Effect of 4-hexylresocinol and Ascorbic Acid on Enzymatic Browning Reduction and Vitamin C Content of Fresh-cut Green Apple

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ABSTRACT

Browning of cut surface is the major problem of fresh-cut green apple. This research aims to study the effect of 4-hexylresocinol (4-HR) and ascorbic acid (AsA) on reducing browning and maintain the quality of fresh-cut green apple. Apple fruits (Granny Smith) were washed with 200 ppm sodium chlorite (NaOCl) for 3 min. Then washed apple fruits were cut into small pieces (6 pieces/fruit). Cut apples were dipped for 1 min in 0.0125% (w/v) 4-HR and 0.5% (w/v) AsA or 0.0125% 4-HR combined with 0.5% AsA. After dipping, apples were packed in plastic tray and sealed with polypropylene (PP) film and stored at 4°C for 8 days. The result showed that the combination of 4-HR (0.0125%, w/v) and AsA (0.5%, w/v) was the most effective in reducing browning of cut surface. Fresh-cut apples dipped in 4-HR combined with AsA treatment showed lower browning score and higher L* value when compared to control, 4-HR, and AsA samples. Apple pieces dipped in 4-HR combined with AsA treatment had higher vitamin C than control and 4-HR samples. The result indicated that the combination of 4-HR and AsA was effective in reducing browning and maintaining the vitamin C content of fresh-cut green apple.

Key Words: fresh-cut, apple, 4-hexylresocinol, ascorbic acid, browning, polyphenol oxidase, peroxidase, polypropylene

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INTRODUCTION

Fresh-cut green apples are popular from consumer's consumption. Therefore, fresh-cut apples are faced many problems with shelf-life cause by the main browning appearance, and vitamin C decrease during storage. An appearance of the color changes on fresh-cut green apple is the main factor to minimize the storage shelf-life (Yan *et al*., 2017). The browning of cutting surface of fresh-cut apples is considering of quality loss and appearance caused by enzymatic browning. Oxidation of endogenous polyphenolic compounds containing an o-dihydroxyl group to co-relationship with o-quinones in the presence of oxygen and the reaction is catalyzed by an oxidizing polyphenol oxidase (PPO) enzyme (Garcia *et al*., 2002). Peroxidase (POD) is another important oxidative enzyme besides PPO. It is performing single electron oxidation of phenolic compounds in presence of hydrogen peroxide from the oxidation. Some phenolics catalyzed by PPO can induce synergistic action between PPO and POD, which involved of POD in browning processes (He and Luo, 2007).

4-hexylresocinol (4-HR) is an anti-browning, an ingredient in oral and sanitizing agents. It is an important for prevention in burn wound and proposed for prevention of black spot (Frankos *et al*., 1991). Furthermore, Alvarez-Parrilla (2007) reported that 4-HR can inhibit enzymatic browning of fresh-cut apples, avocados and potatoes in food industry (Ahn *et al*., 2016).

Ascorbic acid (AsA) or vitamin C is an antioxidant that plays a great role in removing reactive oxygen species (ROS). Moreover, it was also proved that AsA can prevent browning surface of fresh-cut fruits and vegetables (Fang *et al*., 2017). Cutting induces wounding of the plant cell wall and thus stimulates oxidative stress in fresh cuts, consequently leading cell damage or cell death. Jang and Moon (2011) reported that ascorbic acid can reduce *o*-quinones produced by PPO oxidation catalyzed of polyphenols back to dihydroxyl polyphenols. Cocetta (2014) suggested that 1-3% ascorbic acid was effective for prevention of browning of fresh-cut apple. The aim of the study was to study the effect of 4-hexylresocinol and ascorbic acid in reducing browning and maintain vitamin C content of fresh-cut green apple.

MATERIALS AND METHODS

1. Fruit materials and experimental design

Granny Smith apples (*Malus domestica* Borkh) were purchased from commercial market and delivered to Postharvest laboratory, King Mongkut's University and Technology Thonburi, Bangkok, Thailand. Apple fruits were selected on the basis of color, size and absence of pest infection. Green apple fruits were washed with 200 ppm sodium chlorite (NaOCl) for 3 min before processing. Apples were cut into 6 pieces per fruit, dipped in 0.0125% (w/v) 4-HR, 0.5% (w/v) AsA, and combined 0.0125% (w/v) 4-HR with 0.5% (w/v) AsA for 1 min. Green apple pieces were packed in plastic white tray (12 cm x 7 cm x 6 cm) sealed with polypropylene (PP) film and stored at 4 ^{o}C for 8 days. Each tray contains 8 pieces of fresh cut green apples which is considered as one replicate. The experiment

had 4 replicates per treatment. Results were analyzed by one-way analysis of variance (ANOVA) while Duncan's multiple range tests were used to compare means and differences at $P < 0.05$.

2. Browning score and color changes

The color of apple pieces was measured on cut surface by using a colorimeter CR-400 Minolta Chroma Meter. The color changes of fresh-cut green apples were focused on only the L* value. The L* value is necessary to monitor lightness or darkness of fresh-cut produce. L* value of apple pieces were recorded every two days of storage.

Browning score of fresh-cut green apples was described by 10 people. The evaluated score was using a scale of 1-5, where 1 = none, 2 = slight (up to 5% surface affected), 3 = moderate (5-20% surface affected), 4 = moderately severe (20-50% surface affected) and 5 = extreme (> 50% surface affected) for individual pieces (Zahra *et al.,* 2013).

3. Vitamin C content

Ascorbic acid was extracted by homogenizing 5 g of apples tissue in 5% metaphosphoric acid. The homogenized sample the filtered through Whatman no. 1 filter paper. 0.4 mL of supernatant added with 0.2 mL 0f 0.02% indophenol then added 0.4 mL of 2% thiourea with 0.2 mL of 2% 2,4-Dinitrophenl Hydrazine (DNP). Incubated at 50^{o}C for an hour then added 1 mL of 85% sulfuric acid. Ascorbic acid content was determined by spectrophotometer an absorbance at 540 nm modified by (Roe *et al*., 1948).

4. Polyphenol oxidase (PPO) and peroxidase (POD) enzyme activity

The polyphenol oxidase(PPO) enzyme activity was determined by method modified based on Jang and Moon (2011). Green apple tissue was homogenized in 20 mL of 0.005 mol/L sodium phosphate buffer (pH 7.0) consisted 0.5g polyvinylpyrrolidone (PVPP). The extracted solution was filtered through Whatman no. 1 filter paper and centrifuged at 3,000 x g for 15 min at 4 ^{o}C. 0.5 mL of supernatant was mixed with 1.5 mL of sodium phosphate buffer (pH 7.0) and 1 mL of 23N catechol. Finally, the activity of PPO enzyme was measured by spectrophotometer (410 nm). The activity is expressed as units per milligram of protein, one unit presents the amount of enzyme necessary in 0.001/min.

The peroxidase (POD) was extracted by a modified method based on Chen Zhao (2010). POD was extracted from 5 g of green apple tissue by homogenizing consisted of a 20 ml of 0.05 mol/L sodium phosphate buffer (pH 7.0) with 0.5g polyvinylpyrrolidone (PVPP). The homogenate was centrifuged at 3000x g at 4^{o}C for 15 min. The supernatants were collected for the POD activity assay. POD activity was assayed spectrophotometrically in absorbance at 470 nm. The reaction cuvette contained 0.25 mL substrate solution and 1.75 mL of sodium phosphate buffer (pH 7.0) with 1 mL 1% guaiacol. The activity is expressed as units per milligram of protein, one unit presents the amount of enzyme necessary in 0.01/min.

RESULTS AND DISCUSSION

1. Browning score and Color change

L* values have been widely used to indicate the lightness/darkness of the cut surface of apple pieces with browning (Castaner *et al*,.1999). At day 4 of storage, the L* value was significantly declined in the control samples (Figure 1b). The combination treatment containing 0.0125% (w/v) 4-HR with 0.5% (w/v) AsA showed the stable L* values through 8-day-storage. It was significant different ($p \leq 0.01$) comparing to control samples.

Browning score obtained from combination treatment condition showed the least browning (Figure 1a). Mixing 0.0125% (4-HR) with 0.5% (AsA) exhibited high activity to decrease browning that was caused by PPO and POD enzymes. Meanwhile, this mixture also increased antioxidant capacity associated with PAL activity (Malgorzata and Michal, 2018).

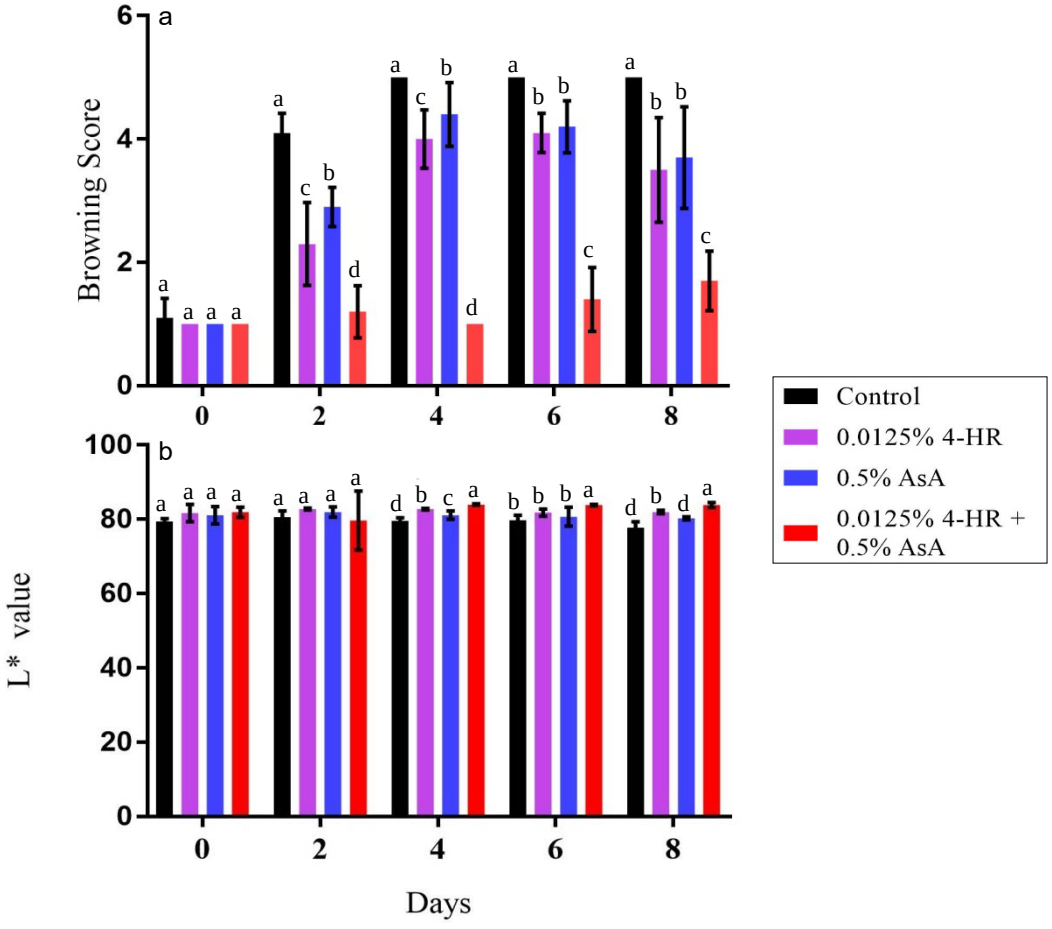

Figure 1 Effect of 4-HR and AsA treatments on the (a) browning score color parameter and (b) L* value of the cut surface of Granny Smith apple slices during storage 8 days at 4°C. Data are the means ± standard deviations of 4 replications.

The combination of 0.0125% 4-HR with 0.5% AsA was the most efficient anti-browning compound in preventing browning of fresh-cut green apple. The samples treated with AsA combined

with 4-HR obtained the lowest appearance score and highest in L* value during cold storage. The L* value was associated with the development of browning. Gorny *et al*., (2002) stated that the increase of antioxidant capacity positively correlated with PPO inhibition. Both of them may prevent cells membrane breakdown, leading to substrates reducing. Thus, fresh-cut green apple color was preserved.

2. Effect of 4-HR and AsA on polyphenol oxidase and peroxidase

The polyphenol oxidase (PPO) enzyme of the treated fresh-cut green apples is presented in Figure 2a. PPO activity is generally considered as the first step in enzymatic browning caused from PPO enzyme which catalyzes monophenols to be *0*-phenols (He and Luo, 2007). Activity of PPO showed a significant difference at $p \leq 0.05$. It showed lower PPO activity in combined with 4-HR and AsA treated samples on day 2 of storage.

Although it has been recognized that PPO is the main enzyme related to enzymatic browning on fresh-cut green apples. It is necessary to study the changes in peroxidase (POD) enzyme in fresh-cut products. The changes of POD enzyme activity in fresh-cut green apples were shown in Figure 2b. POD was significantly different and its highest activity was found at the eighth day of storage in control samples. On the other hand, the combination of 0.0125% (w/v) 4-HR with 0.5% (w/v) AsA treated samples gave significantly low POD activity when compared to the control after day 4 of storage at 4°C.

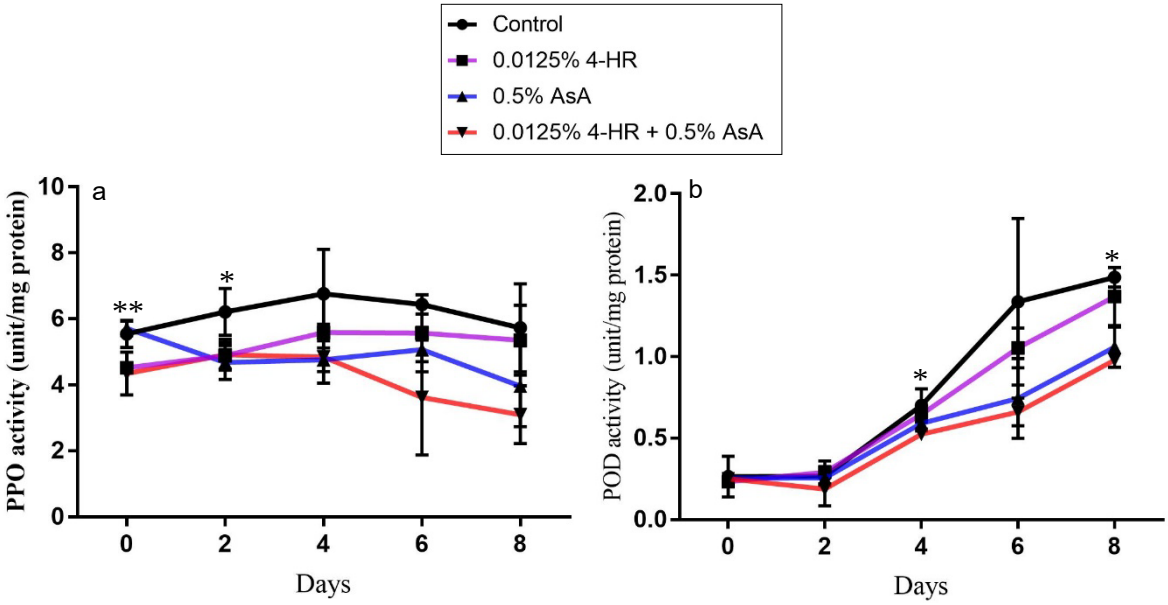

Figure 2 (a) Polyphenol oxidase activity and (b) peroxidase activity of fresh-cut green apples during storage 8 days at 4°C. Data are the means ± standard deviations of 4 replications. Asterisks indicate statistical differences between treatment (* $p \leq 0.05$, ** $p \leq 0.01$).

The combination of 0.0125% (w/v) 4-HR with 0.5% (w/v) AsA treatment effected on reducing the peroxidase (POD) activity in the fresh-cut green apple. The reduced POD activity in the fruits treated with AsA could be the result of a lower oxidative stress on the fruit surface due to the antioxidant ability of AsA. The combination of AsA with 4-HR treatment possibly inhibited the PPO and POD activities by enhanced antioxidant action. Several studies have suggested that the hyper-reactivity of enzymes, such as polyphenol oxidase (PPO) and peroxidase (POD) often cause oxidation in fresh-cut fruits and vegetables. Therefore, the combination of 4-HR and AsA would seem to delay the development of surface browning of green apple slices through inhibition of PPO activity which leads to reduction in the oxidation of polyphenols (Malorzata *et al.*, 2017).

3. Vitamin C contents

Vitamin C (ascorbic acid) is the most important nutrient in fruits and it has mainly biological activities in human body. Changes in vitamin C content of fresh-cut green apples are shown in Figure 3. Vitamin C content of green apple pieces was higher in 4-HR combined with AsA samples at day 0 to day 8 of storage. The ascorbic acid (AsA) and combined treatments showed 17.352 mg vitamin C/100g fresh weight (FW) and 19.529 mg/100mg FW, respectively, at day 6 of storage. While, in control remained only 2.623 mg vitamin C/100g FW at the same storage after day 6. The inhibition of vitamin C loss in the treated samples when compared to control was significantly different ($p \leq 0.01$). All the findings in terms of vitamin C content showed that both AsA and combined of 4-HR with AsA treatments had the ability to maintain the nutritional value of fresh-cut green apple pieces. The increase in vitamin C in AsA and combined 4-HR with AsA treated green apple slices may be due to the inhibitory roles of these compounds on enzymes activity such as ascorbate peroxidase (APX) and ascorbate oxidase (AO), in fresh-cut apple (Perez *et al.*, 1999).

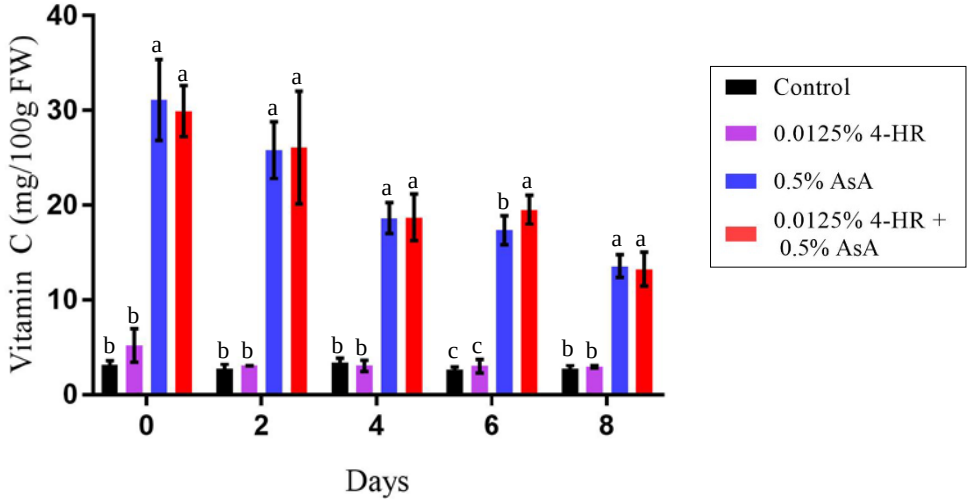

Figure 3 Effect of 4-HR and AsA treatments on vitamin C of fresh-cut green apple during storage 8 days at 4 °C. Data shown are means ± standard deviations of 4 replications.

CONCLUSION

This result suggests that the combination of 4-hexylresocinal and ascorbic acid was effective in reducing browning and maintaining vitamin C contents of fresh-cut green apple. Therefore, the combination of 0.0125% (w/v) 4-hexylresocinol with 0.5% (w/v) ascorbic acid caused the lowest levels of browning in fresh-cut green apples during storage at 4°C for 8 days. This technique can be applied in fresh-cut apple industry.

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