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Browning inhibition and lipid peroxidation change of fresh-cut romaine lettuce by arginine treatment

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ABSTRACT

This study aimed to assess the effect of sodium arginine on browning inhibition and lipid peroxidation in fresh-cut romaine lettuce kept for 15 days at 6±1 °C and 85% relative humidity. The experimental design was a completely randomized design with four treatments: The romaine lettuce was immersed in 0 (distilled water), 1 mM, 10 mM, and 20 mM arginine for 10 min. These results showed that the use of 20mM arginine can inhibit browning compared to other treatments. Reduced levels of phenolic compounds and phenylalanine ammonia lyase were linked to the suppression of browning in romaine lettuce, although polyphenol oxidase activity increased during storage. Additionally, there were studies on the change in lipid peroxidation, which is one of the causes of membrane deterioration. The result indicated that arginine can reduce the process of lipid peroxidation related to the decrease of lipoxygenase activity, malondialdehyde and hydrogen peroxide content compared with the control. The inhibition of stress tolerance by arginine could relate to its influence on metabolic pathways, cellular signaling, or oxidative stress. Arginine enhances stress tolerance, which leads to the production of polyamines that stabilize cell membranes and proteins, scavenge reactive oxygen species, and regulate ion channels, enhancing stress resilience. Hence, these results indicated that arginine treatment inhibited browning symptoms and membrane damage in the process of lipid peroxidation.

Keywords: romaine lettuce; arginine; browning reaction; lipid peroxidation

1. INTRODUCTION

Physiologically, fresh-cut vegetables basically represent damaged tissue. The wound may arise from detachment, grading, cutting, cracking, or breaking during postharvest handling, which leads to changes in physiological and biochemical processes. When vegetables are stored or distributed, they undergo certain physiological and biochemical changes that inevitably reduce their quality and in turn affect consumer acceptance. One consequence of postharvest stress in vegetables is browning associated with wounding. The browning of fresh-cut vegetables is enzymatically regulated and greatly influenced by stresses that occur during storage, including wounds, harsh postharvest conditions, and moisture loss. Substantial evidence has indicated that a set of enzymatic browning responses are significantly influenced by the degree of browning that occurs in fresh-cut vegetables, such as those by polyphenol

oxidase (PPO), phenylalanine ammonia lyase (PAL), and peroxidase (POD). Deterioration of membranes in plant tissue is the typical cause of browning. PAL, the first enzyme in the phenylpropanoid pathway that produces phenolic compounds, is considered to be involved in this process. PPO catalyzes the oxidation of phenolic substances by hydroxylating monophenols into o-diphenols and o-diphenols into quinones, which are then transformed into melanin (Singh et al., 2018).

Arginine is one of the most functionally diverse amino acids with the highest nitrogen to carbon ratio and is a precursor in the biosynthesis of polyamines, agmatine, and proline, as well as the cell-signaling molecules glutamate, amino butyric acid, and nitric oxide (Liu et al., 2006). It has been demonstrated that arginine inhibits tissue browning and lowers lipid peroxidation in mushrooms, strawberries, and sweet cherries. (Li et al., 2019; Shu et al., 2020; Pakkish & Mohammadrezakhani, 2022).

Against this background, this study was implemented to determine how arginine treatment influenced the suppression of enzymatic browning and membrane damage in fresh-cut romaine lettuce preserved at 6±1°C. Browning and lipid peroxidation were measured using the browning score, phenolic compounds, PPO and PAL activities, lipoxygenase (LOX) activity, and malondialdehyde (MDA) and hydrogen peroxide levels.

2. MATERIALS AND METHODS

2.1 Plant materials and treatment

Romaine lettuces were transported to the laboratory of Silpakorn University's Faculty of Animal Sciences and Agricultural Technology from hydroponic farming, Phetchaburi, Thailand. Processing was conducted at 25°C in a cold room. They were chosen, chopped, rinsed in tap water for 5 min, and then submerged for 10 min in solutions of 0, 1, 0, 10, and 20 mM arginine (purified to 99%, food grade). A centrifuge salad spinner was used to remove excess solution from fresh-cut red romaine lettuce, which was then packed into polypropylene plastic boxes (weighing about 55–60 g) and kept at 6±1°C for a period of 15 days. Samples were randomly chosen at 0, 2, 5, 10, and 15 days from each treatment to examine the browning inhibition and oxidation change throughout storage. The remaining portion was frozen and kept at -20°C until being extracted and analyzed, with the sample being kept fresh.

2.2 Browning score and cutting-edge color

To examine the cutting-edge color and browning score, 30 pieces of fresh-cut romaine lettuce were chosen at random from each treatment. The browning score was assessed based on the look of the browning color using a five-point scale; 0 = no browning; 1 = slight browning; 2 = moderate browning; 3 = severe browning; 4 = very severe browning. The cutting-edge color was measured using a color meter (Model Mini Scan EZ, Hunter Lab), and the result was evaluated in terms of a*, hue, and chroma values.

2.3 Total phenolic compounds

One milliliter of 80% ethanol was used to extract the tissue powder (0.5 g), and the mixture was centrifuged at 15,000 rpm for 20 min at 4°C. The assay reaction mixture comprised 150 µL of 7.5% sodium carbonate, 100 µL of 10% Folin–Ciocalteu reagent, and 20 µL of supernatant. A UV-vis microplate reader was used to record the absorbance at 765 nm. A gallic acid standard curve was used to calculate the total amount of phenolic compounds.

2.4 PPO activity and POD activity

PPO and POD enzyme extracts were made by homogenizing 0.5 g of tissue powder with 1 mL of 50 mM potassium phosphate buffer (pH 6.5), which included 0.5% polyvinylpyrrolidone, for 30 min at 15,000 rpm and 4°C. The activity was established by performing enzyme extraction.

In accordance with the procedure reported by Galeazzi and Sgarbieri (1981), PPO activity was measured by monitoring the rise in absorbance at 480 nm for 3 min. After adding 20 µL of enzyme extract, 50 µL of 50 mM potassium phosphate buffer (pH 6.5), and 200 µL of 25 mM catechol solution, the reaction was started. The amount of enzyme responsible for a 0.001 change in absorbance per minute was considered to represent one unit of enzyme activity.

POD activity was measured in accordance with the method reported by Castillo et al. (1984). The reaction mixture contained enzyme extract, 2 mL of hydrogen peroxide, 16 mL of guaiacol, and 50 mL of potassium phosphate buffer (pH 6.5). The absorbance at 470 nm increased for 3 min. An extinction coefficient of 26.6 $mM^{-1}cm^{-1}$ was used to determine POD activity. Unit.mg^{-1} protein was used to express PPO and POD functions. Bovine serum albumin was used as a reference to calculate the protein content, following the Bradford method (Bradford, 1976).

2.5 PAL activity

A modified version of the approach reported by Ke and Saltveit (1986) was used to measure PAL activity. A 0.5 g sample of tissue powder was homogenized using 1 mL of 50 mM borate buffer (pH 8.6) that contained 2% polyvinylpyrrolidone and 5 mM 2-mercaptoethanol. The homogenized mixture was centrifuged at 15,000 rpm and 4°C for 30 min. After adding 10 µL of supernatant, 150 µL of borate buffer extraction, and 40 µL of 10 mM L-phenylalanine, the reaction was monitored at 290 nm using a microplate reader and incubated for 1 h at 30°C. The amount of PAL in mmol of cinnamic acid in 1 h was defined as the unit of enzyme activity.

2.6 LOX activity

In accordance with the procedure reported by Maalekuu et al. (2006), the elevation in absorbance at 234 mM was used to measure the LOX activity. To prepare the enzyme extract, 0.5 g of tissue powder was homogenized with 1 mL of 50 mM potassium phosphate buffer (pH 7.0). This was followed by centrifugation at 15,000 rpm and 4°C for 30 min. The addition of linoleic acid and enzyme extract started the reaction. An extinction coefficient of 25.00 mM^{-1} cm^{-1} was used to determine the LOX activity.

2.7 MDA content

The thiobarbituric acid (TBA) reaction demonstrated that MDA was a byproduct of polyunsaturated fatty

acid oxidation (Ali et al., 2005). On this basis, 0.5 g of tissue powder and 1 mL of 80% ethanol were homogenized, and the mixture was centrifuged at 15,000 rpm for 30 min at 4°C. Then, 1 mL of the supernatant was mixed with 1 mL of 0.65% TBA solution, containing 20% (w/v) trichloroacetic acid (TCA) and 0.01% butylated hydroxytoluene (BHT) or added to a solution containing 20% (w/v) TCA and 0.01% BHT. The samples were centrifuged for 10 min at 15,000 rpm, incubated for 30 min at 95°C, and immediately cooled in an ice bath. Absorbance at 440, 532, and 600 nm was then measured. An extinction coefficient of 157 $mM^{-1}cm^{-1}$ was used to determine the MDA content.

2.8 Hydrogen peroxide content

One milliliter of 1% TCA was used to extract 0.5 g of tissue powder, which was then centrifuged for 30 min at 15,000 rpm. The absorbance at 390 nm was measured, after the supernatant was combined with 50 mM potassium phosphate buffer (pH 7.0) and 1 mM potassium iodide. The outcomes were reported using a hydrogen peroxide standard curve (Velikova et al., 2000).

2.9 Experimental design and statistical analysis

The entire experiment was conducted using a completely randomized design. One-way analysis of variance was used to assess all of the data. Tukey's honestly significant difference test was used to determine the significance of differences between means. Differences were considered to be statistically significant at $p \leq 0.01$.

3. RESULTS AND DISCUSSION

3.1 Browning score (BS), visual appearance, and cutting-area color

As expected, arginine utilization had a dramatic effect on browning inhibition of fresh-cut romaine lettuce, as evaluated by changes in browning score, visual appearance and cutting area color (Figures 1, 2 and 3). Fresh-cut vegetable browning is a key component in evaluating consumer acceptability and influences buying motivation. In selecting a purchase, the consumer considers appearance factors to provide evidence of freshness and flavor quality (Barrett et al., 2010). The results of this study demonstrated that BS in fresh-cut romaine lettuce continuously increased during storage, and there were significant differences among treatments for 5 days until 15 days of storage (Figure 1). The BS can determine a product's quality and shelf life while it is being stored. In comparison to fresh-cut lettuce treated with 1, 10 mM, and non-treated solutions (control treatment), it was found that fresh-cut lettuce treated with 20 mM arginine had the lowest BS. On the final day of storage, the BS values of the control, 1, 10 mM and 20 mM arginine treatments were significantly different 3.67, 3.00, 2.50, and 2.23, respectively. Hence, 20 mM arginine treatment in fresh-cut romaine lettuce displayed the best browning inhibition compared to those other treatments. The decrease in browning violence in wounding areas was a relationship with the increase in arginine concentration, which is certainly evidence that arginine can delay browning. The present results are similar to those reported by Wills and Li (2016) for apple and iceberg lettuce and Li et al. (2019) for white button mushroom (*Agaricus bisporus*).

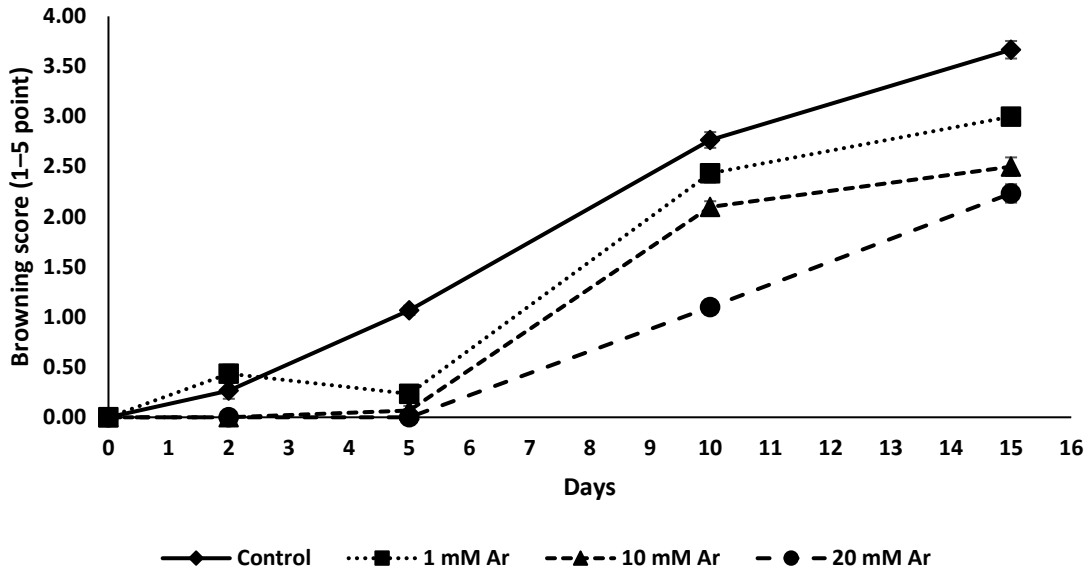

Figure 1. Effects of treatment with different concentrations of arginine on browning score in fresh-cut romaine lettuce stored at 6±1°C for 15 days
Note: Each data point is the mean ± SE of three replicate samples (n = 30 lettuce pieces).

Furthermore, BS was also accorded visual appearance at 10 days of storage and cutting area color, as shown in Figures 2 and 3, respectively. The cutting area color of fresh-cut romaine lettuce was affected by the presence of a change in a*, Hue and Chroma values. The a* value ranges from green (a negative value) to red (a positive value). The a* value in all treatments increased rapidly at 2 days of storage, indicating that the green value in the cutting area of fresh-cut romaine lettuce decreased after storage. Comparing fresh-cut lettuce in control treatment and different concentrations of arginine treatment, the a* value in arginine treatment was clearly the highest at 10 and 15 days of storage. On the contrary, hue and chroma values in control treatment were the lowest compared to all arginine treatments. Hue value is color degree, followed by 90 degrees = yellow and 180 degrees = bluish-green and chroma value is color intensity. Therefore, the low values of hue and chroma can explain why the color in the cutting area is rather yellow-brown.

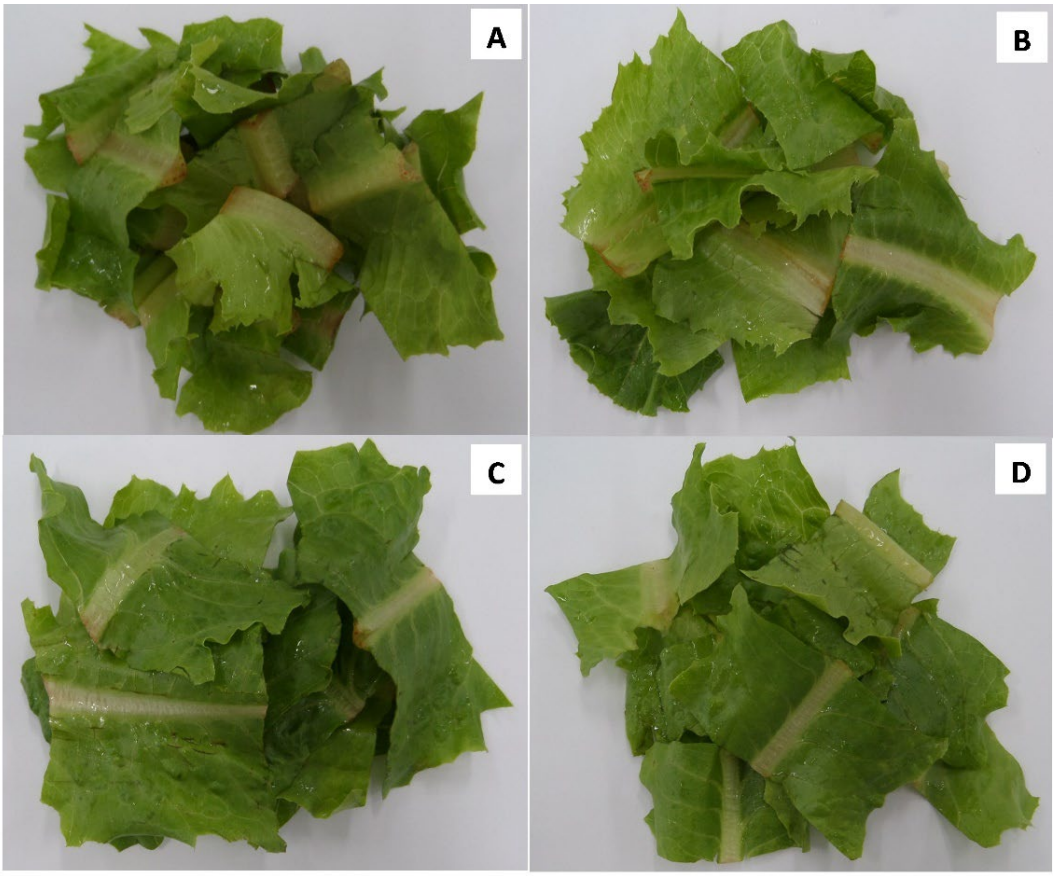

Figure 2. The visual appearance of fresh-cut romaine lettuce treated with different arginine concentrations and stored at 6±1°C for 10 days; (A) Control treatment, (B) 1 mM arginine treatment, (C) 10 mM arginine treatment, and (D) 20 mM arginine treatment

There was a report that arginine plays an important role in the potential to stimulate nitric oxide (NO) synthesis, which NO functions the control of such germination, defense responses and browning inhibition in plants (Arc et al., 2013; Wendehenne et al., 2004). Huque et al. (2013) pointed out that diethylenetriamine nitric oxide (DETANO) and NO gas delayed the development of surface browning in fresh-cut apple slices during storage at 5°C and also resulted in a lower level of total phenols and inhibition of PPO activity. Moreover, arginine plays a significant role in plant physiology not only through its conversion to nitric oxide but also by being a precursor to polyamines. These polyamines are crucial in inhibiting browning in plants, thereby enhancing their post-harvest quality and shelf life. The interplay between NO and polyamines further underscores the complex regulatory networks in plant stress responses and quality control (Tiburcio et al., 2014).

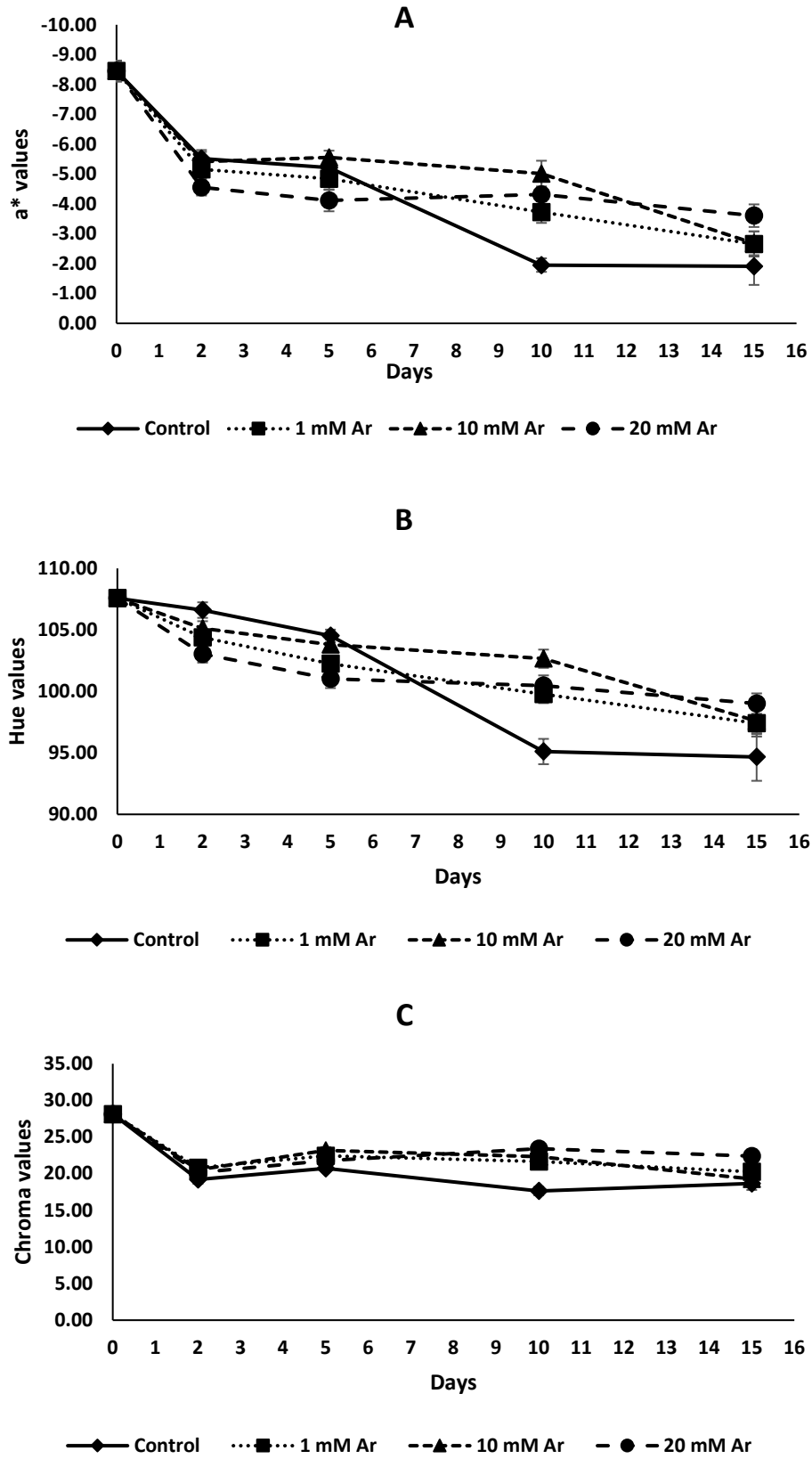

Figure 3. Effects of treatment with arginine at different concentrations on cutting-area color in fresh-cut romaine lettuce stored at 6±1°C for 15 days
Note: A: a* values, B: hue values, and C: chroma values. Each data point is the mean ± SE of three replicate samples (n = 30 lettuce pieces).

3.2 Phenolic compounds, PAL activity, PPO activity and POD activity

The enzymatic browning response, closely connected to the browning symptom caused by wounding on fresh-cut romaine lettuce, is initiated by phenolic compounds. Consequently, reducing phenolic compounds may play an important role in browning protection. Results showed that all solutions treated with arginine were significantly ($p<0.01$) effective in altering phenolic compounds, compared to non-treated solutions with arginine at 10 and 15 days of storage (Figure 4). Fresh-cut romaine lettuce in all solutions treated with L-arginine had lower phenolic compounds than non-treated solutions with arginine (control) at 10 days of storage, while fresh-cut romaine lettuce in treated solutions with 20 mM arginine was not significant as compared with control at 15 days of storage. This finding suggested that browning inhibition may be related to the reduction of phenolic compounds caused by arginine treatment and that the alteration of phenolic compound was time-dependent. According to Tomás-Barberán et al. (1997), lettuce damage triggered an accumulation of soluble phenolic compounds that contributed to browning symptoms. However, the browning reaction occurs in conjunction with PAL and PPO activity as well as the presence of phenolic compounds (Hisaminato et al., 2001).

The production of phenolic compounds, predominantly derived from byproducts of the shikimic pathway, is believed to involve the enzyme PAL. The biosynthesis of most plant phenolic compounds involves the shikimic acid pathway (Tzin & Galili, 2010). As shown in Figure 5, PAL activity increased after 2 days of storage, before maintaining a relatively steady level. Between 2 and 10 days of storage, the control treatment was associated with higher PAL activity than all arginine treatments. The results demonstrate that PAL activity corresponded to changes in these phenolic compounds. The benefit of arginine in reducing browning symptoms may be attributable to a decrease in phenolic compounds and PAL activity. According to Wang et al. (2017), arginine significantly reduces PAL activity in green asparagus (*Asparagus officinalis* L.).

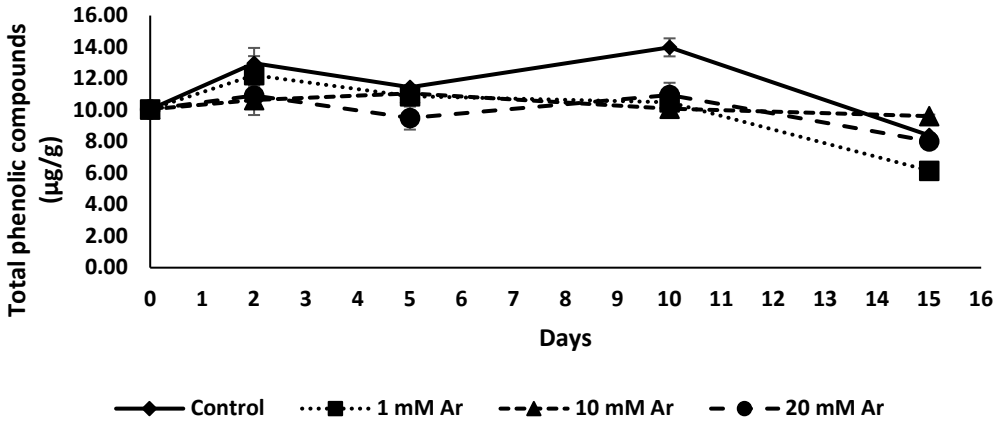

Figure 4. Effect of treatment with different concentrations of arginine on total phenolic compounds in fresh-cut romaine lettuce stored at 6±1°C for 15 days
Note: Each data point is the mean ± SE of three replicate samples.

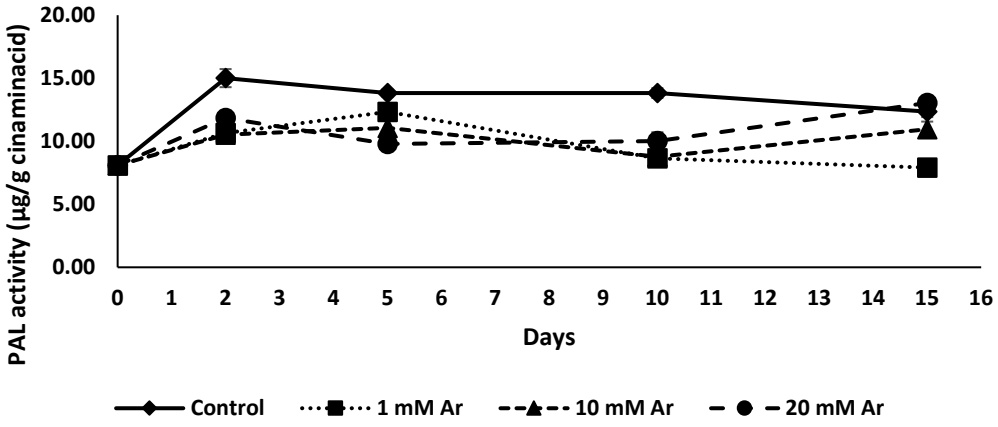

Figure 5. Effect of treatment with different concentrations of arginine on phenylalanine ammonia lyase (PAL) activity in fresh-cut romaine lettuce stored at 6±1°C for 15 days
Note: Each data point is the mean ± SE of three replicate samples.

On the other hand, fresh-cut romaine lettuce in a solution treated with arginine had higher PPO activity than in a non-treated solution with arginine (Figure 6). According to Zhang et al. (2017), arginine treatment increased PPO activity in tomato fruit most of the time after inoculation with *Botrytis cinerea*. However, contrary to the finding of Li et al. (2019), the use of 10 mM arginine inhibited PPO activity in white button mushrooms. PPO activity is one of the key enzymes involved in the enzymatic browning reaction It is possible that the high PPO activity in arginine treatment had no effect on browning because the low levels of phenolic compounds led to a less active reaction. Because there isn't enough substrate for the enzyme to work on, low quantities of phenolic compounds may not result in noticeable browning even with high PPO activity during arginine treatment (Vámos-Vigyázó, 1995).

There is substantial evidence that POD is responsible for the enzymatic browning of fresh-cut products. (Zhan et al., 2012; Kim et al., 2014; Wang et al., 2015). Richard-Forget and Gauillard (1997) described the possible role of enzymatic browning of polyphenols oxidized by POD. The variation in POD activity in fresh-cut romaine lettuce throughout storage is shown in Figure 7. POD activity trended to slightly decrease throughout the storage period. Fresh-cut romaine lettuce in control treatment had higher POD activity than all arginine treatments.

This finding implies that arginine administration can reduce the browning symptom on wounds and has notable potential to decrease phenolic compound buildup and PAL activity. The question of whether the browning symptom in fresh-cut romaine lettuce was directly related to the increase in PPO activity and the decrease in POD activity remained unanswered; however, the change in enzyme activity might be influenced by stress and storage time.

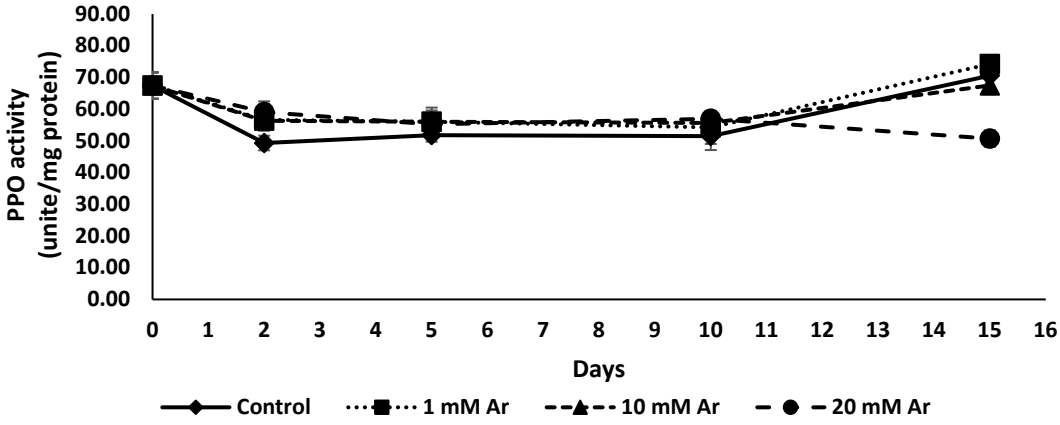

Figure 6. Effects of treatment with different concentrations of arginine on polyphenol oxidase (PPO) activity in fresh-cut romaine lettuce stored at 6°C ± 1°C for 15 days
Note: Each data point is the mean ± SE of three replicate samples.

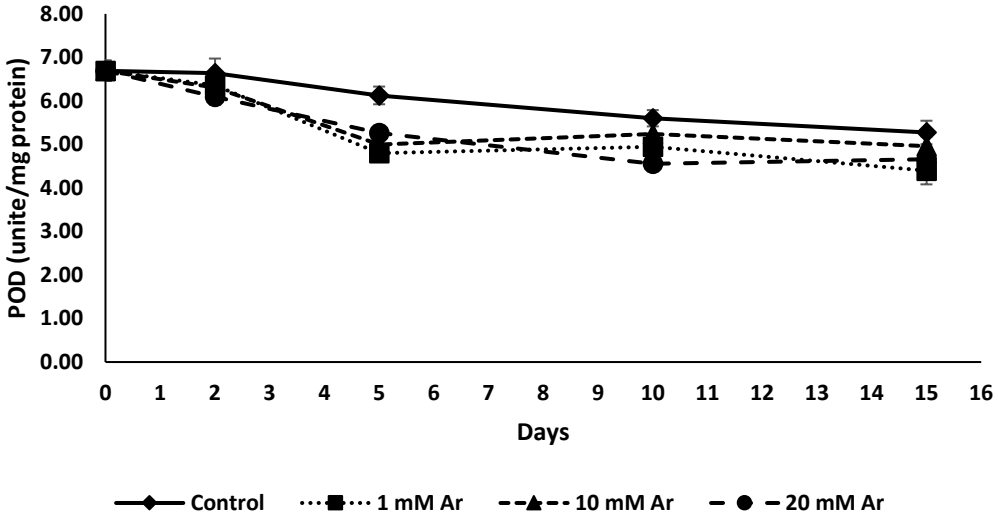

Figure 7. Effects of treatment with different concentrations of arginine on peroxidase activity in fresh-cut romaine lettuce stored at 6±1°C for 15 days
Note: Each data point is the mean ± SE of three replicate samples.

3.3 LOX activity, MDA content, and hydrogen peroxide level

Deterioration during storage is adversely related to metabolic changes, which can increase ROS generation and induce oxidative stress. It has been reported that ROS-induced peroxidation of lipid membranes reflects cell damage (de Bruxelles & Roberts, 2001). In fresh-cut romaine lettuce, browning and senescence during storage are caused by membrane lipid peroxidation. An elevated level of lipid peroxidation is indicated by increases in MDA content, hydrogen peroxide level, and LOX activity.

LOX activity in all arginine treatments decreased rapidly over the first 2 days before stabilizing (Figure 8). All arginine treatments had lower levels of LOX activity compared to the control treatment. This indicates that arginine can prevent membrane lipid peroxidation, thereby reducing physical change during storage. LOX activity is responsible for cell membrane deterioration and peroxidation, which increased the levels of MDA and hydrogen peroxide (Figures 9 and 10). This study showed that arginine prevented lipid oxidation, as evidenced by lower levels of hydrogen peroxide, MDA, and LOX activity compared to the control treatment. Among the treatments, the use of 20 mM arginine proved to be the most effective.

Therefore, the results show that arginine administration prevents membrane lipid peroxidation by decreasing LOX activity, which in turn lowers levels of oxidative stress indicators such as hydrogen peroxide and MDA. This shielding property of arginine may enable it to protect the integrity of cell membranes and prevent them from changing physically during storage or other stressful situations. Arginine is a precursor to NO, which it can lessen oxidative stress and lipid peroxidation (Hussain et al., 2011).

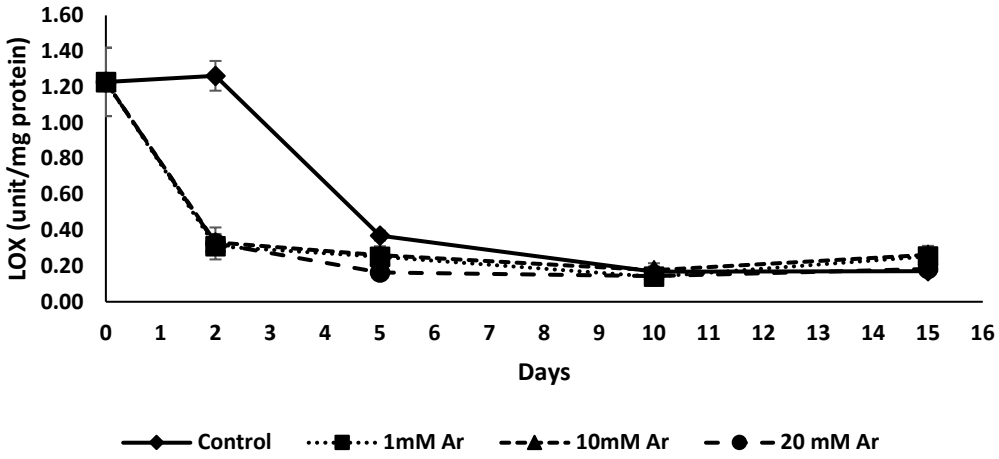

Figure 8. Effects of treatment with different concentrations of arginine on lipoxygenase activity in fresh-cut romaine lettuce stored at 6±1°C for 15 days
Note: Each data point is the mean ± SE of three replicate samples.

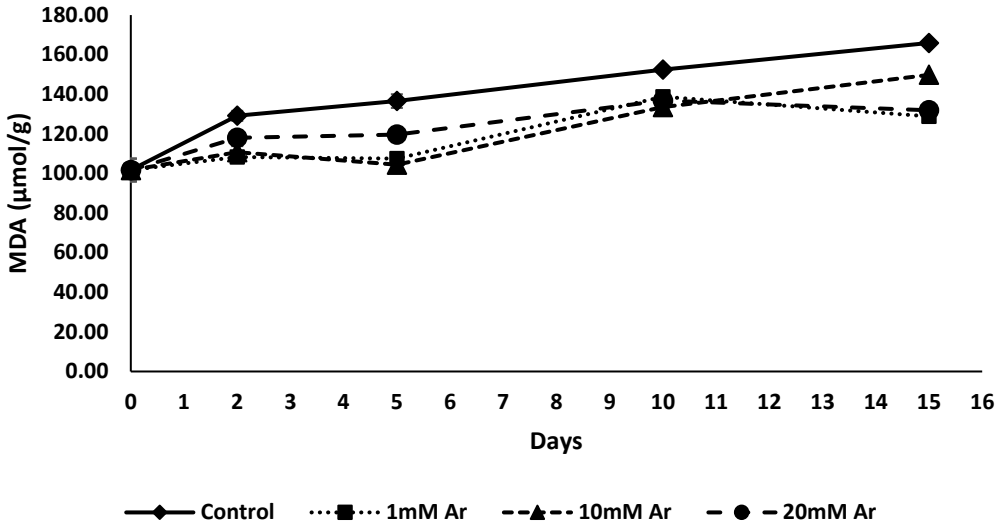

Figure 9. Effects of treatment with different concentrations of arginine on malondialdehyde (MDA) content in fresh-cut romaine lettuce stored at 6±1°C for 15 days
Note: Each data point is the mean ± SE of three replicate samples.

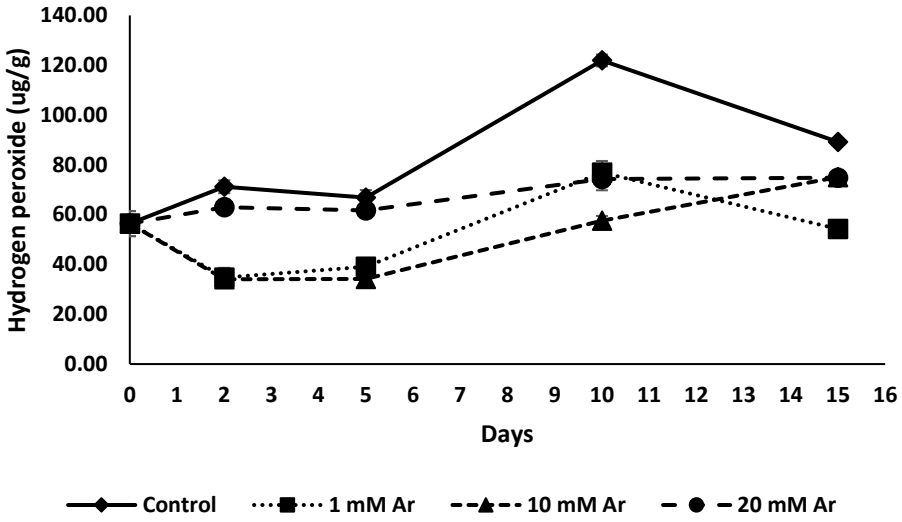

Figure 10. Effects of treatment with different concentrations of arginine on hydrogen peroxide content in fresh-cut romaine lettuce stored at 6±1°C for 15 days
Note: Each data point is the mean ± SE of three replicate samples.

4. CONCLUSION

The browning symptom and lipid peroxidation have been prevented in fresh-cut romaine lettuce treated with arginine. The main indicators used to measure browning symptoms, including the browning index, chroma value, phenolic compounds, and activity of PAL, PPO, and POD, were decreased by the arginine treatment. Additionally, arginine treatment reduced MDA concentration, LOX activity, and hydrogen peroxide during storage compared to the control treatment. Therefore, arginine assists postharvest products in maintaining quality and extending shelf life.

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