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MEMBRANE FATTY ACIDS AND PHYSIOLOGICAL DISORDERS IN COLD STORED 'GOLDEN DELICIOUS' APPLES TREATED WITH 1-MCP AND CALCIUM CHLORIDE

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Abstract

The present research intents to study the evolution of the skin fatty acids and physiological disorders through cold storage in 'Golden Delicious' apples treated with 1-MCP and calcium. Harvested fruit were treated with calcium chloride (Ca), 1-MCP (MCP), Ca+MCP or no treatment (control) then subjected to cold storage at 0.5 ºC for 6 months. Fatty acids composition, Malondialdehyde (MDA) and the physiological disorders bitter pit (BP), superficial scald and diffuse skin browning (DSB) were measured at harvest and after storage plus 7 days shelf-life at room temperature ≈22 ºC. Palmitic acid decreased and linoleic acid increased through time, while oleic and stearic acids had few changes. Unsaturated/saturated fatty acids and MDA increased through time, despite Ca and Ca+MCP were related to lower MDA and lower BP and rotten fruit, after cold storage and shelf-life. In those treatments, the unsaturated/saturated fatty acids were higher, mainly due to higher linoleic acid and lower palmitic acids. Further research is needed to clarify the changes in membrane properties and the effect of some treatments in response to chilling injury through storage.

Keywords: Quality, MDA, Bitter pit, superficial scald, chilling

1. Introduction

To increase postharvest life of fresh fruits, cold storage is the first issue combined or not with controlled atmosphere. During storage, postharvest losses occur due to mechanical damage, diseases and physiological disorders [1,2]. Fresh apple marketing is an important subject

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worldwide. Apples (*Malus domestica* Borkh) can develop many physiological disorders during cold storage at 0 ºC, being one of the most prominent the bitter pit (BP) together with superficial scald, causing significant losses to apple growers worldwide [3,4]. The BP occurs mainly during the period of cold storage and is characterized by black spots in the pulp, which dehydrate with time and form depressions in the skin of the fruit reducing the marketability and quality of apple [5].

The calcium (Ca), in low concentrations in fruits, favors the formation of injuries that progress to death of the tissues, leading to the BP. This is because Ca has a role in the selective permeability, the structure and functionality of the cell membranes, by monogalactosyl diacylglycerols and phospholipids connections on the membrane surface [6]. Thus, the positive effects of Ca in preserving postharvest quality have been attributed to the fact that it is associated with the pectic substances of the middle lamella and cell membranes, stiffening tissues and preserving the characteristics of selective permeability in cell membranes system [5,7].

Scald is also a physiological disorder appearing in the skin after long term cold storage in apples and pears, which is related to the synthesis of α-farnesene and the accumulation of its conjugated trienols in the fruit epidermis and hypodermis. This event causes rupture of the cell membranes, leading to polyphenoloxidase mediated browning of the fruit peel [2,3,8,9].

Fatty acids and lipids are important structural and metabolic constituents of plant/fruit cells. Disturbances in membrane lipid composition often have severe consequences on the ability of the cell to adapt to extreme temperatures and other stress conditions which in fruit, may led to various storage physiological disorders [10,11].

The oxidative damage is the initial response of tissues to chilling. The production and accumulation of reactive oxygen species (ROS) is responsible for the lipid oxidation. The end product of poly-unsaturated fatty acid oxidation is the toxic product malondialdehyde (MDA) [12].

Ethylene, known as the ripening hormone, triggers a series of biochemical changes that culminate in the ripening and senescence of fruits. The volatile compound 1-methylcyclopropene (1-MCP) binds permanently to ethylene receptors in fruit tissue [1,13] and prevents his action [14]. Thus, the postharvest application of 1-MCP can maintain the quality of apples, inhibit the growth of certain physiological disorders during storage such as superficial scald, and demonstrate its positive effect on fruit quality preservation, leading to a delay in ripening and improved firmness retention [4,15].

However, 1-MCP treatment may also cause disorders as bitter pit (BP) [2,16,17], which can be reduced by adding Ca to the 1-MCP treatment [4]. Larrigaudière et al. [18] and Gamrasni et al. [19] reported also a disorder known as Difuse Skin Browning (DSB) in 1-MCP treated fruit, which can be misguidedly interpreted as superficial scald, with the appearance of diffuse browning of the skin, but in this case the skin becomes very rough. Interestingly, this disorder

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appears only in countries that have very hot summers and little rainfall. Further, it was found that by progressive cooling and delaying the time after harvest to 1-MCP application may prevent this disorder [18,19]. It was found no induction [2] or few induction [4] of DSB by 1-MCP when application was delayed 3 days after cooling the fruit.

The objective of this work was to investigate the effect of SmartfreshTM (625 $nl.l^{-1}$ 1-MCP) and calcium chloride (1.5%) on the skin membrane fatty acids and their relation with the development of the physiological disorders during cold storage (0.5ºC in normal atmosphere for 6 months) and subsequent shelf life (7 days) at room temperature ≈22 ºC in 'Golden Delicious' apples.

2. Material and Methods

2.1. Plant material and treatments

Apples of the cultivar Golden Delicious were harvested from 10 orchards, located in the west-centre region of Portugal. All the orchards were under commercial management conditions.

Half of the fruit from each producer (4 crates), were immersed for 2 minutes, in a solution containing 1.5% $CaCl_2$ (Calcium Chloride anhydrous 95% PANREAC) and TECTO 500SC (thiabendazole 42.9% p/p–200 mL/100 L water). The other half of fruits, were immersed for 2 minutes in a solution only with TECTO 500SC (thiabendazole 42.9% p/p –200 mL/100 L water). After, all fruit (no treated or treated with $CaCl_2$) were stored in a cold room at 0.5 ºC and after 3 days of storage, half of the fruit treated with $CaCl_2$ and half of the fruit without calcium treatment, were treated with 625 nL/L 1-MCP using Smartfresh™ during 20 hours. Treatments were as follow: Control (no treatment), Ca (treated with $CaCl_2$), MCP (treated with 1-MCP) and Ca+MCP (treated with $CaCl_2$ and 1-MCP). All fruit were stored at 0.5 ºC in normal atmosphere and relative humidity 90-95% for 6 months.

Sampling dates were at harvest (0 d) and 7 days shelf-life at ≈22ºC, and after 6 months storage at 0.5 ºC (0 d post-storage) and more 7 days of shelf life (7d post-storage) at room temperature (≈22ºC). On these dates, fatty acids identification and quantification as well as MDA content were carried out and the incidence of superficial scald, DSB and BP in the apple exocarp were also observed and registered.

2.2. Fatty Acid Identification and Quantification

Fatty acids derived from the same exocarp tissue sample were extracted according to Meyer and Terry [20]. Briefly, 3g of ground lyophilized exocarp tissue was homogenized with hexane and filtered under vacuum through a Fisherbrand QL 100 filter paper (Fisher Scientific, Leics., UK). The solvent from the lipid-containing filtrate was evaporated under vacuum.

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Fatty acid methyl esters (FAMEs) were produced according to the method prescribed by the International Olive Oil Council (IOOC) with modifications. Briefly, 0.2 mL of methanolic KOH (2 N) was added to 0.1 g of apple oil extract in 2 mL of hexane. Hexane was chosen as the preferred solvent due to improved peak resolution. The mixture was shaken vigorously for 30 s and left to stratify until the upper layer became clear. The hexane layer containing the methyl esters was decanted and kept for no more than 12 h at 5 °C until needed. This solution was diluted 1:100 (v/v) with fresh hexane immediately before injection into a Trace 1300 GC (Thermo Scientific, USA) equipped with a G1540N flame ionization detector (FID) and a 7683B autosampler. The identification and quantification of selected compounds were performed using two capillary columns: TG-17MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Thermo Scientific, USA); TG-1MS (30 m × 0.25 mm i.d., 0.25 µm film thickness; Thermo Scientific, USA). Column temperature was programmed at 55°C for 3 min, and then raised to 175°C at 13°C min $^{-1}$, intervals followed by an isothermal period of 1 min, and increased again to a final temperature of 220°C at 8°C min^{-1}. The carrier gas was He at a constant flow rate of 1.6 mL min^{-1}. The injector and detector temperatures were set at 220 and 250 °C, respectively. The presence and abundance of fatty acids were calculated by comparison of peak area as percentage. The unsaturated/saturated fatty acid ratio was calculated by the formula: (18:1 + 18:2)/ (16:0+18:0) where: 16:0 = Palmitic acid; 18:0 = Stearic acid; 18:1 = Oleic acid; 18:2 = Linoleic acid.

2.3. Malondialdehyde (MDA) content

Lipid peroxidation was determined by measuring the MDA content in frozen ground tissue according to the method of Hodges et al. [21] and Lee et al. [22]. Frozen ground apple skin tissues (1.0 g) were homogenized in 8 mL of 80% (v/v) ice cold ethanol and 5% (w/v) insoluble polyvinylpolypyrrolidone (PVPP) with an Ultra-Turax, then centrifuged at 3000×g at 4 ◦C for 10 min in Microfuge® 18 Centrifuge. One aliquot (0.6 mL) was mixed with 0.6 mL of a solution without thiobarbituric acid (−TBA), which consisted of 20% trichloroacetic acid (TCA) and 0.01% butylated hydroxyltoluene (BHT), while the other aliquot was mixed with 0.6 mL of TBA solution (+TBA), which was comprised of the above with 0.65% TBA. After vigorous mixing, the sample was incubated at 95 ◦C for 25 min, cooled down quickly on ice and then centrifuged at 3000×g at 4 ◦C for 10 min.

The absorbance of the sample was recorded at 440, 532 and 600 nm using a spectrophotometer. MDA equivalents were calculated in the following manner:

(1) $[(\text{Abs } 532_{+TBA}) - (\text{Abs } 600_{+TBA}) - (\text{Abs } 532_{-TBA} - \text{Abs}600_{-TBA})] = A$

(2) $[(\text{Abs } 440_{+TBA} - \text{Abs } 600_{+TBA}) \times 0.0571] = B$

(3) MDA equivalent (nmol g^{-1} FW) = $[(A-B/157{,}000)\times 10^6 \times$ (adjusted sample FW) × (buffer volume)]

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2.4. Physiological disorders and rots

Diffuse skin browning (DSB), superficial scald, bitter pit (BP) and rots were visually evaluated on 100 fruits per orchard per treatment, after 6 months cold storage and 7 d shelf-life. The incidence of each disorder or rot was calculated as the percentage of affected fruits compared to the total number of fruit per orchard.

3. Results and Discussion

Four main fatty acids were identified in the peel apple: palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic (18:2) acids (Figs. 1 and 2). Palmitic acid, a saturated fatty acid (SFA) was the one found in higher percentage in the fruit at harvest, nevertheless after 7 days of shelf-life, there was a significant reduction in its percentage (Fig. 1A). After 6 months cold storage, the palmitic acid reduced and was similar for all treatments, keeping these values during posterior shelf-life, except for Ca treated fruit with significantly lower values. Stearic acid, other SFA, significantly reduced in shelf life after harvest, but did not change through cold storage except that decreases significantly in Ca+MCP treated fruit, nevertheless increasing again after 7 days shelf-life post-storage (Fig. 1B).

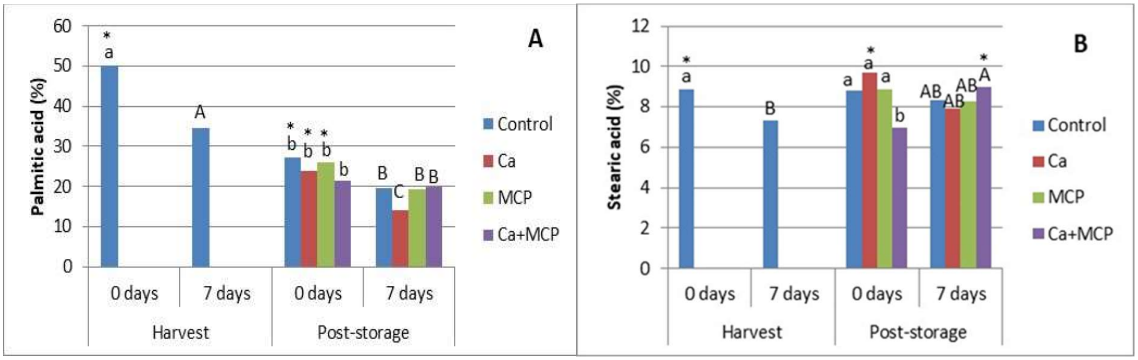

Figure 1. Changes in the saturated fatty acids, palmitic (A) and stearic (B), at harvest and after 6 months storage at 0.5 ºC, and their respective shelf-life (7 days at ≈22 ºC), in 'Golden Delicious' apples subjected to postharvest treatments with calcium chloride (Ca), 1-MCP (MCP), calcium chloride plus 1-MCP (Ca+MCP) and control. Lower case compares treatments at harvest and after 6 months and upper cases compare treatments after shelf-life. Columns with the same lower or upper case are not significantly different by Duncan's Multiple Range Test at $p < 0.05$. *represent significant differences between harvest and its shelf-life or between post-storage and its shelf-life for each treatment, at $p < 0.05$.

The percentage of oleic acid, an important mono-unsaturated fatty acid, increased significantly during the shelf-life after harvest, but it is important to notice that after cold storage, there was a reduction in Ca+MCP fruit, which recovered during the posterior shelf-life as stearic

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acid did (Fig. 2A). The linoleic acid content, an essential poly-unsaturated fatty acid (PUFA), increased after 7 days of shelf-life after harvest (Fig. 2B). After cold storage, the content of linoleic acid continued to increase, being the Ca+MCP treated fruit with higher values than the other treatments. After the shelf-life post-storage, the percentage of linoleic acid continued to increase except for Ca+MCP.

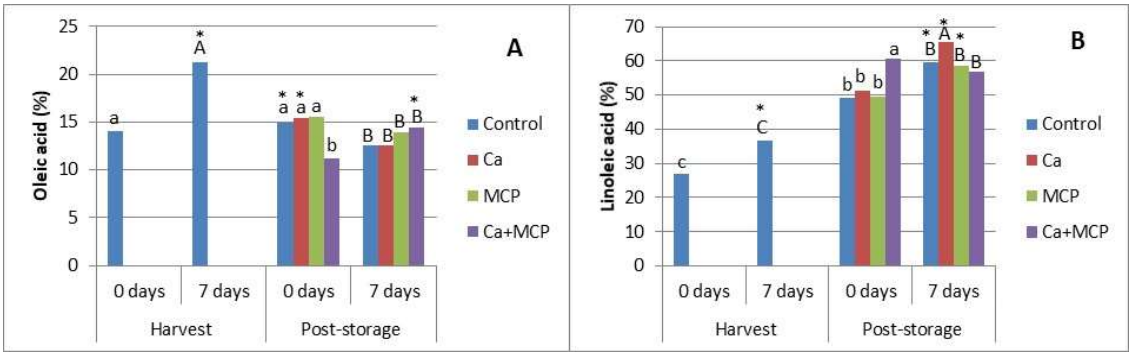

Figure 2. Changes in the unsaturated fatty acids, Oleic (A) and Linoleic (B), at harvest and after 6 months storage at 0.5 ºC, and their respective shelf-life (7 days at ≈22 ºC), in 'Golden Delicious' apples subjected to postharvest treatments with calcium chloride (Ca), 1-MCP (MCP), calcium chloride plus 1-MCP (Ca+MCP) and control. Lower case compares treatments at harvest and after 6 months and upper cases compare treatments after shelf-life. Columns with the same lower or upper case are not significantly different by Duncan's Multiple Range Test at $p < 0.05$. *represent significant differences between harvest and its shelf-life or between post-storage and its shelf-life for each treatment, at $p < 0.05$.

Studies by Wu et al. [23] found that palmitic acid and linoleic acid were the dominant fatty acids in eight commercially harvested apple cultivars, constituting 70–80% of the total fatty acids in the fruits. Similar results were obtained in the present work for 'Golden Delicious' apples, which had at harvest nearly 50% palmitic acid and 27% linoleic acid, nevertheless after shelf-life and after storage, there was an increase in the percentage of linoleic acid and a decrease in the percentage of palmitic acid. As far as the essential fatty acids are concerned, this is of nutritional interest, since the diets rich in unsaturated fatty acids are healthier.

The ratio unsaturated/saturated fatty acids in our experiment increased through time either in storage or shelf-life (Fig. 3A). This is mainly due to the decrease of the saturated palmitic acid and the increase in the PUFA linoleic acid (Figs 1A and 2B). According to our experiments, it appears that Ca treatment has a positive effect on the ratio of unsaturated/saturated fatty acids, since after 6 months storage Ca+MCP showed significantly higher values and after 7 more days the same happened in the Ca treatment (Fig. 3A).

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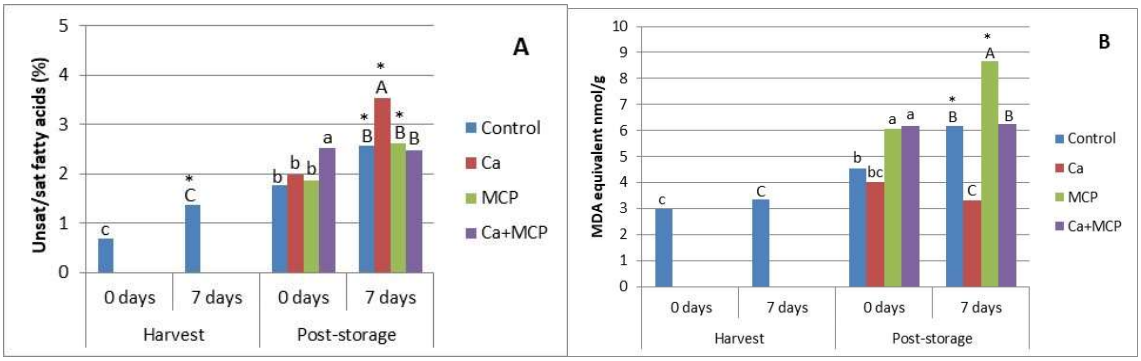

Figure 3. Changes in the unsaturated/saturated fatty acids ratio (A) and MDA equivalent (B) at harvest and after 6 months storage at 0.5 ºC, and their respective shelf-life (7 days at ≈22 ºC), in 'Golden Delicious' apples subjected to postharvest treatments with calcium chloride (Ca), 1-MCP (MCP), calcium chloride plus 1-MCP (Ca+MCP) and control. Lower case compares treatments at harvest and after 6 months and upper cases compare treatments after shelf-life. Columns with the same lower or upper case are not significantly different by Duncan's Multiple Range Test at $p < 0.05$. *represent significant differences between harvest and its shelf-life or between post-storage and its shelf-life for each treatment, at $p < 0.05$.

Phospholipids and fatty acids are metabolic constituents of plant/fruit cells and disturbances in membrane lipid composition frequently have severe consequences on the ability of the cell to adapt to extreme temperatures and other stress conditions leading to several storage physiological disorders [10,11]. Ge at al. [24] and Antunes and Sfakiotakis [25] found increase in unsaturated/saturated fatty acids in cold storage of kiwifruit and bell pepper, respectively. However, in pepper the increase was till 15 days storage at 4 ºC, while chilling injury started to develop after 5 days, nevertheless unsaturated/saturated fatty acids decreased thereafter although chilling injury continued. Gao et al. [26] reported decreased unsaturated/saturated fatty acids as peaches developed chilling injury, while there were no reported unsaturated/saturated fatty acids ratio changes while fruit developing chilling injury in pineapple [27]. It is worthy to notice that the fatty acids used in the formulas to calculate unsaturated/saturated ratio were not always the same in all mentioned researches.

The content of MDA is often used as an indicator of lipid peroxidation stress and cell damage [28,29]. At 7 days shelf life after harvest, apples kept the MDA content similar to that found at harvest, but it increased in storage except for Ca treatment (Fig. 3B). After 6 months storage at 0.5 ºC, MCP and MCP+Ca showed higher MDA content than the other treatments. After 7 days shelf-life post-storage, control and MCP fruit significantly increased their MDA content, being MCP with the highest value and Ca with the lowest. Importantly, treatment with Ca prevented the increase in MDA production by fruits after cold storage and shelf-life, while MCP had the highest increase after storage plus shelf life (Fig. 3B). In almost all cases, the higher value of unsaturated/saturated fatty acids was coincident with the highest production of MDA, which was expected since this end oxidation product results from the PUFA oxidation. Moreover, lower temperatures promote lipid oxidation affecting the structural integrity of the plant

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membrane cells [12]. Interestingly, the higher value of unsaturated/saturated fatty acids obtained after storage plus shelf-life in Ca treatment, was coincident with the lowest MDA (Fig. 3B), which may reveal a positive effect of Ca on the lipid peroxidation.

As in our case, 'Golden Delicious' and 'Fuji' apples in cold storage increased the concentrations of peroxides and MDA [30,31]. However, the latter authors report a reduced MDA content in apples treated with MCP. Nevertheless, those authors reported MDA content on flesh. Our results are on apple peel and the higher MDA found at the end of storage plus shelf-life can be related to the higher BP damage present in fruit treated with MCP (Fig. 3B and Table 1).

Table 1 depicts the effect of the postharvest treatments on the peel physiological disorders occurred after 6 months storage at 0.5 ºC and plus 7 days shelf-life in 'Golden Delicious' apples. As previously stated, 1-MCP treatment significantly increased the percentage of fruit with BP as compared with the other treatments or control [2,4]. Nevertheless, 1-MCP has been applied to apples to increase their storage life and reduce superficial scald as reported before [2,15]. In fact, MCP reduced superficial scald in our apples as compared to the other treatments, although in this experiment superficial scald was not a big problem (Table 1).

Table 1. Physiological disorders and rot in 'Golden Delicious' apples stored for 6 months at 0.5ºC plus 7 days at ≈22 ºC, as percentage of total fruit, subjected to postharvest treatments with calcium chloride (Ca), 1-MCP (MCP), calcium chloride plus 1-MCP (Ca+MCP) and control.

Disorder	Control	Ca	MCP	Ca+MCP
BP (%)	18.61 b	13.95 b	27.21 a	17.74 b
Scald (%)	3.53 a	1.99 a	0.10 a	3.73 a
DSB (%)	2.50 b	5.02 a	0.62 a	1.82 b
Rot (%)	5.56 a	1.15 b	2.56 b	0.80 b

*Rows with the same case are not significantly different by Duncan's Multiple Range Test at $p < 0.05$.

The degree of inhibition of scald by 1-MCP is cultivar dependent. Inhibition is almost total for 'Granny Smith' [32,33]. However, scald inhibition is less consistent for many other cultivars, being affected by factors such as type of storage (air versus controlled atmosphere) and storage length [15,34,35].

Studies by [36] in apples 'Catherine' and 'Fuji' showed that the fruits with BP had lower Ca in the tissues of the skin. Similar results were found in 'Golden Delicious' apples [2,4]. When Ca was added to MCP, the BP was reduced to values similar to that of control (Table 1).

Interestingly, DSB which has been reported in countries with warm summers and low rain fall in fruit treated with 1-MCP [18,19], had higher development in fruit treated only with Ca (Table 1). The same authors report that by decreasing gradually the storage temperature and

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delaying 1-MCP application, this disorder can be avoided. In our experiment, fruit were put in the cold rooms and 1-MCP treatment occurred only after 3 days, which was proved to be effective to reduce DSB development.

A negative correlation between scald and BP was found in our experiment (Table 2) as reported before [37]. There was not found correlation between palmitic, linoleic, oleic, stearic, unsaturated/saturated fatty acids ratio or MDA and any of the peel chilling physiological disorders studied in this experiment (Table 2). Previous authors also, found no correlation between that ratio and chilling in apple [27].

There was a negative correlation between palmitic acid and MDA and a positive correlation between linoleic or unsaturated/saturated fatty acids ratio and MDA (Table 2). A positive correlation between MDA and unsaturated/saturated fatty acids ratio, was observed and expected as above explained. The treatments where calcium was added, significantly increased unsaturated/saturated fatty acids ratio and the MDA content decrease, with a positive effect in reducing BP and rot. Calcium chloride was already considered as having a positive effect on the ROS homeostasis in loquat fruit (*Eribotrya japonica*). The authors suggested that $CaCl_2$ treatment alleviated chilling injury through rising antioxidant enzymes activities and ascorbate-glutathione (AsA-GSH) cycle system to scavenge ROS [38], which could, therefore, prevent the lipid peroxidation.

It can be concluded that there is no clear correlation between the measured fatty acids (palmitic, linoleic, oleic or stearic fatty acids), unsaturated/saturated fatty acids ratio and MDA with the chilling skin physiological disorders BP, scald and DSB in 'Golden Delicious' apples. However, after 6 months storage at 0.5 ºC and plus 7 days shelf-life, treatments with calcium (Ca and Ca+MCP) showed reduced MDA and increased unsaturated/saturated fatty acids ratio due to higher linoleic acid, coincident with reduced BP and rotten fruit. So, there is a positive correlation between unsaturated/saturated fatty acids ratio and MDA, with the exception of the $CaCl_2$ treatment. More research is needed to clarify the properties of the membranes effect on physiological disorders, namely the identification and quantification of other membrane fatty acids evolution through storage.

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Table 2. Pearson's correlations among the different fatty acids, unsat/sat ratio, MDA and chilling physiological disorders

Parameters	Palmitic	Linoleic	Oleic	Stearic	Uns/sat	MDA	BP	Scald	DSB	Rot
Palmitic acid	1	-0.930**	0.106	-0.026	-0.957**	-0.554*	0.151	-0.151	-0.056	-0.083
Linoleic acid	-0.930**	1	-0.440	-0.108	0.944**	0.577*	-0.074	0.076	0.057	0.068
Oleic acid	0.106	-0.440	1	0.239	-0.189	-0.183	-0.166	0.168	0.091	0.118
Stearic acid	-0.026	-0.108	0.239	1	0.014	0.135	0.032	-0.028	0.079	0.066
Unsaturated/saturated (Uns/sat)	-0.957**	0.944**	-0.189	0.014	1	0.639**	-0.199	0.203	0.148	0.178
MDA	-0.554*	0.577*	-0.183	0.135	0.639**	1	0.139	-0.135	0.035	0.004
BP	0.151	-0.074	-0.166	0.032	-0.199	0.139	1	-0.999**	-0.262	-0.450
Scald	-0.151	0.076	0.168	-0.028	0.203	-0.135	-0.999**	1	0.303	0.488*
DSB	-0.056	0.057	0.091	0.079	0.148	0.035	-0.262	0.303	1	0.980**
Rot	-0.083	0.068	0.118	0.066	0.178	0.004	-0.450	0.488*	0.980**	1

*. Significance level $p < 0.05$ (2-tailed).
**. Significance level p <0,01 (2-tailed).

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References

1. Brackmann, A.; Schorr, M.R.W.; Pinto, J.A.V.; Venturi, T.L. Pre-harvest applications of calcium in post-harvest quality of 'Fuji' apples. *Cienc. Rural*, **2010,** 40, 1435-1438. ISSN 0103-8478
2. Gago, C.M.L.; Guerreiro, A.C.; Miguel, G.; Panagopoulos, T.; Sánchez, C.; Antunes M.D.C. Effect of harvest date and 1-MCP (SmartFreshTM) treatment on 'Golden Delicious' apple cold storage physiological disorders. *Postharvest Biol. Technol.,* **2015**, 110, 77–85.
3. Pesis, E.; Ibanez, A.M.; Phu, M.L.; Mitcham, E.J.; Susan, E.; Ebeler, S.E.; Dandekar, A.M.; Superficial scald and bitter pit development in cold-stored transgenic apples suppressed for ethylene biosynthesis. *J. Agric. Food Chem.,* **2009,** 57(7), 2786–2792.
4. Gago, C.M.L.; Guerreiro, A.C.; Miguel, G.; Panagopoulos, T., Silva, M.M.; Antunes M.D.C. Effect of calcium chloride and 1-MCP (SmartfreshTM) postharvest

treatment on 'Golden Delicious' apple cold storage physiological disorders. *Sci. Hortic.,* **2016,** 211, 440–448.

5. Ferguson, I.B.; Watkins, C.B.; Bitter-pit in apple fruit. *Hortic. Rev.,* **1989,** 11, 289-355.
6. Freitas, S.T.; Amarante, C.V.T.; Labavitch, J.M.; Mitcham, E.J. Cellular approach to understand bitter pit development in apple fruit. *Postharvest Biol. Technol.,* **2010,** 57, 6‑13.
7. Poovaiah, B.W.; Glenn, G.M.; Reddy, A.S.N.; Calcium and fruit softening: physiology and biochemistry. *Hortic. Rev.,* **1988,** 10, 107-152. https://doi.org/10.1002/9781118060834.ch4
8. Lurie, S.; Watkins, C.B.; Superficial scald, its etiology and control. *Postharvest Biol. Technol.,* **2012,** 65, 44-60.
9. Gago, C.; Antão, R.; Dores, C.; Guerreiro, A.; Miguel, M.G.; Faleiro, M.L.; Figueiredo, A.C.; Antunes, M.D.; The effect of nanocoatings enriched with essential oils on 'Rocha' pear long storage. *Foods,* **2020,** 9, 240, 1-15.
10. Marangoni, A. G.; Palma, T.; Stanley, D. W.; Membrane effects in postharvest physiology. *Postharvest Biol.Technol.,* **1996,** 7, 193-217.
11. Saquet, A.A.; Streif, J. ; Bangerth, F.; Changes in ATP, ADP and pyridine nucleotide levels related to the incidence of physiological disorders in 'Conference' pears and 'Jonagold' apples during controlled atmosphere storage. *J. Hortic. Sci. Biotechnol.,* **2000,** 75(2), 243-249.
12. Imahori, Y.; Takemura, M.; Bai, J.; Chilling-induced oxidative stress and antioxidant responses in mume (*Prunus mume*) fruit during low temperature storage. *Postharvest Biol.Technol.,* **2008,** 49, 54-60.
13. Hall, A.E.; Findell, J.L.; Schaller, G.E.; Sisler, E.C.; Bleecker, A.B.; Ethylene perception by the ERS1 protein in Arabidopsis. *Plant Physiol.,* **2000,** 123, 1449–1457.
14. Ambaw, A.; Beaudry, R.; Bulens, I.; Delele, M.A.; Q. Ho, T.; Schenk, A.; Nicolaï, B.M.; Verboven, P. Modeling the diffusion–adsorption kinetics of 1-methylcyclopropene (1-MCP) in apple fruit and non-target materials in storage rooms. *J. Food Eng.,* **2011,** 102, 257-265.
15. Rupasinghe, H.P.V.; Murr, D.P.; Paliyath, G.; Skog, L. Inhibitory effect of 1-MCP on ripening and superficial scald development in `McIntosh' and `Delicious' apples. *J. Hort. Sci. Biotechnol*., **2000,** 75(3), 271-276.
16. Calvo, G.; Candan, A.P.; 1-Methylcyclopropene (1-MCP) affects physiological disorders in 'Granny Smith' apples depending on maturity stage. *Acta Hort.,* **2010**, 857, 63-69.
17. Larrigaudière, C.; Ubach, D.; Soria, Y.; Recaenses, I.; Biochemical changes in 1-MCP treated skin tissue during cold storage and their relationship with physiological disorders. *Acta Hort.,* **2008,** 796, 119-123.

18. Larrigaudière, C.; Rosa Vilaplana, R.; Recasens, I.; Soria, Y.; Dupille, E.; 'Diffuse skin browning' in 1-MCP-treated apples: etiology and systems of control. *J. Sci. Food Agric.* **2010,** 90(14), 2379-2385.

19. Gamrasni, D.; Nerya, O.; Tsvilling, A.; Gizis, A.; Maoz-Katz, M.; Ben-Arie, R.; The complexity of preventing diffuse skin browning (DSB) on 1-MCP (Smartfresh) -treated 'GoldenDelicious' apples. *Acta Hort.,* **2010,** 877, 507-511.

20. Meyer, M.D.; Terry, L.A.; Development of a rapid method for the sequential extraction and subsequent quantification of fatty acids and sugars from avocado mesocarp tissue. *J. Agric. Food Chem.,* **2008**, 56(16), 7439–7445.

21. Hodges, D.M.; DeLong, J.M.; Forney, C.F.; Prange, R.K.; Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta*, **1999,** 207, 604–611.

22. Lee, J.; Cheng, L.; Rudell, D.R.; Watkins, C.B.; Antioxidant metabolism of 1-methylcyclopropene (1-MCP) treated 'Empire' apples during controlled atmosphere storage. *Postharvest Biol. Technol.,* **2012,** 65, 79-91.

23. Wu, J.; Gao, H.; Zhao, L.; Liao, X.; Chen, F.; Wang, Z.; Hu, X.; Chemical compositional characterization of some apple cultivars. *Food Chem.,* **2007,** 103(1), 88-93.

24. Ge, W.; Kong, X.; Zhao, Y.; Wei, B.; Zhou, Q.; Ji, S.; Insights into the metabolism of membrane lipid fatty acids associated with chilling injury in post-harvest bell peppers. *Food Chem.,* **2019,** 295, 26-35.

25. Antunes, M.D.C.; & Sfakiotakis, E.M. Changes in fatty acid composition and electrolyte leakage of 'Hayward' kiwifruit during storage at different temperatures. *Food Chem.,* **2008,** 110(4), 891-896.

26. Gao, H.; Lu, Z.M.; Yang, Y.; Wang, D.N.; Yang, T.; Cao, M.M.; Cao, W. Melatonin treatment reduces chilling injury in peach fruit through its regulation of membrane fatty acid contents and phenolic metabolism. *Food Chem.,* **2018,** 245, 659–666.

27. Nukuntornprakit, O.; Chanjirakul, K.; Doorn, K.G.; Siriphanich, J.; Chilling injury in pineapple fruit: Fatty acid composition and antioxidant metabolism. *Postharvest Biol.Technol.,***2015,** 99, 20-26.

28. Smirnoff, N.; Antioxidant systems and plant response to the environment. In N. Smirnoff (Ed.), Environment and plant metabolism: Flexibility and acclimation, Oxford: Bios Scientific Publishers, **1995,** pp. 243–317.

29. Xu, F.; Liu, S.; Control of postharvest quality in blueberry fruit by combined 1-methylcyclopropene (1-MCP) and UV-C irradiation. *Food Bioproc. Technol.,* **2017,** 10, 1695–1703.

30. Du, Z. ; Bramlage, W.J. Peroxidative activity of apple peel in relation to development of poststorage disorders. *HortSci.,* **1995,** 30(4), 205-209.

31. Chen, H-Y.; Jiang, L-F.; Zeng, J-H.; Huo, Y-R.; Li, Y-X.; Combination of carnauba wax-based coating and 1-methylcyclopropene (1-MCP) maintains better “Fuji” apple qualities during storage at low temperature. *J. Food Process Preserv.,* **2020,** 44, 1-8.
32. Lurie, S.; Lers, A.; Shacham, Z.; Sonego, L.; Burd, S.; Whitaker, B.; Expression of α-farnesene synthase AFS1 and 3-hydroxy-3-methylglutaryl-coenzymea reductase HMG2 and HMG3 in relation to α-farnesene and conjugated trienolsin ‘Granny Smith’ apples heat or 1-MCP treated to prevent superficial scald. *J.Am. Soc. Hortic. Sci.,* **2005,** 130, 232–236.
33. Magazin, N.; Gvozdenovic, D.; Keserovic, Z.; Milic, B.; Fruit quality of Granny Smith apples picked at different harvest times and treated with 1-MCP. *Fruits,* **2010,** 65(3), 191 – 197.
34. Bai, J.; Baldwin, E.A.; Kevin L. Goodner, K.L.; Mattheis, J.P.; Brecht, J., K.; Response of four apple cultivars to 1-methylcyclopropene treatment and controlled atmosphere storage. *HortSci.,* **2005,** 40(5), 1534-1538.
35. Watkins, C.B.; Nock, J.F.; Effects of delays between harvest and 1-methylcyclopropene treatment, and temperature during treatment, on ripening of air-stored and controlled atmosphere-stored Apples. *HortSci.,* **2005,** 40(7), 2096-2101.
36. Miqueloto, A.; Amarante, C.V.T., Steffens, C.A.; Santos, A.D., Miqueloto, T.; Silveira, J.P.G.; Physiological, physical, chemical and mineral attributes associated with the occurrence of "bitter pit" in apples. *Pesqui. Agropecu. Bras.,* **2011,** 46(7), 689-696.
37. Shoffe, Y.A.; Nock, J.F.; Baugher, T.A.; Marinic, R.P.; Watkins, C.B.; Bitter pit and soft scald development during storage of unconditioned and conditioned ‘Honeycrisp’ apples in relation to mineral contents and harvest indices. *Postharvest Biol.Technol.,* **2020,** 160, 111044.
38. Hou, Y.; Li, Z.; Zheng, Y.; Jin, P.; Effects of $CaCl_2$ treatment alleviates chilling injury of loquat fruit (*Eribotrya japonica*) by modulating ROS homeostasis. *Foods,* **2021,** 10, 1662.