บทความวิจัย

IMPACTS OF ASCORBIC ACID AND CITRIC ACID ON REDUCING BROWNING OF READY TO COOK COARSE GROUND CHILI

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ABSTRACT

Browning is critical in the preservation of quality and shelf life of fresh cut products. In this study, the effects of ascorbic acid and citric acid to inhibit browning were investigated. Mature green mature chili cv. Supper Hot was prepared by washing with tap water and then the chili stem was removed. The chilies were treated with 1.5% w/v ascorbic acid, 0.5% w/v citric acid, or untreated chilies (control). All samples were then ground using a blender for 30 sec. Afterward the all samples were packed in a foam tray, sealed with PVC film and kept at 4 °C for 6 days. Treatment with ascorbic acid inhibited browning as indicating by lower color changes (L*, a*, b* and hue angle values) and browning index when compared to non-treated sample. In addition, samples treated with 1.5% ascorbic acid showed the increase in antioxidant capacity, vitamin C concentration and the highest acceptance score by consumer evaluations. The samples treated with citric acid showed

the opposite tendency. These results suggest that 1.5% ascorbic acid could be used as an alternative approach to control browning and maintain quality of coarse grinding chili.

Keywords: anti-browning agent, ascorbic acid, browning, *Capsicum annuum* L., citric acid

Introduction

Chili (*Capsicum annuum* L.) belongs to the family Solanaceae. It is recognized as an important tropical vegetable as well as spice crop. It has been widely consumed throughout the world, particularly in South East Asia. There are used for flavoring, colorant and pungency taste for spicy food. Coarse ground chili is one of the minimally processed products and increasingly demand of the supermarket, hotel and restaurant but the product suffers from browning which limits the quality and shelf life. The browning reaction mainly involves the oxidation of phenolic compounds by the action of PPO, resulting in the formation of quinones (Kim et al., 2014). Subsequent polymerization of the quinones results in the formation of brown color pigments (Apintanapong et al., 2007). Various methods have been recently used to inhibit browning reactions in fresh cut fruit and vegetable products, such as UV irradiation (Lante et al., 2015), edible coatings (Saba & Sogvar, 2016; Sharma & Rao, 2015), thermal treatment (Maghoumi et al., 2013), low oxygen storage atmospheres (Chung & Moon, 2009) and anti-browning agents (Du et al., 2009; Lu et al., 2007; Ramos-Villarroel et al., 2015). Within these options, the application of natural anti-browning agents, such as organic acids, is a popular approach for retarding browning in fresh cut products.

Ascorbic acid and citric acid are natural organic acids that are generally recognized as safe (GRAS) for fruit and vegetables (Rojas-Grau et al., 2006). Ascorbic acid has been shown to the effectiveness in inhibiting browning in several fresh cut fruits such as apple (Li et al., 2015), peach (Li-Qin et al., 2009), banana (Apintanapong et al., 2007) and pineapple slices (Gonza´lez-Aguilar et al., 2005). Barbagallo et al. (2012) reported that ascorbic acid inhibited the browning reaction by reducing the conversion of *O*-quinones to diphenols, leading to the formation of colorless compounds. In addition, ascorbic acid could directly inhibit the activity of browning enzymes (Li et al., 2015; Li-Qin et al., 2009; Saba & Sogvar, 2016). Li-Qin et al. (2009) reported that 0.2% ascorbic acid inhibited PPO and POD activities in peach slices and 1.0% ascorbic acid showed effective

inhibition of PPO activity in apple slices (Li et al., 2015). Citric acid has also been used to retard browning in fresh cut fruits and vegetables. Jiang et al. (2004) reported that 0.1 M citric acid markedly extended shelf life and inhibited browning in fresh cut Chinese water chestnut. The mode of action of citric acid was recorded as being the direct inhibition of PPO at concentrations of 0.1 M or higher (Jiang et al., 2004).

The objective of this study was to investigate the effectiveness of ascorbic acid or citric acid in retarding browning and extending shelf life of coarse ground chili.

Methods

Plant materials and treatments

Mature green chili cv. Supper Hot was washed with tap water and then the chili stem was removed. The intact chilies were divided into three groups; the first group was added with 1.5% w/v ascorbic acid (5 ml/10 g fresh weight), the second added with 0.5% w/v citric acid (5 ml/10 g fresh weight), and the third group was untreated as a control. (1.5% ascorbic acid and 0.5% citric acid were selected to use in this experiment because they were found to be the most effective to reducing browning in the preliminary experiment.) Then all samples were ground using a blender (Bosch, E-Nr: MMB 2001/10), for 30 sec. Afterward the samples were packed in a foam tray (50 g/ tray), sealed with PVC film and kept at 4 °C for 6 days. Each treatment consisted of four replications.

Color measurement

The color of the coarse ground chili was measured in every two days during storage by using a colorimeter (CR 300, Minolta, Japan). The data were expressed as a*, b*, L* and hue angle values. Three readings were taken on each sample and the average of the values was calculated.

Evaluation of browning index

Browning index (BI) was calculated using the equation of Zambrano-Zaragoza et al. (2014) as shown below

$$BI = [100(X - 0.31)/0.172$$

Where: $X = (a^* + 1.75L^*)/ (5.645L^* + a^* - 3.012b^*)$

Determination of PPO activity

PPO extraction was generated by the method of Zheng et al. (2012), with slight modifications. Coarse ground chili (5g) was mixed in 20 mL of 100 mM sodium phosphate buffer, pH 6.8, containing 0.4 g of PVPP and then homogenized. The homogenate was centrifuged at 10,000 × g for 25 min at 4 °C, and the supernatant was collected for enzyme assay. The activity of PPO was measured by mixing 0.5 mL of the supernatant with 1.5 mL of 50 mM sodium acetate buffer (pH 6.8) and 200 µL of 4-methylcatechol substrate (containing 50 mM sodium phosphate buffer (pH 6.8) and 100 mM 4-methylcatechol). The absorbance of the mixture was measured at 398 nm with a spectrophotometer. The enzyme activity was expressed as units per mg of protein.

Protein determination

The protein concentration of the samples was determined by the method of Bradford (1976), measuring the optical density at 595 nm, with bovine serum albumin as a standard.

Determination of antioxidant capacity using DPPH assay

Coarse ground chili (3 g) was mixed with 25 mL of methanol and homogenized, then the homogenate was centrifuged at 15,000 x g for 20 min at 4 °C. The DPPH assay was conducted according to the method of Brand-Williams et al. (1995) with slight modifications. The stock solution of 24 mg in 2,2 diphenyl-l-picrylhydrazyl (DPPD) dissolved in 100 mL methanol was prepared and stored at -20 °C until required. The working solution was obtained by mixing 10 mL stock solution with 45 mL methanol to obtain an absorbance of 1.1 ± 0.02 units at 515 nm using a spectrophotometer. The mixture consisted of 300 µL of the sample extract and 2,700 µL of the DPPH solution and then incubated at room temperature for 30 min in the dark condition. The control solution was prepared by mixing ethanol (300 µL) and DPPH solution (2,700 µL). Absorbance was measured at 515 nm. The result was expressed as percentage inhibition of free radical DPPH and was determined using the following equation:

$$\text{Inhibition of free radical DPPH (\%)} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

Determination of total ascorbic acid concentration

Total ascorbic acid was determined by the method of Terada et al. (1978), with slight modifications. A 5 g sample of coarse ground chili was homogenized in 20 mL of 5% metaphosphoric acid. The homogenate was centrifuged at 12,000 × g for 30 min at 4 °C. Total ascorbic acid was measured using 0.5 mL of supernatant extract mixed with 0.2 mL of 0.02% 2,6-dichlorophenol-indophenol sodium salt. It was then incubated at room temperature for 1 h. Afterward, 0.5 mL of 2% thiourea in 5% meta-phosphoric acid and 0.25 mL of 2% 2,4-dinitrophenylhydrazine in 9 N sulfuric acid was added. The mixture was incubated at 60 ºC for 1 h, then cooled for 15 min. The reaction was stopped by adding 1.25 mL of 90% sulfuric acid. Then kept at room temperature for 30 min before reading the absorbance at 540 nm using a spectrophotometer (UV-1800, Shimadzu, Japan). The ascorbic acid concentration was calculated using a standard curve generated using commercial L-(+)-ascorbic acid and then expressed as mg per 100 g fresh weight (FW).

Sensory evaluation

The sensory panel consisted of 10 untrained members. Sensory attributes evaluated by visual characteristics included visual color and overall acceptance of quality. A rating score, based on a 9-Point Hedonic Scale, where 9 = like extremely, 1 = dislike extremely, was used.

Statistical analysis

The results were analyzed by one-way analysis of variance using the general linear models procedure of SAS (SAS Institute, Cary, N.C., USA) for completely randomized design experiments. The data are presented as the means ± standard errors of the means.

Results

Browning index

Browning incidence were detected immediately after grinding and the severity of browning increased during storage. After grinding about 2 hr. the browning index were detected, the result found that the browning index was significantly highest in the control treatment (39.9), followed by 0.5% citric acid treatment (31.6) and the 1.5% ascorbic acid treatment (25.3), respectively. There were no differences in browning index among treatments on day 2 of storage but on days 4 and 6, 1.5% ascorbic acid treatment was

the most effective at inhibiting browning while 0.5% citric acid showed similar level to the control (Figure 1A).

PPO activity

PPO activity in all treatments increased rapidly during the first two days of storage and then remained constant (Figure 1B). Samples treated with 1.5% ascorbic acid showed low PPO activity when compared to the other treatments, while, samples treated with 0.5% citric acid showed the significant higher activity than the control.

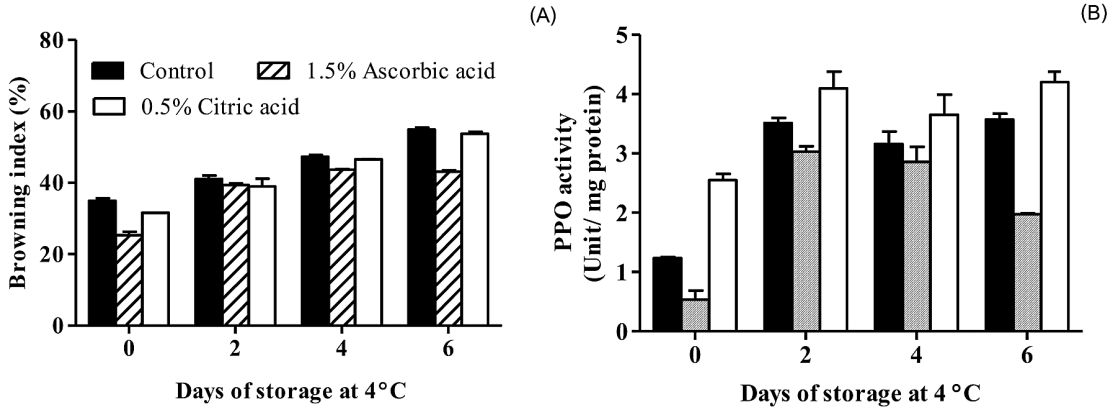

Figure 1 Browning index (A) and PPO activity (B) of course ground chili treated with 1.5% ascorbic acid, 0.5% citric acid or untreated (control) during storage at 4 ºC for 6 days. Vertical bars represent ± SE for triplicate samples.

Color change

The change of color in coarse grinding chili was indicated by a*, b*, L* and Hue angle values. Usually, the decrease in L* and Hue angle values and the increase in a* and b* values could indicate browning. The treatment with 1.5% ascorbic acid maintained the L* value throughout storage, while the both 0.5% citric acid and control treatments showed low L* values (Figure 2A). Similarly, samples treated with 1.5% ascorbic acid maintained a high Hue angle value, but it were not significantly different from the control (Figure 2B). In contrast, a* value of all treatments increased (i.e., became less negative) during storage time, especially in samples treated with 1.5% ascorbic acid (Figure 2C). However, b* value of 1.5% ascorbic acid treated sample was higher than that of 0.5% citric acid and the control treated sample (Figure 2D). The results indicated that 1.5% ascorbic acid showed the potential to maintain the color of coarse ground chili.

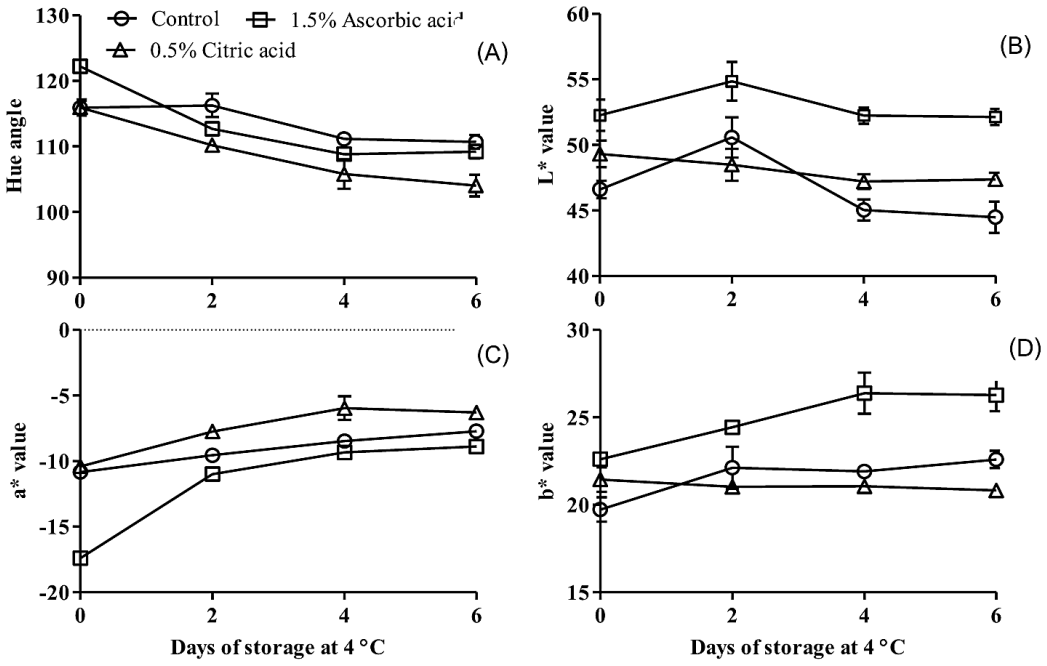

Figure 2. Color change in coarse ground chili: a* (A), b* (B), L* value (C) and hue angle (D) when treated with 1.5% ascorbic acid, 0.5% citric acid or untreated (control) during storage at 4 ºC for 6 days. Vertical bars represent ± SE for triplicate samples.

DPPH radical scavenging capacity

The antioxidant capacity in coarse ground chili after treatment was highest in samples treated with 1.5% ascorbic acid which was 94.1%, while that of 0.5% citric acid and control treated samples were 65.7% and 56.8%, respectively. Thereafter, the DPPH scavenging capacity in the 1.5% ascorbic acid treated samples decreased during storage while in the other treatments it remained constant (Figure 3A).

Vitamin C contents

The concentration of vitamin C in coarse ground chili increased rapidly after treatment with 1.5% ascorbic acid and maintained high levels throughout the storage period. In contrast, vitamin C concentration in samples treated with 0.5% citric acid and in the control treatment were similar, and obviously lower than 1.5% ascorbic acid treated samples (Figure 3B).

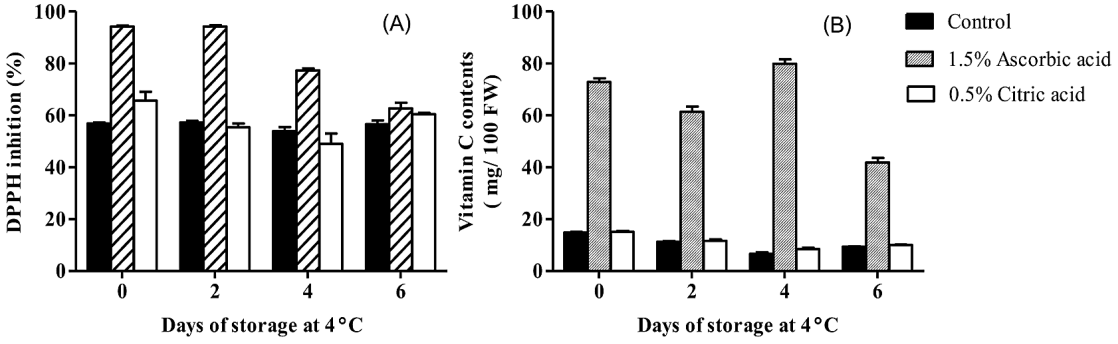

Figure 3 DPPH radical scavenging activity (A) and vitamin C contents (B) of coarse ground chili when treated with 1.5% ascorbic acid, 0.5% citric acid or untreated (control) during storage at 4 ºC for 6 days. Vertical bars represent ± SE for triplicate samples.

Sensory evaluations

The best visual color and overall acceptance scores were found in the 1.5% ascorbic acid treatment, followed by the control treatment and then the sample treated with 0.5% citric acid. Scores below the acceptable limit (score 5) were observed for the samples treated with 0.5% citric acid and the control on days 2 and 4 of storage, respectively. In comparison, the sample treated with 1.5% ascorbic acid had an extended acceptance score until day 6 of storage (Figure 4A-4B).

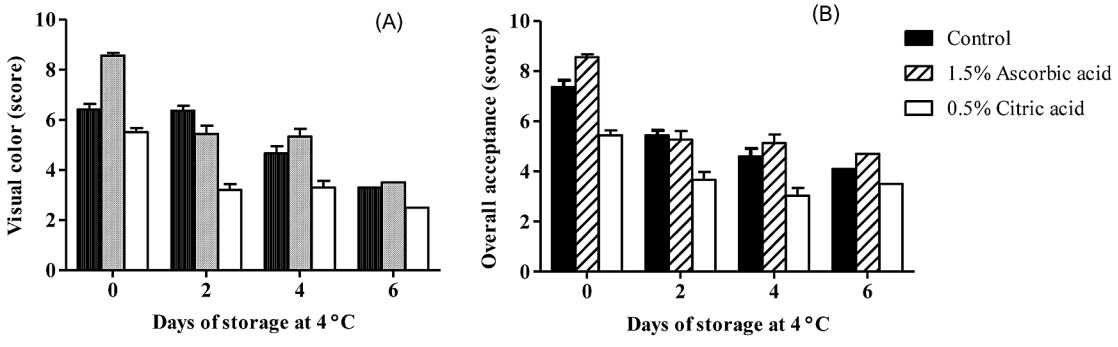

Figure 4. Visual color (A) and overall acceptance (B) of coarse ground chili when treated with 1.5% ascorbic acid, 0.5% citric acid or untreated (control) during storage at 4 ºC for 6 days. Vertical bars represent ± SE for triplicate samples.

Discussion and Conclusion

Browning is the main problem of coarse ground chili. It was evident immediately after the chilies were ground and increased thereafter. However, the application of 1.5% ascorbic acid markedly reduced the browning index in the prepared samples. The action of ascorbic acid on the inhibition of browning is related to the chemical reduction of *o*-quinones to diphenols, leading to the formation of uncolored compounds (Barbagallo et al., 2012). Moreover, ascorbic acid can inhibit PPO activity, which it is the main enzyme in the browning reaction (Amodio et al., 2011). This agreed with Li-Qin et al. (2009) reported that 0.2% ascorbic acid could inhibit PPO activity in peach slices and a mixed solution of 1.0% ascorbic acid and 0.1% citric acid showed effective inhibition of PPO activity in apple slices (Li et al., 2015). Apintanapong et al. (2007) suggested that ascorbic acid inhibited PPO activity by chelating copper from the active site of the PPO enzyme. In addition, Suttirak & Manurakchinakorn (2010) indicated that a solution of organic acids at pH values below 4 inactivated the PPO enzyme. On the other hand, this result found that 0.5% citric acid exhibited higher activated PPO activity in comparison to the control treatment. These findings may be explained by high concentration of citric acid induced the oxidative damage as shown in fresh cut apple and fresh cut artichokes (Chen et al., 2016; Amodio et al., 2011). Similar result of Jiang et al. (2004) found that 1% citric acid induced PPO activity and showed the highest degree of browning when compared with other treatments. However, Suttirak & Manurakchinakorn (2010) reported that the efficiency of organic acids to inhibit browning in fresh cut fruits and vegetables depends on their concentrations. This correlated with the result of Pizzocaro et al. (1993) who indicated that citric acid at 0.02 - 0.1% was shown to activate PPO activity, while a concentration at 0.2% or higher inhibited PPO activity in apple slices.

The measurement of color (L*, a*, b* and hue angle values) can be used to confirm the degree of browning in fresh cut fruits and vegetables. In general, higher a* and lower L* values indicate browning incidence (Kim et al., 2014). The current results showed that L* value in all samples decreased with increasing storage period, while a*, b* and hue angle values increased, indicating an increase in brown colour formation. However, the samples treated with ascorbic acid maintained the highest L* value and showed the lowest a*, b* and hue angle values. Thus, these results confirmed that ascorbic

acid treatment effectively inhibited browning and maintained color in the coarse ground chili.

Visual color and overall acceptance are considered as the most important factor determining marketability. The score of visual color and overall acceptance declined rapidly with development of browning during storage. The treatment of ascorbic acid was the most effective treatment to delay the reduction of acceptance score.

Moreover, this study investigated the antioxidant capacity of coarse ground chili using the DPPH radical scavenging method. The radical scavenging correlated with the concentration of ascorbic acid. Sogvar et al. (2016) reported that antioxidant capacity closely correlated with the presence of efficient oxygen radical scavengers, such as vitamin C and phenolic compounds. In this study, the antioxidant capacity was also significantly correlated with the concentrations of vitamin C. These results are similar to those reported by Liu et al. (2015) who found that vitamin C and phenolic compounds are the main compounds with antioxidant properties in peach fruit pulp.

In conclusion, this study indicated that the application of ascorbic acid retarded browning in coarse ground chili, which was associated with a reduction of PPO activity. Furthermore, ascorbic acid increased total ascorbic acid and antioxidant capacity. These results suggested that ascorbic acid may be a feasible approach controlling browning and extending the shelf life of coarse ground chili.

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