Effect of combined application of 1-MCP and low oxygen treatments on alleviation of chilling injury and lipid oxidation stability of avocado (*Persea americana* Mill.) under low temperature storage

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Abstract -- **Introduction**. The avocado (*Persea americana* Mill.) fruit is sensitive to chilling injury (CI) when exposed to low temperatures. High lipid content in avocado pulp makes it prone to oxidation, resulting in rancidity and subsequent production of undesirable flavours and quality loss during storage. **Materials and methods**. Avocado fruit (cv. Becon) were treated with 1 $\mu L \cdot L^{-1}$ 1-MCP for 24 h at 20 °C, and thereafter stored at 4 °C for 21 d under low oxygen atmosphere (3.5% O_2), followed by transferring to 20 °C for 14 d to simulate the shelf life. The CI index, firmness, peel colour, relative electrical conductivity (EC), respiration, ethylene evolution, malondialdehyde (MDA) content, peroxidase (POD) activity, peroxide value and iodine value were measured throughout the storage period. **Results and discussion**. The CI incidence and severity of fruit treated with a combination of 1-MCP and low oxygen were significantly lower and delayed compared with the other treatments. The combined application of 1-MCP and low oxygen treatment was effective and delayed the onset of climacteric peaks of respiration and ethylene production. The delay was associated with reductions in fruit softening and cell membrane permeability as expressed by EC. The results of MDA content and POD activity of fruit treated with a combination of 1-MCP and low oxygen were significantly reduced. Moreover, significantly lower peroxide values and higher iodine values suggested that a combination of 1-MCP and low oxygen treatment effectively controlled the lipid oxidation in avocado fruit pulp. **Conclusion**. Overall, the results support the hypothesis that combined treatment (as compared with either 1-MCP or low oxygen treatment) was the most effective for alleviation of CI, lipid oxidation stability and extending the shelf life of avocado fruit under low temperature storage.

Japan / *Persea americana* / fruits / cooling / plant growth substances / controlled atmosphere storage / lipid peroxidation

Effet de l'application combinée de 1-MCP et d'une faible teneur en oxygène sur la réduction des dégâts dus au froid et la stabilité de l'oxydation des lipides chez l'avocat (*Persea americana* Mill.) stocké à basse température.

Résumé -- **Introduction**. Les fruits de l'avocatier (*Persea americana* Mill.) sont sensibles au froid lorsqu'ils sont exposés à de basses températures. Les hautes teneurs en lipides de la pulpe d'avocat sont sujettes à de l'oxydation ce qui induit du rancissement accompagné de saveurs indésirables et d'une baisse de qualité tout au long du stockage. **Matériel et méthodes**. Des avocats (cv. Becon) ont été traités avec 1 $\mu L \cdot L^{-1}$ 1-MCP pendant 24 h à 20 °C, puis stockés à 4 °C pendant 21 jours sous atmosphère pauvre en oxygène (3,5 % de O_2) avant d'être transférés à 20 °C pendant 14 jours afin de simuler leur durée de conservation. L'indice de sensibilité au froid, la fermeté, la couleur de la peau, la conductivité électrique relative (CE), la respiration, l'évolution de l'éthylène, le malondialdéhyde (MDA), l'activité peroxydase (POD), l'indice peroxyde, et l'indice d'iode ont été mesurés tout au long de la période de stockage. **Résultats et discussion**. L'incidence et la gravité de la sensibilité au froid des fruits traités de façon combinée avec du 1-MCP et une faible teneur en oxygène ont été significativement plus faibles et retardés par rapport aux autres fruits ayant subi les traitements sans combinaison. L'application combinée de 1-MCP et d'une faible teneur en oxygène a été efficace et elle a retardé l'apparition des pics climactériques de la respiration et de la production d'éthylène. Ce retard a été associé à des réductions du ramollissement des fruits et de la perméabilité de la membrane cellulaire exprimée par la conductivité électrique. La teneur en MDA et l'activité POD des fruits traités avec la combinaison de 1-MCP et d'une faible teneur en oxygène ont été considérablement réduites. En outre, l'indice peroxyde significativement plus faible et l'indice d'iode supérieur ont suggéré que la combinaison de 1-MCP et d'une faible teneur en oxygène contrôlaient effectivement l'oxydation des lipides dans la pulpe des fruits d'avocat. **Conclusion**. L'ensemble des résultats soutiennent l'hypothèse que le traitement combiné (par rapport à soit du 1-MCP seul, soit un traitement à faible teneur en oxygène seul) a été le plus efficace pour réduire les dégâts dus à la sensibilité au froid, pour maintenir la stabilité d'oxydation des lipides et pour étendre la durée de vie d'avocats stockés à basse température.

Japon / *Persea americana* / fruits / refroidissement / substance de croissance végétale / stockage en atmosphère contrôlée / péroxydation des lipides

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1. Introduction

The avocado (*Persea americana* Mill.) is an important fruit with a high market value, which has a relatively short storage life. Low temperature storage is utilised to extend its shelf life, but it induces chilling injury (CI) symptoms, as expressed by pulp browning, as well as external damage [1, 2].

Electrolyte leakage and disrupted ion balance resulting from ultra-structural changes in the membranes have been proposed as some of the causes of the development of CI symptoms [3]. Biochemical mechanisms of browning involve the oxidation of phenolic substrates, mediated by polyphenol oxidase (PPO) and peroxidase (POD), resulting in the production of brown pigments [4, 5]. Low temperatures increase the permeability of the cellular reservoir, allowing ions to leak out during exposure to chilling, a phenomenon that is characteristic of many plant tissues that exhibit CI symptoms [6, 7].

High lipid content (15% to 30% depending on the variety) is one of the distinguishing features of avocado fruit [8]. The shelf life of avocado pulp is severely limited by oxidative processes, which affect both lipidic and aqueous fractions. Avocado pulp is sensitive to oxidative processes, resulting in rancidity and subsequent production of undesirable flavours and quality loss during storage [9].

Recently, the ethylene action inhibitor, 1-methylcyclopropene (1-MCP), was found to be effective in overcoming the effects of ethylene in a range of perishable fruits [10]. Application of 1-MCP at low concentrations (average dose 0.1 $\mu L \cdot L^{-1}$) was found to effectively inhibit ethylene-induced ripening of avocado fruit. 1-MCP works by beating ethylene to bind the receptors. 1-MCP binds to the receptors for a substantially longer period than ethylene. Furthermore, unlike ethylene, 1-MCP does not inactivate the receptors. As a result, the cells do not break down, which prolongs the freshness of flowers and fruit after harvest, extends their shelf life, reduces waste and, ultimately, benefits producers and consumers [11].

The lipid oxidation rate and course of reaction are influenced by light, local oxygen concentration, high temperature, the presence of catalysts (generally transition metals such as iron and copper) and water activity. Control of these factors can significantly reduce the extent of lipid oxidation in foods. Controlled atmosphere (CA) storage reduces the lipid oxidation and prolongs the shelf life of avocado [12, 13]. Furthermore, CA can prolong the impact of 1-MCP on both physical and sensory responses of fruit, and both technologies generally are more effective when they are used in combination [14, 15].

The overall objective of the present study was to elucidate the effectiveness of combination of 1-MCP application and low oxygen atmosphere storage on alleviation of CI and control of lipid oxidation of avocado under low temperature storage.

2. Materials and methods

2.1. Fruit preparation and treatments

Avocado fruit (cv. Becon) were provided from the commercial growers Wakayama Prefecture, Japan. Fruit were harvested at their commercial harvest maturity. Then they were transported to the Graduate School of Life and Environmental Sciences at the University of Tsukuba one day after harvest. The fresh weight and volume of individual fruit were measured immediately after arrival and the fruit were divided randomly into four groups according to the following treatments: Group 1: 1-MCP, Group 2: Low oxygen; Group 3: Combined (1-MCP + Low oxygen); Group 4: Control.

The experiment was conducted on a total of 180 samples, with 45 samples for each group. Group 1 was treated with 1-MCP immediately by exposing them to 1-MCP (SmartFresh, Rohm and Hass Japan K.K., Japan, 0.14% active ingredient) at 1 $\mu L \cdot L^{-1}$ concentration for 24 h by placing an open wide-mouth bottle of an aqueous solution of the powder at 30 °C in the bottom of a 59-L glass chamber which was promptly sealed. The chamber was incubated at 20 °C and 90–95% RH; after incubation, fruit were ventilated and stored at 4 °C, and 65–70% RH for

3 weeks. Then fruit were transferred to 20 °C for 2 weeks to simulate shelf life.

The fruit in Group 2 were stored in a 59-L glass chamber and the chamber was stored in a refrigerator at 4 °C and connected to a flowing gas system. Through this gas system, 3.58% O_2 and 96.42% N_2 mixture was applied into the headspace of the chamber at a 100 mL·min^{-1} flow rate. After 3 weeks of storage at 4 °C, the chamber was opened and fruit were ventilated and transferred to 20 °C for 2 weeks to simulate shelf life.

The fruit in Group 3 were immediately treated with 1-MCP at 1 µL·L^{-1} concentration for 24 h at 20 °C. Then, fruit were stored at 4 °C under a low oxygen flowing gas system. After 3 weeks of storage at 4 °C, fruit were transferred to 20 °C for 2 weeks to simulate shelf life.

The control fruit (Group 4) were stored at 4 °C and 65–70% RH without 1-MCP or low oxygen treatments for 3 weeks, and then transferred to 20 °C for 2 weeks to ripen.

Observations were performed on one sample of fruit with three replicates per treatment at 7-day intervals during cold storage and 4-day intervals during room temperature storage. The studied parameters were fruit firmness, peel colour, relative electrical conductivity (EC) of peel, CI index, respiration and ethylene evolution rate, malondialdehyde (MDA) content, peroxidase (POD) activity, and peroxide and iodine values.

2.2. Fruit quality criteria

Fruit firmness was determined by the required pressure to penetrate the avocado fruit through the pulp using the Rheometer (Model NRM-2002J, Fudokogyu Co., Ltd., Japan) connected to a 5-mm-diameter conical-tip plunger, which was individually penetrated to a depth of 10 mm and compressed at a crosshead travelling speed of 50 mm·min^{-1}.

The peel colour of fruit was measured using the Minolta Chroma Meter (Model CR-400, Minolta, Japan). The results were expressed as H° (where the value of 90° represents a totally yellow colour and 180° a totally green colour) [16]. Duplicate measurements were taken of three different positions on each avocado fruit and the average of each position was used; three measurements were taken at the circumference of the avocados.

The membrane integrity was measured by relative electrical conductivity of fruit peel tissues [17]. Three replicated samples were taken from each treatment. Plugs of 20 discs were removed from each fruit with a 10-mm-diameter stainless steel cork borer from various locations of the fruit. Cortex cells were removed with a stainless steel razor blade (the thickness of each was 2 mm). Twenty discs were provided for each fruit; a total of 60 discs were used per treatment. The discs were put into 30 mL of aqueous 0.8 mM mannitol and incubated for 2 h. The conductivity of the solution (EC_o) was measured using a conductivity meter (Model CM-30E, Japan), and the discs were then boiled for 10 min. After cooling, the solution was re-adjusted to a volume of 30 mL before the total conductivity of the solution (EC_T) was measured. The relative electric conductivity (%) was expressed by $[(EC_o / EC_T) \times 100]$ %.

The internal and external chilling injuries (CI) were assessed visually. The CI index in the fruit peel and pulp was scored using % of discoloured surface area on fruit peel and pulp of a cross-section area separately according to a 5-point scale: 0: absent (0%), 1: slight, acceptable marketability (1–25%), 2: moderate, limited marketability (26–50%), 3: high (51–75%), and 4: severe, almost entire fruit surface brown (76–100%). Twenty fruit were randomly sampled each time and the CI index was calculated by multiplying the number of fruit in each category by the respective score, summing the products and dividing by the total number of fruit.

2.3. Determination of respiration and ethylene evolution rate

Individual fruit were weighed and placed in a 1-L airtight container at 20 °C for 1 h. Headspace gas samples were withdrawn with a 1-mL syringe.

The carbon dioxide concentration in the gas samples was determined with a gas chromatograph (Model Shimadzu GC-8A) equipped with a WG-100 column and a thermal conductivity detector. Helium (He) was employed as a carrier gas. Injector and column temperatures were 150 °C and 70 °C, respectively. The carbon dioxide production was expressed as CO_2 mL·kg^{-1} FW·h^{-1}.

To determine the ethylene evolution rate, fruit were weighed and sealed in a 1-L airtight container for 1 h at 20 °C and ethylene evolution was analysed by injecting 1 mL of headspace gas into a gas chromatograph (Model Shimadzu GC-18A) equipped with a Porapak Q (Mesh 60/80) column and flame ionisation detector. The carrier gas was helium (He). Injector, column and detector temperatures were 75 °C, 75 °C and 120 °C, respectively. The ethylene evolution was expressed as C_2H_4 µL·kg^{-1} FW·h^{-1}.

2.4. Determination of malondialdehyde (MDA) content

Thiobarbituric acid-reactive compounds were determined using the thiobarbituric acid reaction (TBA) [18, 19]. Ten grams of tissue were collected from each replicate and homogenised in 25 mL of 100 mM sodium phosphate buffer (pH 6.4%) containing 0.5 g polyvinylpolypyrrolidone (PVPP). The homogenate was filtered through cotton cloth and then centrifuged at 27,000 × *g* for 50 min at 4 °C and the resulting supernatants were used directly for assay. The MDA content was determined by adding 2 mL of 0.5% TBA in 15% trichloroacetic acid (TCA) to a 1-mL sample. The mixture was heated at 95 °C for 20 min and cooled immediately, and the absorbance of the supernatant was read at 532 nm and 600 nm. The level of TBA equivalents (nmol·g^{-1} FW) was equal to {[(A532–A600) / 155,000] × 106}.

2.5. Extraction and assay of peroxidase (POD)

For analysis of enzymatic activities, flesh and pulp tissues (5.0 g) from each fruit were homogenised with 12 mL of 50 mM potassium phosphate buffer (pH 7) containing 1% (w/v) polyvinylpolypyrrolidone. After centrifugation for 30 min at 200,000 × *g* and 4 °C, the supernatant was collected and used as the crude enzyme extract.

Peroxidase (POD) activity using guaiacol as a substrate was assayed by the method of Chen and Wang [20] in a reaction mixture (3 mL) containing 0.05 mL enzyme solution, 2.75 mL of 50 mM phosphate buffer (pH 7.0), 0.1 mL of 1% H_2O_2 and 0.1 mL of 4% guaiacol. The increase in absorbance at 470 nm due to the guaiacol oxidation was recorded for 2 min. One unit of enzymatic activity was defined as the amount of the enzyme that caused a change of 0.001 in absorbance per minute. The specific POD activity was expressed as units·g^{-1} FW.

2.6. Avocado oil extraction and assay of peroxide and iodine values

Avocado pulp was heated to 60 °C for 30 min and periodically stirred to improve mechanical and enzymatic destruction of oil cells. Next, avocado paste was centrifuged at 22,100 × *g* for 30 min at 4 °C. The supernatant phase was separated from the aqueous phase, and oil samples were filtered to remove impurities.

Peroxide value was assayed according to the method proposed by Garcia *et al.* [21] with a slight modification for avocado oil. Two grams of avocado oil sample from each replicate were placed in a 250-mL Erlenmeyer flask, which was previously purged with nitrogen. The sample was shaken and dissolved in 25 mL of an [acetic acid:chloroform] solution (3:2, v/v). Next, 1 mL of saturated potassium iodide (KI) solution was added, and the flask was placed in darkness for 5 min. After that period, 75 mL of distilled water were added, and the mixture was titrated with 0.005 N sodium thiosulphate with a 1% (w/v) starch indicator solution. Results were expressed in milli-equivalents of oxygen per kilogram of avocado oil (mEq of O_2·kg^{-1} oil).

Iodine value was assessed using the Wijs method [22]. An accurately weighed 200-mg oil sample was dissolved in a 300-mL glass-stoppered flask containing 15 mL of carbon

tetrachloride, and 25.0 mL of Wijs reagent was pipetted into the flask. The flask was swirled and put in darkness for 1 h. Subsequently, 20 mL of 10% (w/v) KI and 150 mL of distilled water were added. The excess iodine was titrated with 0.1 N sodium thiosulphate using a 1% (w/v) starch indicator solution. Results were expressed in grams of iodine per 100 g of avocado oil.

2.7. Statistical analysis

The treatments were arranged in a completely randomised design (CRD). Data were analysed statistically by ANOVA and difference between means was compared using the least significant difference (LSD) at $P \leq 0.05$. Each treatment was composed of three replicates except for the chilling injury index, for which 20 individual fruits per treatment were used.

3. Results and discussion

3.1. Firmness

When the experiment was started, firmness of fruit was higher than 20 N (*figure 1*). During storage at 4 °C for 3 weeks, fruit firmness decreased slowly. When fruit were transferred to 20 °C, the control fruit softened markedly. However, fruit treated with 1-MCP, low oxygen and combined treatment maintained a high level of firmness after being transferred to 20 °C. When compared with 1-MCP and low oxygen treatment, combined treatment was most effective on firmness retention of the 'Becon' avocado cultivar to delay the ripening.

3.2. Colour

The 'Becon' avocado cultivar was a yellow-green colour when the experiment was started (*figure 1*). During 3 weeks of storage at 4 °C and following 8 days of storage at 20 °C, the H° value decreased slightly, but the differences among 1-MCP, low oxygen, combined treatment and control fruit were not significant ($P \leq 0.05$). In the later

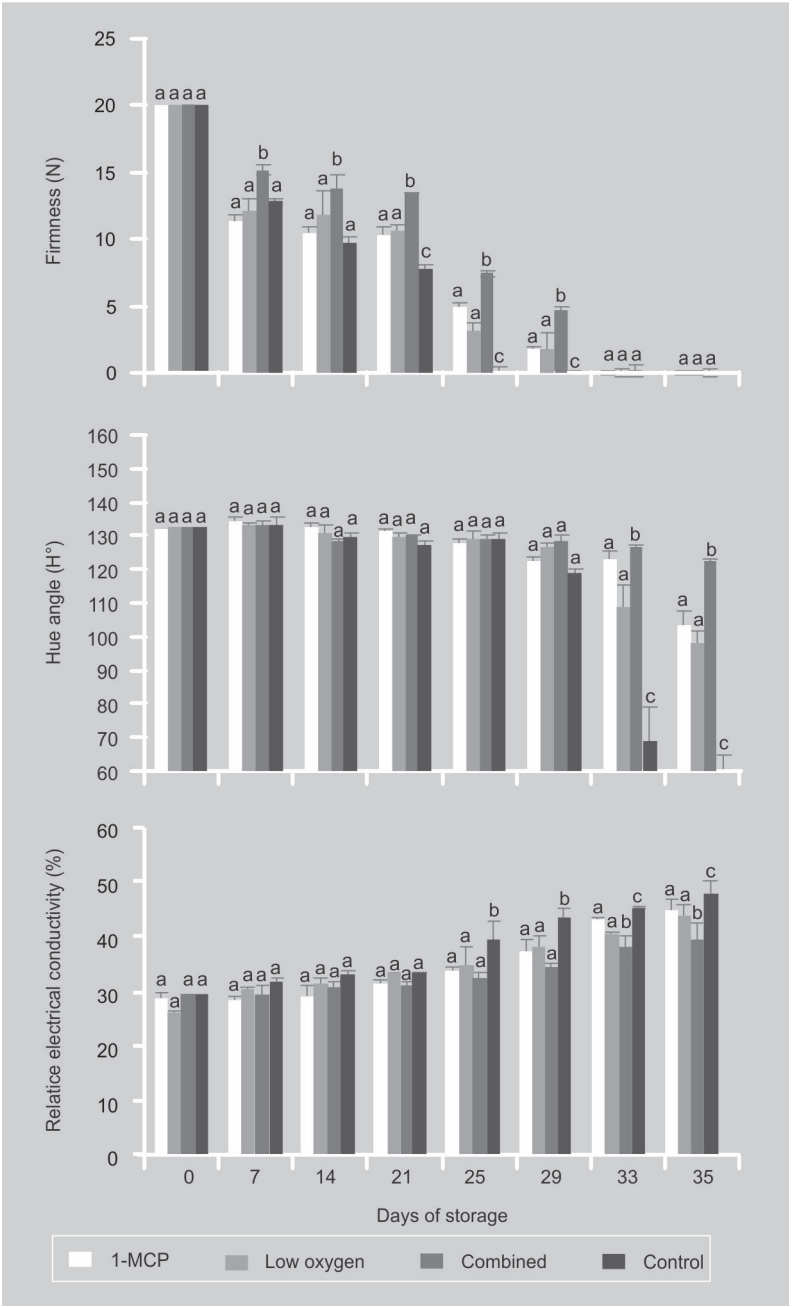

Figure 1.
Fruit firmness, Hue angle value of peel colour and relative electrical conductivity of peel of 1-MCP, low oxygen, combination of 1-MCP and low oxygen treatments and untreated control 'Becon' avocado cultivar stored for 3 weeks at 4 °C, followed by 2 weeks at 20 °C. Different letters indicate significant differences among means according to the least significant difference (LSD) at $P \leq 0.05$; vertical bars represent standard deviation (± SD, $n = 3$).

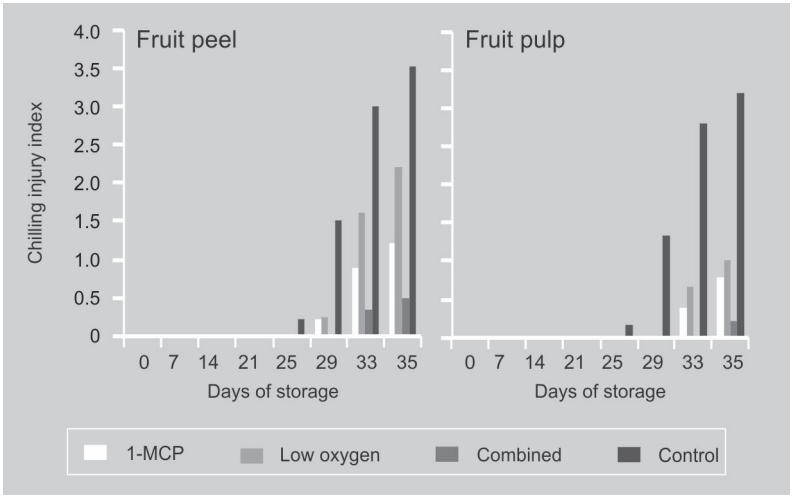

Figure 2.
Chilling injury index of fruit peel and pulp of 1-MCP-treated, low oxygen-treated, combination of 1-MCP and low oxygen-treated and untreated control 'Becon' avocado cultivar stored for 3 weeks at 4 °C, followed by 2 weeks at 20 °C.

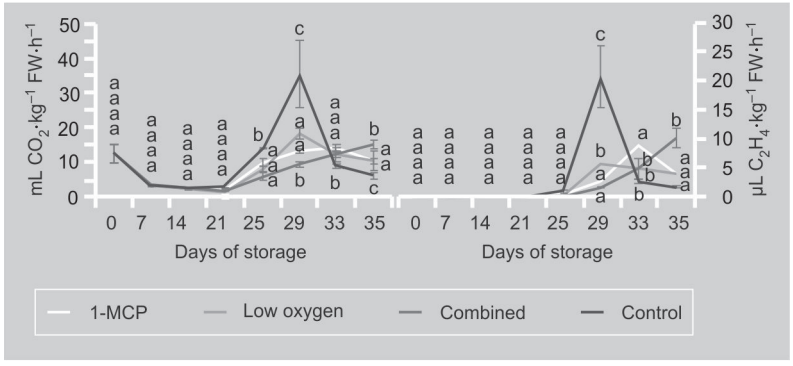

Figure 3.
Respiration rate and ethylene evolution rate of 1-MCP, low oxygen, combination of 1-MCP and low oxygen treatments and untreated control 'Becon' avocado cultivar stored for 3 weeks at 4 °C, followed by 2 weeks at 20 °C. Different letters indicate significant differences among means according to the least significant difference (LSD) at $P \leq 0.05$; vertical bars represent standard deviation (± SD, $n = 3$).

part of the storage period (days 33 and 35), the H° value was decreased dramatically in untreated control fruit due to development of severe chilling injury on the peel. However, significantly higher H° values were retained in combined treatment fruit as compared with 1-MCP- and low oxygen-treated fruit.

3.3. Electrical conductivity

The relative EC of the fruit tissue continually increased after harvest (*figure 1*), suggesting a gradual loss of cell membrane integrity. This increase in conductivity was slightly slowed down by 1-MCP application, low oxygen and combined treatments during storage at 4 °C. After the cold storage period, when fruit were transferred to 20 °C, untreated control fruit's relative EC was markedly increased. However, fruit treated with 1-MCP, low oxygen and combined treatment maintained a lower level of relative EC after being transferred to 20 °C. The EC of the fruit tissue showed a negative correlation with fruit firmness.

3.4. Chilling injury

External CI symptoms (skin blackening) and internal CI symptoms (pulp browning) were manifested at day 25 in untreated fruit (*figure 2*). The maximum shelf life of control fruit was 7 d at 20 °C. CI symptoms on the peel and pulp of fruit treated with 1-MCP or low oxygen atmosphere were manifested at days 29 and 31, respectively. The maximum shelf life of 1-MCP- and low oxygen-treated fruit was (14 and 12) d at 20 °C, respectively. Furthermore, CI symptoms in the peel and pulp of combined treatment fruit were manifested at days 33 and 35, respectively. The maximum shelf life of combined treatment fruit was 22 d at 20 °C. It is apparent that the combined treatment was the most effective in alleviation of CI and extends the shelf life of avocado as compared with individual application of 1-MCP or low oxygen atmosphere treatment.

3.5. Respiration and ethylene evolution rates

Respiration rates of both treated and untreated avocado fruit were steadily maintained at a low level for 2 weeks of storage at 4 °C, then increased immediately after transferring to 20 °C in all treatments including untreated control fruit (*figure 3*). These results are complementary to those of other studies on avocado [23, 24]. However, a significantly lower respiration rate was observed in the combined treatment than in 1-MCP or low oxygen treatment. Further, the climacteric peak was more delayed in the combined treatment than in 1-MCP, low oxygen and control treatments.

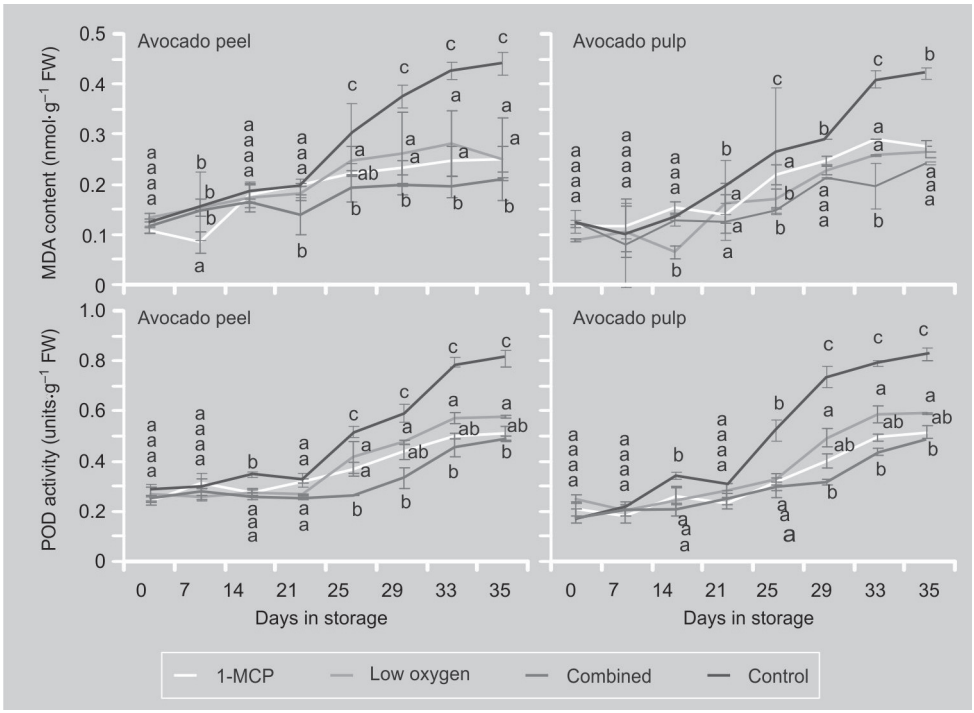

Figure 4.
Malondialdehyde (MDA) content and peroxidase (POD) activity of avocado peel and pulp of 1-MCP, low oxygen, combination of 1-MCP and low oxygen treatments and untreated control 'Becon' avocado cultivar stored for 3 weeks at 4 °C, followed by 2 weeks at 20 °C. Different letters indicate significant differences among means according to the least significant difference (LSD) at $P \leq 0.05$; vertical bars represent standard deviation (± SD, $n = 3$).

The characteristic climacteric ethylene evolution commenced at 4 d after transferring to 20 °C and reached the climacteric peak rapidly in untreated control fruit (*figure 3*). The climacteric increase in ethylene evolution for the 1-MCP, low oxygen and combined treatment fruit was delayed. However, there was a significantly lower ethylene evolution rate and the climacteric ethylene evolution peak was more delayed in the combined treatment than in the other treatments. In our study, combination of 1-MCP and low oxygen treatment significantly delayed the onset of the climacteric ethylene evolution and respiratory patterns in the 'Becon' avocado cultivar.

3.6. Malondialdehyde content

The oxidation of polyunsaturated fatty acids results in peroxide ions and malondialdehyde (MDA). The extent of lipid peroxidation activation depends on the degree of cold stress and is correlated to the extent of chilling injury (CI). Therefore, accumulation of MDA is often taken as an indicator of CI [25]. The pattern of MDA content of avocado during storage at 4 °C followed by shelf life at 20 °C trended to increase in both fruit peel and pulp (*figure 4*). The MDA content in both the peel and pulp of combined treatment fruit was significantly lower than in other treatments. This result indicates that combined treatment was more effective than 1-MCP or low oxygen treatment in alleviation of CI.

3.7. Peroxidase activity

The peroxidase (POD) activity in all treatments increased significantly during the shelf life at 20 °C (*figure 4*). Relatively higher POD activity was observed in control fruit and lower activity was observed in the fruit treated with combination of 1-MCP and low oxygen atmosphere. These results support the relationship between chilling sensitivity of fruit peel and pulp and the induction of POD enzymes during storage. Similar results were reported by Setha *et al.* [26] on papaya storage at 5 °C and 15 °C for up to 30 d. Chilling injury symptoms were found to be related to increases in POD activities in avocado, which indicates that the mechanism of tissue browning in avocado fruit involves the actions of POD.

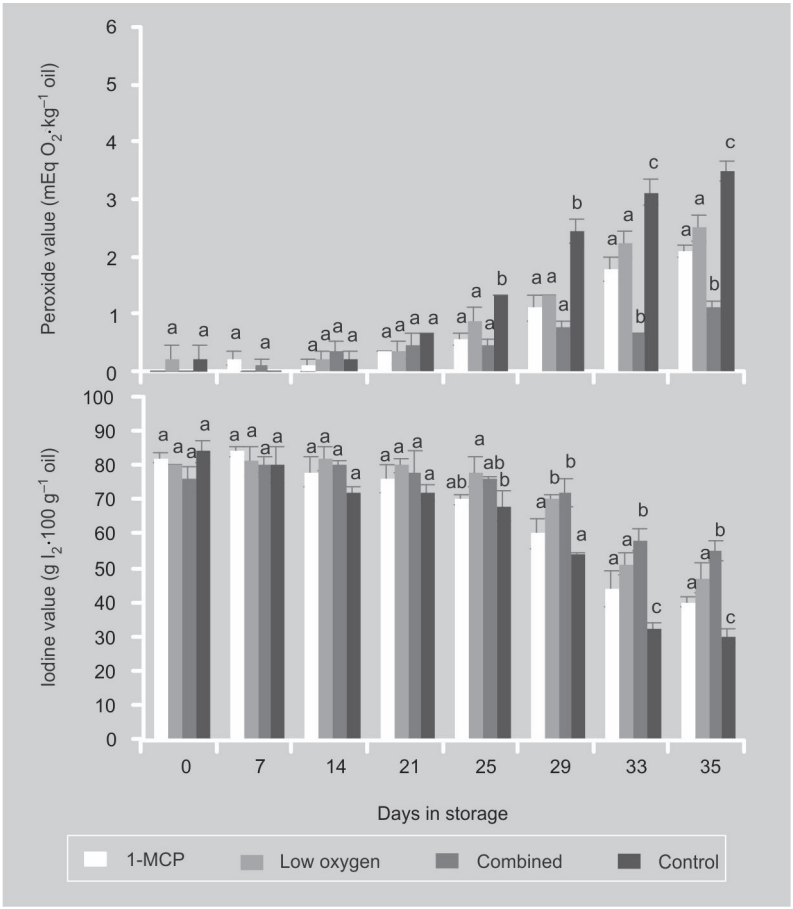

Figure 5.
Peroxide index and iodine value of 1-MCP, low oxygen, combination of 1-MCP and low oxygen treatments and untreated control 'Becon' avocado cultivar stored for 3 weeks at 4 °C, followed by 2 weeks at 20 °C. Different letters indicate significant differences among means according to the least significant difference (LSD) at $P \leq 0.05$; vertical bars represent standard deviation (± SD, $n = 3$).

3.8. Peroxide index

The avocado lipid fraction is very rich in monounsaturated fatty acids, especially oleic acid, whereas it has a low content of saturated fats [27]. Hydroperoxide formation in a crude oil can serve as an indicator of the oxidative processes and, in turn, of the oil quality. Thus, a rapid hydroperoxide formation evidences the initiation of the oxidative reactions that precede rancidity [28]. The peroxide value of fresh avocado puree ranged from (0 to 0.2) mEq O_2·kg^{-1} oil (*figure 5*). Peroxide values increased slowly during the fruit storage at 4 °C for 3 weeks, but increased rapidly after transferring to 20 °C. Untreated fruit exhibited a significantly higher peroxide value; meanwhile, the combined treatment exhibited the significantly lowest peroxide value during the storage at 20 °C. It seems that combined treatment reduces the lipid oxidation more remarkably than 1-MCP or low oxygen treatment.

3.9. Iodine values

The iodine value can provide information about the saturation of conjugated double bonds of fatty acids, although it loses accuracy for oils containing a small proportion of saturated fatty acids. The fatty acids profile of avocado is dominated by oleic acid, followed by linoleic acid, palmitic acid and palmitoleic acid [29, 30]. Combined treatment avocados exhibited almost unchanged iodine values of (76.21 and 55.43) g I_2·100 g^{-1} oil at days 0 and 35, respectively (*figure 5*). However, untreated fruit had values of (84.12 and 30.06) g I_2·100 g^{-1} oil at days 0 and 35, respectively (64.26% depletion), indicating a rapid and massive saturation of conjugated double bonds of the fatty acid chains. During the storage period at 20 °C, iodine value rapidly decreased, which negatively correlated with the increase in the peroxide value. Therefore, untreated avocado fruit may undergo autoxidation, forming peroxides and secondary oxidation products that contribute to the variation in the oxidation indices.

4. Conclusion

Our data provide information about the individual effect of pre-storage application of 1-MCP and low oxygen atmosphere in cold storage as compared with combination of the above 1-MCP and low oxygen treatments on alleviation of chilling injury (CI) and lipid oxidation stability of avocado. The data importantly show that combination of 1-MCP and low oxygen treatments dramatically delays ripening, alleviates CI and retards lipid oxidation of avocado under low temperature storage. Significantly lower CI index, respiration rate, ethylene evolution rate, malondialdehyde content, peroxidase activity, relative electrical conductivity and peroxide value, and higher fruit firmness and iodine value in the combined treatment proved the hypothesis of

this experiment: compared with 1-MCP or low oxygen treatments, combined treatment is the most effective in alleviation of chilling injury, lipid oxidation stability and prolonging the shelf life of avocado. In addition, storage life of the combined treatment could be increased by an additional 15 d more than untreated control fruit and 9 d more than 1-MCP- or low oxygen-treated fruit at 20 °C. The effectiveness of combination of 1-MCP and low oxygen treatments in delaying the occurrence of CI may be due to reduction of ethylene production, ethylene action and cell membrane degradation enzyme activity during 20 °C storage. However, further research is needed on the effect of 1-MCP on controlling lipid peroxidation of fruit pulp for better understanding of how 1-MCP can reduce lipid oxidation in stored avocado fruit.

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Efecto de la aplicación combinada de 1-MCP con un ligero contenido de oxígeno en la reducción de los estragos causados por el frío y la estabilidad de la oxidación de los lípidos en el aguacatero (*Persea americana* Mill.) almacenado a baja temperatura.

Resumen - Introducción. Los frutos del aguacatero (*Persea americana* Mill.) son sensibles al frío cuando se exponen a bajas temperaturas. Los altos contenidos de lípidos de la pulpa del aguacatero están sujetos a la oxidación, lo que conduce a rancidez y sabores indeseados y a una bajada de calidad a lo largo de todo el almacenamiento. **Material y métodos**. Se trataron aguacates (cv. Becon) con 1 µ 1·MCP·L^{-1} durante 24 h a 20 °C, luego se almacenaron a 4 °C durante 21 días bajo atmósfera pobre en oxígeno (3,5% de O_2), antes de ser traspasado a 20 °C durante 14 días con el fin de simular su duración de conservación. A lo largo del periodo de almacenamiento, se midieron el índice de sensibilidad al frío, la firmeza, el color de la cáscara, la conductividad eléctrica relativa (CE), la respiración, la evolución del etileno, el malondialdehído (MDA), la actividad peroxidasa (POD), el índice peróxido, y el índice de yodo. **Resultados y discusión**. Se debilitaron y retrasaron significativamente tanto la incidencia como la gravedad de la sensibilidad al frío de los frutos tratados de modo combinado con 1-MCP con un ligero contenido de oxígeno, en relación con los otros frutos sometidos los tratamientos sin combinación. La aplicación combinada de 1-MCP con un ligero contenido de oxígeno fue eficaz y retrasó la aparición de picos climatéricos de la respiración y de la producción de etileno. Este retraso se asoció a reducciones de ablandamiento de los frutos y de la permeabilidad de la membrana celular expresada por la conductividad eléctrica. Se redujeron considerablemente el contenido de MDA y la actividad POD de los frutos tratados con la combinación de 1-MCP con un ligero contenido de oxígeno. Por otro lado, el índice peróxido significativamente más bajo y el índice de yodo superior sugirieron que la combinación de 1-MCP con un ligero contenido de oxígeno controlaba, efectivamente, la oxidación de lípidos en la pulpa de los frutos de aguacate. **Conclusión**. El conjunto de los resultados sostiene la hipótesis según la cual el tratamiento combinado (en relación tanto con el 1-MCP individualmente, como con un tratamiento con un ligero contenido de oxígeno solo) fue más eficaz para reducir los estragos causados por la sensibilidad al frío, para mantener la estabilidad de oxidación de los lípidos y para alargar la duración de la vida de los frutos en almacenamiento a baja temperatura.

Japón / *Persea americana* / frutas / enfriamiento / sustancias de crecimiento vegetal / almacenamiento atmósfera controlada / peroxidación