Changes in lipid oxidation stability and antioxidant properties of avocado in response to 1-MCP and low oxygen treatment under low-temperature storage

*Prabath Pathirana, U.A., Sekozawa, Y., Sugaya, S. and Gemma, H.

*Laboratory of Pomology, Graduate School of Life and Environmental Sciences,
University of Tsukuba, Tsukuba City, 305-8572, Japan*

Abstract

Avocado (*Persea americana* Mill.) pulp is rich in unsaturated fatty acids, including oleic, linoleic, and palmitoleic acids, which tend to oxidize during storage. Oxidation of avocado oil in pulp can lead to the formation of volatile compounds responsible for off-flavors and to nutritional losses. Antioxidant systems in cells inhibit lipid oxidation and thus may contribute to protection against oxidative deterioration of fatty acids. The aim of this study was to determine the fatty acid profile of avocado pulp and to evaluate the extent of lipid oxidation and antioxidant properties of avocado pulp resulting from treatment with 1-methylcyclopropane (1-MCP) prior to low O_2 treatment under low-temperature storage conditions. The avocado (cv. Fuerte) fruit was treated with 1 µL L-1 1-MCP for 24 h at 20ºC, stored at 5ºC for 21 days under a low O_2 atmosphere (3.5% O_2 and 96.5% N2), and then stored at 20ºC for 14 days to simulate shelf storage conditions. We found that combined treatment of avocados with 1-MCP and low O_2 and under low-temperature storage conditions lowered peroxide levels and increased iodine levels, which effectively controlled lipid oxidation and ripening. In the sensory evaluation test, off-flavors were not identified in either the treated or control avocado pulp at the early stages of ripening but were identified in the control at the over-ripe stage. Avocado fruit treated with 1-MCP and low O_2 had higher saturated fatty acid (palmitic acid) and lower mono-unsaturated fatty acid (oleic acid and palmitoleic acid) than controls. In control fruit, total antioxidant activity, SOD activity, and α-tocopherol content decreased under low-temperature and room temperature storage conditions. Under the same conditions, fruit treated with 1-MCP and low O_2 had increased total antioxidant activity, SOD activity, and α-tocopherol content. Taken together, these results show that pre-storage treatment of avocado fruit with a combination of 1-MCP and a low O_2 atmosphere effectively reduces lipid oxidation by enhancing the antioxidant activity of avocado pulp.

Introduction

Avocado (*Persea americana* Mill.) is a highly caloric fruit rich in vitamins, minerals, folates, potassium, and fiber, with a unique lipid composition (Slater *et al*., 1975). Avocado oil is rich in monounsaturated fatty acids, especially oleic and palmitoleic acids, and is low in saturated fats compared with other vegetable oils (Swisher, 1988). A high dietary intake of oleic and palmitoleic acid is related to a decreased risk of cardiovascular disease because these fatty acids preserve levels of high-density lipoproteins and act as antioxidants (Lopez *et al*., 1996; Richard *et al*., 2008). Furthermore, of all commonly eaten fresh fruit, avocado has the highest level of β-sitosterol, which has been shown in clinical trials to reduce blood levels of low-density

cholesterol by blocking cholesterol absorption in the intestine (Heinemann *et al*., 1993). Thus, avocado is considered a highly desirable addition to a healthy diet. Avocado fruit also contains high levels of lipophilic bioactive phytochemicals, including vitamin E, carotenoids, and sterols, which possess antioxidant and radical scavenging activities (Lee *et al*., 2004). Factors that affect the antioxidant capacity of fruit include cultivar, agronomic conditions, postharvest conditions, and the stage of fruit ripeness (Kevers *et al*., 2007).

Avocado pulp is sensitive to oxidative process during postharvest storage resulting in rancidity and subsequent production of undesirable flavors and reduction in quality. Oxidative changes in the lipid phase are due mostly to auto-oxidation, which in most vegetable oils accounts for development of

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*Corresponding author.
Email: *uparuna@yahoo.com*

the carbonyl compounds responsible for rancid off-flavors (Gunstone and Norris, 1982). The chlorophyll content of avocado oil is higher than most other vegetable oils, such as olive oil (Werman and Neeman, 1986). Chlorophyll can act as a pro-oxidant by stimulating photo-oxidation. This fact, combined with a relatively low content in natural antioxidants, especially α-tocopherol, makes avocado oil highly susceptible to oxidation. Werman and Neeman (1986) studied the effectiveness of natural antioxidants, such as propyl gallate and α-tocopherol, in stabilizing the fatty fraction of avocado pulp. Tocopherols contain an unsaturated aromatic ring with a hydroxyl group that was found to act as a hydrogen donor and thus retard the formation of free radicals during the initiation stage of oxidative processes (Werman and Neeman, 1986). Ascorbic acid also acts as a powerful antioxidant in the lipid fraction of avocado pulp (Soliva et al., 2001).

There are some indications that exposure of fruit to 1-methylcyclopropane (1-MCP) or low O_2 treatment can stabilize lipid oxidation and enhance post-harvest quality attributes. 1-MCP acts by binding tightly to ethylene receptors and thereby blocking the effects of ethylene (Sisler and Serek, 1997). 1-MCP also inhibits ethylene production (Hans et al., 1999). Low O_2 levels induced through low O_2 atmospheres or hypobaric atmospheres can influence post-harvest physiology and quality of fruit either directly or indirectly via altered CO_2 and C_2H_4 production rates (Kader, 1985). Similar results were observed by Edna et al. (1994), in that pre-storage treatment of Fuerte avocado fruit with a low O_2 atmosphere (3% O_2 and 97% N_2) for 24 hours at 17°C, significantly reduced chilling injury symptoms after storage at 2°C for 3 weeks. The mechanism underlying inhibition of ethylene production by low O_2 levels is thought to involve depletion of ACC synthase protein (Mathooko et al., 1995), reduction in the efficiency of conversion of ACC to ethylene (Rothan and Nicolas, 1994), and reduction in the level of ATP caused by inhibition of respiration (Hans et al., 1999). Exposure of fruit to high O_2 levels can damage plant tissue and thus decrease quality attributes by increasing the level of free radicals (Fridovich, 1986).

Our previous study using 'Becon' avocado fruit showed that combined treatment was more effective than individual treatment of 1-MCP or low O_2 application under low temperature storage in reducing chilling injury and stabilizing lipid oxidation (Prabath et al., 2011). Lipid oxidation was lowest with a combination of exposure to 1-MCP and low O_2 treatment. Most research to date on lipid oxidation has been done on avocado puree or oil.

In the present study, we investigated the effects of exposure to 1-MCP prior to low O2 treatment under low-temperature storage on lipid oxidation stability, fatty acid profile, and antioxidant activity in 'Fuerte' avocado fruit pulp.

Materials and methods

Fruit sampling and treatments

The avocado fruit (cv. Fuerte) was obtained from Wakayama Prefecture, Japan and transported in refrigerated trucks (5-7°C) to Tsukuba University, Tsukuba, Japan within 2 days after harvest. Upon arrival, fruit was selected according to size, weight, color, and external appearance, and then washed with water and left to dry at room temperature for about 1 h. A random selection of 50 fruit was immediately exposed to 1-MCP (1 μL L-1) for 24 h by placing an aqueous solution of 1-MCP at 30°C made up in an open wide-mouth bottle in the bottom of a sealed 59-L glass chamber with the fruit. The chamber was maintained at 20°C and a relative humidity (RH) of 90% - 95%. After the incubation period, the chamber was ventilated and the fruit was stored in the 59-L glass chamber at 5°C with a mixture of 3.58% O_2 and 96.42% N_2 applied into the headspace of the chamber at a 100 mL min-1 flow rate. After 3 weeks, the chamber was opened and fruit was transferred to 20°C for 2 weeks to simulate shelf storage conditions. The control fruit was stored at 5°C and 65% - 70% RH without 1-MCP or low O_2 treatment for 3 weeks, and then transferred to 20°C for 2 weeks to ripen. Physicochemical, physiological, and biological analyses were performed on three replicates of three fruit samples per replicate at 7-day intervals during cold storage and 4-day intervals during room temperature storage.

Determination of ethylene evolution, respiration rate and firmness

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 ethylene evolution was analyzed 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 ionization detector (FID). 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 μL C2H4•kg⁻¹ FW•h⁻¹.

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 (TCD).

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 mg•kg^{-1} FW•h^{-1}.

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}.

Sensory evaluation test

Fruit samples for the sensory evaluation test were prepared on the basis of stage of ripening. Fruit at the climacteric peak stage (day-4 for control fruit and day-12 for treated fruit stored at 20ºC) and over-ripened stage (day-8 for control fruit and day-16 for treated fruit stored at 20ºC) were evaluated. The unilateral paired comparison test was used to analyze results from a sensory evaluation test conducted to determine whether off- flavor could be detected in avocado pulp. The sensory panel was composed of 30 untrained participants. Testing was conducted in the absence of extraneous odors and sound. Slices of peeled avocado fruit samples were placed onto numerically coded plates. Two avocado fruit samples, coded with random three-digit numbers, were presented to each participant along with water. Two of the samples were identical. Participants were instructed to evaluate the samples by chewing the fruit in the given order, clearing their palate using water, and indicating which one had a more off- flavor. The participants were allowed to re-test the samples and to make a choice.

Oil extraction

Avocado fruit was cut vertically into two equal sections. The stone and peel were removed manually and the flesh was sliced and immediately snap-frozen in liquid nitrogen and held at -40ºC before being freeze-dried in a lyophilizer for 2 days. The dry weight (DW) was determined and freeze-dried samples were ground to a powder before being returned to – 40ºC for storage until required for further analysis.

Fatty acids derived from the pulp tissue sample were extracted and quantified as described by Meyer and Terry (2010). Briefly, 1 g of ground powdered avocado pulp was homogenized with hexane and filtered under vacuum through a 110 mm filter (ADVANTEC paper, Toyo Roshi, Japan). The solvent from the lipid-containing filtrate was evaporated under vacuum. The recovered oil was weighed and kept at – 20ºC for subsequent analysis.

Quantification of fatty acids

Fatty acids were analyzed as fatty acid methyl esters (FAMEs) and quantified by gas chromatography with flame ionized detection, as described by Meyer and Terry (2010). Briefly, 0.2 mL of methanolic KOH (2 N) was added to 0.1 g of avocado oil extract in 2 mL of hexane. 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 ºC until needed. This solution was diluted 1:100 (v/v) with fresh hexane immediately before injection into a gas chromatograph (GC) (Agilent 6890N GC, Agilent Technologies, Cheshire, U.K.) equipped with a G1540N flame ionization detector (FID). Identification and quantification of selected compounds was performed on a CP-Sil 88 fused silica capillary column (50 m × 0.25 mm i.d., 0.2 μm film thickness; Varian, Palo Alto, CA). Column temperature was programmed at 55ºC for 3 min, and then raised to 175ºC at 13ºC min-1 intervals followed by an isothermal period of 1 min, and increased again to a final temperature of 220ºC at 8 ºC 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 250ºC, respectively. The fatty acid profile was calculated as a percentage of a total of the six detected FAMEs, after comparison of peak areas of samples and peak areas of standards of known composition.

Peroxide and iodine assays

Peroxide values were measured according to the method of Garcia *et al.* (1996) with a slight modification for avocado oil. Two grams of avocado oil extracted from each replicate sample was placed in a 250-mL Erlenmeyer flask that was previously purged with nitrogen. Each 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 was added, and the mixture was titrated with 0.005 N sodium thiosulphate with a 1% (w/v) starch indicator solution. Results were expressed in milliequivalents of oxygen per kilogram of avocado oil (meq O_2/kg oil).

Iodine value was assessed using Wijs method (Anon, 1988). Samples of extracted avocado oil were weighed to 200 mg and mixed with 15 mL of carbon tetrachloride in a 300-mL glass-stoppered flask. Wijs reagent (25.0 mL) was then added to the flask and the solution was mixed by swirling and placed 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 thiosulfate using a 1% (w/v) starch indicator solution. Results were expressed in grams of iodine per 100 g of avocado oil (g I2/100 g oil).

Total phenolic content

The total phenolic content was determined following the method described by Singleton and Rossi (1965) with slight modifications. A centrifuge tube containing 0.5 mL of methanolic extract was mixed with Folin-Ciocalteu's reagent (0.5 mL) and saturated sodium carbonate solution (1 mL). The volume of the mixture was adjusted to 10 mL using distilled water. The sample was allowed to stand at ambient temperature for 45 min until the blue color developed and was then centrifuged at 4,000 g for 5 min. An absorbance of the clear supernatant was measured at 725 nm using a spectrophotometer (Jasco V-550, Tokyo, Japan). The total phenolic content of each extract was expressed as mg galic acid equivalent/1 g dry weight of the defatted sample (mg GAE /g DW).

Total antioxidant capacity

Antioxidant activity in the samples was measured using the ferric reducing ability of plasma (FRAP) assay (Benzie and Strain, 1996) using a spectrophotometer (Jasco V-550, Tokyo, Japan). The FRAP method measures the absorption change that appears when the TPTZ (2,4,6-tri-pyridyl-s-triazine)-Fe3+complex is reduced to the TPTZ-Fe2+form in the presence of antioxidants. This reduction is monitored by measuring the absorption change at 593 nm. Briefly, 3 mL of working FRAP reagent prepared daily was mixed with 100 µL of diluted sample; the absorbance at 593 nm was recorded after a 30-min incubation at 37 ºC. FRAP values were obtained by comparing the absorption change in the test mixture with those obtained from increasing concentrations of Fe^{3+} and expressed as mmol of Fe^{2+} equivalents per kg fresh weight.

Superoxide dismutase (SOD) assay

Avocado pulp samples were homogenized on ice in 0.1 M phosphate buffer, pH 7.0, containing 1% polyvinyl polypyrrolidone (PVP) in a ratio of 1:4 (w/v). Homogenates were centrifuged, and the cleared supernatant was used immediately as the enzyme source. SOD activity was determined by the method of Ukeda *et al.* (1997) based on the inhibition of a tetrazolium salt reduction by superoxide generated from the xanthine–xanthine oxidase reaction. The assay mixture contained 0.1 mL of the enzyme extract,

2.3 mL of sodium phosphate buffer, 0.1 mL of 1M nitro blue tetrazolium (NBT), 0.1 mL of 4 mM xanthine, and 0.1 mL of 0.15% bis-trimethylsilyl-acetamide (BSA). Water instead of the enzyme extract was used for the blank control. The reaction was initiated by the addition of 0.1 mL of xanthine oxidase and the reaction mixture incubated on a water bath at 30ºC for 20 min. The reaction was stopped by the addition of 0.2 mL of 8 mM $CuCl_2$. In this assay, xanthine oxidase was used to generate superoxide radicals in vitro. Activity of superoxide radical production was determined by the conversion of NBT to an insoluble formazan product detected by measuring the absorbance at 560 nm in a spectrophotometer (Jasco V-550, Tokyo, Japan) at 25ºC. Activity of SOD in the enzyme extract was expressed in terms of its ability to inhibit superoxide radical production from the xanthine oxidase activity.

Ascorbic acid (AA) analysis

Ascorbic acid was determined spectrophotometrically (Hüppe et al., 1992; Vazquez et al., 1994). Samples (100-1000 mg) were mixed with 5 mL of metaphosphoric acid (4.5 g/100 mL) and vigorously shaken for 1 min. After centrifugation at 3500 g for 15 min, the supernatant was transferred into a volumetric flask, and the extraction was repeated twice. The volume of the combined extracts was increased to 20 mL with meta-phosphoric acid, and 200 µL of this extract was then mixed with 300 µL 50 g L^{-1} trichloroacetic acid. After centrifugation (20,000g for 30 min), 300 µL of the supernatant was mixed with 100 µL 2,4-dinitrophenylhydrazine reagent and heated to 60ºC for 1 h. Subsequent to cooling in an ice bath for 5 min, 400 µL of 15.75 mol L^{-1} sulphuric acid was added. After 20 min, the absorbance at 520 nm was measured using a spectrophotometer (Jasco V-550, Tokyo, Japan).

α-Tocopherol quantification

The tocopherol content was analyzed by an HPLC system (Jasco, Tokyo, Japan) equipped with a FP-2020 fluorescence detector, CO-2065 intelligent column oven, and PU-2089 quaternary gradient pump. Separation was achieved with a reverse-phase CrestPak C18s column (150 mm length × 4.6 mm i.d., 5 µm particle diameter) (Jasco, Tokyo, Japan). The mobile phase was methanol/acetonitrile/methylene chloride (25:22:3 v/v/v) and the flow rate was 1 mL min-1. Peaks were registered at 290 and 330 nm as excitation and emission wavelengths, respectively.

Statistical analysis

Statistical analyses were performed using SPSS

software version 19 (SPSS Inc., Chicago, USA) and graphs were produced using Prism v.2.0 (Graph Pad Inc., San Diego, USA) and Microsoft Excel 2007. The data were analyzed by one-way analysis of variance (ANOVA). Mean separations were performed by Duncan's multiple range test. Differences were considered significant when $P < 0.05$. The significant differences for sensory evaluation data were determined according to the unilateral paired-comparison test (one-tailed, $P = 1/2$) (Roessler *et al.*, 1978).

Results and discussion

Ethylene evolution, respiration rate and fruit firmness

The rate of respiration and ethylene evolution of avocado fruit exhibited a typical climacteric pattern during storage (Figure 1A and 1B). The climacteric respiratory peak of control fruit was observed at 4 d after transferring to 20ºC, and respiration rate decreased rapidly afterward. Combined application of 1-MCP and low O_2 significantly inhibited the peak value of respiration rate, suppressed ethylene production, and delayed the peak of ethylene. This suppression in respiration rate and ethylene evolution by 1-MCP has been reported in other fruits including plum (Abdi *et al.*, 1998) and persimmon (Lou, 2007). Ethylene, as a phytohormone, plays important roles in ripening and senescence of climacteric fruits. A number of reports showed that 1-MCP inhibited softening, alleviated chilling injury (CI), and decreased senescence in avocado (Jeong *et al.*, 2002) and Loquat fruit (Cai *et al.*, 2006) by blocking the ethylene action. Control and combined application of 1-MCP and low O_2 treated avocado fruit exhibited similar trends of softening during the first week of storage (Figure 1C). After 1 week, softening trends of control and combined application of 1-MCP and low O_2 treated fruit diverged markedly. The final firmness values of control fruit and those treated with 1-MCP and low O_2 averaged 1 N and 5 N, respectively.

Possible explanation for the observed delay in ripening through the inhibition of ethylene production by low oxygen application are (1) depletion of ACC synthase protein due to degradation and/or inhibition of synthesis, or inactivation of ACC synthase activity (Mathooko *et al.*, 1995), but without interference with the ethylene receptor; (2) reduction of the efficiency of conversion of ACC to ethylene. This can lead to inhibition of ethylene production when ACC content is low (Rothan and Nicolas, 1994); and (3) reduced ATP level by inhibition of respiration as ATP is involved in the conversion of ACC to
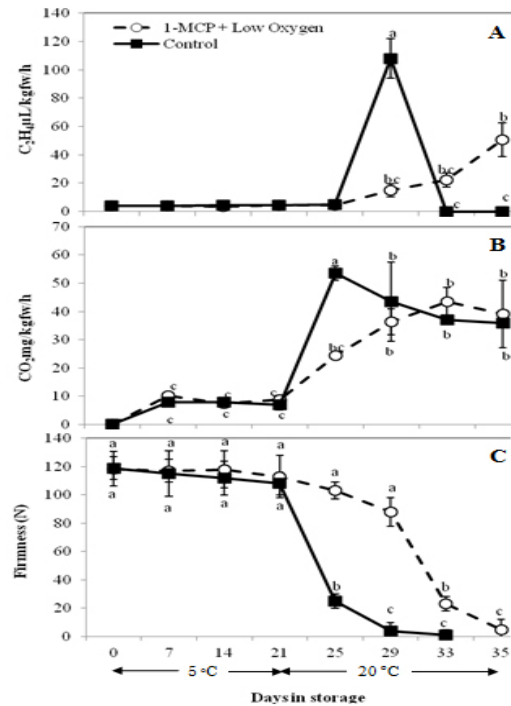

Figure 1. Effects of treatment with 1-MCP and low O_2 on ethylene evolution rate (A), respiration rate (B) and fruit firmness (C) of avocado (cv. Fuerte) stored at 5ºC for 3 weeks and at 20ºC for 2 weeks. Vertical bars indicate standard error of means (± SE, n = 3). Mean separated by DMRT, 5 %.

ethylene. The inhibitory effect of CO_2 on ethylene production could be mediated by the effect of CO_2 on respiration. This could be explained as follows: an inhibition of respiration results in reduced ATP production. ATP is involved in the conversion of ACC to ethylene. Inhibitors of electron transfer and oxidative phosphorylation reduced ATP levels and inhibited the conversion of ACC to ethylene (Apelbaum *et al.*, 1981). Gorny and Kader (1996) men tioned the possibility that reduced ATP pools affects the protein phosphorylation that might be necessary for activation of ACC oxidase.

Sensory evaluation

A unilateral paired comparison of results from the sensory evaluation test was used to identify differences in flavor between control avocado pulp samples and those exposed to 1-MCP and low O_2 levels at the climacteric peak stage and the over-ripening stage (Figure 2). The sensory evaluation test conducted for avocados at the climacteric peak stage (day-4 for control fruit and day-12 for treated fruit at 20 ºC) revealed no significant difference in flavor between treated and control fruit. By comparison, for avocados at the over-ripening stage (day-12 for control fruit and day-20 for treated fruit at 20ºC) there was a significant difference between treated and control fruit (Figure 2). Off-flavor was not detected
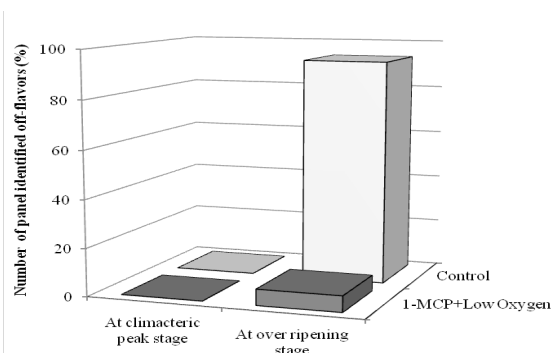
Figure 2. Quantitative descriptive analysis (off-flavor development) of untreated control avocados (cv. Fuerte) and those treated with 1-MCP before low O_2 storage at 5ºC for 3 weeks and storage at 20°C for 2 weeks.

Table 1. Fatty acid composition of avocado mesocarp durig storage period (% of total fatty acids)[a]

Fatty acid	Treatment	Days in storage					
		0	7	14	21	28	35
Palmitic (16:0)	1-MCP+Low-O₂	10.75±0.06a	10.83±0.09a	10.94±0.03a	11.24±0.53a	11.29±0.01a	11.74±0.04ab
	Control	10.59±0.08a	10.82±0.04a	10.85±0.17a	11.41±0.23a	11.96±0.03ab	12.75±0.07b
Stearic (18:0)	1-MCP+Low-O₂	0.564±0.07a	0.613±0.22a	0.559±0.05a	0.59±0.18a	0.592±0.34a	0.648±0.06a
	Control	0.551±0.09a	0.58±0.03a	0.561±0.14a	0.612±0.21a	0.657±0.72a	0.697±0.08a
Palmitoleic (16:1)	1-MCP+Low-O₂	4.506±0.21a	4.481±0.51a	4.466±0.02a	4.455±0.42b	4.459±0.26b	4.454±0.02b
	Control	4.502±0.31a	4.486±0.19a	4.468±0.03a	4.461±0.13a	4.453±0.22b	4.424±0.08c
Oleic (18:1)	1-MCP+Low-O₂	76.32±0.23a	76.25±0.05a	76.41±0.21a	76.17±0.05a	76.29±0.42a	75.88±0.03ab
	Control	76.44±0.03a	76.4±0.08a	76.48±0.33a	76±0.01a	75.59±0.32a	74.95±0.02c
Linoleic (18:2)	1-MCP+Low-O₂	7.871±0.22a	7.702±0.23ab	7.54±0.11b	7.597±0.05b	7.508±0.07bc	7.483±0.35c
	Control	7.858±0.04a	7.596±0.41b	7.562±0.33b	7.661±0.09b	7.548±0.14bc	7.536±0.31bc
Linolenic (18:3)	1-MCP+Low-O₂	0.826±0.01a	0.952±0.13a	0.909±0.23a	0.8±0.02a	0.709±0.33a	0.662±0.24ab
	Control	0.885±0.05a	0.936±0.26a	0.904±0.63a	0.737±0.03a	0.689±0.21ab	0.603±0.61b

[a] Values are the mean of three indipendents ± standard deviation. Different lower case letters in the same fatty acid indicate significant differences by ANOVA (P<0.05).

in treated and control fruit at the climacteric peak stage; it was detected by almost all the sensory panel (93.33%) in control samples at the over-ripening stage. Earlier, sour and rancid flavors were identified as the critical descriptors of avocado pulp samples (Jacobo and Hernandez, 2011) most likely as a result of the formation of hydroperoxides preceding oxidation reactions that cause oil rancidity (Werman and Neeman, 1986).

Fatty acid methyl ester profile

The fatty acid composition of avocado pulp is shown in Table 1. Oleic (C 18:1) acid was found to be the major fatty acid in avocado pulp, representing around 76% of total fatty acids quantified. Also, avocados were rich in palmitic (C 16:0), linoleic (C 18:2), and palmitoleic (C 16:1) acids (10%, 7%, and 4% of total fatty acids quantified, respectively), whereas stearic (C 18:0) and linolenic (C 18:3) acids were present in only small amounts. Monounsaturated, polyunsaturated, and saturated fatty acids represented about 81%, 8%, and 11%, respectively, of total fatty acids quantified. The relative amounts of monounsaturated, polyunsaturated, and saturated fatty acids was calculated because it is considered nutritionally desirable to have a low intake of saturated fats. The ratio of monounsaturated to saturated and of monounsaturated plus polyunsaturated to saturated fatty acids was 7.3% and 8.09%, respectively. These results are in agreement with those reported by others for avocado (Ozdemir and Topuz, 2004; Vekiari *et al.*, 2004).

We did not find a significant difference in avocado fatty acid except for palmitoleic acid and linoleic acid content following 21 days of storage at 5ºC; however, during 2 weeks storage at 20ºC for ripening we found a significant decrease in unsaturated fatty acid and significant increase in saturated fatty acid content that was related to the oxidative degradation of fatty acids. By comparison, the level of oleic acid decreased (2%) in the control samples, but remained unchanged in samples exposed to 1-MCP and a low O_2 atmosphere. Although the content of the rest of the fatty acids (palmitoleic and linoleic acid) significantly decreased in both the control and treated samples over the 35-day storage period, there was no significant difference in fatty acid content between control and treated samples. The only exception was the palmitoleic acid content after 35 days of storage. Of the three main groups of fatty acids, the monounsaturated and polyunsaturated fatty acids gradually decreased during the storage period, whereas the saturated fatty acids slightly increased or remained unchanged. These changes are most likely related to the oxidative degradation of fatty acids (Richard *et al.*, 2008). The proportion of some fatty acids of avocado showed a statistically significant change. In contrast, there were no significant differences in the proportion of other fatty acids, such as oleic acid, and there were no numerically important changes in terms of nutrition value (Ozdemir *et al.*, 2004).

Peroxide and iodine values

Rapid hydroperoxide formation initiates the oxidative reactions that lead to off-flavor development. Peroxide values indicate the extent of intermediate hydroperoxide formation and are expressed as milliequivalents of active oxygen per kilogram of avocado oil sample. The lipid fraction of fresh avocado pulp has peroxide values ranging from 1.66 to 7.33 meq O_2/kg oil (Figure 3A). This result is consistent with the report by Werman and Neeman (1986) that crude avocado oil had an initial peroxide content of 5.85 meq O_2/kg. Initial peroxide level variations can be attributed to many factors including variety, agronomic practices, and the action of lipolytic enzymes released from the fruit
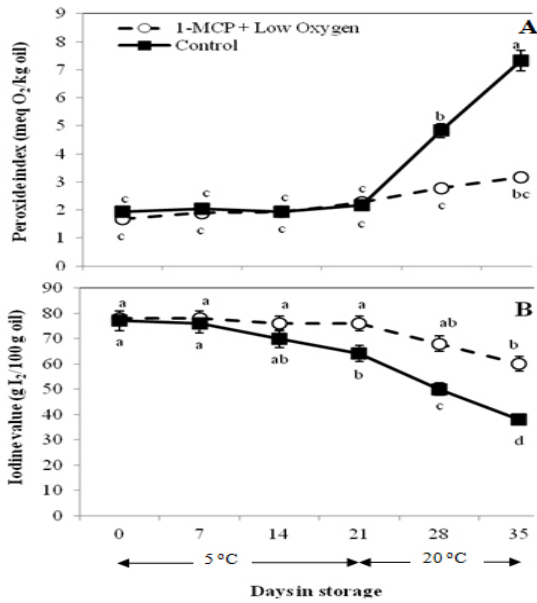
Figure 3. Effects of treatment with 1-MCP and low O_2 on peroxide value (A) and iodine value (B) of avocado (cv. Fuerte) stored at 5ºC for 3 weeks and at 20ºC for 2 weeks. Vertical bars indicate standard error of means (± SE, n = 3). Mean separated by DMRT, 5 %.
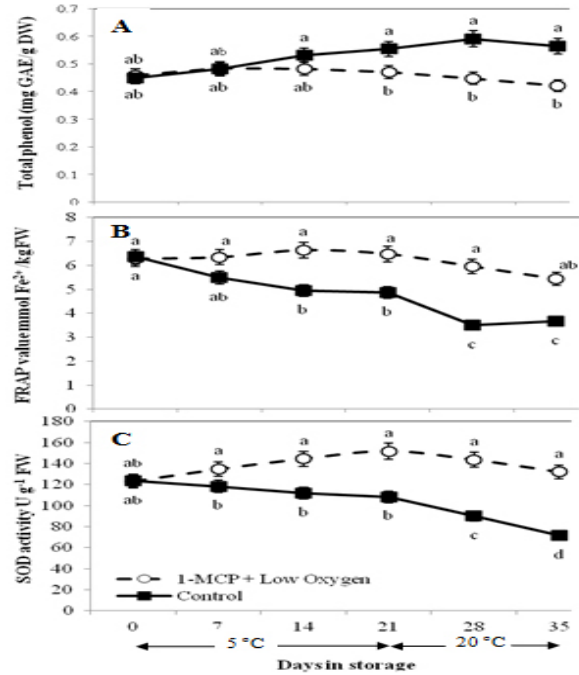

Figure 4. Effects of treatment with 1-MCP and low O_2 on total phenolic content (A) total antioxidant capacity (B) and SOD activity (C) of avocado (cv. Fuerte) stored at 5ºC for 3 weeks and at 20ºC for 2 weeks. Vertical bars indicate standard error of means (± SE, n=3). Mean separated by DMRT, 5 %.

cells during processing. We found that the peroxide value increased slowly during the 3-week storage period at 5ºC and then increased rapidly over the storage period at 20 ºC. A comparison of peroxide values shows that peroxide formation is suppressed by exposure of avocados to 1-MCP and low O_2 levels under low-temperature storage conditions. Kirk and Sawyer (1991) reported that a rancid taste was noticeable when the peroxide value was between 20 and 40 meq O_2/kg oil in oily products. However, our study showed that the limit for detection of off-flavors was between 4.8 and 7.3 meq O_2/kg oil, which was in the range where results from the sensory evaluation test and peroxide values could be compared.

Because of the transitory nature of peroxide, we also used iodine values to assess lipid oxidation in avocado pulp. The iodine value also gives information about secondary products formed during lipid oxidation. Determination of the iodine value is used to ascertain the extent to which the bonds in oil can be regarded as unsaturated. Iodine values serve as a guide of the overall oxidation of oil and are fairly accurate for samples not containing a large proportion of conjugated double bonds (Martinez et al., 2007). There was a negative correlation between peroxide and iodine values (Figure 3A and 3B) and we found a significant difference in iodine values between treated and control pulp samples after the avocados were transferred from storage at 5ºC to storage at 20ºC. Fruit treated with 1-MCP and low O_2 under cold storage conditions underwent a small reduction in iodine values of 23% from initial values.

By comparison, untreated control samples underwent a decrease in iodine values of 50.64%.

Total phenol and antioxidant activity

Phenols are bioactive compounds that can ameliorate oxidative damage and the risk of chronic diseases in the human body due to their ability to reduce free radical formation and to scavenge free radicals (González et al., 2010). Ayala-Zavala et al. (2011) reported to contain great amounts of phenolic compounds and to display a higher antioxidant activity in the pulp of avocado fruit. Figure 4A shows the changes in total phenol activity in 'Fuerte' avocado mesocarp during storage. Compared with controls, treatment with 1-MCP and low O_2 under cold storage conditions significantly reduces the total phenolic content in avocado mesocarp. The total phenolic content in untreated control avocados increased over the storage period with the highest values recorded on day 28 (0.591 mg GAE/g DW) and the lowest on day 0 (0.45 mg GAE/gDW). A similar increase in phenolic compounds was reported for ripened tropical fruit (Mahattanatawee et al., 2006). This increase in total phenolic compounds is the result of ethylene-induced increases in the enzyme phenylalanine ammonia lyase (PAL), which is involved in the biosynthetic pathway of phenolic compounds (Martinez and Whitaker, 1995). However, treatment of avocados with 1-MCP and low O_2 under cold storage conditions underwent

a reduction in total phenolic content of 7.86% from initial values. These findings are consistent with the results of a previous report showing that lowered O_2 or increased CO_2 causes a decrease (16%) in total soluble phenolic compounds during controlled atmosphere (CA) storage of mango fruit (Youngmok *et al.*, 2007).

The initial antioxidant capacity of mature green avocados ranged from 6.28 to 6.35 mmol Fe^{2+} /kgFW (Figure 4B). Over the 35-day storage period the total antioxidant capacity of treated and control avocados decreased by 13.21% and 42.36%, respectively. There was no change in the total antioxidant capacity of treated avocados during storage at 5 ºC and at the end of storage period the total antioxidant capacity was significantly higher than controls. Antioxidant capacity and free radical scavenging ability is a desirable health benefit of many fruit that can be compromised with storage. We show here that storage of avocados without treatment can reduce these health benefits. Our findings are consistent with the studies conducted by Egeal *et al*. (2010) showing that 1-MCP conferred a greater resistance to oxidation stress in apricot during storage at low temperature. Also, rice stored under low O_2 was shown to retain antioxidant activity and bioactive compounds otherwise lost during storage (Nyein et al., 2010). We show here that pre-storage treatment with 1-MCP and low O_2 has potential in stabilizing antioxidant capacity in avocado during storage.

Superoxide dismutase activity

Antioxidant enzymes, like SOD, play a crucial role in antioxidant defense during cold stress. As shown in Figure 4C, SOD activity in control fruit decreased gradually throughout storage at 5ºC and 20ºC. By comparison, SOD activity in avocados treated with 1-MCP and low O_2 increased by 28% throughout storage at 5ºC then declined during storage at 20ºC. The changes in SOD activity during the ripening period at 20ºC were similar in control and treated fruit, but SOD activity at the end of the 35-day storage period was significantly higher in treated fruit than in the controls. These findings are consistent with results reported by Singh and Dwivedi (2008) who showed that treatment with 1-MCP induced a decrease in the level of lipid peroxidation, concomitant with increased SOD activity compared with controls. They also showed that low O_2 (5% O_2) storage reduced chilling injury and delayed the reduction in antioxidant enzyme activity. These observations, together with our results, suggest that the ripening-induced decrease in SOD activity can be ameliorated by pre-storage treatment of fruit with
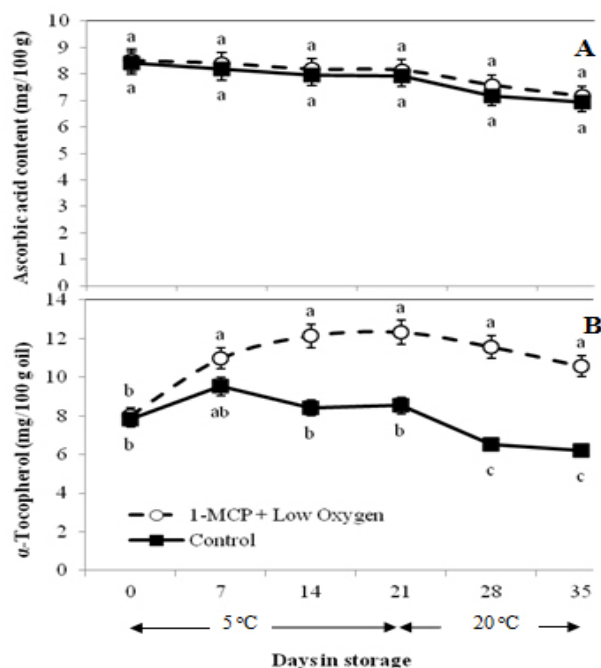

Figure 5. Effects of 1-MCP and low O_2 treatments on total ascorbic acid (A) α-Tocopherol (B) content of avocado (cv. Fuerte) stored at 5ºC for 3 weeks and at 20ºC for 2 weeks. Vertical bars indicate standard error of means (± SE, n = 3). Mean separated by DMRT, 5 %.

1-MCP and low O_2. Superoxide anions generated either by auto-oxidation processes or by enzymes, produce other kinds of cell-damaging free radicals and oxidizing agents. Due to a substantial increase in respiration of this climacteric fruit, oxygen free radical generation probably increases with ripening. The concomitant decline in SOD activity would thus contribute to accumulation of O^{2-} and thus an increase in oxidative stress with ripening.

Ascorbic acid concentration

Total ascorbic acid concentrations declined in both untreated and treated avocados during storage (Figure 5A). The total ascorbic acid concentration in avocado pulp was not significantly affected by treatment with 1-MCP and low O_2. However, overall ascorbic acid concentrations were slightly higher in treated avocado than in untreated avocados, averaging 7.99 and 7.76 mg/100g FW, respectively. Surprisingly, little information is available on changes in ascorbic acid concentration in avocados during storage, especially under low O_2 conditions, and even less is known about the effect of 1-MCP. Vilaplana *et al*. (2006) found that the concentration of ascorbic acid was lower in 'Golden Smoothee' apples treated with 1-MCP than in untreated controls after a month of air storage. The reason for the decrease in ascorbic acid concentration during storage and for the greater decrease following 1-MCP treatment is unclear. In contrast, Li *et al*. (2011) reported significantly higher levels of ascorbic acid in melon fruit treated with 1-MCP. Although

ascorbic acid is generally considered to be important in nutrition (Davey *et al*., 2000), it represents a minor component of the total antioxidant activity of apples (Eberhardt *et al*., 2000). Ascorbic acid, however, is a critical component of antioxidative processes in plant cells because it interacts enzymatically and non-enzymatically with damaging oxygen radical and reactive oxygen species (Davey *et al*., 2000).

α-Tocopherol concentration

α-Tocopherol has a phenolic structure and is an important natural antioxidant because it retards the formation of free radicals during the initiation stage of oxidative processes by acting as a hydrogen donor. In control avocados, the α-tocopherol content, expressed in mg/100 g of oil, was unchanged during storage at 5ºC, but declined during storage at 20ºC (Figure 5B). By comparison, in treated avocados, the α-tocopherol content increased throughout storage at 5ºC (53.8% increase), then declined during storage at 20ºC. The changes in α-tocopherol content in control and treated fruit were similar during the ripening storage period at 20ºC, but the α-tocopherol content was significantly higher in treated fruit than in controls. The increase in α-tocopherol in treated fruit suggests that exposure to 1-MCP and low O_2 under low-temperature storage conditions accelerates the biosynthesis of α-tocopherol in avocados. Considering the fact that α-tocopherol contributes to the stability of oil by protecting unsaturated fatty acids against free radical attack (Papadopoulos and Boskou, 1991), the increase in α-tocopherol content could be linked to the increase in unsaturated fatty acids that occurs during ripening (Boskou, 1996).

Conclusions

Lipid oxidation and the subsequent production of unfavorable quality attributes in avocado pulp were delayed by the combined treatment with 1-MCP and low O_2 under low-temperature storage. This was most likely because of the induction of antioxidant activity. The reduction of total phenolic compounds with the combined treatment did not compare well with the level of antioxidant activity. Further research is needed to fully understand the mechanisms underlying the effect of 1-MCP and low O_2 on antioxidant activity in avocados.

Acknowledgments

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