

Research Article

Effectiveness of 1-Methylcyclopropene (1-MCP) Combination with Nisin-EDTA Treatment of Fresh-cut Kiwifruit for Prevention

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Abstract: A comprehensive study was made to determine the effects of 1-MCP (1 μ L L⁻¹) pretreated combined with Nisin-EDTA (0.02 M) on fresh-cut kiwifruit slices. Physico-chemical attributes of kiwifruit slices were evaluated every 3 days during storage. The fresh-cut kiwifruits pre-treated with 1-MCP, Nisin-EDTA coating and their combination showed better preservation quality and compared with the control slices during the entire storage period. 1-MCP showed an ability to relieve the pulp softening extent and inhibit total chlorophyll content decrease. Lower Polyphenol Oxidase (PPO) and higher ascorbic acid were observed in Nisin-EDTA treated samples which also showed a less total bacteria count compared with the control in the end storage. Moreover, the combination of 1-MCP and Nisin-EDTA was more effective than individual treatment on maintaining the quality of fresh-cut kiwifruit, which suggested that this may be a healthy alternative method for fresh-cut kiwifruit.

Keywords: 1-MCP, fresh-cut, kiwifruit, nisin-EDTA, quality

INTRODUCTION

Kiwifruit (Chinese gooseberry) is a popular fruit, characterized by its significant attracting green color and amount of ascorbic acid (Fiorentino *et al.*, 2009). Fresh-cut kiwifruit, as one kind of the kiwifruit products, is recognized as an important component of a healthy diet for its fresh, healthy and natural (Soliva-Fortuny and Martin-Belloso, 2003). However, fresh-cut products are wounded tissues essentially, wounding and other minimal processing procedures would cause physiological effects, including ethylene production, loss of chlorophyll and enzymatic browning, susceptibility to microbiological spoilage (Rico *et al.*, 2007).

Tissue browning, which can be a major defect of fruits, depends upon the concentration of Chlorophyll (Chl), phenolic compounds, the activity of Polyphenol Oxidase (PPO) and the content of antioxidants such as ascorbic acid in the tissue (Brandelli and Lopes, 2005). Changes in color of the fresh include a loss of chlorophylls and an increase in yellow components including carotenoids, are totally ethylene-dependent (Flores *et al.*, 2001). PPO activity has been extensively reported to be the main factor involved in kiwifruit browning, could be enhance by ethylene, metal ions and microbial infection (Rocha and Morais, 2002). Additionally, the bacterial soft rot would also accelerate discoloration of kiwifruit (Saltveit, 1999).

1-Methylcyclopropene (1-MCP) has been added to the list of agents for extending the shelf life and quality of plant products, which can restrain ethylene action by blocking its receptor so as to extend periods

(Blankenship and Dole, 2003). In fresh-cut fruits, 1-MCP prevented or delayed chlorophyll degradation and various types of color changes, reduced respiration rates or delayed increase in respiration (Budu and Joyce, 2003). Ethylenediamine Tetraacetic Acid (EDTA), one common metal chelating agents, can act as an inhibitor of PPO activity by chelating the copper in the prosthetic group of the enzyme (Sapers *et al.*, 1989). Nisin is a pentacyclic heterodetic subtype A lantibiotic peptide synthesized by *Lactococcus lactis* subsp. *Lactis* in fruit and vegetables (Ukuku *et al.*, 2001). Both of them had received GRAS (Generally Recognized as Safe) status in the United States and were approved for use in some processed cheese and juice spreads to prevent the outgrowth of clostridia spores and toxin production (Benkerroum and Sandine, 1988) and approved by China government from 1990. Before package, physical and chemical treatments are usually used in fruit processing to eliminate or reduce the presence of pathogenic and spoilage microorganisms (Ray, 1992). Washing or dipping is one of the very first processing operations to which a fruit or vegetable is subjected. The combination of EDTA and Nisin were tested as a washing treatment were significantly more effective than washing with water alone for reducing bacteria and yeast and molds (Ukuku and Fett, 2002).

The main goal of the present study was to evaluate the effect of 1-MCP and applied on the posterior behavior of ripe kiwifruit prepared as fresh-cut and treated with Nisin-EDTA after cutting, on the color, ascorbic acid and total bacteria count as well as other important quality parameters and their possible changes during the shelf life.

MATERIALS AND METHODS

Plant material and preparation: Fresh unripe Kiwifruit (*Actinidia chinensis* Planch cv. Lushanxiang) were taken from Baoshan borough, Shandong province in the autumn of 2012. At this stage, the initial SSC and firmness were 6.8% and 33.4 N, respectively. Fruits of uniform color, size and absence of defects were selected for studies. There are four treatments fresh-cut kiwifruit were separated for treatments as follows: the Control fruit (CK), 1-MCP fumigation (TR1), dipped in Nisin-EDTA (TR2), 1-MCP combination with Nisin-EDTA (TR3). The TR1 and TR3 were treated with 1 μ L/L 1-MCP (Rohm Haas, Shanghai, China) for 24 h in closed containers with a circulation system and a CO₂ trapping system (NaOH) at room temperature (~20°C), while TR2 samples were placed in the same system with 1-MCP (Peilong *et al.*, 2013). All of the fruits were cross-cut into four slices of uniform thickness using a sterile knife and then TR2 and TR3 were dipped for 2 min with 10 μ g/mL Nisin-EDTA (0.02 M), while TR1 and control were dipped in deionized water. The slices were placed in 120 μ m polypropylene bags (Pingyang Foreign Trade Co., Ltd., China), through which a continuous, humidified, ethylene-free air stream was passed at a rate of 100 mL/min. Bags containing the pieces were stored at 4°C for up to 15 days. All reagents were of analytical grade brought from Sigma. Chemical Co., St. Louis, USA. At each storage time, four trays per treatment (3 replicates) were randomly taken for analysis. Color parameters (CIELAB units L^* , H^*), total chlorophyll content, PPO, ascorbic acid content and total bacteria count were determined every 3 days.

Color change: A Chroma meter CR-400 series (CE Minolta, Japan), was used to measure flesh color quantified in the CIE L^* , a^* and b^* color space. The L^* value indicates lightness (black = 0 and white = 100) and Hue (H^*) = $180^\circ + \arctan(b^*/a^*)$, ranging from green- (180°) to yellow- (90°) (Antunes *et al.*, 2010).

Total chlorophyll content: Determination of extinction coefficients of Chl a and b in various solvents. Two samples of 1 cm diameter were taken from the equatorial zone of each fruit, with a weight of 1.00 ± 0.01 g, were kept at -16°C for 24 h, then were defrosted and underwent the protocol to extract chlorophyll established by Porra *et al.* (1989). Chlorophyll was extracted with 10 mL of 80% (v/v) acetone as a blank, centrifuged and light absorption by the supernatant was measured at wave lengths of 646.6, 663.6 and 750 nm, respectively in a visible light spectrophotometer Shimadzu Model UV-1700. Readings at 646.6 and 663.6 nm were corrected subtracting light absorption at 750 nm. Chlorophyll a and b concentrations were calculated with the following equations:

$$\text{Chlorophyll } a \text{ (g/mL)} = 12.25 A_{663.6} - 2.55 A_{646.6}$$

$$\text{Chlorophyll } b \text{ (g/mL)} = 20.31 A_{646.6} - 4.91 A_{663.6}$$

$$\text{Chlorophyll total (g/mL)} = 17.76 A_{646.6} + 7.34 A_{663.6}$$

Polyphenol Oxidase (PPO) activities: PPO activity was determined according to Rocha and Morais (2002). In an external ice bath, one kiwifruit slice per replicate was homogenized with 25 mL 0.2 M phosphate buffer (pH 6.5) and 0.8 g Polyvinylpyrrolidone (PVPP) during 3 with 1 min interval after each min. The mixture was centrifuged at $5000 \times g$ for 15 min at 4°C. The volume of the centrifugate was recorded. An aliquot (0.10 mL) of the centrifugate was added to the 2.80 mL substrate solution (1.1 g catechol in 50 mL 0.05 M phosphate buffer, pH 6.5) just before the assay and the rate of increase in absorbance at 420 nm was monitored using a spectrophotometer (Shimadzu UV-Vis 1700, Japan). The substrate solution (2.90 mL) was used as a reference blank. The linear section on of the activity curve as a function of time was used to determine the enzyme activity (U/g fresh weight/min). The Unit (U) for the PPO activity was defined as a change of 0.001 in absorbance at the conditions of the assay.

Ascorbic acid content: Ascorbic acid content was determined with Liquid Chromatography (HPLC). Briefly, kiwifruit tissue (2-3 g) was added to 10 mL of 4.5% metaphosphoric acid and vortexed. Extracts were centrifuged at $5000 \times g$ for 15 min at 4°C. The supernatant was filtered through a Whatman no. 1 filter and diluted to 15 mL with the 4.5% metaphosphoric acid. Analysis was performed using a Varian 325 HPLC pump equipped with tunable absorbance detector (Varian, CA). The flow rate was 0.5 mL/min and the detection wavelength was 254 nm. Sample aliquots were filtered through a 0.45 μ m poly (tetrafluoroethylene) filter prior to injection. Ascorbic acid content was expressed as mg/g Fresh Weight (FW). All samples were run in triplicate.

Total bacteria count analyses: A total of 5 g kiwifruit tissues randomly taken from different cylinders were blended with 45 mL of 2% peptone water for 2 min under sterile conditions. Flesh adhering to the rind plugs was trimmed off with a sterilized stainless steel knife. Decimal dilutions of the sample were made with 0.1% peptone water and aliquots (0.1 mL) were plated in duplicate on bismuth sulfite agar (BSA; BBL/Difco). A series of dilutions were prepared as needed according to standard procedures (Luna-Guzman and Barrett, 2000). The enumeration of particular microbial groups was performed by using the following media and culture conditions: Plate Count Agar (PCA, Difco Baton Dickinson, Sparks, MO) with incubation at 28°C for 48 h was used for enumeration of mesospheric aerobes. All microbial counts were reported as log cfu/g (colony forming units per gram of sample).

Statistical analysis: Data were subjected to a repeated measures two ways Analysis of Variance (ANOVA) to determine the significance of differences between treatments that consisted in the storage. Least Significant Difference at 5% level (LSD) was calculated to compare differences between means following a significant ANOVA effect. The comparison was carried out to evidence differences among different storage modality. For analysis of correlation between antioxidant capacity and antioxidant substances, the regression analysis was carried out.

RESULTS AND DISCUSSION

Color change: The color L^* value of all samples tended to decrease with time through storage as showed in Fig. 1A. Compared with the control, L^* values of the TR3 were higher than other three groups, especially after the 3rd day, while the L^* in TR1 and TR2 fluctuation decrease over the storage time. It mirrored that treatment of Nisin-EDTA dipping application of 1-MCP before processing containing anti-browning agents was most effective for inhibiting the browning on the surface of fresh-cut kiwifruit. The first period was characterized as a rapid increase in L^* range from 3rd to 6th day, attributed to the fast precipitation of unstable particles, such as soluble solid and Vitamin C, after a maximum at 4th day the L^* values decreased exponentially due to the oxidative darkening (McKenzie and Beveridge, 1988).

The initial hue (H^*) angle of kiwifruit was about 108.1°, which represents a color in the very slightly green-predominantly yellow region (when $180^\circ > \text{hue} > 90^\circ$) of the color solid dimensions. Both Nisin-EDTA-treated fruits (TR2 and TR3) were greener (higher h°) than controls during the storage (Fig. 1B). Yellow-brown, or loss of green color, is normally considered the major consequence of Chlorophyll (Chl) degradation in kiwifruit (Heaton and Marangoni, 1996; Matile *et al.*, 1999).

Total chlorophyll content: The total Chl showed similar decreases to those of flesh color assessments (h^* value), where chlorophyll content of the untreated fruit were low throughout storage (Fig. 2). From day 3, the chlorophyll content in fruit treated with 1-MCP and Nisin-EDTA were significantly higher, compared with the initial groups ($p < 0.05$). The change of total Chlorophyll content is thought to begin with the degradation of Chl into phytol, Mg^{2+} and a primary cleavage derivative of the porphyrin moiety in three consecutive steps (Rodoni *et al.*, 1997). As Chlase activity decreases during kiwifruits degreening, Chl degradation may be degraded via the Kinetin and POD-hydrogen peroxide pathway, which appears to be closely regulated by ethylene (Gong and Mattheis, 2003; Sabater and Rodrguez, 1978). The Mg^{2+} from the

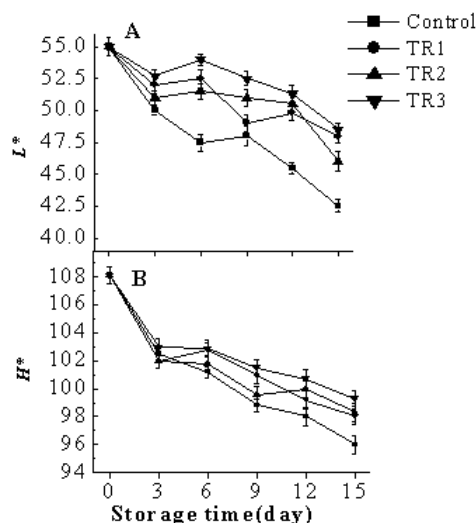


Fig. 1: Changes in fresh color (L^* and H^* value), bars represent means \pm S.D. (n = 3, $p < 0.05$)

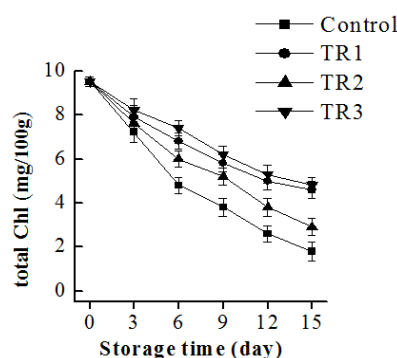


Fig. 2: Changes in fresh total chlorophyll content, bars represent means \pm S.D. (n = 3, $p < 0.05$)

Chl chelation under EDTA would cause the green decrease of the kiwifruit, because its chelation, which accelerates the degradation reaction of Chl and the production of pheophytin (Jacob-Wilk *et al.*, 1999).

Polyphenol Oxidase (PPO) activities: PPO activities were an important reason leading to enzymatic browning. The color alteration in fresh-cut fruits and vegetables is the direct consequence of PPO action on polyphenols. Oxidation of phenolic substrates by PPO is believed to be a major cause of browning of many fruits and vegetables, including kiwifruit (Nguyen *et al.*, 2003). Activity of enzymes involved in the oxidation of phenolic compounds (Concellon *et al.*, 2004; Haard *et al.*, 1974). PPO catalyze the oxidation of phenolic to quinone using oxygen as a final electron acceptor, while for peroxidases the final electron acceptor is hydrogen peroxide. After quinone formation, secondary non-enzymatic reactions result in the accumulation of melanin-like pigments, giving a brown undesirable appearance to the tissues.

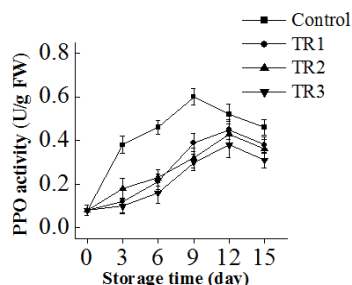


Fig. 3: Changes in fresh PPO activity, bars represent means±S.D. (n = 3, p<0.05)

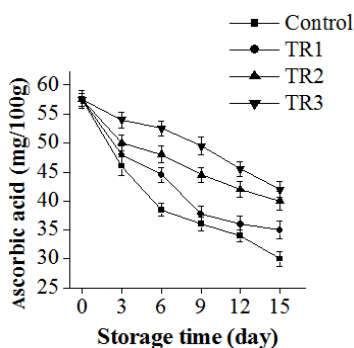


Fig. 4: Changes in fresh ascorbic acid content, bars represent means±S.D. (n = 3, p<0.05)

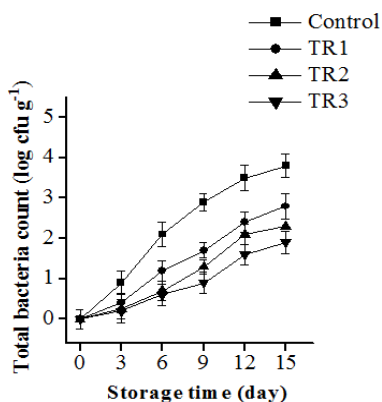


Fig. 5: Changes in fresh total bacteria count, bars represent means±S.D. (n = 3, p<0.05)

Figure 3 showed the PPO activity of fresh-cut kiwifruits during storage. TR3 showed the lowest PPO activity among all treatments during all storage. Furthermore, the high PPO activity of the control on day 2 and 10 associated with the low level of total phenolic may be due to the unprotected state of the sample towards enzymatic reactions. Rocha and Lee (2012) found L^* values of apple cubes and peach cultivars during storage to be moderately correlated to PPO activity. Metal chelating agents, such as EDTA, can act as an inhibitor of PPO activity by chelating the copper in the prosthetic group of the enzyme, so as to

inhibit the browning of polyphenols in the tissue (Sapers *et al.*, 1989).

Ascorbic acid content: Ascorbic acid could not influence polyphenol oxidase directly but acts as a reducing compound and reduces the orthoquinones to dehydroxyphenols (Sapers and Douglas, 1987). As the concentration of ascorbic acid is decreased, the quinone concentration increases and causes the formation of the brown pigments. The degradation of ascorbic acid could change the color of fresh-cut products, including cause a reduction in the H^* . The rapid rate of ascorbic acid decline in kiwifruit slices was significantly delayed by the 1-MCP and EDTA treatment. The ascorbic acid levels in the initial fruit after 12th day were reached almost as twice time as Nisin-EDTA treated fruit and the TR3 get the highest content of Ascorbic acid at the end of storage (Fig. 4), which suggested that Nisin-EDTA can inhibit the degradation of ascorbic acid.

As a highly effective inhibitor of enzymatic browning, ascorbic acid have ability to reduce quinones back to phenolic compounds. However, once the ascorbic acid has been completely oxidized to dehydroascorbic acid by this reaction, quinones can accumulate and induce browning again (Chen *et al.*, 2000). The enzymes, such as ascorbic acid oxidase and peroxidase, would also decompose the ascorbic acid content, the activities of which were promoted by the ethylene produced during the storage (Baur and Yang, 1969). In addition, ascorbic acid was converted to dehydroascorbic acid when PPO activity was increased. Ascorbic acid oxidase (AAO, EC 1.10.3.3) is a Cu-containing enzyme that catalyses the oxidation reaction of ascorbate to Dehydroascorbic Acid (DHA) with the concomitant reduction of molecular oxygen to water. The concentration of ethylene was reduced by 1-MCP fumigating to restrain the activity of AAO. Moreover, the Nisin-EDTA can protect the ascorbic acid from being dehydroascorbic acid, which is easily oxidized by endogenous enzymes or by copper-catalyzed autoxidation (Deutsch, 1998). 1-MCP treatment inhibited the accumulation of O_2^- and H_2O_2 during storage; therefore oxidative damage was alleviated and senescence was delayed (Larrigaudiere *et al.*, 2009).

Total bacteria count analyses: Minimally processed fresh fruits and vegetables provide a good substrate for microbial growth. PPO were induced by infection of microflora to play a defending a territory from disease-causing microorganisms (Avdiushko *et al.*, 1993). Total aerobic Plate Count (TPC) showed that, on day 0, the total number of aerobic microorganisms contained in all kiwifruit samples were below the detection limit of the viable counting method (2 log cfu/g) (Fig. 5). However, on day 6 there were a significantly higher number of counts in the untreated sample (control) and TR1

compared to other treatments. The bacteriostatic effect in TR2 and TR3 treated by Nisin-EDTA were better than samples fumigated with 1-MCP.

EDTA is recognized to directly inhibit the growth of bacteria in fruit through disruption of the integrity of bacterial membrane and cell wall by its chelation with cations, which has well been proved against Gram negative, the Gram-positive bacteria and yeast (Brul and Coote, 1999; Hansen *et al.*, 2001). Nisin is a pentacyclic heterodetic subtype A lantibiotic peptide synthesized by *Lactococcus lactis* subsp. *lactis*. It is an effective inhibitor of gram-positive bacteria and bacterial spores. In several cases, Nisin used in combination with a chelating agent has been reported to exhibit a bactericidal effect toward both gram-positive and gram-negative bacteria (Cutter and Siragusa, 1995). The adsorbing effect on the membrane of Nisin could be promoted by EDTA for its chelation.

CONCLUSION

In conclusion, treatments with 1-MCP before fresh-cut processing are an effective method in color maintenance of kiwifruit slices. The presence of Nisin-EDTA in dip solutions after 1-MCP treatment had a marginal effect in decaying the growth of micro-flora in slices. Dip treatment leads to partial metal ion loss from the fruit, which prevented browning in slices by inhibiting PPO activity and microbial growth. The total chlorophyll content was protected from depredating treated with 1-MCP for inhibiting chlorophyllase activity. However, the Mg^{2+} in chlorophyll was chelated in present with Nisin-EDTA induced the chlorophyll change into phaeophytin, which could accelerate the kiwifruit's color become from green to yellow. Although Nisin-EDTA presence has little bad influence on the chlorophyll preservation of fruit slices, it also had other positive effects such as antioxidant. So TR3 treatments with 1-MCP before and Nisin-EDTA after fresh-cut processing would maintenance the color and ascorbic acid of fresh-cut kiwifruit during the period.

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