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Effect of 1-Methylcyclopropene Treatment on Chilling Injury, Fatty Acid and Cell Wall Polysaccharide Composition in Loquat Fruit

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Loquat fruit was treated with 2.32 nmol L^{-1} 1-methylcyclopropene (1-MCP) for 24 h at 20 °C, and then stored at 1 °C for 35 days to investigate the effect of 1-MCP treatment on chilling injury (CI) and fatty acid and cell wall polysaccharide composition. Loquat fruit developed CI, manifested as increased fruit firmness, internal browning and decreased extractable juice. These CI symptoms were reduced by 1-MCP treatment. 1-MCP-treated fruit exhibited higher levels of linoleic and linolenic acid and a higher unsaturated/saturated fatty acid ratio than control fruit during storage. The treatment also markedly delayed increase in alcohol insoluble residue, i.e. hemicellulose and cellulose. Meanwhile, the level of water- and CDTA-soluble pectins in treated fruit was higher than that in control. Our result suggested modifications of fatty acid and cell wall polysaccharide composition are associated with CI develpoment in loquat and 1-MCP treatment modulates the changes that seem to regulate the strength of cell wall and so to alleviate CI.

KEYWORDS: Loquat; 1-MCP; chilling injury; fatty acid; cell wall polysaccharide

INTRODUCTION

Low temperature storage is a postharvest technology used widely to extend the postharvest life of loquat fruit, but the fruit is susceptible to chilling injury (CI) when exposed to temperatures below 1 °C, depending upon variety. Fruit of red-fleshed loquat cultivars developed chilling disorders including flesh leatheriness and internal browning (*1*). Some methods that are effective to alleviate CI of loquat fruit are low temperature conditioning and application of polyamine, salicylic acid, methyl jasmonate (MeJA) or 1-methylcyclopropene (1-MCP) (*2*−*6*).

Alterations in biomembrane conformation and structure are considered the first events of CI having an effect on membrane permeability (*7*). The chemical composition of the membranes, in particular the fatty acid composition, can affect the fluidity of membranes and the changes from a gel phase to a liquid crystalline phase. This transition is suggested to be the basis of alteration of membrane permeability (*7*). A higher proportion of unsaturated fatty acids in polar lipids generally results in increasing membrane permeability (*8*). Reduction of the damaging effects of chilling in resistant fruit may be related to their higher ratio of unsaturated to saturated fatty acids, as has been reported, for example in banana and pomegranate fruit (*9*, *10*).

It has been reported that the development of CI in loquat fruit has been related to lignification and biochemical modification of cell wall polysaccharides (specifically pectin, hemicelluose and celluose) (*1*). Though some work has been done on regulation of lignification during the development of CI in loquat fruit after 1-MCP treatment (*3*), there have been very few reports dealing specifically with these cell wall polysaccharide modifications.

As an inhibitor of ethylene perception, 1-MCP has been reported to regulate various processes, such as ripening, softening, senescence and chilling tolerance in horticultural crops (*11*−*15*). Previous study has shown that application of 1-MCP could reduce the development of CI in loquat fruit (*3*). However, the mechanism by which 1-MCP alleviates CI in loquat fruit have not clearly elucidated. Moreover, there are no published data on the effect of exogenous 1-MCP treatment on fatty acid and cell wall polysaccharide composition in relation to CI in loquat fruit. The objective of this work was to study the changes of cell wall polysaccharide and fatty acid in control and 1-MCP treated loquat fruit stored at chilling temperatures.

MATERIALS AND METHODS

Fruit and Treatment. Loquat fruit (*Eriobotrya japonica* L. cv. Fuyang) was picked at commercial maturity from Suzhou, China, and transported within 4 h to our laboratory. Fruit of uniform shape, size, color, weight and absence of mechanical damage was randomly divided into two groups. Both groups of fruit were placed in sealed 40 L plastic tanks and treated with 0 (control), 2.32 nmol L^{-1} 1-MCP for 24 h in the dark at 20 °C and 80−85% RH. Following treatment, the containers were opened and ventilated, and the fruit was stored at 1 °C and 80−90% RH for 35 days. There were three replicates of 5 kg of fruit each per treatment, and the experiment was conducted twice. Fruit samples were taken after 1-MCP treatment (time 0) and at 7-day intervals during

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storage for measurements of CI, fatty acid and cell wall polysaccharide composition.

Evaluation of CI in Loquat Fruit. Fruit firmness, internal browning and extractable juice rate were used to evaluate the development of CI in loquat fruit. Fruit firmness was measured on two paired sides of 10 fruits from each replicate (skin removed) with a TA-XT2i texture analyzer (Stable Micro System Ltd., U.K.) with a 5 mm diameter probe at a speed of 1 mm s^{-1}. Extractable juice rate was estimated from the weight loss from placental tissue plugs in response to low-speed centrifugation. Four plugs (7 mm wide and 10 mm thick) were placed over sterile cotton in a 50 mL centrifuge tube and centrifuged for 10 min at 1700*g* at 25 °C. The results were expressed as fresh weight loss of the tissue plugs after centrifugation. Internal browning (IB) index manifested as browning near the core was evaluated visually using 10 fruits from each replicate after cutting the fruits longitudinally in half. For each fruit, IB was scored according to a 5-grade scale, where 0 = none; 1 = slight; 2 = moderate; 3 = moderately severe; 4 = severe. Results were expressed as an IB index calculated using the following formula: IB index (between 0 and 4) = [$\sum$(IB level) × (number of fruits at the IB level)]/(total number of fruits in the treatment).

Fatty Acid Quantification. Total lipids were extracted according to Cao et al. (*6*). Briefly, 20 g of flesh tissue was homogenized in 10 mL of chloroform:methanol:0.1 N HCl (200:100:1), and then 10 mL of 0.1 N HCl was added before centrifugation at 4000*g* for 10 min. The organic phase was collected and taken to dryness. Methylation of fatty acids was carried out by adding 1 mL of 14% (v/v) boron trifluoride in methanol at boiling temperature for 10 min. Methylated fatty acids were extracted with hexane, taken to dryness and redissolved in 200 μL of chloroform before injection. Fatty acids were separated and quantified according to Mirdehghan et al. (*9*) by a gas chromatography (Hewlett-Packard Co, Palo Alto, CA), coupled to a flame ionization detector. Identification and quantification of fatty acids were performed by comparing retention times and peak areas with authentic standards (Sigma Chemical Co., St. Louis, MO). The unsaturated/saturated fatty acid ratio was calculated by the formula (18:1 + 18:2 + 18:3)/(16:0 + 18:0), where 16:0 = palmitic acid; 18:0 = stearic acid; 18:1 = oleic acid; 18:2 = linoleic acid; 18:3 = linolenic acid.

Extraction and Analysis of Cell Wall Polysaccharides. Alcohol insoluble residues (AIR) were prepared according to Cao et al. (*16*). Two hundred grams of flesh tissue was homogenized in 80 mL of cold 95% alcohol over ice for 3 min, which was then boiled for 25 min. The suspension was filtered under vacuum through glass fiber filters (GF/C, Whatman) and washed with 100 mL of 95% ethanol. The AIR was transferred to 100 mL of chloroform−methanol (1:1, v/v) and stirred for 30 min at room temperature. The AIR was filtered under vacuum through glass fiber filters (GF/C, Whatman) and washed with 100 mL of 100% acetone. AIR samples were dried in an oven at 40 °C for 5 h and weighed. Results were expressed as mg 100 g^{-1} fresh weight (FW). Then, the AIR (50 mg) was fractionated using the methods described previously (*16*). The uronic acid content in the water, CDTA and Na_2CO_3 fractions was determined by the *m*-hydroxydiphenyl method (*17*). Galacturonic acid (Fluka) was used as standard. Results were expressed as mg of galacturonic acid per 100 mg of AIR. The cellulose and hemicelluloses contents were quantified using a phenol−sulfuric acid method (*18*). Glucose was used as standard for these assays. Results were expressed as mg of glucose per 100 mg of AIR.

Data Analysis. Experiments were performed using a completely randomized design. All statistical analyses were performed with SPSS (SPSS Inc., Chicago, IL). The data were analyzed by one-way analysis of variance (ANOVA). Mean separations were performed by Duncan's multiple range tests. Differences at $p < 0.05$ were considered as significant.

RESULT

Effect of 1-MCP Treatment on CI. Loquat fruit became firmer during storage, with the process delayed by 1-MCP (**Figure 1A**). Firmness of the control and treated fruit was 120.1% and 33.6% that of the original levels, respectively, after 35 days. IB index increased to 3.56 from harvest values during 35 days of storage in control loquat fruit (**Figure 1B**). The increase of IB index was inhibited by 1-MCP treatment. IB index was 45.5% lower than that in control fruit after 35 days of storage. Extractable juice in

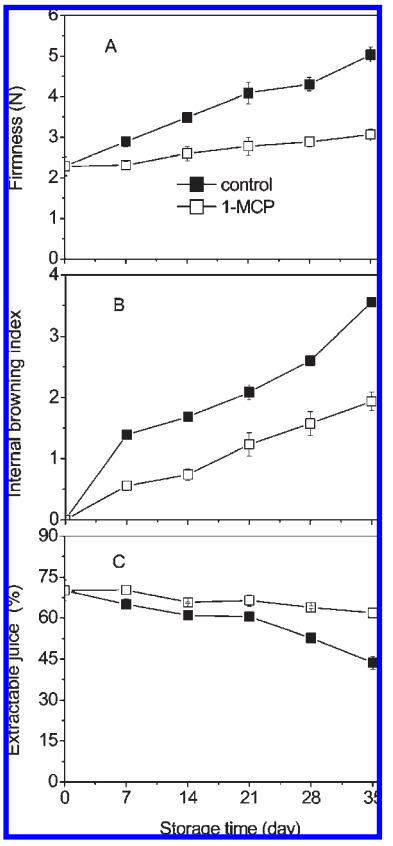

Figure 1. Effect of 1-MCP on fruit firmness (**A**), internal browning index (**B**) and extractable juice (**C**) of loquat fruit during storage at 1 °C. Values for firmness and internal browning are the means ± SE of three replicates. Values for extractable juice are the means ± SE of three replicates. Vertical bars represent the standard errors of the means.

control loquat fruit decreased over time in storage (**Figure 1C**). However, fruit treated with 1-MCP maintained higher extractable juice than that in control fruit. By day 35, the volume extracted from 1-MCP treated fruit was 41.8% higher than that in control fruit. The control fruit exhibited severe chilling symptoms manifested as flesh leatheriness and IB after 21 days of storage at 1 °C. Treatment with 1-MCP significantly ($p < 0.05$) inhibited the increases in fruit firmness, IB and maintained higher extractable juice rate, thereby delaying the development of CI in cold-stored loquat fruit.

Effect of 1-MCP Treatment on Fatty Acid Composition. The major fatty acids in the flesh of loquat fruit were palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3) acids. During the whole storage period at 1 °C, the levels of palmitic, stearic, and oleic acids increased (**Figure 2A−C**), while contents of linoleic, linolenic acid decreased (**Figure 2D**,**E**). As a consequence, there was a decrease in unsaturated/saturated fatty acids ratio in loquat fruit (**Figure 2F**). 1-MCP treatment reduced the increase in palmitic and stearic acid, as well as the decrease in linoleic and linolenic acid. 1-MCP treatment maintained a higher unsaturated/saturated fatty acids ratio than control fruit.

Effect of 1-MCP Treatment on Cell Wall Polysaccharide Composition. The changes of cell wall polysaccharide (pectin, hemicellulose and cellulose) were summarized in **Figure 3**. As shown in **Figure 3A**, AIR content increased gradually with storage time. Treatment with 1-MCP significantly ($p < 0.05$) inhibited the increase. AIR increased to 139.8% and 129.6% of the initial values, respectively, in control and 1-MCP treated fruit after 35 days of storage. Levels of water- and CDTA-soluble

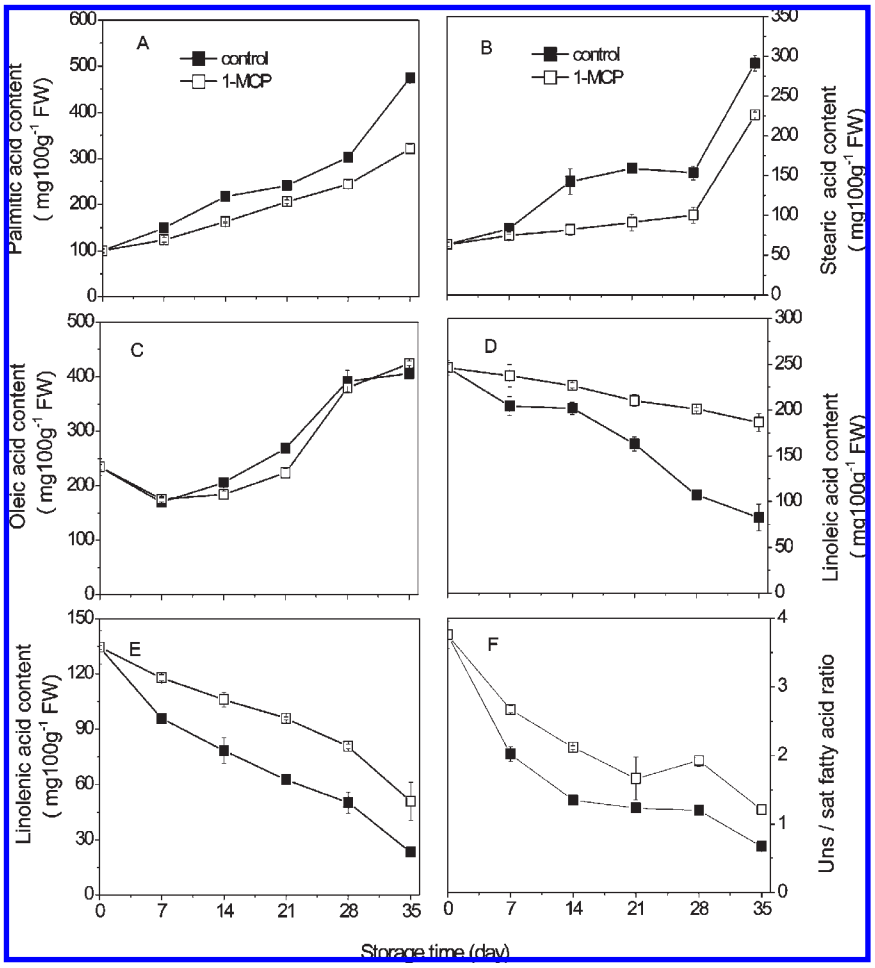

Figure 2. Effect of on contents of palmitic (**A**), stearic (**B**), oleic (**C**), linoleic (**D**), linolenic (**E**) acid and unsaturated/saturated fatty acid ratio (**F**) of loquat fruit during storage at 1 °C. Values are the means ± SE of three replicates. Vertical bars represent the standard errors of the means.

pectin decreased over time (**Figure 3B**,**C**). 1-MCP treatment maintained significantly ($p < 0.05$) higher levels of these two pectin fractions. The contents of water- and CDTA- soluble fractions were 31.8% and 47.3% higher, respectively, than that in control fruit at 35 days of storage. Na_2CO_3-soluble pectin increased steadily during storage (**Figure 3D**). The content in control was 250.2% higher than the level at harvest after 35 days of the storage. 1-MCP significantly ($p < 0.05$) inhibited the accumulation of this pectin fraction, and the value was 217.1% higher than the level at harvest. For contents of hemicellulose and cellulose, a significant increase was observed in control loquat fruit during storage; the levels were significantly ($p < 0.05$) lower in 1-MCP treated fruit (**Figure 3E**,**F**). Contents of hemicellulose and cellulose were 46.7% and 12.6% lower, respectively, than that in control fruit at 35 days of storage.

DISCUSSION

The primary event leading to CI may be membrane damage due to the formation of a gel phase. A higher ratio of unsaturated to saturated fatty acids provides higher tolerance to low temperature, as it lowers the temperature at which gel is formed (*7*, *19*). In the present study, we found that membrane lipid composition changed during storage with significant loss in linoleic and linolenic acids (two unsaturated fatty acids) and increase in palmitic and stearic acids (two saturated fatty acids) in control fruit, while in 1-MCP treated fruit, maintenance for these two unsaturated fatty acids occurred. Thus, fruit from 1-MCP treatment exhibited a higher ratio of unsaturated to saturated fatty acids which may be associated with the higher resistance to CI in loquat fruit after 1-MCP treatment.

CI in cold-stored loquat fruit is characterized by increase in fruit firmness and loss of juice due to a cell wall polysaccharide metabolism disorder (*1*). In the present study, we observed an accumulation of AIR concomitantly with increased CI in loquat fruit during cold storage. Previous studies have shown that cell wall synthesis was important in fruit softening as well as cell wall breakdown in apple and tomato (*20*, *21*), however, the speed of synthesis was more slow than breakdown in tomato fruit, leading to a clear reduction in AIR as ripening progress (*21*). Contrarily, our results here might suggest that when stored at chilling temperature, the cell wall synthesis in loquat fruit is more rapid than degradation, which resulted in an increase in AIR content. The lightening of CI symptoms by 1-MCP treatment may be related to inhibited AIR accumulation.

Cell wall analysis reveals that pectins, as indicated by uronic acid content, are solubilized from the wall during softening in an array of fruit (*22*). The water-soluble fraction is typically thought to be included polymeric material that has been solubilized from the cell wall metabolic processes or is only loosenly associated with the wall prior to softening, whereas the CDTA and Na_2CO_3-soluble fractions are generally considered to be enriched for ionically and covalently bound pectins, respectively. Softening-related increases in water- and CDTA-soluble fractions have been shown to be reflected in a decrease in the Na_2CO_3-soluble fraction (*23*, *24*). However, in our study, a decrease in water- and CDTA-soluble polysaccharides was accompanied by an

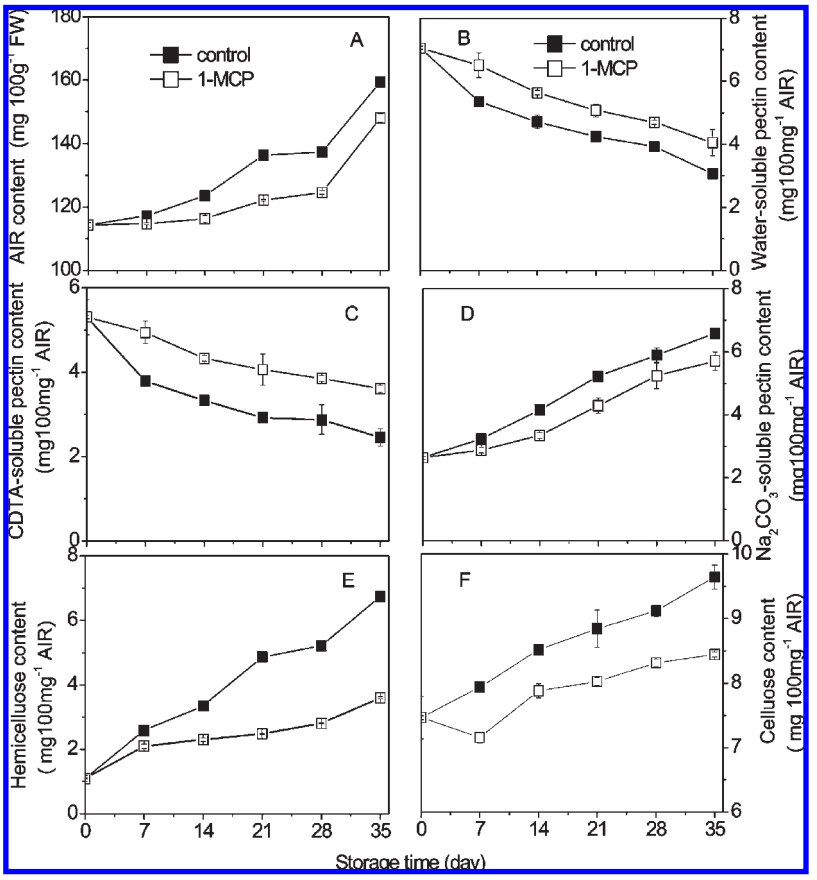

Figure 3. Effects of 1-MCP on contents of AIR (**A**), water- (**B**), CDTA- (**C**), Na_2CO_3-soluble pectins (**D**), hemicellulose (**E**) and cellulose (**F**) in loquat fruit during cold storage at 1 °C. Values are the means ± SE of three replicates. Vertical bars represent the standard errors of the means.

increase in the yields of polysaccharide extracted in Na_2CO_3. These findings were correlated to an increase in fruit firmness and CI in cold-stored loquats. As compared to untreated fruit, higher levels of water- and CDTA-soluble polysaccharides in 1-MCP-treated fruit indicated that a higher degree of pectic polymer solubilization was maintained by fruit in this treatment. It was reported that the architectural strength of cell wall can be increased by the cross-linking of pectin, and an association between pectin and extensin proteins has been suggested as a possible mechanism for cell wall strengthening (*25*). In addition, there is evidence for the existence of covalent links between pectin and extensin proteins and for a significant proportion of xyloglucan to be covalently attached to the pectic network (*26*). As the amount of covalently bound pectin (Na_2CO_3-soluble fraction) increased in loquat fruit, it probably cross-linked to other cell wall polymers and strengthened the cell wall, which was manifested as the increase in fruit firmness. 1-MCP treatment maintained higher contents of water- and CDTA-soluble pectins and inhibited the accumulation of Na_2CO_3-soluble pectin, thereby decreasing fruit firmness and CI in loquat fruit.

The cell wall composition reflects the balance between synthesis and hydrolysis, both of which occur throughout development and ripening (*27*). Previous study has shown that there is continuing synthesis of hemicellulose and cellulose during ripening in tomato (*28*). The increase in cellulose level can result from an enhancement of cellulose synthase, an inhibition of 1,4-β-glucanase, or both (*29*). In the current study, such inhibition of synthesis of hemicellulose and cellulose by 1-MCP treatment could account for the lower fruit firmness and CI incidence in loquat fruit.

Lignin is thought to play important roles in cross-linking cell wall components (*30*). Since lignin when bound to the cell wall polysaccharide confers rigidity and compression resistance to the cell wall, its accumulation results in low insolubilization of cell wall polysaccharide (*31*). Previous study reported that lignin accumulation occurred during loquat CI and ethylene could induce this process in loquat fruit (*4*, *6*). In this respect, 1-MCP, as a inhibitor of ethylene perception, might inhibit the lignin accumulation in loquat fruit at chilling temperature, and thus modulate the cross-linking of cell wall polysaccharide and alleviate CI. However, such a mechanism needs to be supported by further investigation.

Our results suggest that CI development in loquat fruit is associated with a reduction in unsaturated/saturated fatty acid ratio and 1-MCP treatment decreases this modification, thereby alleviating CI. The treatment also reduces abnormal cell wall polysaccharide insolubilization, which plays important roles in inducing acclimation to chilling stress.

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