Effect of One-methylcyclopropene (1-MCP) and chlorine dioxide (ClO_2) on preservation of green walnut fruit and kernel traits

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Abstract The effect of the ethylene receptor competitor 1-methylcyclopropene (1-MCP) and the legally approved disinfectant chlorine dioxide (ClO_2) on preservation of the green walnut fruit during storage was investigated. Green Chinese walnut fruit cv. Xilin No.2 was harvested on commercial maturity and stored at 0–1 °C after the fruit was treated by water (control), 80 mg L⁻¹ ClO_2 (ClO_2), 0.5 μL L⁻¹ 1-MCP (1-MCP), or combination treatment of 80 mg L⁻¹ ClO_2 with either 0.1 μL L⁻¹ 1-MCP (0.1 1-MCP+ ClO_2) or 0.5 μL L⁻¹ 1-MCP (0.5 1-MCP+ ClO_2). During storage, respiration, ethylene production, phenolics content, antioxidative activity, weight changes, decay of the fruit and kernel traits of acid value, peroxide value, free fatty were measured. All treatments decreased postharvest respiration intensity in different degrees and inhibited ethylene production peak. ClO_2 increased the total phenol and flavonoid content of the green fruit compared with other treatments and the control ($P<0.05$), but not did the total antioxidant activity for this treatment. After 42-day storage, ClO_2 remained higher fresh weight and lower decay index than control, while 1-MCP increased the fruit decay index. Final acid values of kernel from ClO_2 , control and 0.1 1-MCP+ ClO_2 were not different from their initial values, which from 0.5 1-MCP increased. Final peroxide value for kernel from ClO_2 showed no change during storage but increased at least 1.0-fold for other treatments. ClO_2

preserved 99.9 % of initial free fatty acid, similar to that for the control (99.8 %), whereas 0.5 1-MCP preserved only 95.7 %. ClO_2 is of potential in decay retardation and kernel traits maintenance of green walnut fruit, whereas the 1-MCP has a negative effect for decay control on walnut.

Keywords Green walnut fruit · Preservation · 1-MCP · ClO_2 · Kernel traits

Walnut (*Juglans regia* L.) is of nutritional value because its kernel is rich in unsaturated fatty acids, protein, minerals etc, which helps to lower cholesterol levels and to keep the brain healthy (Abbey et al. 1994; Pribis et al. 2012). It is in increasing demand as a food source as well as a “health food” and its economic importance is increasing in global markets (Liebson 2004; Mexis et al. 2009). Traditionally, walnut is eaten as a dried nut, but since the 1990s, it has been recognized that fresh walnuts are more nutritious than dried ones, and the unique flavor of the fresh nut is appreciated in some countries (Cannella and Dernini 2006). However, preservation of fresh walnut is still problematic.

Considerable research has been directed toward the preservation of peeled fresh walnuts, with limited success due to development of mildew 50–60 days after harvest, regardless of the type of packaging, chemical treatment, or radiation treatment used (Ma et al. 2010). The potential to prolong the storage life of fresh walnut by adding a “pre-storage” treatment during which the green husk is retained has also been researched. Gao et al. (2008) showed that retention of the green husk preserved the walnut 40 days longer than without the pre-storage at 1–5 °C, but longer storage was prevented by the onset of decay. Ma et al. (2012) found that modified-atmosphere packaging with 10.1–13.0 % O_2 , 4.3–6.5 % CO_2 extended the life of green walnut fruit to

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approximately 60 days at 0–1 °C; thereafter, decay of the green husk occurred rapidly. Therefore, decay prevention of the husk is of primary consideration in the preservation of green walnut fruit.

Ripening of the green husk of harvested walnut fruit is characterized by a respiratory climacteric (Ma et al. 2012) and may be induced by ethylene autocatalysis production, decay in turns occurs (Yang et al. 2009; Fawbush et al. 2009). One-methylcyclopropene (1-MCP), which blocks the ethylene receptor and prevents ethylene binding (Sisler and Serek 1997), may preserve the green husk by inhibiting ethylene-dependent senescence. Also of interest to husk preservation is another compound, chlorine dioxide (ClO_2), a disinfectant with a broad and high biocidal activity that does not react with organic compounds to produce toxic chlorinated by products (Han et al. 2000; Gómez-López et al. 2007). ClO_2 use is legal in China and the United States to control food-borne pathogens and other microorganisms in fruit and vegetables (Hubbard 1998; Ministry of Health of the People's Republic of China 2008). Rare Literature refers to the combined use of 1-MCP and ClO_2 to increase the storage period of fresh produce. In this study, we investigated the effects of 1-MCP or ClO_2 , individually and in combination, on the preservation of green walnut fruit.

Materials and methods

Fruit material, treatments, and storage

Green Chinese walnut fruit *Juglans regia* L.cv. Xilin No.2 was harvested in a walnut orchard in Zhouzhi county, Shaanxi province in China at commercial maturity of August late, 2011. Immediately after picking, fruit were packaged in towel paper padded corrugated boxes (17×25×25 cm) and transported to a laboratory at Northwest Agriculture and Forestry University. Upon arrival, the fruit was stored at 0~1 °C for 24 h. Fruit of uniform size and free from mechanical damage, pests, or diseases was selected and divided into five groups:

- (1) Control: Fruit was immersed in deionized water.
- (2) ClO_2 : Fruit was immersed in 80 $\text{mg}\cdot\text{L}^{-1}\text{ClO}_2$ only.
- (3) 0.1 1-MCP+ ClO_2 : Fruit was treated with 0.1 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP and immersed in 80 $\text{mg}\cdot\text{L}^{-1}\text{ClO}_2$.
- (4) 0.5 1-MCP+ ClO_2 : Fruit was treated with 0.5 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP and immersed in 80 $\text{mg}\cdot\text{L}^{-1}\text{ClO}_2$.
- (5) 0.5 1-MCP: Fruit was treated with 0.5 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP only.

Solution of 80 $\text{mg}\cdot\text{L}^{-1}\text{ClO}_2$ was prepared by dissolving accurate amount ClO_2 (powdered formula, Shandongzhaoguan Pharmaceutical Co., Ltd., Shandong, China) in deionized water. According to the regulation of FDA (Hubbard 1998), ClO_2

is limited not to exceed 3 $\text{mg}\cdot\text{kg}^{-1}$ residue in raw agricultural commodities like fruits and vegetables when ClO_2 is used as an antimicrobial treatment. Thus, a potable water rinse shall be followed after the treatment. In this study the rinse was not conducted after ClO_2 immersion since the walnut husk will not serve for eating.

Treatment with 1-MCP was performed as follows. Fruit was placed in a plastic chamber (0.23 m^3 volume). The accurate amount of 1-MCP powder (0.14 % a.i., presented by Rohm and Haas China Shanghai Rep. Office, AgroFresh™ Technology, Philadelphia, Pennsylvania, USA.) was placed into a beaker inside the chamber. Five milliliters of water were added to the beaker to release gaseous 1-MCP, and the chamber was immediately hermetically closed for 24 h at 25 °C in the dark. For treatments (3) and (4), in which 1-MCP and ClO_2 were both supplied, the fruit was treated with 1-MCP as described, followed by the ClO_2 immersion. All immersions were done for 20 min at 25 °C, after which the fruit was air-dried at 25 °C.

Fruit of each group was placed in a plastic basket and covered with plastic film (6- μm thickness, permeability of $1.35\times 10^4\text{ mL O}_2/\text{m}^2\cdot\text{d}\cdot\text{atm}$, $6.53\times 10^4\text{ mL CO}_2/\text{m}^2\cdot\text{d}\cdot\text{atm}$), stored at 0~1 °C and 70–80 % relative humidity (RH) in the dark for 45 days. The groups were arranged in a completely randomized design with three replicates, each with 200 fruits. Five fruit were sampled from each basket at each sampling time. For “green husk” samples, the 1 mm surface layer was removed and for “kernel” samples the skin was removed. Samples were either analyzed immediately or frozen in liquid N_2 and stored at –80 °C.

Respiration intensity and ethylene production rate

Respiration was measured every 6 days for 42 days at storage temperature. The five fruit from each replication were weighed and placed in a 1-L glass jar to measure CO_2 production with the principle-of-airflow method (Loomis and Schull 1973). The jar was connected to a respiration analyzer (GXH-1050, Beijing Junfang Chemical Institute of Technology, Beijing, China) for the outlet and to CO_2 -free air for the inlet and keep air flow rate of 0.4 $\text{L}\cdot\text{min}^{-1}$. Read the balanced CO_2 concentration (C) inside the jar on the infrared CO_2 detection meter of the analyzer when data on the meter became stable. Carbon dioxide production by the fruit was calculated as follows:

$$(\mu\text{molCO}_2\text{ kg}^{-1}\text{h}^{-1}) = \frac{F \times 60 \times C}{22.4} \times \frac{1}{W} \times 10^{-3} \\ \times \frac{273}{273 + T}$$

Where F is air flow rate ($\text{L}\cdot\text{min}^{-1}$), C is balanced CO_2 concentration in the respiration jar ($\mu\text{L}\cdot\text{L}^{-1}$), W is the weight

of fruit (kg), T is the measuring temperature (°C), 22.4 is millimoles of CO₂.

After respiration measurement, each sample was sealed in a glass desiccator with gas sampling ports at 0–1 °C and 70–80 % RH for 2 h. Gas samples (5 mL) were withdrawn with a syringe from the headspace of the desiccators and stored with the drainage method in a 10-mL bottle until they were measured. Ethylene fraction of the samples was quantitatively analyzed by gas chromatography (GC; Trace GC VitrA, USA) equipped with a 2-m stainless-steel packed column (1.8 m×2 mm) and a flame ionization detector. The temperatures of injector, column, and detector were 70 °C, 70 °C, and 150 °C, respectively. Injection volume was 1 mL.

Total phenolics, flavonoids and antioxidant activity

Fruit samples ($n=5$) were randomly collected every 9 days for 45 days for determination of total phenols, total flavonoids, and total antioxidant activity in the green husk.

Total phenolics and flavonoids were extracted and determined according to the Folin-Ciocalteu procedure of Toor and Savage (2005).

Two-gram aliquots of the homogenized sample were weighed and transferred to a centrifuge tube. The volume was adjusted to 20 mL with deionized water and the sample was stirred for 10 min at 4,024 g in an orbital shaker. Total antioxidant activity of the supernatant was determined by the ferric reducing antioxidant power assay (Benzie and Strain 1996).

Weight changes and decay measurement

Weight changes and decay during storage was measured in 100 fruits per sample. Weight changes were calculated as the percentage change in fresh weight. Decay was visually evaluated and expressed as decay index calculated using the following formula:

Decay index

$$= \frac{\sum (\text{decay grade} \times \text{fruit number of the grade})}{\text{total fruit} \times \text{highest grade}} \times 100\%$$

The grade of decay was defined as the area affected by visible decay. Grades assigned were 0–5 and corresponded to affected areas of 0 %, ≤20 %, 21–40 %, 41–60 %, 61–80 %, and >80 %, respectively (Zheng et al. 2005).

Kernel traits

To evaluate the influence of treatments on preservation of kernel traits, acid value, peroxide value and free fatty acid content of kernel were measured. The three attributes

indicate an accumulation change of kernel trait; therefore they were assayed only before and after storage to compare differences between treatments.

Acid value

The acid value was determined by the official GB 5530-2005 method. Two grams fresh weight of kernels were ground into a homogenate, transferred to a 250-mL flask, suspended in 50 mL mixed with neutral ether-ethanol solution, and shaken for 1 min. Two to three drops of phenolphthalein indicator were added and the suspension was titrated to the end point with 0.1 M KOH. The acid value in this assay is related to KOH neutralization in the titration.

Peroxide value

Walnut oil was extracted from 5 g fresh kernels according to the method described by Vanhanen and Savage (2006) with some modification. Ground kernels were transferred into a separatory funnel with 50 mL petroleum ether and 5 mL deionized water. The mixture was agitated for five minutes and left to equilibrate for 24 h. Twenty-five milliliters of the upper solution was transferred to a crystallizing dish and the petroleum ether was evaporated in a water bath at 70 °C. The extracted oil was dried in an oven at 105 °C for 3 min and the residue was used to determine the peroxide value according to the official GB/T 5538-2005 method.

Free fatty acid

Total lipid was extracted from the walnut kernel using the method described by Xie et al (2003). Free fatty acid in the extraction fraction was measured by GC (Agilent Technologies 7890A GC System, USA). The gas chromatograph was equipped with an SGE 00088A23 capillary column (30 m×320 μm, 0.5 μm). The injector temperature was 260 °C and the split ratio was 20:1. The carrier gas was high-purity helium at a flow rate of 1.5 mL·min⁻¹. The initial temperature, maintained for 2 min, was 200 °C and increased at increments of 10 °C·min⁻¹ to 240 °C, maintained for 7 min. The relative content of each component was calculated using the peak area normalization method.

Statistical analysis

All statistically analyses were performed by using the software SPSS 17.0 (IBM Corp., Chicago, IL, USA) for Windows. Data was analyzed for variance among treatments. There were three replications of the five treatments with 1 observation per replication for respiration intensity,

Fig. 1 Changes in Respiration intensity (a), Ethylene production rate (b), Total phenolics content (c), Total flavonoids content (d), Total anti-oxidative activity (e), Fresh weight (f) and Decay index (g) of green walnut fruit during storage at $0-1^\circ\text{C}$ and 70–80 % RH ($n=3$), which have accepted one of five treatments: deionized water (Control),

$80 \text{ mg L}^{-1} \text{ ClO}_2$ only (ClO_2), $0.1 \mu\text{L L}^{-1}$ 1-MCP and $80 \text{ mg L}^{-1} \text{ ClO}_2$ (0.1 1-MCP+ ClO_2), $0.5 \mu\text{L L}^{-1}$ 1-MCP and $80 \text{ mg L}^{-1} \text{ ClO}_2$ (0.5 1-MCP+ ClO_2), $0.5 \mu\text{L L}^{-1}$ 1-MCP only (0.5 1-MCP). Values are the means $\pm$ Standard Deviation (SD) of triplicate assays

ethylene production rate, fresh weight percentage, decay index, free fatty acid and with two subsampling (observations) per

replication for total phenol, total flavonoids, total antioxidant ability, acid value, peroxide value. Mean separations were

performed by Duncan's multiple-range tests. Differences at $P \leq 0.05$ were considered significant.

Results and discussion

Effect of 1-MCP and ClO_2 on respiration intensity and ethylene production of green walnut fruit

As shown in Fig. 1(a), the respiration intensity (RI) of fruit was significantly weakened in all treatments at the beginning of the storage in comparison with the control; ClO_2 group showed the lowest level; and then they all declined until day 6. During storage, fruit showed a two-peak respiratory drift, which is in agreement with a previous report (Ma et al. 2012). In fruit treated with 0.5 1-MCP+ ClO_2 , the first peak appeared later than it did in the control. Although the first peak for fruit from the other treatments coincided with that for the control, the peak values of individual ClO_2 and 0.5 1-MCP treatment were significantly lower than the control ($P < 0.05$). The second peak in RI of fruit treated with 0.5 1-MCP was delayed so late that it was not detected. These results indicate that both ClO_2 and 1-MCP treatments inhibited RI of walnut fruit and 1-MCP inhibited the second peak in RI. This latter effect of 1-MCP was compromised in the presence of ClO_2 .

The ethylene production rates of fruit from each treatment were determined every 6 days until 42 days of storage, as shown in Fig. 1(b). There was a sharp peak in ethylene production in the control on the 24th day of storage. Only a small peak appeared in the 0.5 1-MCP+ ClO_2 treatment and this was delayed compared to the control, to day 36. The other three treatment groups produced constant low levels of ethylene with no significant difference ($P > 0.05$) throughout storage. The data indicate that both 1-MCP and ClO_2 treatment inhibited ethylene production rate of the fruit significantly ($P < 0.01$), but the inhibition was less effective when 1-MCP and ClO_2 were combined.

A two-peak type respiration drift was also found in honey peaches and cherimoya (Gong et al. 2000; Rafael et al. 1994). Gong et al. pointed out that the first peak may be a sign of ripening, whereas the second peak may indicate the initiation of senescence. For green walnut fruit, the second RI peak occurred 24 days after the first (Fig. 1(a)) and followed the maximum rate of ethylene production (Fig. 1(b)). These senescence-related events and their timing support Gong et al.'s explanation of the significance of the second respiration peak in green walnut fruit.

Effect of 1-MCP and ClO_2 treatments on total phenolics and flavonoids content

Changes in the total phenolic content of the green husk of walnut fruit are shown in Fig. 1(c). Total phenolic content

was significantly less in all 1-MCP included treatments at the beginning of storage compared with the control ($P < 0.05$), whereas the value of fruit treated with individual ClO_2 was not significantly different from the control. During storage, the phenolic content increased first then decreased a little for individual ClO_2 treatment and was significantly higher ($P < 0.05$) than the control and all other treatments within the first 18 days. After day 18, the total phenolic content of the other treatment waved irregularly except for ClO_2 treatment kept a stable level. In the end of 45 days no significant difference ($P > 0.05$) showed between groups. The data indicate that ClO_2 treatment promoted the postharvest synthesis of phenolics in green husk of walnut fruit during the first period of storage, whereas 1-MCP enhanced the degradation right after its application and repressed the protective effect of ClO_2 on phenolic content during storage.

Some phenolics act as phytoalexins and participate in the defense reaction against microorganisms (Taiz and Zeiger 2006). The increase in phenolic content of the green walnut husk observed in response to ClO_2 treatment may benefit the preservation of the fruit.

The total flavonoid content of the green husk of walnut fruits shown in Fig. 1(d). Overall, the changes in each treatment are similar to those of the total phenolic content during storage. The total flavonoids represent only approximately 10 % of the phenolics at each storage time. This result indicates that a large proportion of the phenols in green husk are not flavonoids. Treatment with ClO_2 enhanced the postharvest synthesis of flavonoids and non-flavonoids, an effect opposite to that of 1-MCP. ClO_2 is a strong oxidizing agent that effectively retards physiological senescence, inhibits rot, and improves the quality of fresh-cut lotus root, green bell pepper, and apple fruit (Du et al. 2007, 2009; Chen and Zhu 2011; Zhao et al. 2011). Chen and Zhu (2011) found increased total phenolics in ClO_2 -treated plum fruit. The current study is also the first report on the application of ClO_2 to walnut fruit. The result is consistent with previous reports on the physiological effects and decay control by this compound. Moreover, ClO_2 significantly promoted the synthesis of phenolics during pre-storage. However, in this study, 1-MCP effects were opposite to those of ClO_2 application.

Effect of 1-MCP and ClO_2 on total anti-oxidative ability

Total anti-oxidative (TAA) ability increased in the 0.5 1-MCP+ ClO_2 , 0.5 1-MCP and ClO_2 treatments on day 9 and 36, whereas in the control and 0.1 1-MCP+ ClO_2 treatments, TAA remained low throughout storage (Fig. 1(e)). The data suggest that both the 1-MCP and ClO_2 treatments caused significantly greater TAA of green husk of walnut fruit in comparison with control ($P < 0.01$, $P < 0.05$, respectively)

and 1-MCP was more effective than ClO_2 . The highest TAA detected was in fruit treated with 0.5 1-MCP+ ClO_2 on day 9. To date, researchers have found either no effect or a negative effect of 1-MCP on the postharvest phenol content of apple, blueberry, and Chinese kale (Hoang et al. 2011; Chiabrando and Giacalone 2011; Sun et al. 2012); however, Hassan and Mahfouz (2012) reported that 1-MCP treatment increased the total antioxidant capacity of coriander leaves. Ethylene induced the defense mechanism and increased the phenolics and lignin contents in postharvest 'Navelate' fruit (Cajuste and Lafuente 2007). Polyphenols are bioactive molecules in plant species that play important roles in plant resistance to parasites and the response to environmental stress (Bahorun et al. 2004). This implies that the effect of ClO_2 on decay prevention is due to its effect on increasing phenolic content. However, if ClO_2 acts on phenol synthesis, the different pathway from ethylene may be followed because it inhibited the increase of ethylene throughout the storage, while increased phenolic content. In this study, when 1-MCP was applied, regardless of the concentration, the effect of ClO_2 on decay was reduced. The application of 1-MCP may reduce the postharvest synthesis of phenolics through counteracting ethylene, and therefore would not be reversed by ClO_2 . The correlation between phenolic content and total anti-oxidative activity is different in different fruit, as is the relationship between storability of fruit and the fruit's TAA (Hoang et al. 2011; Torres-Rodríguez et al. 2011).

Effect of 1-MCP and ClO_2 on the preservation of green walnut fruit

Weight changes

Fresh weight change during storage was expressed as the percentage of the fresh weight of the fruit measured immediately after treatment. This value decreased slightly until day 27 for the control and all fruits that received 1-MCP. However, for all fruits, the fresh weight increased from day 27–30, sharply decreased from day 30–36, and increased again from day 36–39. The changes in percentage fresh weight were smaller for the fruit receiving only ClO_2 than for the other treatments and fruit receiving only ClO_2 had significantly higher final fresh weight than the other groups on day 36 ($P<0.05$) and a final higher fresh weight than other groups (Fig. 1(f)). The data suggest that water metabolism of green walnut fruit became active during middle-to-late storage.

Decay index

Green walnut fruit are perishable. As shown in Fig. 1(g), decay occurred 18–24 days after the start of storage and

increased with time in storage. Treatment with ClO_2 significantly inhibited the incidence of decay. It showed the lowest decay index throughout storage and was significantly different than the control until 42 days after storage ($P<0.05$). In contrast, the 0.5 1-MCP treatment significantly increased the incidence of decay. In this treatment, decay was detected 6 days earlier than in any other treatment and these fruits had the highest decay index throughout storage, which was significantly higher than the control from day 30 until the end of storage ($P<0.05$). The decay index from the treatments in which ClO_2 and 1-MCP were applied together were intermediate between the control and the ClO_2 treatment and not significantly ($P>0.05$) different from either, indicating that 1-MCP reduced, but did not prevent, the effect of ClO_2 on preservation of the fruit. The consistent result with previous surveys on other plants, dealing with the role of 1-MCP on respiration and ethylene production has revealed in above text, but a negative effect of 1-MCP on decay of walnut fruit was observed. Pre-storage with 1-MCP reduces the respiration rate, ethylene production, improves the quality of a number of horticultural products (Watkins 2006; Luo et al. 2009; Ma et al. 2009; Massoloa et al. 2011; Sun et al. 2012) and retards decay in blueberry and jujube (Chiabrando and Giacalone 2011; Zhang et al. 2012). 1-MCP was also effective in reducing incidence of scald, greasiness, and core flush in apple, but increased bitter pit of apple during storage and the shelf rot of pear (Sun et al. 2003). Earlier researches had suggested that the effect of 1-MCP varies with species and cultivars (Sun et al. 2003; Blankenship and Dole 2003). Therefore, practical application of 1-MCP should be carefully tested for each horticultural product.

Literature had reported that plastic film package (control in this study) led to a storage of 40 days for green walnut fruit of cv.Liaoning No.1 and Xilin No.2 (Gao et al. 2008). We observed the control fruit of cv.Liaoning No.4 initiate to deteriorate on day 30. Although variety differences in response to a treatment exist, a significant decrease in decay index makes ClO_2 treatment practical in green walnut preservation.

Effect of 1-MCP and ClO_2 on kernel traits

Acid value

The initial acid value of kernel in green walnut fruit was $3.71 \pm 0.02 \text{ mg g}^{-1}$ fresh weight. After 42 days of storage it increased significantly for all treatments ($p<0.01$). The 0.5 1-MCP treatment resulted the highest final acid value of $6.24 \pm 0.09 \text{ mg g}^{-1}$ fresh weight, which was significant higher than value from 0.5 1-MCP+ ClO_2 treatment ($4.85 \pm 0.05 \text{ mg g}^{-1}$);

ClO_2 treatment and the control gave statistically the same level of final acid value of 4.52 ± 0.06 , $4.49 \pm 0.07 \text{ mg g}^{-1}$, respectively, being significant higher than 0.1 1-MCP+ ClO_2 treatment ($3.99 \pm 0.06 \text{ mg g}^{-1}$). Acid values represent the amount of free fatty acid in the walnut kernel. Increase during storage is due to the enzymatic hydrolysis of lipids, which may adversely affect the flavor of the fruit. It may be that $0.5 \mu\text{L} \cdot \text{L}^{-1}$ 1-MCP treatment decreased the level of phytoalexin in the green husk, which caused a higher acid value.

Peroxide value

Peroxide values represent the oxidation degree of polyunsaturated fatty acid and determines the organoleptic properties of oil (Vanhanen and Savage 2006). The initial peroxide value of walnut kernels were very low, $0.0039 \pm 0.0008 \text{ meq O}_2 \text{ kg}^{-1} \text{ oil}$. It increased significantly to 0.0313 ± 0.0011 , 0.0237 ± 0.001 , 0.0153 ± 0.0006 , for 0.5 1-MCP, 0.5 1-MCP+ ClO_2 treatment and control, respectively after 42 days of storage ($P < 0.01$). The highest final peroxide value was obtained in 0.5 1-MCP. The next highest value was in the 0.5 1-MCP+ ClO_2 treatment. The control treatment had an intermediate value. The 0.1 1-MCP+ ClO_2 treatment had a least increase in final peroxide value, twice as high as the initial. The ClO_2 treatment gave a final peroxide value of 0.0040 ± 0.0007 , being significantly the same level as the initial value. These findings indicate that ClO_2 treatment prevents the deterioration of unsaturated fatty acid in the kernel of green walnut fruit during refrigerated storage, whereas $0.5 \mu\text{L} \cdot \text{L}^{-1}$ 1-MCP enhanced the deterioration. Adding ClO_2 to the 1-MCP

treatment reduced the deterioration caused by 1-MCP alone. If this finding is considered along with the data in Figs. 1(c) and (d), the protection of ClO_2 on phenolics production may inhibit the fatty acid deterioration by repressing oxidation of the acid.

Free fatty acid

The main constituents of fatty acid in walnut kernel are unsaturated fatty acids (Martínez et al. 2011). As show in Table 1, before storage, the content of unsaturated fatty acids in the total fatty acid was 90.43 % and linoleic acid accounted for 70.90 %. The final content of unsaturated fatty acids decreased significantly for the 0.5 1-MCP treatment ($P < 0.01$) and the combination of 0.5 1-MCP+ ClO_2 ($P < 0.05$), whereas no significant decreases were observed for ClO_2 treatment. The highest final content of total fatty acid was observed in the ClO_2 treatment, and the control group had the second highest value, followed by the 0.1 1-MCP+ ClO_2 treatments, and the 0.5 1-MCP+ ClO_2 treatment. The 0.5 1-MCP treatment had the lowest value. Final content of total free fatty acid for each treatment was negatively correlated with their acid value ($R^2 = 0.891$) and peroxide value consistent ($R^2 = 0.710$). These results confirm that unsaturated fatty acids are preserved well in walnut kernels where less oxidative deterioration develops. Treatment with 1-MCP had the highest final acid value but the lowest free fatty acid content, indicating that some unidentified acid was formed. Considering unsaturated fatty acids, the polyunsaturated fatty acid linoleic acid(18:2) and

Table 1 Relative content of different fatty acids in walnut fruit treated with 1-MCP and chlorine dioxide (ClO_2) before and after storage for 42 days

Free fatty acid	Initial content (%)	Final content (%)				
		Control	0.11-MCP+ ClO ₂	0.51-MCP+ ClO ₂	0.51-MCP	ClO ₂
Saturated fatty acids						
Palmitic acid	5.12±0.06	5.68±0.05	5.61±0.05	4.97±0.05	5.31±0.06	5.08±0.05
Stearic acid	4.26±0.04	3.80±0.04	3.46±0.03	3.62±0.04	3.20±0.03	3.71±0.04
Arachidic acid	0.19±0.002	0.21±0.002	0.20±0.001	0.22±0.002	0.19±0.001	0.21±0.002
Summation	9.57±0.09	9.70±0.07	9.27±0.11	8.80±0.009	8.70±0.09	8.99±0.08
Unsaturated fatty acids						
Palmitoleic acid		0.09±0.0007	0.28±0.002	0.35±0.003	0.47±0.005	0.20±0.002
Hexadecadienoic acid		0.062±0.001	0.59±0.006	0.051±0.0004	0.06±0.0005	0.09±0.0008
Oleic acid	9.50±0.09	13.86±0.012	13.02±0.011	14.28±0.013	10.88±0.011	13.74±0.013
Linoleic acid	70.90±0.65	66.35±0.60	66.85±0.63	64.39±0.62	67.12±0.62	64.57±0.61
Linolenic acid	10.03±0.10	9.72±0.11	8.99±0.08	9.40±0.09	8.45±0.07	12.32±0.12
Summation	90.43±0.14	90.09±0.25	89.73±0.18	88.46±0.23	86.97±0.26	90.92±0.21
Total	100.00 ^a ±0.013	99.79 ^b ±0.024	99.00 ^c ±0.019	97.26 ^d ±0.022	95.68 ^e ±0.021	99.91 ^b ±0.014

Fruits accepted one of five treatments: deionized water immersion (control), 80 mg L^{-1} ClO_2 only (ClO_2), $0.1 \mu\text{L L}^{-1}$ 1-MCP and 80 mg L^{-1} ClO_2 (0.1 1-MCP+ ClO_2), $0.5 \mu\text{L L}^{-1}$ 1-MCP and 80 mg L^{-1} ClO_2 (0.5 1-MCP+ ClO_2), $0.5 \mu\text{L L}^{-1}$ 1-MCP only (0.5 1-MCP). Values represent the means $\pm$ SD of triplicate assays ($n=3$)

linolenic acid (18:3) decreased by 3.78–6.51 and 0.33–1.58 percentage points, respectively, while monounsaturated fatty acid of oleic acid(18:1) increased by 1.38–4.78 percentage points, palmitoleic acid and hexadienoic acid increased from undetectable levels to 0.09 % and 0.06 %, respectively. This revealed the conversion happened from polyunsaturated acid to monounsaturated acid. Moreover, linonlenic acid (18:3) content increased instead of decreased for ClO_2 treatment, showing an additional preservation effect of ClO_2 on polyunsaturated acid, which may ascribed to less oxidation related to phenolic protection reported in above text. ClO_2 treatment prevented the increase in acid value and peroxide value of kernel in this study, though it decreased TAA. 1-MCP in combination with ClO_2 alleviated the increase in acid value and peroxide value, which suggests that ClO_2 protects kernel lipids from hydrolysis and oxidation. Phenolic content increased in response to ClO_2 treatment, but not when it was combined with 1-MCP, suggesting that promotion of postharvest synthesis of phenolics is not the only mechanism for maintaining kernel traits by ClO_2 .

Conclusion

This study investigated the effect of individual ClO_2 and the combination of ClO_2 with 1-MCP on the preservation of green walnut fruit as well as kernel traits at 0~1 °C. ClO_2 is of potential value in the preservation of green walnut fruit. Using ClO_2 at the concentration of 80 mg·L⁻¹ postponed the physiological senescence of green walnut fruit through decreasing its respiration activity, ethylene production and enhanced total phenolic and total flavnoid content during storage. ClO_2 reduced decay of the fruit for 45 days of the storage period. Kernel traits were maintained almost unchanged after 45 days of storage when the decay index of the fruit was less than 20 %, as was achieved with 80 mg L⁻¹ ClO_2 .

The effect of 1-MCP was too inconsistent to warrant recommending it to be proper for commercial application for decay control. 0.5 1-MCP treatment increased the decay the fruit and resulted the highest decay index throughout the storage, though 1-MCP treatment also decreased respiration activity and ethylene production of the fruit. Kernel traits were adversely affected by additional 1-MCP treatment and were not reversed by the combination treatment with ClO_2 . Effects of ClO_2 on inhibition of microorganisms, defense mechanism, and nutrients of the fresh walnut also need to be studied.

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