



## Original Article

# Impacts of sodium chlorite combined with calcium chloride, and calcium ascorbate on microbial population, browning, and quality of fresh-cut rose apple



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## ABSTRACT

Microbial activity and browning were minimized and fresh-cut rose apple quality was maintained using sodium chlorite (SC) combined with calcium chloride (CC) and calcium ascorbate (CaAs) and by investigating the optimal concentration and dipping time of SC for inhibiting microbial activity and browning. Fresh-cut rose apple samples were dipped in SC solution at 100 mg/L and 200 mg/L for 1 min and 3 min, with filtered water and non-dipped samples as controls. All samples were kept at  $4 \pm 2$  °C for 9 d. The results showed that 200 mg/L SC for 3 min was the best treatment to inhibit microbial growth (total bacteria, yeast and molds, *Escherichia coli* and coliforms), delay browning and polyphenol oxidase (PPO) activity of fresh-cut rose apples, but could not maintain the fresh firmness. A firmness experiment was conducted by dipping fresh-cut rose apples in 200 mg/L SC and in 200 mg/L SC combined with 20 g/L CC and 20 g/L CaAs (SC + CC + CaAs) for 3 min before storage at  $4 \pm 2$  °C for 9 d. Samples immersed in filtered water were used as the control. The combined treatment delayed microbial contamination and browning by reducing the PPO activity and the accumulation of phenolic content, and maintained the fresh firmness of fresh-cut rose apples. Thus, the combination treatment of SC + CC + CaAs solution can protect fresh-cut rose apples against microbial contamination and delay browning and maintain firmness.

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## Introduction

Fresh-cut produce (minimally processed products) first arose in the 1990s, initially offering lettuce, cabbage, carrot and some other vegetables (Colle et al., 2013). Thereafter, the fresh-cut studies expanded to fruits such as mango (Dea et al., 2010), apple (Luo et al., 2011; Wu et al., 2012), pear (Xiao et al., 2010, 2011) and rose apple (Worakeeratikul et al., 2007a, 2007b; Supapvanich et al., 2012). In Thailand, the fresh-cut fruit business commenced earlier than the fresh-cut vegetable business, with fresh-cut fruit originally sold in canteens at schools, universities, working offices and often sold on carts which traveled around villages and communities (James and Ngarmsak, 2010). At present, popular fresh-cut fruits such as ripe

and unripe mangoes, ripe and unripe papayas, guava, pineapple, water melon, cantaloupe, jackfruit, pomelo and rose apple are predominantly sold in markets (Zagory, 1999; Sa-nguanpuag et al., 2007; James and Ngarmsak, 2010).

Rose apple (*Syzygium samarangense* L.) is a tropical fruit which was originally cultivated in Southeast Asia (Vara-Ubol et al., 2006). Recently, the demand for fresh-cut products has increased in many countries due to the busy lifestyle of people, especially in cities as consumers need convenient and portable foods but still require high quality, safety, nutrition and freshness similar to that associated with intact fruits (Beirão-da-Costa et al., 2006). However the peeling, cutting and slicing processes are the main causes of rapid deterioration in fresh-cut fruit and can accelerate the respiration rate and ethylene production, which lead to rapid loss of water, firmness, aroma and flavor (Rolle and Chism, 1987; Oms-Oliu et al., 2010). Many cases have been reported where the most important problems of fresh-cut fruit are browning and microbial contamination with food-borne pathogens and spoilage microbes, which

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lead to a short shelf life and reduced consumer safety (McEvily et al., 1992; Watada et al., 1996; Hedges and Toivonen, 2008).

Browning of fresh-cut produce is mainly caused by polyphenol oxidase (PPO), which oxidizes the phenols to quinines in the presence of oxygen (Walker and Wilson, 1975; Friedman, 1996). Enzymatic browning can be a significant problem as it limits the shelf life of fresh-cut fruits, but there is very limited information about browning inhibition of fresh-cut rose apple. Worakeeratikul et al. (2007a) reported that hydro cooling rose apple fruits before dipping in 6% calcium ascorbate and 0.2% chitosan solution could delay browning, PPO activity and maintain the firmness. A low storage temperature at  $4 \pm 2$  °C could reduce browning of fresh-cut rose apple without any significant effects on quality when compared with storage at  $12 \pm 2$  °C (Supapvanich et al., 2011). Browning inhibition of fresh-cut rose apple using an edible coating has been reported with chitosan (Worakeeratikul et al., 2007b) and konjacglucomannan coating incorporated with pineapple fruit extract (Supapvanich et al., 2012).

Sodium chlorite (SC) is elucidated as having the dual effect of being an anti-browning and anti-microbial agent (Lu et al., 2006, 2007; He et al., 2008; Guan and Fan, 2010). SC is accepted by the US Food and Drug Administration (USFDA) as a sanitizing agent for food and fresh-cut produce as it is a powerful oxidizing agent. It has been used to clean utensils, disinfect water and to remove biofilms on the food preparation surface (Anonymous, 2000; Lu et al., 2006). Researchers have demonstrated that SC effectively inactivates PPO and microbial growth on fresh-cut apple (Lu et al., 2007; Luo et al., 2011; Li et al., 2015), pear (Xiao et al., 2011), cilantro (Allende et al., 2009), broccoli (Renumarn et al., 2012) and lettuce (Hengphum et al., 2015).

Softening is another problem of fresh-cut produce that needs to be considered. It is known that the rate of fruit softening is related to calcium levels in the plant tissue (Fallahi et al., 1997) and cell-wall-degrading enzymes; especially polygalacturonase (PG) and pectin methyl esterase (PME) (Chisari et al., 2007; Silveira et al., 2011). Calcium application in fresh-cut produce can help to stabilize the cell membrane and form Ca-pectates, which increase rigidity of the middle lamella and plant cell wall resulting in resistance to attack from cell-wall-degrading enzymes (Luna-Guzman et al., 1999). However, calcium salts, particularly calcium chloride (CC) and calcium ascorbate (CaAs) have been reported to maintain firmness, inhibit browning, and extend the shelf life of fresh produce and fresh-cut produce such as apples (Wang et al., 2007; Aguayo et al., 2010; Tardelli et al., 2013; Li et al., 2015), melons (Luna-Guzman et al., 1999; Luna-Guzman and Barrett, 2000; Silveira et al., 2011) and eggplant (Barbagallo et al., 2012). Luo et al. (2011) stated that SC caused tissue softening of fresh-cut apple. Thus, SC mixed with CC was used to solve the tissue softening problem. Improving the texture of fresh-cut apple could be obtained by treating with CC at 20 g/L, where no bitter taste was observed. Calcium ascorbate (CaAs) has been industrially used to control the browning and to maintain the quality of fresh-cut produce (Luo et al., 2011). However, its effect depends on the concentration used. Wang et al. (2007) stated that 50 g/L CaAs resulted in strong browning inhibition, but it is expensive and therefore more commonly used at lower, less effective concentrations, while CC is inexpensive. Thus, the incorporation of a low level of CaAs (20 g/L) with CC (20 g/L) was investigated in the current study.

The literature cited earlier reported that the strong, dual anti-browning and anti-microbial effect of sodium chlorite might also improve the quality and extend the shelf-life of rose apple when it was incorporated with calcium salts. Therefore, the aim of this work was to investigate the effectiveness of sodium chlorite with calcium chloride and calcium ascorbate in inhibiting the microbial

population and browning, and maintaining the firmness of fresh-cut rose apples.

## Materials and methods

### Fresh-cut fruit preparation

Rose apple fruits from the Good Agricultural Practices (GAP) orchard, Ratchaburi province, Thailand were harvested at 45 d after full bloom. Non defect fruits were selected and washed with tap water and then disinfected with 50 parts per million sodium hypochlorite solution for 3 min. Fresh-cut rose apple was prepared by cutting the fruit into four pieces, with the core and ends of each fruit removed.

### Sodium chlorite treatment

Fresh-cut rose apple samples were immersed in 100 mg/L or 200 mg/L sodium chlorite solution (SC) for 1 and 3 min at room temperature. Fresh-cut samples immersed in filtered water were used as the control treatment. All samples were dried under ambient conditions. Six pieces of fresh-cut samples were randomly selected and placed on a polypropylene tray (14 mm × 19 mm × 2.5 mm), top heat sealed with polypropylene film (anti-fog), and then kept at  $4 \pm 2$  °C for 9 d. Each treatment had four replicates (trays). The microbial population, quality and biochemical changes of fresh-cut rose apple were investigated at 3-day intervals.

### Combination treatment

The fresh-cut samples were immersed in 200 mg/L sodium chlorite for 3 min or 200 mg/L sodium chlorite combined with 20 g/L calcium chloride and 20 g/L calcium ascorbate (SC + CC + CaAs) for 3 min. The sample immersed in filtered water served as the control treatment. All samples were dried, packed and stored under the same conditions as previously described. Each treatment had four replicates (trays). The microbial population, quality and biochemical changes of fresh-cut rose apple were investigated at 3-day intervals.

### Microbiological analysis

A 25 g sample of fresh-cut rose apple was homogenized with 225 mL of 1% sterile peptone water using a stomacher (IUL Instruments Masticator; Barcelona, Spain) for 1 min. Ten-fold dilution series were made in sterile peptone water as required for plating. Plate count agar (PCA; HiMedia; Mumbai, India), eosin methylene blue agar (EMB, HiMedia, India), and potato dextrose agar (PDA, HiMedia, India) were used to enumerate total bacteria, *Escherichia coli*, and yeast and molds respectively. PCA plates and EMB plates were incubated at 37 °C for 1–2 d, whereas PDA plates were incubated at ambient temperature ( $26 \pm 2$  °C) for 5 d. Four replicates were analyzed and expressed as log colony forming units per gram fresh weight (CFU/g FW).

### Biochemical analysis

The phenolic compound content was determined as described by Ikram et al. (2009). A 5 g sample of tissue was homogenized with 10 mL of 80% ethanol in a blender. The homogenate sample was centrifuged at  $12,000 \times g$  for 20 min at 4 °C. A 20 µL of supernatant was mixed with 1.58 mL of distilled water before adding 100 µL of 1 N Folin-Ciocalteu phenol reagent and 300 µL of 2.5 M Na<sub>2</sub>CO<sub>3</sub>. The sample solutions were mixed and then incubated in a water bath at 40 °C for 30 min, and then allowed to incubate at room temperature

for 20 min. The sample solution was measured using a spectrophotometer (model UV-1800; Shimadzu; Kyoto, Japan) at 765 nm. Distilled water was used as the blank. Data were reported as grams per 100 g fresh weight.

Polyphenol oxidase (PPO) was determined according to the method of [Lichter et al. \(2000\)](#). A 2 g sample of fresh-cut rose apple was homogenized in 20 mL of 0.1 M sodium phosphate buffer (pH 6.6) and 0.5 g polyvinylpolypyrrolidone. The sample was centrifuged twice at 8000 × g, for 10 min and 20 min, respectively, at 4 °C. A 0.75 mL sample of supernatant was mixed with 0.12 mL of 23 mM 4-methyl catechol. The activity of PPO was measured every 10 s for 1 min using the spectrophotometer at 410 nm. The total protein content of the samples was analyzed using the method of [Bradford \(1976\)](#) with Coomassie Brilliant Blue. The PPO activity was presented as specific activity, defined as the increase in absorbance per minute per 1 mg enzyme of reaction mixture ( $\Delta OD_{410\text{nm}}$  per mg protein per min).

#### Quality determination

Changes in the pulp color of fresh-cut rose apple were determined using a Minolta DP-301 colorimeter (Konica Minolta; Tokyo, Japan) and were expressed as  $L^*$ ,  $a^*$  and  $b^*$  values. The browning index (BI) was calculated as described by [Palou et al. \(1999\)](#) using  $X = (a^* + 1.75L^*)/(5.645L^* + a^* - 3.012b^*)$ , and  $BI = (X - 0.31)/0.17$ . The difference in total color of fresh-cut rose apple during storage was calculated according to [Maskan \(2001\)](#) using equation (1):

$$\Delta E^* = \left[ (L^*_{\text{F}} - L^*)^2 + (a^*_{\text{F}} - a^*)^2 + (b^*_{\text{F}} - b^*)^2 \right]^{1/2} \quad (1)$$

where,  $L^*$ ,  $a^*$  and  $b^*$  are the color values on the initial day, and  $L^*_{\text{F}}$ ,  $a^*_{\text{F}}$  and  $b^*_{\text{F}}$  were the color values after 3, 6 or 9 d storage.

The shear force was measured using a texture analyzer (model TA-XT Plus; Stable Micro System Co. Ltd.; Goldalming, England) equipped with a 7 cm × 12 cm knife blade with a 45° chisel. The data were recorded and expressed in newtons.

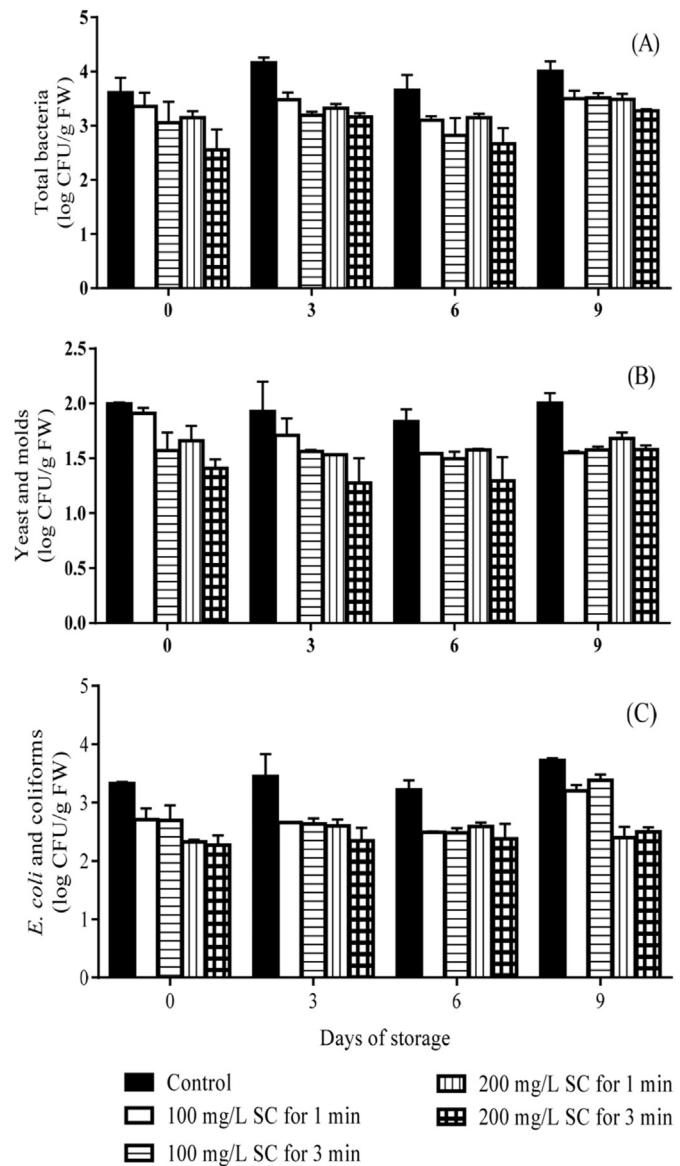
#### Statistical analysis

Data were analyzed using the ANOVA procedure in the general linear models procedure of SAS 9 (SAS Institute; Cary, NC, USA) for completely randomized design experiments. Data were presented as mean  $\pm$  SE. Each treatment contained four replicates and each replicate consisted of six pieces of fresh-cut rose apple. Significance was tested at  $p \leq 0.05$  using Duncan's multiple range test.

## Results

#### Effects of sodium chlorite on microbial populations

Treatment with SC solution at 100 mg/L and 200 mg/L for 1 and 3 min reduced the total bacteria, yeast and molds, and *E. coli* and coliforms, during storage at 4 °C ([Fig. 1](#)). The level of 200 mg/L SC for 3 min had the highest inhibitory effect on the reduction of microorganisms. After treatment (day 0), total bacteria reduced from 3.61 log CFU/g FW (control) to 2.55 log CFU/g FW, respectively. However, SC treatment at 200 mg/L for 3 min showed the greatest reduction of total bacteria of 0.73–1.06 log CFU/g FW reduction during storage, whereas treatment with 100 mg/L SC for 1 min or 3 min showed a lower reduction (0.26–0.97 log CFU/g FW) after treatment, which varied significantly compared to the control from day 3 to day 9 of storage ([Fig. 1A](#)).



**Fig. 1.** Total bacteria (A), yeast and molds (B), and *E. coli* and coliforms (C) of fresh-cut rose apple immersed in filtered water (control), sodium chlorite (SC) at 100 mg/L or 200 mg/L for 1 and 3 min. Treated samples were stored at 4 °C for 9 d. Data shown are mean values of  $n = 3$  and the error bars represent standard errors of the means using Duncan's multiple range test at  $p \leq 0.05$ . CFU/g FW = colony forming units per gram fresh weight.

The level of yeast and molds of non-treated, fresh-cut rose apples was 1.99 log CFU/g FW on day 0. The SC treatment at 200 mg/L for 3 min had the greatest inhibitory effect on yeast and molds compared to the other treatments. Although there was no significant inhibitory effect between SC treatments at all concentrations, it seemed that the higher concentration showed a greater effect than the lower concentration. The yeast and molds populations in fresh-cut rose apples treated with 200 mg/L SC for 3 min were in the range 1.27–1.57 log CFU/g FW, whereas with 100 mg/L SC for 3 min, the levels were higher, with a range of 1.49–1.57 CFU/g FW ([Fig. 1B](#)).

The *E. coli* and coliforms counts of fresh-cut rose apples treated with 100 mg/L and 200 mg/L SC for 1 and 3 min were significantly lower than the control. A 200 mg/L SC for 3 min reduced *E. coli* and coliforms by 1.06–1.32 log CFU/g FW followed by 100 mg/L SC for 1 min (0.62–1.23 log CFU/g FW), 100 mg/L SC for 3 min (0.35–0.81

log CFU/g FW) and 100 mg/L SC for 1 min (0.52–0.80 log CFU/g FW), respectively (Fig. 1C).

#### Effects of sodium chlorite on browning

Fresh-cut rose apple normally became brown at the cut surface within a few minutes after cutting. The color change of fresh-cut rose apple was measured at the cut surface and reported as a lightness ( $L^*$ ) value and browning index (Fig. 2A and B). The  $L^*$  value of the control decreased from the beginning (65.42) to the last day of storage (61.46), on the samples treated with 100 mg/L SC for 1 min. The treatments of 100 mg/L SC for 3 min and 200 mg/L SC for 1 and 3 min maintained the  $L^*$  value of fresh-cut rose apples throughout storage. The treatment of 200 mg/L SC for 3 min

showed the greatest anti-browning of fresh-cut rose apple (Fig. 2A). This result correlated with the browning index (BI). The lowest BI was found in fresh-cut rose apples treated with 200 mg/L SC for 3 min, whereas the highest BI was found in non-treated samples (Fig. 2B). PPO is the key enzyme related to browning in fruits and vegetables and the PPO activity of fresh-cut rose apple in all treatments increased during storage (Fig. 2B). The SC treatment slightly inhibited PPO activity. The lowest PPO activity was found in fresh-cut rose apples treated with 200 mg/L SC (0.034–0.069  $\Delta OD_{410nm}$  per mg protein per min), while the control was 0.044–0.108  $\Delta OD_{410nm}$  per mg protein per min (Fig. 2C).

#### Effects of sodium chlorite on firmness

The firmness of fresh-cut rose apple was expressed as the shearing force. At the beginning of storage, the firmness ranged between 41.19 N and 42.17 N; afterward, it decreased to 33.83–35.03 N (Fig. 2D). The treatment with SC slightly maintained the firmness of fresh-cut rose apple compared to the control sample.

#### Effects of combination treatment on microbial counts

The effect of SC on the inhibition of microbial counts was improved when integrated with the CC and CaAs treatments. Total bacteria and yeast and molds in the SC- and SC + CC + CaAs-treated samples noticeably declined in comparison to the control. Counts of total bacteria, yeast and molds in the combined treatment samples showed reductions in the range 1.0–1.66 and 0.97–1.75 log CFU/g FW, respectively, whereas, the SC treatment showed a reduction in the range 0.46–1.41 and 0.71–0.74 log CFU/g FW, respectively, during 9 d storage (Fig. 3A and B). The *E. coli* and coliforms counts in the control samples were in the range 2.85–3.16 log CFU/g FW during storage. Dipping fresh-cut rose apple samples in SC solution decreased the *E. coli* and coliforms count significantly by 0.70–1.15 log CFU/g FW and 0.80–1.16 log CFU/g FW in SC + CC + CaAs solution, respectively (Fig. 3C).

#### Effects of combination treatment on browning

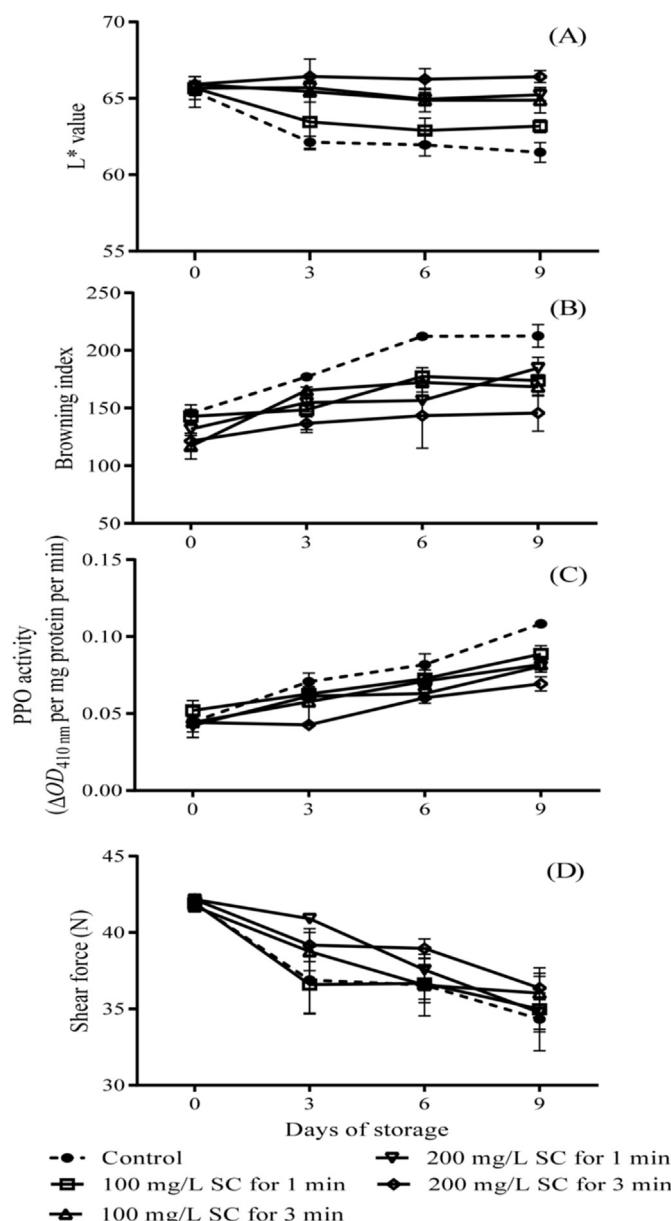
The  $L^*$  value of the control sample declined during storage, whereas the SC-treated sample and its combination with CC + CaAs treatment significantly increased from 62.99 to 63.17 to 64.28–65.63 (Fig. 4A). This was related with color change ( $\Delta E$ ). Fresh-cut rose apple samples treated with SC + CC + CaAs significantly delayed the increase in  $\Delta E$  (Fig. 4B), the browning index (Fig. 4C) and the PPO activity compared to the control samples (Fig. 5A). The PPO activity of non-treated fresh-cut rose apple samples increased rapidly with storage time. No significant differences in the phenolic contents were found in any treatment (Fig. 5B).

#### Effects of combination treatment on firmness

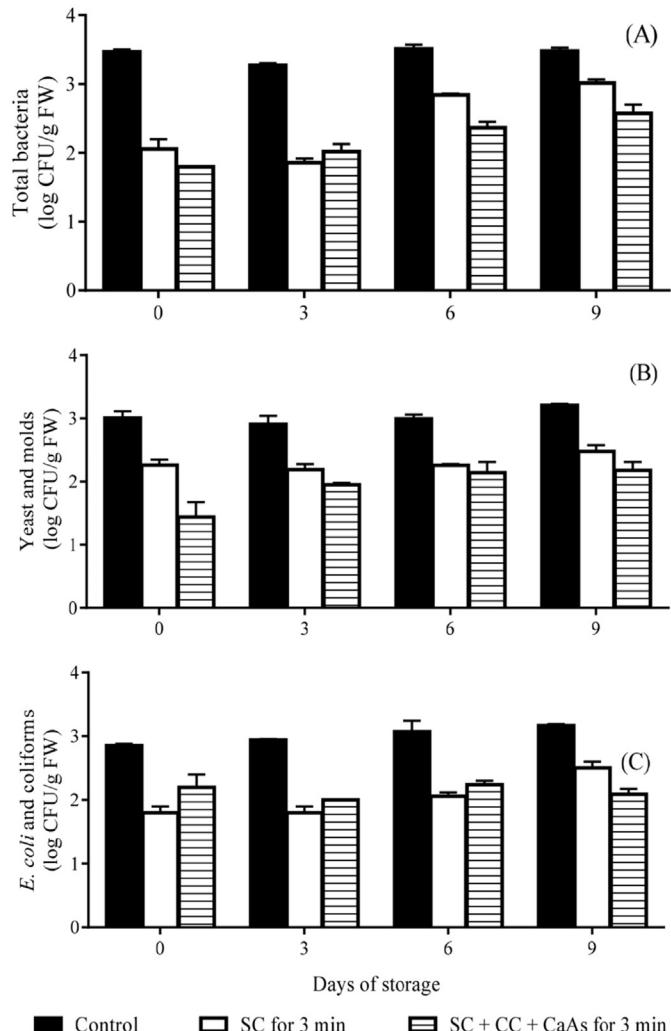
On the initial day of storage, the firmness of the fresh-cut rose apple samples was in the range 44.28–47.35 N (Fig. 5C); afterward, the firmness of non-treated samples and SC treated samples decreased to 31.60 N and 34.03 N, respectively, at the end of storage, whereas SC combined with CC and CaAs maintained firmness at 38.68 N.

#### Discussion

SC is a strong oxidizing agent, which can generate chlorine dioxide ( $ClO_2$ ) under acidic conditions (Lu et al., 2007). The



**Fig. 2.**  $L^*$  value (A), browning index (B), PPO activity (C), and shear force (D) of fresh-cut rose apple treated with filtered water (control) or with sodium chlorite (SC) at 100 mg/L or 200 mg/L for 1 and 3 min. Treated samples were stored at 4 °C for 9 d. Data shown are mean values of  $n = 3$  and the error bars represent standard errors of the means using Duncan's multiple range test at  $p \leq 0.05$ .

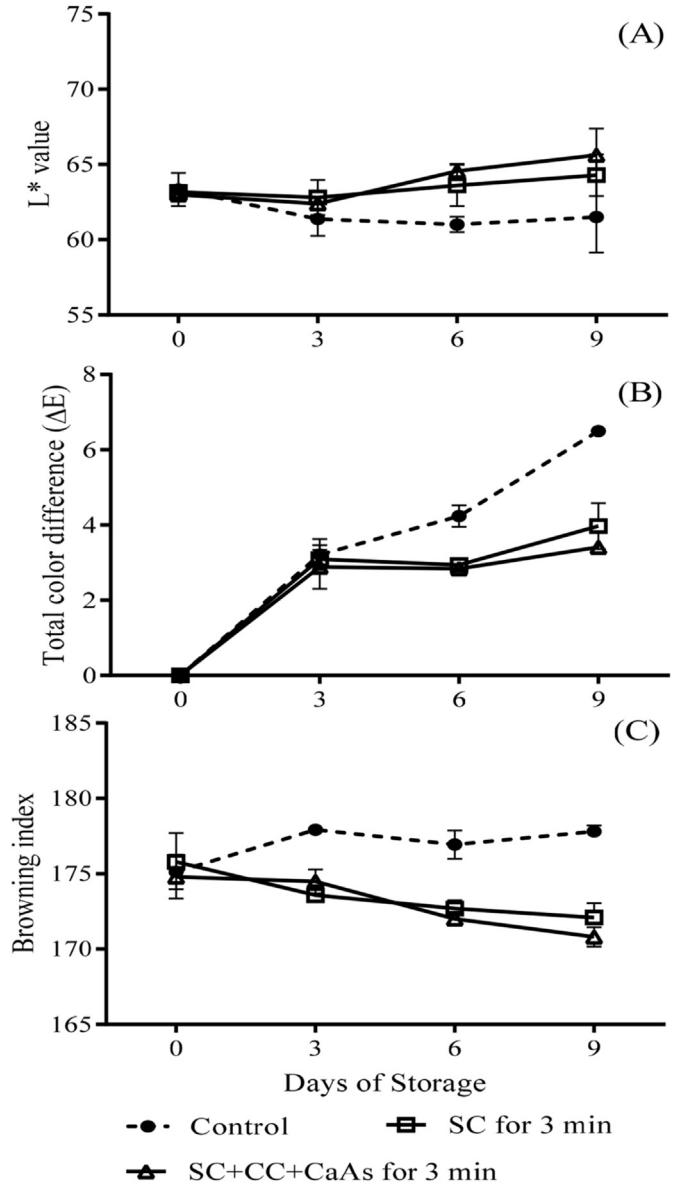


**Fig. 3.** Total bacteria (A), yeast and molds (B), and *E. coli* and coliforms (C) of fresh-cut rose apple treated with filtered water (control) for 3 min, 200 mg/L sodium chloride (SC) for 3 min, or 200 mg/L SC mixed with 20 mg/L calcium chloride (CC) and 20 mg/L calcium ascorbate (CaAs). Treated samples were stored at 4 °C for 9 d. Data shown are mean values of  $n = 3$  and the error bars represent standard errors of the means using Duncan's multiple range test at  $p \leq 0.05$ . CFU/g FW = colony forming units per gram fresh weight.

mechanism of microbial inactivation by  $\text{ClO}_2$  has been proposed, but the most commonly accepted strategy of  $\text{ClO}_2$  is inhibition of microbial protein synthesis (Trinetta et al., 2012). SC in the range 0.5–1.2 g/L is approved to use on raw fruit and vegetables by the USFDA (Anonymous, 2000). In the current study, SC at 200 mg/L was used to inhibit microbial populations and browning development on fresh-cut rose apple. Thus, it is safe for human consumption.

The current results showed that SC was the most powerful disinfectant to reduce total bacteria, yeast and molds, and *E. coli* and coliforms in fresh-cut rose apple samples throughout storage. The effectiveness of SC depended on the concentration and immersion time. The higher the concentration and the longer the immersion time of SC, the more effective the inhibition of microbial counts, in contrast to the lower concentration and a shorter immersion time. Similarly, Xiao et al. (2011) reported that a higher SC concentration resulted in a stronger inactivation of *E. coli* O157:H7 compared to a lower SC concentration.

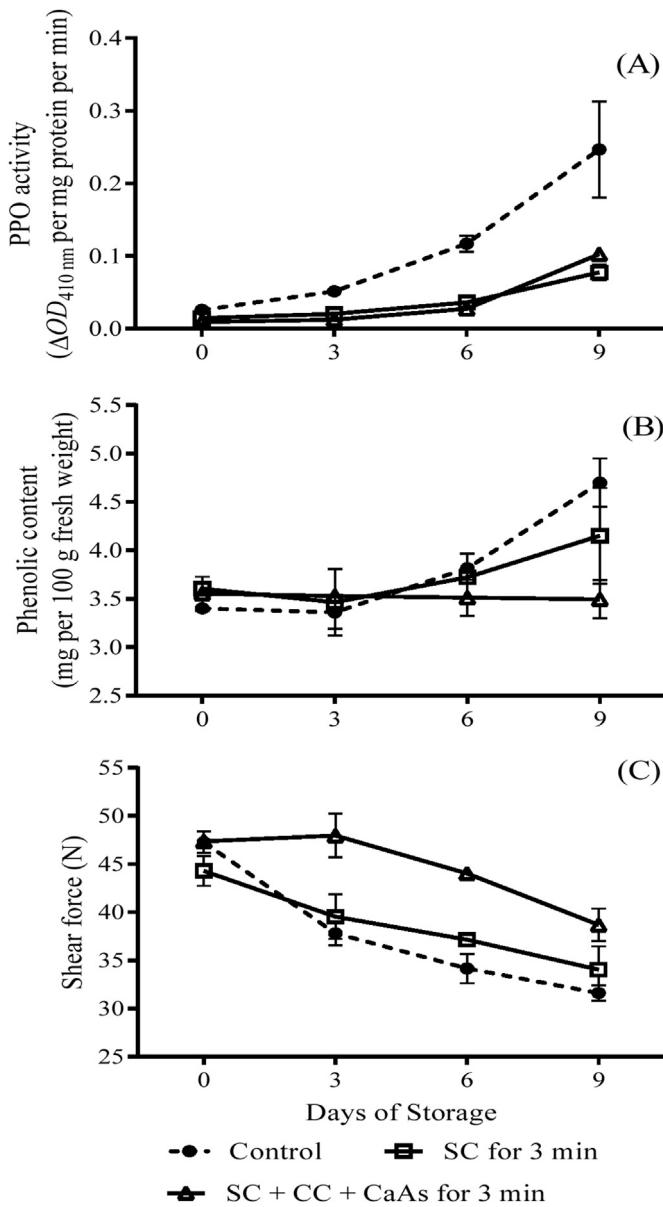
SC combined with CC and CaAs showed a greater effect in delaying microbial activity than did SC alone. Inactivation of *E. coli*



**Fig. 4.**  $L^*$  value (A), total color difference ( $\Delta E$ ) (B), and browning index (C) of fresh-cut rose apple treated with filtered water (control) for 3 min, 200 mg/L sodium chloride (SC) for 3 min, or 200 mg/L SC mixed with 20 mg/L calcium chloride (CC) and 20 mg/L calcium ascorbate (CaAs). Treated samples were stored at 4 °C for 9 d. Data shown are mean values of  $n = 3$  and the error bars represent standard errors of the means using Duncan's multiple range test at  $p \leq 0.05$ .

by SC and by SC combined with CC was reported by Luo et al. (2011), where SC and SC + CC treatments significantly reduced *E. coli* population in apple slices. SC + CC had slightly more effectiveness than SC alone. This may have been due to the antimicrobial effect of SC (Allende et al., 2009; Luo et al., 2011) and some indirect effect of CC or CaAs. Although, CaAs has no antimicrobial effect (Gorny, 2003), both calcium salts may reduce water activity, which can lead to delayed microbial growth (Luna-Guzman and Barret, 2000). Silveira et al. (2011) reported that calcium salt treatments can reduce microbial growth in fresh-cut 'Galia' melon.

SC treatment can inhibit the browning reaction of fresh-cut rose apple due to the low activity of PPO. Increasing the concentration of SC and the immersion time resulted in increased inhibition of the PPO activity as was also reported by He et al. (2008), as SC acts as the inhibitor of PPO activity which is the key enzyme associated



**Fig. 5.** PPO activity (A), phenolic content (B), and shear force (C) of fresh-cut rose apple treated with filtered water (control) for 3 min, 200 mg/L sodium chloride (SC) for 3 min, or 200 mg/L SC mixed with 20 mg/L calcium chloride (CC) and 20 mg/L calcium ascorbate (CaAs). Treated samples were stored at 4 °C for 9 d. Data shown are mean values of  $n = 3$  and the error bars represent standard errors of the means using Duncan's multiple range test at  $p \leq 0.05$ .

with the browning reaction (He and Luo, 2007). Lu et al. (2007) proposed that the effect of SC in lightening the color of fresh-cut pears was caused by its bleaching property, while He et al. (2008) presumed that copper exists at the active site of PPO were essential for enzymatic reaction. The copper helps control the balance between enzyme- $\text{Cu}^{2+}$  and enzyme- $\text{Cu}^{+}$  during enzymatic browning. SC might oxidize copper which alters the catalyzing PPO activity resulting in the activity of PPO being inactivated through the increase in the  $\text{Cu}^{2+}$  concentration (Kertész et al., 1972).

Combined treatment of SC + CC + CaAs promoted lightness on the cut surface of the fresh-cut rose apple samples. Color change as well as the browning index was suppressed. The PPO activity slightly increased during storage compared to the control sample. The combination treatment showed higher effectiveness at inhibiting browning than the SC treatment. These results might have

been caused by the anti-browning property of ascorbic acid. McEvily et al. (1992) reported that ascorbic acid and its derivatives are known to reduce quinones back to the original colorless diphenol. Thus, the anti-browning effect of ascorbic acid increased when it was synergistic with SC. The phenolic contents significantly affected the surface color of the rose apple samples. The phenolic content was low when fresh-cut rose apple was treated with SC + CC + CaAs solution. Its level was also related with the PPO activity. Similar results were demonstrated by Worakeeratikul et al. (2007a) in fresh-cut rose apple treated with CaAs.

SC treatment caused the softening of fresh-cut apple (Luo et al., 2011) although there was no significant difference in firmness between the SC-treated samples and non-treated samples in this experiment. The firmness of fresh-cut rose apple increased when dipped in SC + CC + CaAs solution. Additionally, Silveier et al. (2011) found that the flesh firmness of fresh-cut melon treated with CC increased significantly compared to non-treated melon. CC has been reported to maintain fruit firmness and extend the shelf life of fresh-cut produce (Luo et al., 2011). Calcium is generally found in the cell wall and middle lamella and it improves the structural integrity of plant tissue (Van-Buren, 1979). Therefore, calcium treatment (CC and CaAs) may influence tissue firmness by contributing to the cell wall strength. The current study clearly showed the dual effects of SC against microbial growth and browning through delaying the PPO activity and accumulating phenolic compounds. The combination of the SC treatment with CC and CaAs could strengthen the plant cell wall and maintain the firmness of fresh-cut rose apple. These data suggested that a SC + CC + CaAs solution could be an alternative technology or a multi-chemical formula that may be used to maintain the quality and extend the shelf life of fresh-cut rose apples.

#### Conflict of interest

The authors declare that there are no conflicts of interest.

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#### References

- Aguayo, E., Requejo-Jackman, C., Stanley, R., Woolf, A., 2010. Effects of calcium ascorbate treatments and storage atmosphere on antioxidant activity and quality of fresh-cut apple slices. *Postharvest Biol. Technol.* 57, 52–60.
- Allende, A., McEvoy, J.L., Tao, Y., Luo, Y., 2009. Antimicrobial effect of acidified sodium chlorite, sodium chlorite, sodium hypochlorite, and citric acid on *Escherichia coli* O157:H7 and natural microflora of fresh-cut cilantro. *Food Control* 20, 230–234.
- Anonymous, 2000. Acidified sodium chlorite solutions. In: Code of Federal Regulations 21 CFR 173.325. Food and Drugs. Office of the Federal Register. US Government Printing Office, Washington, DC, USA, pp. 144–146.
- Barbagallo, R.N., Chisari, M., Caputa, G., 2012. Effects of calcium citrate and ascorbate as inhibitors of browning and softening in minimally processed 'Birgh' eggplants. *Postharvest Biol. Technol.* 73, 107–114.
- Beirão-da-Costa, S., Steiner, A., Correia, L., Empísi, J., Moldão-Martins, M., 2006. Effects of maturity stage and mild heat treatments on quality of minimally processed kiwifruit. *J. Food Eng.* 76, 616–625.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254.
- Chisari, M., Barbagallo, R.N., Spagna, G., 2007. Characterization of polyphenol oxidase and peroxidase and influence on browning of cold stored strawberry fruit. *J. Agric. Food Chem.* 55, 3469–3476.

Colle, I.J.P., Lemmens, L., Buggenhout, S.V., Met, K., Loey, A.M.V., Hendrickx, M.E., 2013. Processing tomato pulp in the presence of lipids: the impact on lycopene bioaccessibility. *Food Res. Int.* 51, 32–38.

Dea, S., Brecht, J.K., Nunes, M.C.N., Baldwin, E.A., 2010. Occurrence of chilling injury in fresh-cut 'Kent' mangoes. *Postharvest Biol. Technol.* 57, 61–71.

Fallahi, E., Conway, W.S., Hickey, K.D., Sams, C.E., 1997. The role of calcium and nitrogen in postharvest quality and disease resistance of apples. *Hort. Sci.* 32, 831–835.

Friedman, M., 1996. Food browning and its prevention: an overview. *J. Agric. Food Chem.* 44, 631–653.

Gorny, J.R., 2003. Reducing contamination risks in fresh-cut apple. *Fresh Cut.* 2, 5–7.

Guan, W., Fan, X., 2010. Combination of sodium chloride and calcium propionate reduces enzymatic browning and microbial population of fresh-cut "Granny Smith" apples. *J. Food Sci.* 75, 72–77.

He, Q., Luo, Y., 2007. Enzymatic browning and its control in fresh-cut produce. *Stewart Postharvest Rev.* 6, 1–7.

He, Q., Luo, Y., Chen, P., 2008. Elucidation of the mechanism of enzymatic browning inhibition by sodium chloride. *Food Chem.* 110, 847–851.

Hengphum, D., Uthairatanakij, A., Boonyaritthongchai, P., Pongprasert, P., Jitareerat, P., 2015. Effects of sodium chloride on browning and microbial growth of fresh-cut 'green oak' lettuce. *J. Food Nutr. Sci.* 3, 171–176.

Hodges, D.M., Toivonen, P.M.A., 2008. Quality of fresh-cut fruits and vegetables as affected by exposure to a biotic stress. *Postharvest Biol. Technol.* 48, 155–162.

Ikram, E.H.K., Eng, K.H., Jalil, A.M.M., et al., 2009. Antioxidant capacity and total phenolic content of Malaysian underutilized fruits. *J. Food Comp. Anal.* 22, 388–393.

James, J.B., Ngarmsak, T., 2010. Processing of Fresh-cut Tropical Fruits and Vegetables: A Technical Guide. Food and Agriculture Organization of the United Nations Regional Office for Asia and the Pacific, Bangkok, Thailand.

Kertész, D., Rotillio, G., Brunori, M., Zito, R., Antonini, E., 1972. Kinetics of reconstitution of polyphenoloxidase from apoenzyme and copper. *Biochem. Biophys. Res. Commun.* 49, 1208–1215.

Li, Y., Wills, R.B.H., Golding, J.B., 2015. Sodium chloride, a cost effective partial replacement of calcium ascorbate and ascorbic acid to inhibit surface browning on fresh-cut apple slices. *LWT Food Sci. Technol.* 64, 503–507.

Lichter, A., Dvir, O., Rot, I., et al., 2000. Hot water blushing: an alternative method to  $\text{SO}_2$  fumigation for color retention of litchi fruits. *Postharvest Biol. Technol.* 18, 235–244.

Lu, S., Luo, Y., Feng, H., 2006. Inhibition of apple polyphenol oxidase activity by sodium chlorite. *J. Agric. Food Chem.* 54, 3693–3696.

Lu, S., Luo, Y., Turner, E., Feng, H., 2007. Efficacy of sodium chlorite as an inhibitor of enzymatic browning in apple slices. *Food Chem.* 104, 824–829.

Luna-Guzmán, I., Barrett, D.M., 2000. Comparison of calcium chloride and calcium lactate effectiveness in maintaining shelf stability and quality of fresh-cut cantaloupes. *Postharvest Biol. Technol.* 19, 61–72.

Luna-Gutzman, I., Cantwell, M., Barrett, D.M., 1999. Fresh-cut cantaloupe: effects of  $\text{CaCl}_2$  dips and heat treatments on firmness and metabolic activity. *Postharvest Biol. Technol.* 17, 201–213.

Luo, Y., Lu, S., Zhou, B., Feng, H., 2011. Dual effectiveness of sodium chlorite for enzymatic browning inhibition and microbial inactivation on fresh-cut apples. *LWT Food Sci. Technol.* 44, 1621–1625.

Maskan, M., 2001. Kinetics of colour change of kiwifruits during hot air and microwave drying. *J. Food Eng.* 48, 169–175.

McEvily, A.J., Iyengar, R., Steven, W.S., 1992. Inhibition of enzymatic browning in foods and beverages. *Crit. Rev. Food Sci. Nutr.* 32, 253–273.

Oms-Oliu, G., Rojas-Grau, M.A., Gonzalez, L.A., et al., 2010. Recent approaches using chemical treatments to preserve quality of fresh-cut fruit. *Postharvest Biol. Technol.* 57, 139–148.

Palou, E., Lopez-Malo, A., Barbosa-Canovas, G.V., Welti-Chanes, J., Swanson, B.G., 1999. Polyphenoloxidase activity and color of blanched and high hydrostatic pressure treated banana puree. *J. Food Sci.* 64, 42–45.

Renumarn, P., Jitareerat, P., Srilaong, V., Uthairatanakij, A., Kanlayanarat, S., 2012. Efficacy of sodium chloride in reducing microbial populations and improving the quality of fresh-cut broccoli florets. *Acta Hort.* 943, 223–229.

Rolle, R.S., Chism, G.W., 1987. Physiological consequences of minimally processed fruits and vegetables. *J. Food Qual.* 10, 157–177.

Sa-nguanpuag, K., Kanlayanarat, S., Tanprasert, K., 2007. Trends of fresh cut produce in Thai retail markets for identification of packaging for shredded green papaya. *Acta Hort.* 746, 481–483.

Silveira, A.C., Aguayo, E., Chisari, M., Artés, F., 2011. Calcium salts and heat treatment for quality retention of fresh-cut 'Galia' melon. *Postharvest Biol. Technol.* 62, 77–84.

Supapvanich, S., Prathaan, P., Tepsorn, R., 2011. Physiochemical changes in fresh-cut wax apple (*Syzygium samarangense* [Blume] Merrill & L. M. Perry) during storage. *Food Chem.* 127, 912–917.

Supapvanich, S., Prathaan, P., Tepsorn, R., 2012. Browning inhibition in fresh-cut rose apple fruit cv. Taaptimjaan using konjacglucomannan coating incorporated with pineapple fruit extract. *Postharvest Biol. Technol.* 73, 46–49.

Tardelli, F., Guidi, L., Massai, R., Toivonen, P.M.A., 2013. Effects of 1-methylcyclopropene and post-controlled atmosphere air storage treatments on fresh-cut Ambrosia apple slices. *J. Sci. Food Agric.* 93, 262–270.

Trinetta, V., Morgan, M., Linton, R., 2012. Chlorine dioxide for microbial decontamination of food. In: Demirci, A., Ngadi, M.O. (Eds.), *Microbial Decontamination in the Food Industry: Novel Methods and Applications*. Woodhead Publishing Series in Food Science, Technology and Nutrition, Cambridge, UK, pp. 533–562.

Van-Buren, J.P., 1979. The chemistry of texture in fruits and vegetables. *J. Texture Stud.* 10, 1–23.

Vara-Ubol, S., Chambers, E., Kongpensook, V., Oupadissakoon, C., Yenket, R., Retiveau, A., 2006. Determination of the sensory characteristics of rose apples cultivated in Thailand. *J. Food Sci.* 71, 547–552.

Walker, J.R.L., Wilson, E.L., 1975. Studies on the enzymatic browning of apples. Inhibition of apple  $\text{o}$ -diphenol oxidase by phenolic acids. *J. Sci. Food Agric.* 26, 1825–1831.

Wang, H., Feng, H., Luo, Y., 2007. Control of browning and microbial growth on fresh-cut apples by sequential treatment of sanitizers and calcium ascorbate. *J. Food Sci.* 72, 1–7.

Watada, A.E., Ko, N.P., Minott, D.A., 1996. Factors affecting quality of fresh-cut horticultural products. *Postharvest Biol. Technol.* 9, 115–125.

Worakeeratkul, W., Srilaong, V., Uthairatanakij, A., Jitareerat, P., 2007a. Effects of hydrocooling and chitosan coating on browning and physiological changes in fresh-cut rose apple. *Acta Hort.* 746, 427–434.

Worakeeratkul, W., Srilaong, V., Uthairatanakij, A., Jitareerat, P., 2007b. Effect of whey protein concentrate on quality and biochemical changes in fresh-cut rose apple. *Acta Hort.* 746, 435–442.

Wu, Z.S., Zhang, M., Wang, S., 2012. Effects of high pressure argon treatments on the quality of fresh-cut apples at cold storage. *Food Control* 23, 120–127.

Xiao, C., Zhu, L., Luo, W., Song, X., Deng, Y., 2010. Combined action of pure oxygen pretreatment and chitosan coating incorporated with rosemary extracts on the quality of fresh-cut pears. *Food Chem.* 121, 1003–1009.

Xiao, Z., Luo, Y., Luo, Y., Wang, Q., 2011. Combined effect of sodium chlorite dip treatment and chitosan coatings on the quality of fresh-cut d'Anjou pears. *Postharvest Biol. Technol.* 62, 319–326.

Zagory, D., 1999. Sanitation Concerns in the Fresh-cut Fruit and Vegetable Industry. University of California. Davis food processors sanitation workshop. Davis Fresh Technologies, Davis, CA, USA.