However, during CA storage, a dissonance was observed between LP and EL, as well as with these variables and CI development.

Therefore, both sorbitol and CA could have induced the control of the metabolic activity of the fruit, leading to lower ROS production and, consequently, less toxic compounds. This would also justify the lower PPO activity associated with the reduced vector size in relation to CI.

In contrast, under ambient conditions, both peel and pulp had intensified CI development, which is associated with H_2O_2, LP, EL, and PPO activity. It is likely that the higher temperature increased ROS production, which elicited LP in relation to MDA accumulation because of the fatty acid degradation (Sevillano et al., 2009). This caused structural damage to the membranes and increased EL. The loss of membrane integrity allowed the PPO located in the plastids to react with the phenolic compounds released from the vacuole (Jiang et al., 2016). This resulted in tissue browning mainly in mangoes from the negative control, control + CA, and 0.1 % sorbitol + CA treatments. Previous reports have demonstrated clear relationships between CI and LP, EL (Ruan et al., 2015; Khaliq et al., 2016; Bhardwaj et al., 2021), increasing PPO activity (Wang et al., 2008; Chidtragool et al., 2011) and ROS accumulation in mangoes stored at temperatures below 13 °C.

Although significant results were obtained in the univariate analysis (supplementary material), the overall understanding of the relationship between the treatments with sorbitol and CA and CI development in association with oxidative metabolism was improved using the PCA. It was possible to relate the 2.5 % sorbitol + CA treatment with the antioxidant defense metabolism and the control and/or alleviation of CI (Figure 1S). The dissociations of the enzymatic defense system (SOD, CAT, and APX) in the peel and APX in the pulp with CI indicated that this was the main mechanism of CI reduction in mangoes irrespective of the treatment. Although reports have associated the activities of SOD, CAT, and APX with lower oxidative damage caused by CI in mangoes (Chongchatuporn et al., 2013; Junmatong et al., 2015; Rosalie et al., 2018), the non-enzymatic defense system (AsA and TP) can also be considered, since low oxidative damage was reported in the peel of mangoes throughout the storage period withCA. This result is related to the higher concentrations of AsA and TP in the peel than in the pulp (Sivankalyani et al., 2016; Thiruchelvam et al., 2020; Sousa et al., 2021).

5. Conclusions

The multivariate analysis the relationships between physicochemical variables and the enzymatic and non-enzymatic defense metabolisms with the development of CI symptoms in 'Palmer' mangoes stored at 8 ºC under a CA (5% O_2 + 5% CO_2) for 30 d. It was possible to identify that is associated with ROS accumulation as a response to low storage temperature.

Fruit immersed in 2.5 % sorbitol and stored under a CA (5% O_2 + 5% CO_2) showed activation of the enzymatic and non-enzymatic defense antioxidant metabolisms, resulting in the control and/or alleviation of CI, without compromising fruit quality.

In the epicarp, antioxidant damage control was correlated with the non-enzymatic mechanism (AsA and total polyphenols), whereas in the mesocarp, the enzymatic (SOD, CAT, and APX) activities were more responsive. In general, the activation of these defense mechanisms against H_2O_2 accumulation reduced CI in mangoes.

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Figures

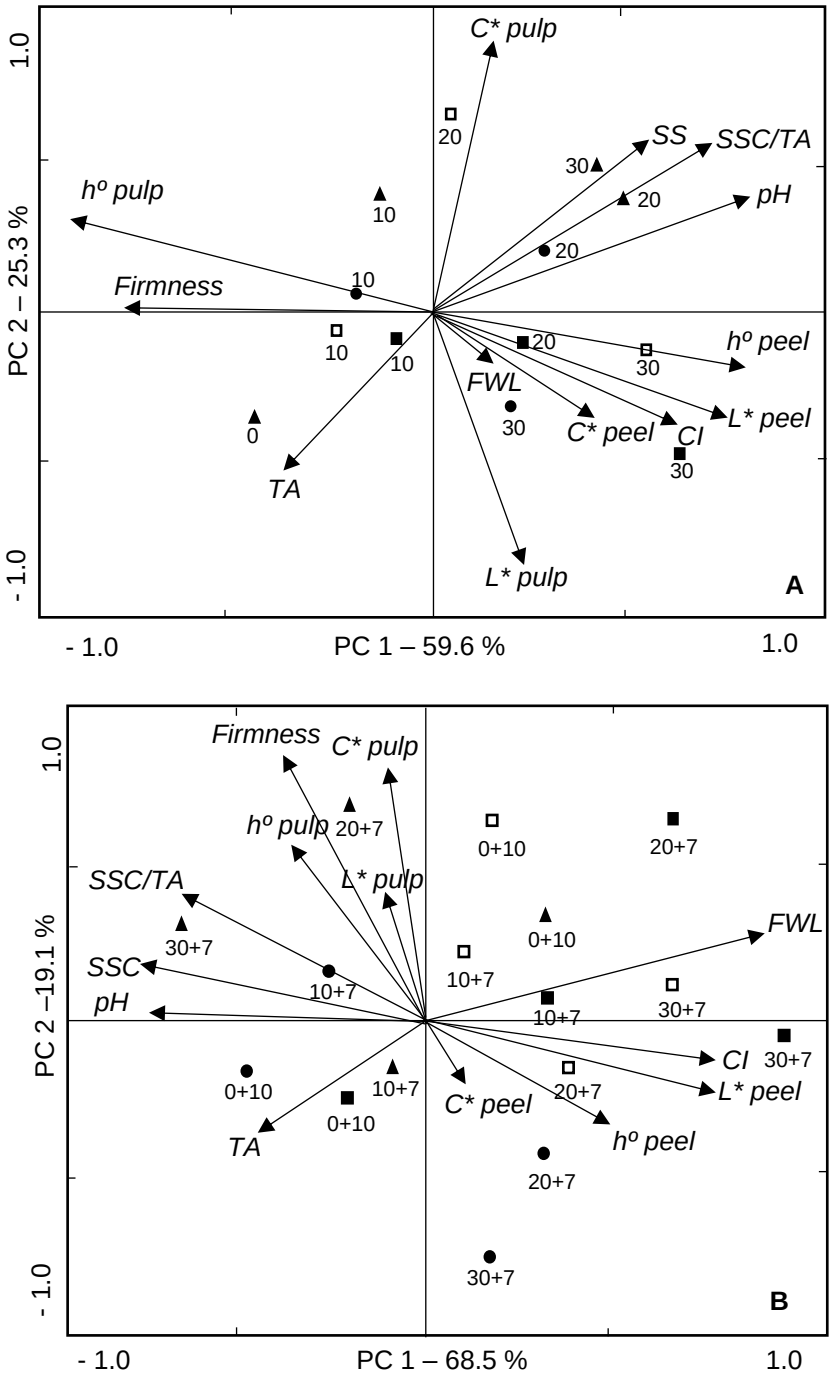


Figure 1. First and second principal component (PCA) scores plot of 'Palmer' mango samples (peel and pulp) stored at 8 ºC for 30 d (A) and transfer to ambient (23 ºC) for 7 d (B). Firmness, luminosity (L*), hue angle (hº), chromaticity(C*), soluble solids content (SSC), titratable acidity (TA), ratio (SSC/TA) and pH. Treatments: negative control (■), control + CA (□), 01 % sorbitol + CA (●), and 2.5 % sorbitol + CA (▲).

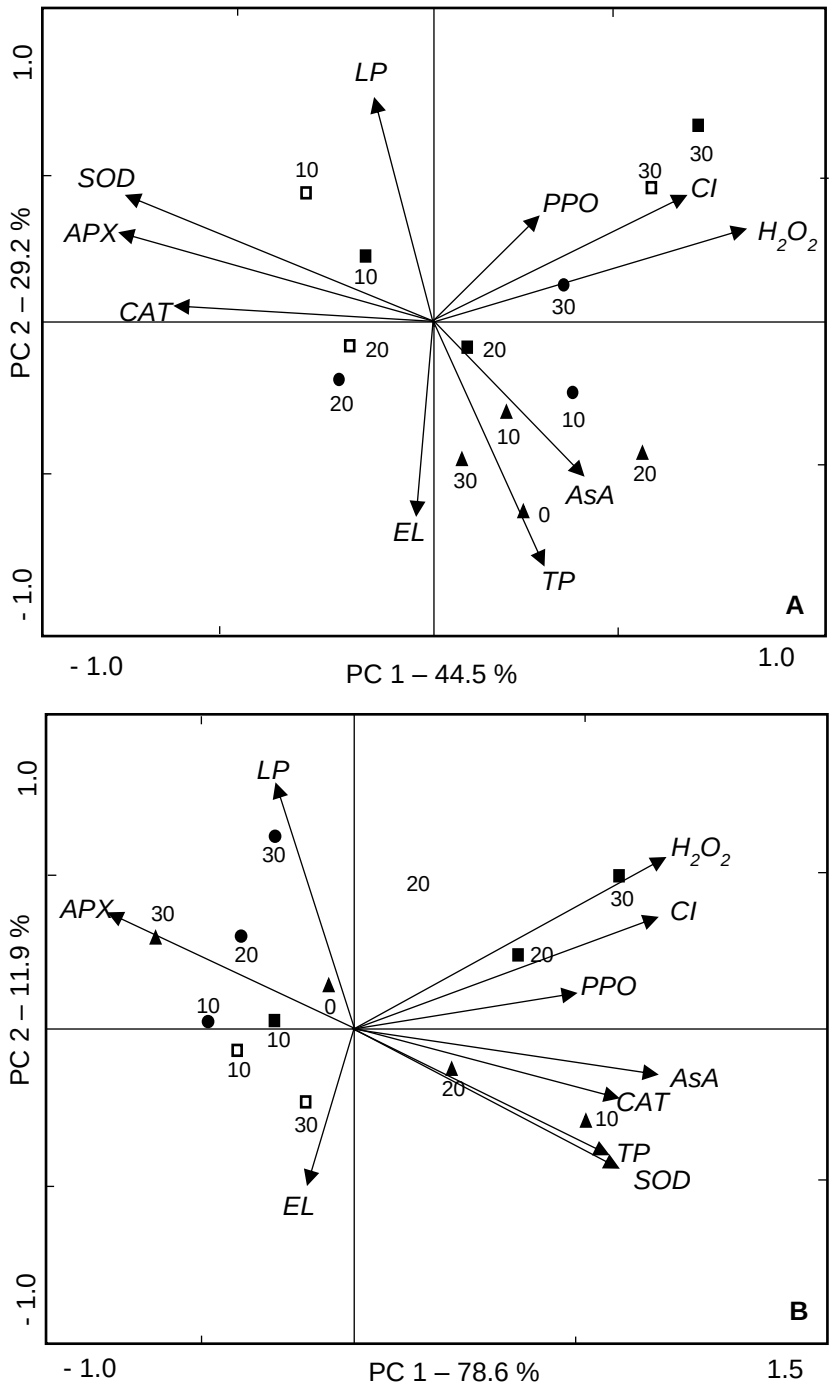


Figure 2. First and second principal component (PCA) scores plot of 'Palmer' mango samples (peel – A and pulp B) stored at 8 ºC for 30 d (0, 10, 20, and 30 d. Lipid peroxidation (LP), electrolyte leakage (EL), hydrogen peroxide (H_2O_2), vitamin C (AsA), total phenols (TP), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and polyphenol oxidase (PPO). Treatments: negative control (■), control + CA (□), 01 % sorbitol + CA (●), and 2.5 % sorbitol + CA (▲).

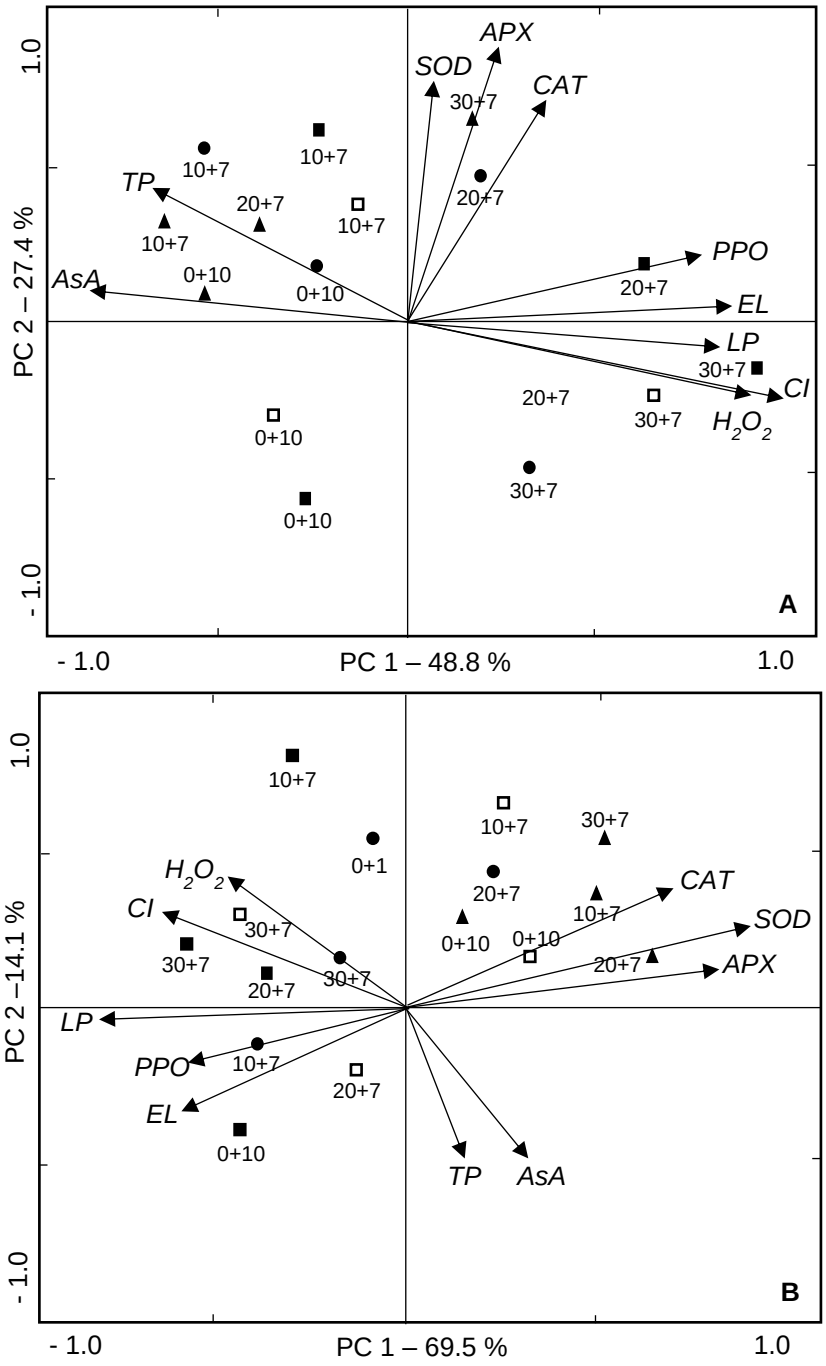


Figure 3. First and second principal component (PCA) scores plot of 'Palmer' mango samples (peel – A and pulp B) stored at 8 ºC for 30 d and transfer to ambient (23 ºC) for 7 d (0+7, 10+7, 20+7, and 30+7 d. Lipid peroxidation (LP), electrolyte leakage (EL), hydrogen peroxide (H_2O_2), vitamin C (AsA), total phenols (TP), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and polyphenol oxidase (PPO). Treatments: negative control (■), control + CA (□), 01 % sorbitol + CA (●), and 2.5 % sorbitol + CA (▲).

Supplementary material

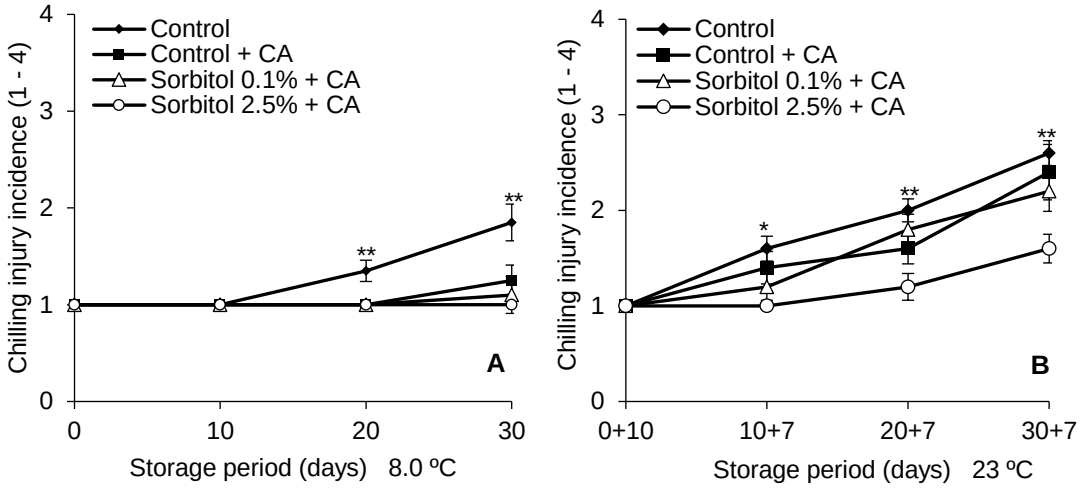
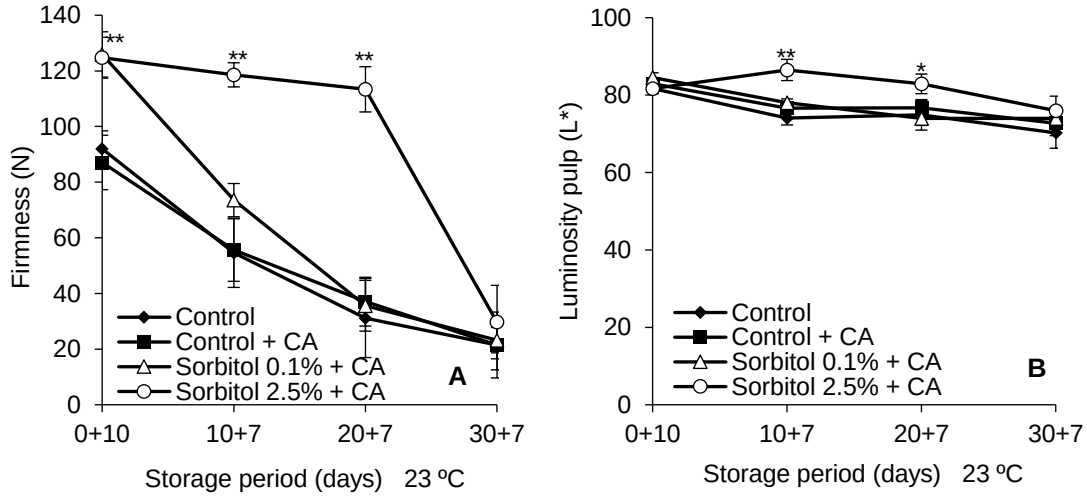


Figure 1. Interaction between treatments and storage period for chilling injury (CI) symptoms in 'Palmer' mangoes treated with sorbitol (0.1 and 2.5 %) during controlled atmosphere (CA) storage at 8.0 ± 0.5 ºC for 30 d (A) and transfer to ambient (23 ± 2 ºC) for 7 more d (B). The means within treatments were different at P<0.05 (**) and P<0.01 (*) for the storage period.



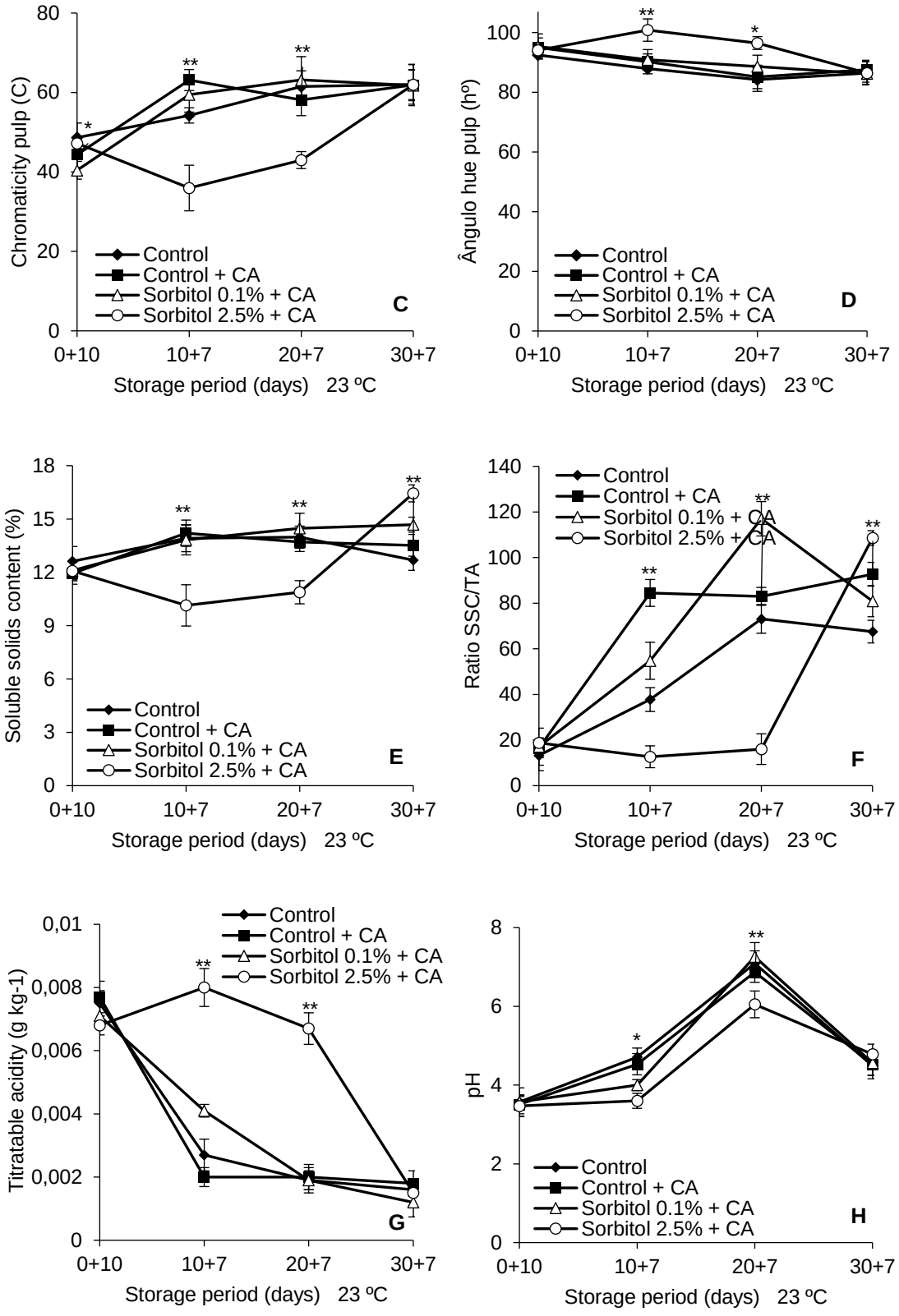


Figure 2. Interaction between treatments and storage period for firmness (A), luminosity (B), chromaticity (C), hue angle (D), soluble solids content (E), ratio (F), titratable acidity (G) and pH (H) of 'Palmer' mangoes treated with sorbitol (0.1 and 2.5 %) during controlled atmosphere (CA) storage at 8.0 ± 0.5 ºC for 30 d

and transfer to ambient (23 ± 2 ºC) for 7 more days. The means within treatments were different at P<0.05 (**) and P<0.01 (*) for the storage period.

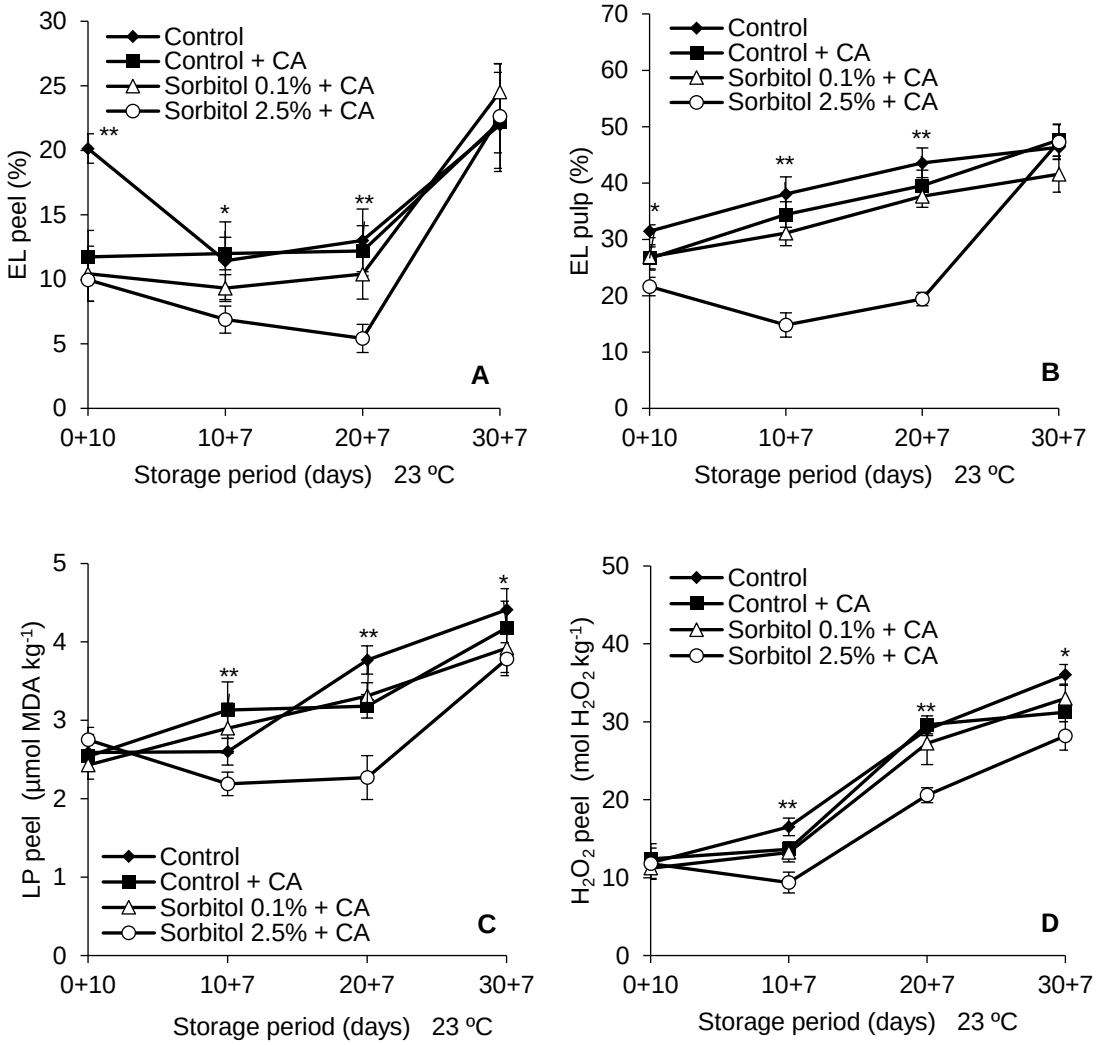


Figure 3. Interaction between treatments and storage period for electrolyte leakage (A and B), lipid peroxidation (C), and hydrogen peroxide (D) of 'Palmer' mangoes tissues (peel and pulp) treated with sorbitol (0.1 and 2.5 %) during controlled atmosphere (CA) storage at 8.0 ± 0.5 ºC for 30 d and transfer to ambient (23 ± 2 ºC) for 7 more days. The means within treatments were different at P<0.05 (**) and P<0.01 (*) for the storage period.

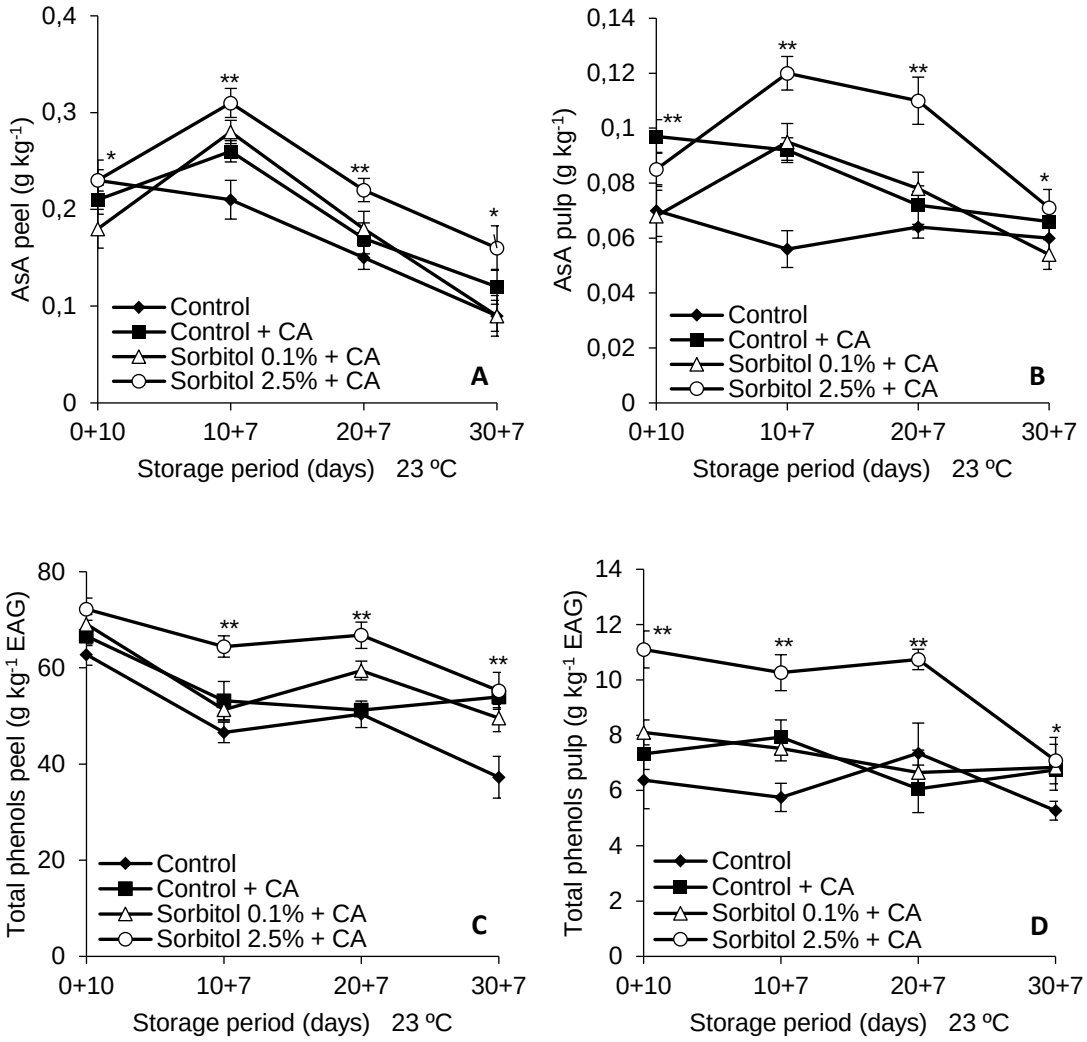


Figure 4. Interaction between treatments and storage period for vitamin C (A and B) and total polyphenols (C and D) of 'Palmer' mangoes tissues (peel and pulp) treated with sorbitol (0.1 and 2.5 %) during controlled atmosphere (CA) storage at 8.0 ± 0.5 ºC for 30 d and transfer to ambient (23 ± 2 ºC) for 7 more days. The means within treatments were different at P<0.05 (**) and P<0.01 (*) for the storage period.

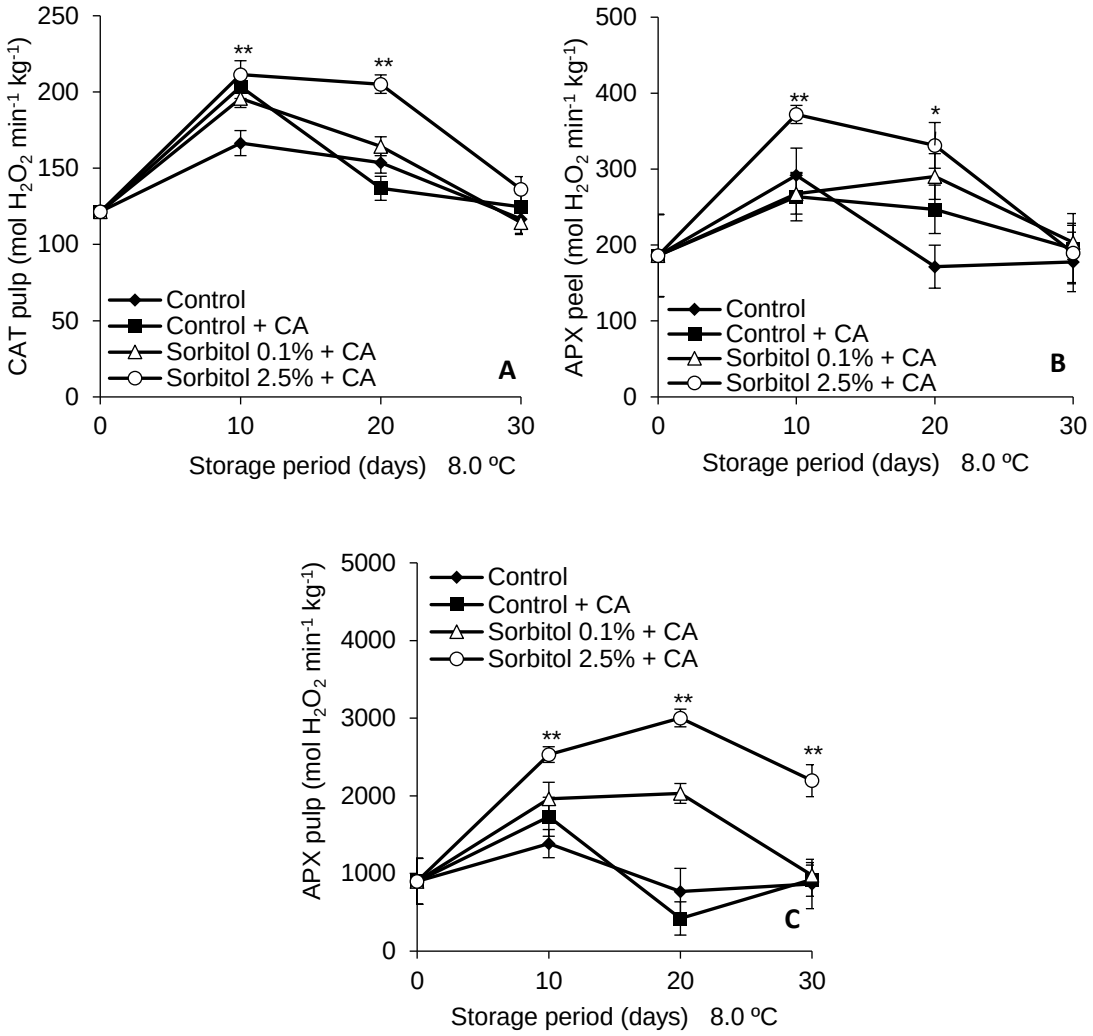


Figure 5. Interaction between treatments and storage period for catalase (A) and ascorbate peroxidase APX (B and C) of 'Palmer' mangoes tissues (peel and pulp) treated with sorbitol (0.1 and 2.5 %) during controlled atmosphere (CA) storage at 8.0 ± 0.5 ºC for 30 d. The means within treatments were different at P<0.05 (**) and P<0.01 (*) for the storage period.

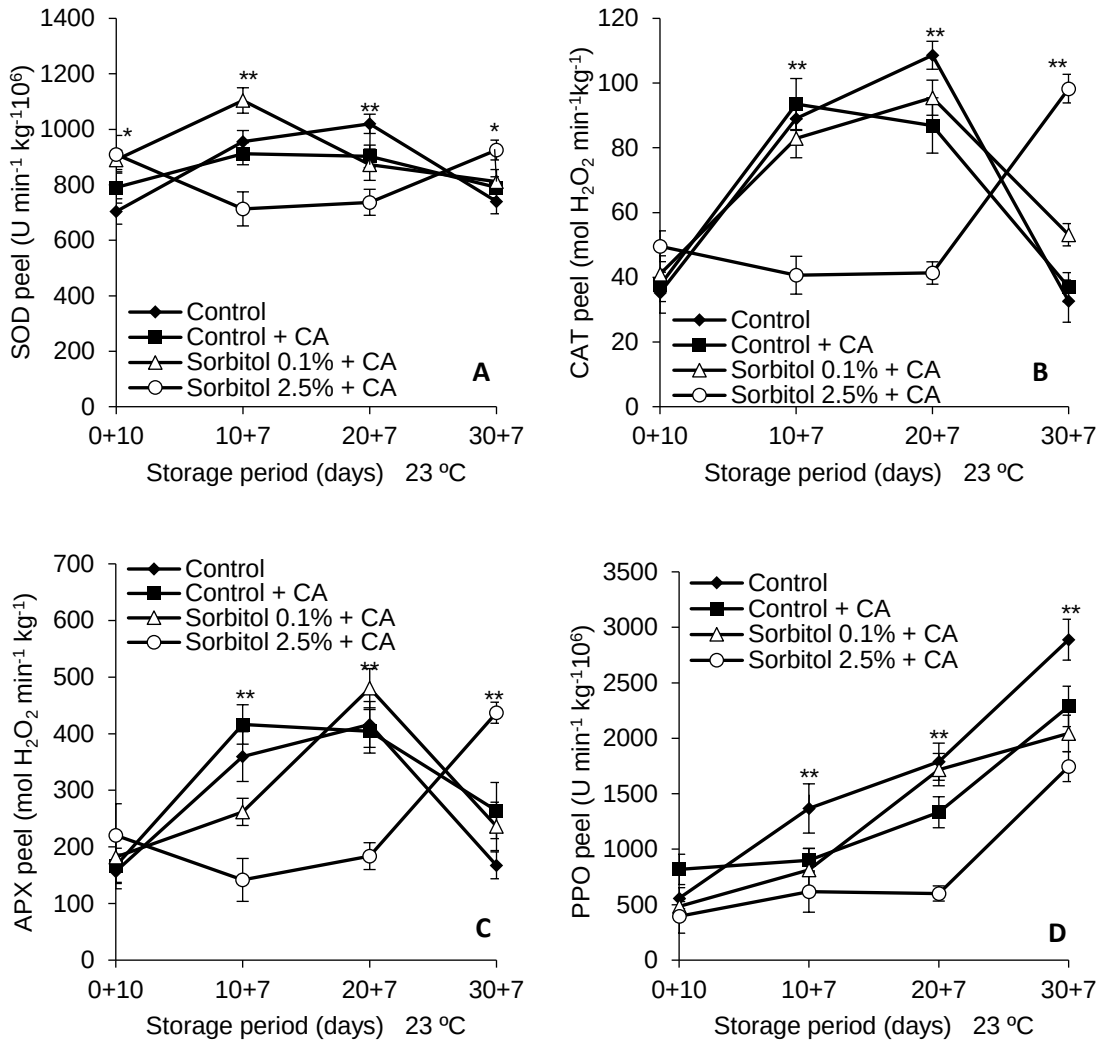


Figure 6. Interaction between treatments and storage period for superoxide dismutase (A), catalase (B), ascorbate peroxidase (C) and polyphenol oxidase (D) of 'Palmer' mangoes peel treated with sorbitol (0.1 and 2.5 %) during controlled atmosphere (CA) storage at 8.0 ± 0.5 ºC for 30 d and transfer to ambient (23 ± 2 ºC) for 7 more days. The means within treatments were different at P<0.05 (**) and P<0.01 (*) for the storage.

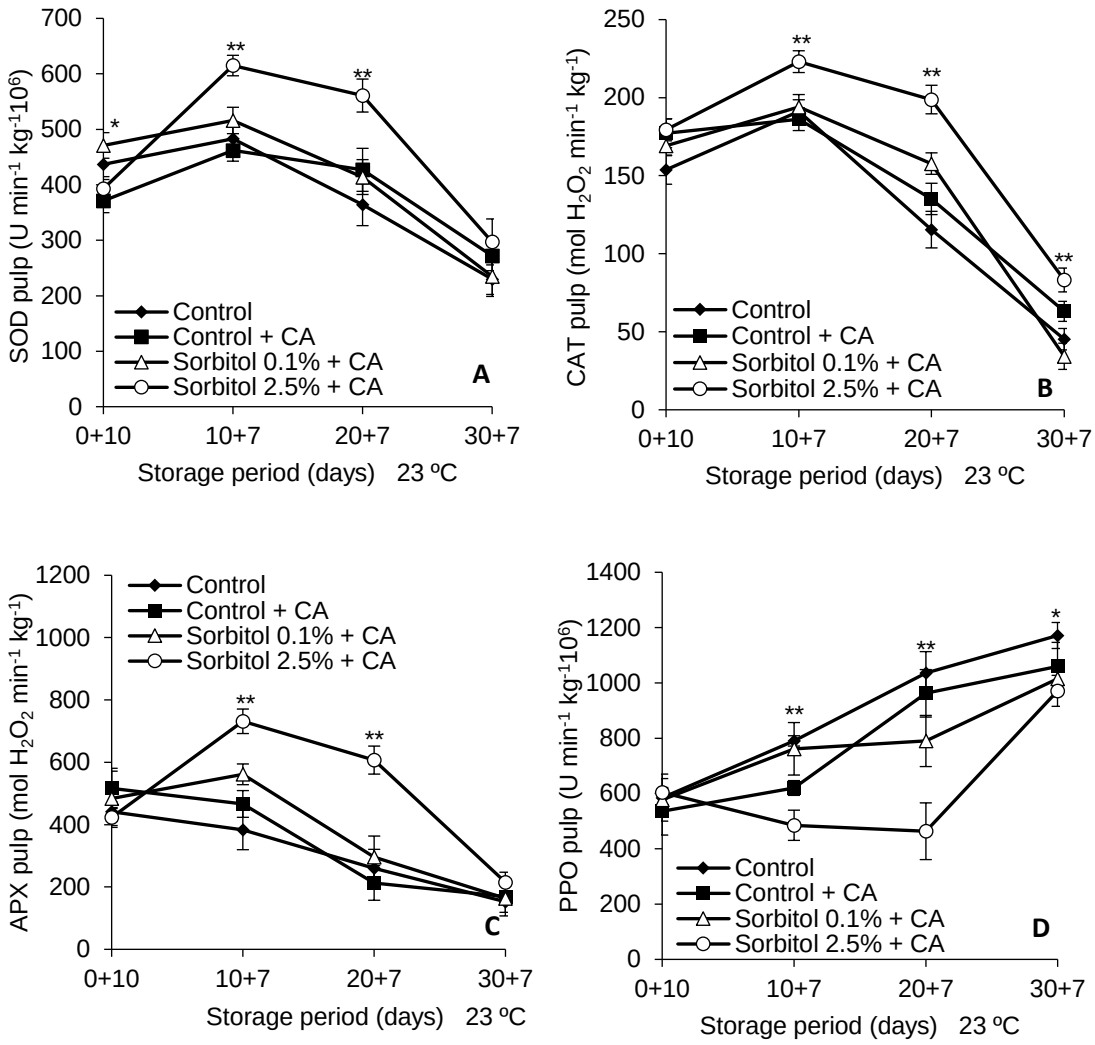


Figure 7. Interaction between treatments and storage period for superoxide dismutase (A), catalase (B), ascorbate peroxidase (C) and polyphenol oxidase (D) of 'Palmer' mangoes tissues pulp treated with sorbitol (0.1 and 2.5 %) during controlled atmosphere (CA) storage at 8.0 ± 0.5 ºC for 30 d and transfer to ambient (23 ± 2 ºC) for 7 more days. The means within treatments were different at P<0.05 (**) and P<0.01 (*) for the storage.

Tables

Table 1. Effect of sorbitol (0.1 and 2.5%) immersion and controlled atmosphere (CA) on firmness, luminosity (L*), hue angle (hue), and chromaticity (C*) of 'Palmer' mango tissues (peel and pulp) stored at 8.0 ± 0.5 ºC for 30 d.

Principal effects	Firmness (N)	Color peel			Color pulp		
		L*	hue	C*	L*	hue	C*
Treatments (A)							
Control	126.45 ± 3.33 a	39.85 ± 3.43 b	89.01 ± 4.16 b	21.35 ± 4.02 b	86.04 ± 0.78 a	101.43 ± 0.58 a	33.97 ± 4.86 a
Control + CA	126.57 ± 2.80 a	40.61 ± 2.77 b	90.32 ± 3.03 b	21.19 ± 3.16 b	85.58 ± 0.69 a	101.71 ± 0.39 a	34.56 ± 3.93 a
Sorbitol 0.1% + CA	126.94 ± 1.94 a	40.73 ± 3.94 b	87.87 ± 3.91 b	19.79 ± 3.71 b	85.44 ± 1.12 a	101.63 ± 0.92 a	34.46 ± 4.31 a
Sorbitol 2.5% + CA	127.19 ± 2.84 a	45.03 ± 2.21 a	96.89 ± 3.17 a	26.58 ± 2.23 a	85.94 ± 0.83 a	101.78 ± 0.54 a	34.80 ± 1.14 a
Storage – days (B)							
0	127.48 ± 2.84 a	44.03 ± 2.51 a	96.30 ± 4.01 a	18.55 ± 3.17 b	85.05 ± 1.01 c	102.49 ± 0.88 a	35.72 ± 4.06 a
10	127.48 ± 2.84 a	41.04 ± 1.20 b	91.43 ± 6.23 ab	21.83 ± 2.01 ab	87.11 ± 1.12 a	102.95 ± 0.56 a	32.80 ± 3.05 a
20	127.48 ± 2.84 a	38.74 ± 2.09 b	83.86 ± 8.12 ab	22.89 ± 1.91 a	86.14 ± 0.89 b	101.37 ± 0.19 b	34.57 ± 3.92 a
30	124.79 ± 1.76 b	38.43 ± 1.11 b	80.50 ± 4.03 b	18.64 ± 2.64 b	84.71 ± 0.91 c	99.78 ± 0.21 c	34.69 ± 2.18 a
Interactions							
AxB	NS	NS	NS	NS	NS	NS	NS

Means followed by the same letter within each column do not differ statistically from each other by the Tukey test (P<0.05). Non-significant interaction (NS), significant interaction at P<0.05 (**) and significant interaction at P <0.01 (*).

Table 2. Effect of sorbitol (0.1 and 2.5%) immersion and controlled atmosphere (CA) on soluble solids content (SSC), titratable acidity (TA), ration (SSC/TA), and pH of 'Palmer' mangoes stored at 8.0 ± 0.5 ºC for 30 d.

Principal effects	**SSC (%)**	**TA (g kg^{-1})**	**SSC/TA**	**pH**
Treatments (A)				
Control	7.47 ± 0.55 b	0.0057 ± 0.0003 a	13.65 ± 1.34 b	3.43 ± 0.14 a
Control + CA	7.70 ± 0.76 b	0.0060 ± 0.0004 a	13.87 ± 3.15 b	3.46 ± 0.10 a
Sorbitol 0.1% + CA	7.86 ± 0.44 b	0.0059 ± 0.0002 a	14.60 ± 1.96 b	3.42 ± 0.16 a
Sorbitol 2.5% + CA	9.04 ± 0.51 a	0.0056 ± 0.0002 a	16.96 ± 2.31 a	3.51 ± 0.11 a
Storage – days (B)				
0	7.20 ± 1.10 c	0.0071 ± 0.0005 a	10.13 ± 3.05 b	3.25 ± 0.12 b
10	7.00 ± 0.64 c	0.0063 ± 0.0004 b	11.31 ± 2.01 b	3.49 ± 0.09 a
20	8.17 ± 0.53 b	0.0053 ± 0.0009 c	18.12 ± 1.96 a	3.56 ± 0.11 a
30	9.80 ± 0.42 a	0.0046 ± 0.0004 c	17.82 ± 2.12 a	3.53 ± 0.13 a
Interactions				
AxB	NS	NS	NS	NS

Means followed by the same letter within each column do not differ statistically from each other by the Tukey test (P<0.05). Non-significant interaction (NS), significant interaction at P<0.05 (**) and significant interaction at P <0.01 (*).

Table 3. Effect of sorbitol (0.1 and 2.5%) immersion and controlled atmosphere (CA) on firmness, luminosity (L*), hue angle (hue), and chromaticity (C*) of 'Palmer' mango tissues (peel and pulp) stored at 8.0 ± 0.5 ºC for 30 d and transfer to ambient (23 ± 2 ºC) for 7 more days.

Principal effects	Firmness (N)	Color peel			Color pulp		
		L*	hue	C*	L*	hue	C*
Treatments (A)							
Control	49.81 ± 3.87 b	36.33 ± 2.15 b	82.38 ± 8.19 ab	22.68 ± 3.12 a	74.71 ± 2.19 c	88.87 ± 1.16 b	56.60 ± 2.07 a
Control + CA	50.30 ± 4.67 b	36.61 ± 2.53 b	79.18 ± 4.93 b	22.86 ± 4.18 a	77.26 ± 1.59 bc	88.89 ± 0.67 b	56.87 ± 1.19 a
Sorbitol 0.1% + CA	61.32 ± 2.13 b	37.32 ± 2.00 b	89.11 ± 3.16 ab	22.92 ± 2.67 a	78.14 ± 1.18 b	89.76 ± 1.34 b	53.71 ± 1.85 a
Sorbitol 2.5% + CA	96.59 ± 5.11 a	41.86 ± 2.03 a	91.47 ± 1.65 a	23.75 ± 3.03 a	81.04 ± 0.94 a	94.45 ± 0.83 a	47.04 ± 2.01 b
Storage – days (B)							
0+10	104.58 ± 4.21 a	37.40 ± 2.04 b	84.09 ± 4.01 b	18.05 ± 2.19 c	82.72 ± 1.13 a	94.71 ± 1.09 a	45.16 ± 1.10 c
10+7	74.62 ± 6.11 b	37.53 ± 3.01 b	91.53 ± 5.27 a	21.63 ± 1.93 b	79.05 ± 1.07 b	91.18 ± 0.78 b	53.20 ± 2.01 b
20+7	55.60 ± 3.04 c	39.43 ± 2.42 ab	85.29 ± 3.19 b	23.68 ± 2.11 b	77.12 ± 2.01 b	88.71 ± 2.63 bc	56.45 ± 2.94 ab
30+7	23.21 ± 4.72 d	41.81 ± 2.19 a	80.38 ± 5.93 b	28.86 ± 3.81 a	73.25 ± 2.69 a	87.87 ± 1.16 c	59.43 ± 3.56 a
Interactions							
AxB	8.77**	NS	NS	NS	6.02**	7.31**	6.64**

Means followed by the same letter within each column do not differ statistically from each other by the Tukey test (P<0.05). Non-significant interaction (NS), significant interaction at P<0.05 (**) and significant interaction at P <0.01 (*).

Table 4. Effect of sorbitol (0.1 and 2.5%) immersion and controlled atmosphere (CA) on soluble solids content (SSC), titratable acidity (TA), ration (SSC/TA), and pH of 'Palmer' mangoes stored at 8.0 ± 0.5 ºC for 30 d and transfer to ambient (23 ± 2 ºC) for 7 more days.

Principal effects	**SSC (%)**	**TA (g kg^{-1})**	**SSC/TA**	**pH**
Treatments (A)				
Control	13.87 ± 0.42 a	0.0034 ± 0.0002 b	59.40 ± 6.18 a	4.76 ± 0.19 a
Control + CA	13.34 ± 0.66 a	0.0033 ± 0.0001 b	66.22 ± 7.21 a	4.91 ± 0.19 a
Sorbitol 0.1% + CA	13.27 ± 0.84 ab	0.0036 ± 0.0003 b	58.89 ± 8.04 a	4.84 ± 0.14 a
Sorbitol 2.5% + CA	12.38 ± 0.11 b	0.0058 ± 0.0002 a	38.98 ± 6.82 b	4.53 ± 0.21 b
Storage – days (B)				
0+10	12.18 ± 0.74 c	0.0073 ± 0.0001 a	16.38 ± 5.18 d	3.53 ± 0.21 d
10+7	13.02 ± 1.05 bc	0.0042 ± 0.0003 b	47.41 ± 7.29 c	3.93 ± 0.17 c
20+7	13.26 ± 0.83 b	0.0030 ± 0.0001 c	72.28 ± 6.05 b	6.87 ± 0.24 a
30+7	14.40 ± 0.61 a	0.0017 ± 0.0002 a	87.42 ± 5.91 a	4.72 ± 0.18 b
Interactions				
AxB	10.21**	15.89**	11.21**	7.24**

Means followed by the same letter within each column do not differ statistically from each other by the Tukey test (P<0.05). Non-significant interaction (NS), significant interaction at P<0.05 (**) and significant interaction at P <0.01 (*).

Table 5. Effect of sorbitol (0.1 and 2.5%) immersion and controlled atmosphere (CA) on electrolyte leakage (EL), lipid peroxidation (LP) and hydrogen peroxide (H_2O_2) of 'Palmer' mango tissues (peel and pulp) stored at 8.0 ± 0.5 ºC for 30 d.

Principal effects	EL peel (%)	EL pulp (%)	LP peel (µmol MDA kg^{-1})	LP pulp (µmol MDA kg^{-1})	H_2O_2 peel (mol H_2O_2 kg^{-1})	H_2O_2 pulp (mol H_2O_2 kg^{-1})
Treatments (A)						
Control	7.76 ± 0.98 a	15.01 ± 1.31 a	2.01 ± 0.11 a	1.73 ± 0.11 a	18.74 ± 2.59 a	8.53 ± 0.68 a
Control + CA	7.02 ± 1.39 ab	13.71 ± 0.93 ab	1.98 ± 0.17 a	1.72 ± 0.09 a	16.28 ± 2.11 ab	7.85 ± 0.93 a
Sorbitol 0.1% + CA	6.85 ± 1.13 b	14.84 ± 1.02 ab	1.84 ± 0.21 a	1.68 ± 0.13 a	15.13 ± 1.96 b	7.91 ± 1.02 ab
Sorbitol 2.5% + CA	5.91 ± 1.26 c	13.20 ± 1.27 b	1.79 ± 0.16 a	1.64 ± 0.16 a	14.50 ± 1.62 b	6.78 ± 0.91 b
Storage – days (B)						
0	2.65 ± 1.56 c	7.49 ± 1.61 c	1.74 ± 0.15 b	1.46 ± 0.11 c	19.67 ± 1.81 a	6.61 ± 0.93 c
10	6.97 ± 1.03 b	13.93 ± 1.44 b	1.63 ± 0.18 c	1.41 ± 0.13 c	10.68 ± 2.32 b	4.67 ± 0.52 d
20	8.50 ± 0.63 a	15.59 ± 1.11 b	1.81 ± 0.11 b	1.89 ± 0.11 b	12.53 ± 2.61 b	9.30 ± 0.37 b
30	9.02 ± 1.12 a	17.95 ± 0.91 a	2.19 ± 0.13 a	2.05 ± 0.10 a	21.28 ± 2.41 a	10.72 ± 0.60 a
Interactions						
AxB	NS	NS	NS	NS	NS	NS

Means followed by the same letter within each column do not differ statistically from each other by the Tukey test (P<0.05). Non-significant interaction (NS), significant interaction at P<0.05 (**) and significant interaction at P <0.01 (*).

Table 6. Effect of sorbitol (0.1 and 2.5%) immersion and controlled atmosphere (CA) on electrolyte leakage (EL), lipid peroxidation (LP) and hydrogen peroxide (H_2O_2) of 'Palmer' mango tissues (peel and pulp) stored at 8.0 ± 0.5 ºC for 30 d and transfer to ambient (23 ± 2 ºC) for 7 more days.

Principal effects	EL peel (%)	EL pulp (%)	L peel (µmol MDA kg^{-1})	LP pulp (µmol MDA kg^{-1})	H_2O_2 peel (mol H_2O_2 kg^{-1})	H_2O_2 pulp (mol H_2O_2 kg^{-1})
Treatments (A)						
Control	16.64 ± 1.13 a	44.83 ± 3.18 a	3.36 ± 0.23 a	3.35 ± 0.30 a	24.65 ± 2.15 a	22.61 ± 1.83 a
Control + CA	14.54 ± 2.65 a	35.52 ± 2.85 b	3.27 ± 0.13 a	3.23 ± 0.21 ab	20.15 ± 1.93 b	20.47 ± 2.68 ab
Sorbitol 0.1% + CA	16.18 ± 2.07 a	35.81 ± 3.05 b	3.11 ± 0.15 b	3.05 ± 0.24 ab	19.40 ± 1.26 b	19.67 ± 2.01 b
Sorbitol 2.5% + CA	11.23 ± 1.94 b	25.78 ± 3.17 c	2.75 ± 0.21 c	2.86 ± 0.21 b	14.89 ± 2.04 c	17.48 ± 2.03 c
Storage – days (B)						
0+10	13.07 ± 3.17 b	31.61 ± 4.76 b	2.58 ± 0.31 c	2.61 ± 0.25 c	21.47 ± 2.04 b	11.83 ± 3.46 a
10+7	9.14 ± 1.52 c	30.85 ± 3.18 b	2.75 ± 0.26 bc	3.08 ± 0.17 b	14.86 ± 1.12 c	12.43 ± 2.63 a
20+7	12.77 ± 2.16 bc	36.78 ± 5.24 ab	3.08 ± 0.38 b	3.17 ± 0.28 b	20.54 ± 1.87 b	26.61 ± 3.52 b
30+7	22.84 ± 3.04 a	42.70 ± 5.66 a	4.07 ± 0.21 a	3.72 ± 0.19 a	32.20 ± 3.16 a	29.36 ± 2.61 b
Interactions						
AxB	5.14**	4.63**	2.94*	NS	3.41**	NS

Means followed by the same letter within each column do not differ statistically from each other by the Tukey test (P<0.05). Non-significant interaction (NS), significant interaction at P<0.05 (**) and significant interaction at P <0.01 (*).

Table 7. Effect of sorbitol (0.1 and 2.5%) immersion and controlled atmosphere (CA) on vitamin C (AsA), total polyphenols (TP), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and polyphenol oxidase (PPO) 'Palmer' mango peel stored at 8.0 ± 0.5 ºC for 30 d.

Principal effects	AsA (g kg^{-1})	TP (g kg^{-1} GAE)	SOD (U min^{-1} $kg^{-1}10^6$)	CAT (mol H_2O_2 min^{-1} kg^{-1})	APX (mol H_2O_2 min^{-1} kg^{-1})	PPO (U min^{-1} $kg^{-1}10^6$)
Treatments (A)						
Control	0.33 ± 0.016 a	71.82 ± 2.46 c	633.98 ± 17.89 a	46.25 ± 3.03 a	206.81 ± 20.12 c	74.34 ± 6.12 a
Control + CA	0.34 ± 0.011 a	74.41 ± 1.95 b	618.68 ± 20.50 a	48.27 ± 4.61 a	223.02 ± 19.78 bc	71.59 ± 3.08 a
Sorbitol 0.1% + CA	0.34 ± 0.013 a	73.03 ± 1.69 c	645.39 ± 33.06 ab	49.02 ± 5.17 a	236.88 ± 25.02 b	69.41 ± 4.14 a
Sorbitol 2.5% + CA	0.37 ± 0.015 a	78.27 ± 2.01 a	668.53 ± 28.91 b	53.71 ± 4.89 a	269.72 ± 28.33 a	58.35 ± 5.72 b
Storage – days (B)						
0	0.37 ± 0.016 a	85.65 ± 1.36 a	646.28 ± 9.11 b	41.86 ± 4.60 b	186.15 ± 28.07 b	45.94 ± 5.18 c
10	0.36 ± 0.015 a	85.51 ± 2.48 a	595.84 ± 31.16 c	54.81 ± 3.79 a	298.74 ± 35.85 a	49.85 ± 4.70 c
20	0.38 ± 0.012 a	86.17 ± 1.24 a	551.33 ± 56.95 c	55.53 ± 2.01 a	260.01 ± 31.87 a	72.61 ± 4.64 b
30	0.37 ± 0.018 a	70.19 ± 2.50 b	773.63 ± 41.52 a	45.07 ± 4.18 b	191.54 ± 24.18 b	103.98 ± 3.11 a
Interactions						
AxB	NS	NS	NS	NS	5.92**	NS

Means followed by the same letter within each column do not differ statistically from each other by the Tukey test (P<0.05). Non-significant interaction (NS), significant interaction at P<0.05 (**) and significant interaction at P <0.01 (*).

Table 8. Effect of sorbitol (0.1 and 2.5%) immersion and controlled atmosphere (CA) on vitamin C (AsA), total polyphenols (TP), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and polyphenol oxidase (PPO) 'Palmer' mango pulp stored at 8.0 ± 0.5 ºC for 30 d.

Principal effects	AsA (g kg^{-1})	TP (g kg^{-1} GAE)	SOD (U min^{-1} $kg^{-1}10^6$)	CAT (mol H_2O_2 min^{-1} kg^{-1})	APX (mol H_2O_2 min^{-1} kg^{-1})	PPO (U min^{-1} $kg^{-1}10^6$)
Treatments (A)						
Control	0.085 ± 0.009 a	9.01 ± 1.43 a	709.27 ± 35.53 a	139.57 ± 4.20 c	979.45 ± 184.91 c	45.25 ± 3.72 a
Control + CA	0.087 ± 0.011 a	10.21 ± 1.07 a	712.39 ± 48.03 a	146.61 ± 5.19 b	993.94 ± 201.27 c	40.51 ± 2.96 b
Sorbitol 0.1% + CA	0.089 ± 0.009 a	10.95 ± 2.03 a	710.61 ± 21.05 a	149.01 ± 5.63 b	1467.61 ± 269.74 b	39.73 ± 3.07 b
Sorbitol 2.5% + CA	0.091 ± 0.013 a	11.21 ± 1.83 a	767.00 ± 38.12 b	168.54 ± 7.07 a	2157.11± 180.60 a	35.50 ± 2.80 c
Storage – days (B)						
0	0.077 ± 0.010 b	11.67 ± 0.40 a	743.34 ± 56.96 a	121.42 ± 6.02 c	899.97 ± 103.61 d	22.34 ± 2.18 c
10	0.095 ± 0.014 a	11.82 ± 0.63 a	826.27 ± 78.32 a	194.29 ± 7.17 a	1902.09 ± 210.43 a	26.25 ± 4.03 c
20	0.094 ± 0.007 a	10.35 ± 0.27 a	681.43 ± 56.95 b	165.05 ± 5.94 b	1555.68 ± 343.08 b	45.34 ± 3.11 b
30	0.086 ± 0.009 a	10.06 ± 0.53 a	648.24 ± 41.52 b	123.05 ± 4.73 c	1240.36 ± 192.06 c	67.89 ± 3.62 a
Interactions						
AxB	NS	NS	NS	4.73**	7.14**	NS

Means followed by the same letter within each column do not differ statistically from each other by the Tukey test (P<0.05). Non-significant interaction (NS), significant interaction at P<0.05 (**) and significant interaction at P <0.01 (*).

Table 9. Effect of sorbitol (0.1 and 2.5%) immersion and controlled atmosphere (CA) on vitamin C (AsA), total polyphenols (TP), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and polyphenol oxidase (PPO) 'Palmer' mango peel stored at 8.0 ± 0.5 ºC for 30 d and transfer to ambient (23 ± 2 ºC) for 7 more days.

Principal effects	AsA (g kg^{-1})	TP (g kg^{-1} GAE)	SOD (U min^{-1} $kg^{-1}10^6$)	CAT (mol H_2O_2 min^{-1} kg^{-1})	APX (mol H_2O_2 min^{-1} kg^{-1})	PPO (U min^{-1} $kg^{-1}10^6$)
Treatments (A)						
Control	0.14 ± 0.011 c	57.91 ± 0.83 c	919.49 ± 35.53 a	68.38 ± 4.52 a	275.01 ± 23.03 a	1650.73 ± 2129.06 a
Control + CA	0.20 ± 0.015 b	60.82 ± 1.04 b	849.04 ± 18.23 a	66.70 ± 5.11 a	296.83 ± 31.06 a	1264.52 ± 208.73 b
Sorbitol 0.1% + CA	0.18 ± 0.016 b	63.83 ± 1.72 a	854.93 ± 19.11 a	63.12± 6.07 a	291.18 ± 29.65 a	1334.65 ± 184.17 b
Sorbitol 2.5% + CA	0.23 ± 0.013 a	62.42 ± 0.90 a	812.64 ± 21.06 b	57.45 ± 4.90 b	245.75 ± 27.32 b	839.96 ± 151.11 c
Storage – days (B)						
0+10	0.21 ± 0.018 ab	67.67 ± 0.76 a	823.58 ± 10.76 b	40.81 ± 5.19 c	181.45 ± 30.65 c	563.87 ± 104.93 d
10+7	0.24 ± 0.009 a	65.61 ± 1.52 a	921.31 ± 17.04 a	77.53 ± 6.08 a	294.88 ± 23.34 b	924.72 ± 203.18 c
20+7	0.18 ± 0.021 b	62.70 ± 0.87 b	882.93 ± 11.16 a	83.08 ± 5.95 a	371.16 ± 37.41 a	1360.09 ± 161.04 b
30+7	0.11 ± 0.015 c	49.00 ± 1.17 c	817.03 ± 13.49 b	55.24 ± 5.81 b	276.26 ± 27.04 b	2241.00 ± 121.99 a
Interactions						
AxB	5.25**	8.20**	7.91**	5.84**	6.17**	8.63**

Means followed by the same letter within each column do not differ statistically from each other by the Tukey test (P<0.05). Non-significant interaction (NS), significant interaction at P<0.05 (**) and significant interaction at P <0.01 (*).

Table 10. Effect of sorbitol (0.1 and 2.5%) immersion and controlled atmosphere (CA) on vitamin C (AsA), total polyphenols (TP), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and polyphenol oxidase (PPO) 'Palmer' mango pulp stored at 8.0 ± 0.5 ºC for 30 d and transfer to ambient (23 ± 2 ºC) for 7 more days.

Principal effects	**AsA (g kg^{-1})**	**TP (g kg^{-1} GAE)**	**SOD (U min^{-1} $kg^{-1}10^6$)**	**CAT (mol H_2O_2 min^{-1} kg^{-1})**	**APX (mol H_2O_2 min^{-1} kg^{-1})**	**PPO (U min^{-1} $kg^{-1}10^6$)**
Treatments (A)						
Control	0.065 ± 0.006 c	6.86 ± 0.67 c	378.50 ± 6.91 b	121.43 ± 10.75 c	309.15 ± 26.08 b	895.60 ± 75.90 c
Control + CA	0.083 ± 0.008 b	7.93 ± 0.42 b	383.64 ± 8.00 b	140.37 ± 7.61 b	341.32 ± 36.93 b	794.36 ± 93.65 b
Sorbitol 0.1% + CA	0.076 ± 0.006 b	6.88 ± 0.73 c	389.26 ± 5.81 b	132.56 ± 9.18 c	358.71 ± 31.11 b	785.88 ± 67.07 b
Sorbitol 2.5% + CA	0.095 ± 0.010 a	9.79 ± 0.81 a	469.33 ± 7.13 a	171.10 ± 11.40 a	494.20 ± 43.00 a	631.00 ± 74.83 a
Storage – days (B)						
0+10	0.083 ± 0.012 b	8.22 ± 0.74 a	418.71 ± 9.71 c	167.38 ± 9.15 b	466.27 ± 33.25 b	575.41 ±86.04 c
10+7	0.095 ± 0.007 a	7.87 ± 0.37 b	508.03 ± 11.63 a	198.62 ± 7.28 a	518.08 ± 28.92 a	664.19 ± 104.91c
20+7	0.081 ± 0.008 b	7.73 ± 0.61 b	435.29 ± 8.84 b	143.02 ± 11.01 c	342.92 ± 41.67 c	812.96 ± 100.62 b
30+7	0.060 ± 0.013 c	6.80 ± 0.59 a	258.50 ± 10.02 d	56.44 ± 6.86 d	175.12 ± 57.21 d	1054.28 ± 85.03 a
Interactions						
AxB	8.89**	5.45**	6.07**	6.63**	5.18**	7.88**

Means followed by the same letter within each column do not differ statistically from each other by the Tukey test (P<0.05). Non-significant interaction (NS), significant interaction at P<0.05 (**) and significant interaction at P <0.01 (*).

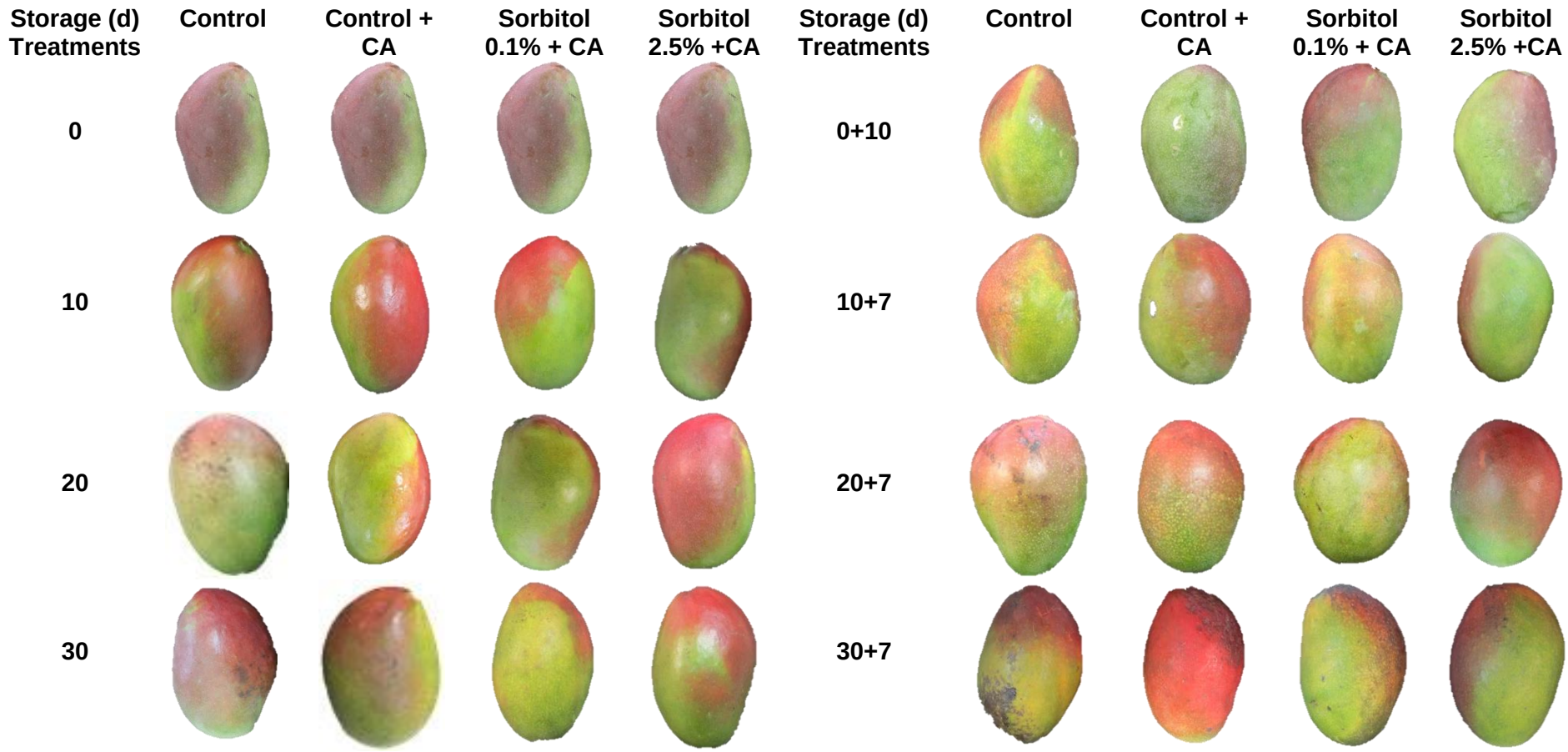


Figura 1S1: Development of chilling injury (CI) in 'Palmer' mangoes (epicarp) treated with sorbitol (0.1 and 2.5%), stored in a controlled atmosphere and kept under refrigeration (8.0 ± 0.5 ºC) for 30 days followed by transfer to the environment (23 ± 2 ºC) for 7 days.

CAPÍTULO 6 – Considerações finais

O tratamento por imersão de mangas ‘Palmer’ em soluções contendo polióis, especificamente o sorbitol (0,1 e 2,5 %) reduz a incidência de injúrias pelo frio (IF) durante o armazenamento a baixas temperaturas (1,0 e 8,0 ºC), sem comprometer a qualidade das frutas. Esse potencial de conservação pode ser atribuído ao papel umectante, texturizante e edulcorante exercido por este aditivo reconhecido como seguro e amplamente utilizado na indústria alimentícia.

Neste estudo, a tolerância ao frio nas frutas tratadas com sorbitol foi atribuída a redução na perda de massa fresca e na ativação dos mecanismos de defesa antioxidante. Na teoria, a função umectante e texturizante do sorbitol teriam preservado a integridade das membranas restringindo a perda de água e retardando processos fisiológicos causados pelo estresse da baixa temperatura. No metabolismo oxidativo, o sorbitol, como uma molécula de açúcar, é um excelente osmólito e pode ter atuado como fonte de energia e ou sinalização ao acumulo de ERO através da ativação dos mecanismos de defesa antioxidante (enzimático e não enzimático) reduzindo com isso a incidência de IF (Figura 2).

A associação do sorbitol (2.5 %) com a atmosfera controlada (5 % O_2 e 5% CO_2) inibiu o desenvolvimento da IF durante o armazenamento refrigerado (8,0 ºC) por 30 dias e atrasou o amadurecimento sob condições ambiente (~23 ºC) por até 20+7 dias. Na prática, esse efeito combinado (sorbitol + AC) abre novas perspectivas para o prolongamento do armazenamento pós-colheita da manga resultando em benefícios tecnológicos, socioambientais e econômicos na cadeia de comercialização da fruta.

Todavia, como o foco da pesquisa foi o metabolismo oxidativo, pesquisas futuras devem ser direcionadas para compreender como o efeito sinérgico dessas tecnologias de conservação coordenam mecanismos fisiológicos e bioquímicos relacionados ao amadurecimento dos frutos da mangueira. Além disso, seria útil no futuro realizar a quantificação do sorbitol nos tecidos da casca e polpa, testar concentrações maiores e utilizar abordagens de proteômica, metabolômica, atividade de enzimas e expressão de genes envolvidos no metabolismo de açúcar, para tentar compreender como este poliol está envolvido na ativação desses mecanismos

antioxidantes e na indução da tolerância ao resfriamento afim de embasar de maneira sólida essa tecnologia na pós-colheita de frutos e hortaliças.

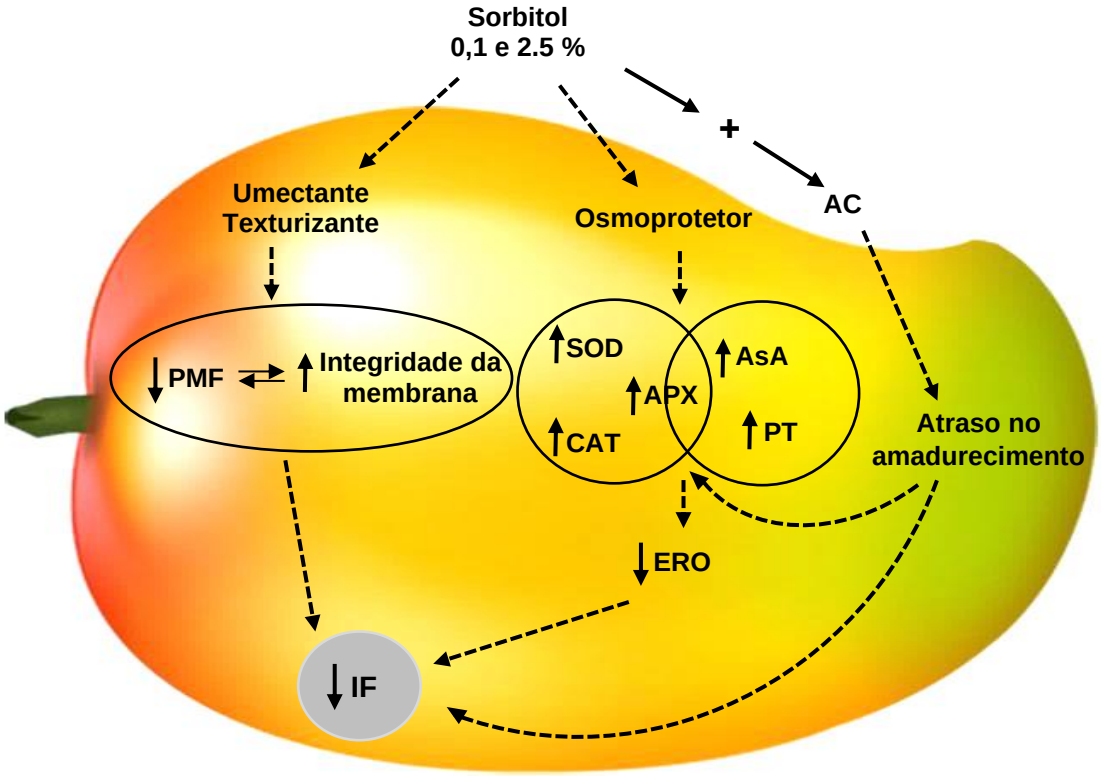


Figura 2. Modelo proposto para explicar o mecanismo de ação do sorbitol (0,1 e 2,5 %) e da associação com a AC (5% O_2 e 5 % CO_2) sobre o metabolismo antioxidante e a incidência de lesões pelo frio em mangas 'Palmer' armazenadas a 1,0 e 8,0 ºC por 28 dias seguido da seguido da transferência para o ambiente (23 ± 2 ºC) por mais 7 dias. AC: atmosfera controlada; PMF: perda de massa fresca; SOD: superóxido dismutase; APX: ascorbato peroxidase; CAT: catalase; AsA: ascorbato; PT: polifenóis totais; ERO: espécies reativas de oxigênio; IF: injúria pelo frio.