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Exogenous arginine treatment for inhibiting browning symptom and improving the quality of fresh-cut red cabbage

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Abstract

Fresh-cut red cabbage was immersed with 0, 50 and 200 mm arginine and stored at 6±1 ˚C for 15 d to study the effect of exogenous arginine treatment on the browning symptom and quality of this vegetable. Browning score, phenolic compounds, polyphenol (PPO) activity, phenylalanine ammonia lyase (PAL) activity, weight loss, anthocyanin and leaf colour were measured at 2, 5, 10 and 15 d of storage. Results showed that arginine treatment affected leaf colour and reduced browning score, phenolic compounds, PPO activity, PAL activity, weight loss and anthocyanin content. The 50 mm arginine treatment was more efficient in inhibiting browning symptom than the 200 mm arginine and control treatments. The inhibitory effect of arginine on browning was directly associated with a decrease in phenolic compounds, PPO activity and PAL activity. The leaf colour was red-purple in the arginine treatment group and red in the control group. The change in leaf colour may be correlated with the lower anthocyanin content in the arginine treatment group compared with the control treatment group. In addition, the fresh-cut red cabbage treated with arginine had a lower weight loss than the control one. Physical characteristics such as browning symptom, leaf colour and freshness are the primary considerations of consumers when buying red cabbage. This study showed that exogenous 50 mm arginine treatment inhibited browning and maintained quality, which can be attributed to the reduction in weight loss.

Keywords: Red cabbage, Arginine, Postharvest quality, Enzymatic browning reaction

1. Introduction

Red cabbage (*Brassica oleracea* var. rubra) is a common leafy vegetable belonging to the Brassicaceae or Cruciferae family. Red cabbage is popularly used to process fresh-cut salad or mixed salad. Processing operations such as washing, scrubbing, peeling, trimming, cutting and shredding during the initial stages of fresh-cut preparation cause mechanical injury (wounding) to plant tissues, leading to biochemical changes, especially browning symptom in leaf and edge-cut areas. Polyphenol oxidase (PPO) is characterised by the melanisation pathway and involves the hydroxylation of monophenol into *o*-diphenol and *o*-diphenol into quinones, leading to the formation of pigments. Aside from PPO and phenolic compounds, phenylalanine ammonialyase (PAL) also plays an important role in the stimulation of browning [1]. Wounding not only has a negative effect on the appearance but also accelerates the biochemical change and senescence of plant tissues. Therefore, browning symptom is an important problem in fresh-cut salad processing.

L-arginine is an amino acid implicated in plant development and in stress response. Arginine plays an important role in polyamine (PA) and nitric oxide (NO) syntheses [2-4]. In the first mechanism of arginine metabolism, nitric oxide synthase catalyses arginine to produce NO. Secondly, arginine is hydrolysed by arginine decarboxylase to produce agmatine, and arginase catalyses arginine to produce ornithine and urea; agmatine and ornithine are precursors of the PA synthesis pathway. NO and PAs are involved in plant development and in plant resistance to biotic and abiotic stresses [5]. Limited information is available about the role of arginine biosynthesis in plant response, but most evidence focuses on the mechanisms of PAs and NO in plant resistance. Wills and Li [6] showed that treatment with 10-200 mm arginine in fresh-cut lettuce and apple can reduce browning symptom and extend postharvest life.

NO controls oxidative phosphorylation and photosynthesis and defends cell organelles from oxidative damage to delay senescence and cell death [7]. Leshem et al. [8] reported that fumigating broccoli with 0.25 $\mu mol.L^{-1}$ NO for 5 h can maintain its green colour and firmness and can extend postharvest life compared with the air control. Moreover, exposure to 10 $\mu L.L^{-1}$ NO can reduce surface browning in apple slices by reducing phenolic compounds and PPO activity [9]. A postharvest study applied NO in the form of gas and solution as diethylenetriamine nitric oxide (DETANO) and sodium nitroprusside [10-12]. The study found that NO contributes to browning inhibition in fresh-cut fruits and vegetables. Therefore, utilisation of arginine in postharvest handling, which is a precursor to NO synthesis, may lead to browning inhibition during storage.

Hence, the present study aimed to determine the effect of arginine treatment on the enzymatic browning and quality of fresh-cut red cabbage stored at 6±1 ˚C. The measured parameters of browning symptom and postharvest quality included browning score, phenolic compound, PPO and PAL activities, weight loss, leaf colour and anthocyanin content.

2. Materials and methods

2.1 Plant material and treatment

Red cabbage was obtained from a commercial market in Thailand and transported by truck at room temperature to the Faculty of Animal Sciences and Agricultural Technology, Silpakorn University. The dimension of red cabbage ranged from 12 cm to 15 cm (36 heads). Processing was managed in a cold room at 20 ˚C. Red cabbage samples were selected, cut, washed in tap water for 5 min and then immersed in 0, 50 and 200 mm arginine (purify > 99%, food grade) for 10 min. Fresh-cut red cabbages were centrifuged to remove excess solution using a centrifuge salad spinner, packed (approximately 60 g) in plastic boxes (size box 12 cm × 15 cm × 6 cm, total 108 boxes) and then stored at 6 ˚C and 85% RH for 15 d. Each treatment was subdivided into three replicates of 108 boxes. The samples were randomly chosen from each treatment at 0, 2, 5, 10 and 15 d to determine the biochemical assay. Part of the sample was kept fresh, while the remainder was frozen and stored at -20 ˚C prior to extraction and analysis. All parameters were performed with three replicates.

2.2 Browning score

Thirty pieces of fresh-cut romaine lettuce were randomly selected in each treatment to analyse browning score (10 pieces/replicate). The browning score was evaluated on the basis of the appearance of browning by sensory method using a five-point scale as follows: 0 = no browning, 1= slight browning, 2 = 1/2 browning, 3 = moderate browning (1/4-1/2 browning), 4 = poor quality and 5 = very poor quality.

2.3 Total phenolic compounds

Total phenolic content was determined as previously described by Ketsa and Atantee [13]. The tissue (0.5 g) was extracted with 1 mL of 80% ethanol and then centrifuged at 15,000 rpm for 20 min at 4 ˚C. Reaction mixtures contained 20 μL of supernatant, 100 μL of 10% Folin-Ciocalteu reagent and 150 μL of 7.5% sodium carbonate. The absorbance was determined on a microplate reader at 765 nm. Total phenolic compounds were determined based on a standard curve of gallic acid.

2.4 Polyphenol oxidase

Polyphenol oxidase (PPO) activity was determined in accordance with the method described by Galeazzi and Sgarbieri [14] with some modifications. A 0.5 g tissue was homogenised with 4 mL of 50 mm potassium phosphate

buffer pH 6.5 containing 0.5% polyvinylpyrrolidone. The homogenised mixture was later centrifuged at 15,000 rpm for 30 min at 4 ˚C. The reaction mixture contained 20 µL of supernatant with 50 µL of potassium phosphate buffer and 200 µL of 25 mm catechol solution and was measured on a microplate reader at 480 nm for 3 min. One unit of enzyme activity is defined as the amount of enzyme causing a change of 0.001 in absorbance per minute. The activity of PPO was expressed as µmol $min^{-1}mg^{-1}$ protein. Protein content was determined using the Bradford method (1976) with bovine serum albumin as a standard.

2.5 Phenylalanine ammonia lyase

PAL activity was measured in accordance with the method described by Siriphanich and Kader [15] with some modifications. A 0.5 g tissue was homogenised with 1 mL of 50 mm borate buffer pH 8.6 containing 5 mm 2-mercaptoethanol and 2% polyvinylpyrrolidone. The homogenised mixture was centrifuged at 15,000 rpm for 30 min at 4 ˚C. The reaction was assayed with the addition of 10 µL of supernatant with 150 µL of borate buffer extraction and 40 µL of 10 mm L-phenylalanine and then incubated at 30 ˚C for 1 h. Absorbance was determined on a microplate reader at 290 nm. One unit of enzyme activity is defined as the amount of PAL mmol of cinnamic acid in 1 h.

2.6 Weight loss

Each fresh-cut red cabbage was weighed to calculate the percentage of weight loss with respect to the initial weight.

2.7 Leaf colour

The instrumental measurement of colour was analysed with a colorimeter (Hunterlab, USA), and the results were expressed to standard illuminant D65. The colorimeter measures colour base on the amount of light reflected from the surface.

2.8 Anthocyanin content

Anthocyanin content was determined by the modified method of Ranganna [16]. A 0.5 g of tissue was homogenised with 1 mL of 95% ethanol:1.5 N hydrochloric acid (85:15). The homogenised mixture was later centrifuged at 15,000 rpm for 30 min at 4 ˚C. Absorbance was determined at 535 nm on a microplate reader. Anthocyanin content was calculated and expressed as mg/100 g FW.

2.9 Experimental design and statistical analysis

Completely randomised design was used throughout the experiment. Data were analysed with one-way ANOVA. Mean separations were performed by post-hoc Tukey's honestly significant difference. Statistical significance was considered at $p \leq 0.01$. Statistical analysis was executed using program R version 2.15.1.

3. Results and discussion

3.1 Influence of arginine to browning symptom

Fresh-cut vegetables are subject to various processing operations that result in the wounding of the plant tissue, causing browning symptom. Browning symptom causes quality loss and significant revenue loss of fresh-cut products. The change in characteristic browning of fresh-cut red cabbage was detected by browning score, where a significant difference was observed between the tested treatments (Figure 1). Fresh-cut red cabbage exposed to arginine showed lower browning inhibition than the control treatment. The effects of different concentrations of arginine treatment on the browning score were compared, and results showed that the 50 mm arginine treatment was more efficient than the 200 mm arginine treatment in inhibiting browning symptom. Excessively high arginine concentration resulted in a reduce of browning inhibition efficiency, it is possible that arginine contributes to higher metabolic processes. The value in browning score was directly accorded to visual quality as shown in Figure 2. These results indicate that arginine can inhibit browning symptom. Previous studies implied that arginine is the immediate precursor of NO, which plays a role in plant responses, such as improving plant resistance, inhibiting

senescence, increasing antioxidant capacity and reducing PAL activity [17-19], which may be a cause of browning symptom inhibition on fresh-cut red cabbage. Wills and Li [6] reported that arginine exposure can extend the shelf life and inhibit browning symptom of apple and lettuce. These results suggest that NO plays a key role in the defence mechanism in response to abiotic stress and browning inhibition in plants [9,20].

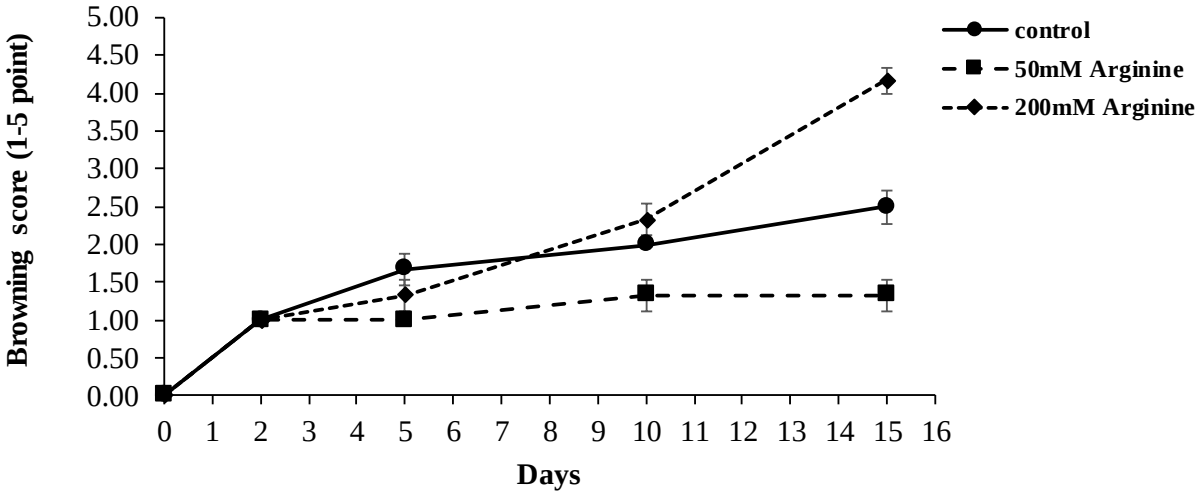

Figure 1 Effect of different concentrations of arginine treatment on the browning score of fresh-cut red cabbage stored at 6 ˚C. Each data point is the mean ± SE of three replicates.

Figure 2 Visual quality of arginine treatment in fresh-cut red cabbage stored at 6 ˚C, 15 d. A = Control treatment; B = 50 mm arginine treatment; C = 200 mm arginine treatment; D = control treatment in edge-cutting; E = 50 mm arginine treatment in edge-cutting; F = 200 mm arginine treatment.

Browning symptom due to enzymes can be analysed by changes in the activities of phenolic compounds PPO and PAL. As a result, the total phenolic compounds were constant throughout the storage (Figure 3). Comparison of treatments showed that the control treatment induced significantly higher phenolic compounds than the arginine treatment, and no significant difference was noted between the 50 and 200 mm arginine treatments. Normally, the browning reaction is a consequence of the oxidation of phenolic compounds by PPO, which triggers the integration of molecules and the generation of dark pigments. The decrease in phenolic compounds in the fresh-cut red cabbage treated with arginine may be attributed to the production of NO, considering that arginine is a primary precursor of NO biosynthesis. The role of NO in browning inhibition in fresh-cut apple slices treated with DETANO is that it decreases total phenol content and PPO activity compared with the control group [9].

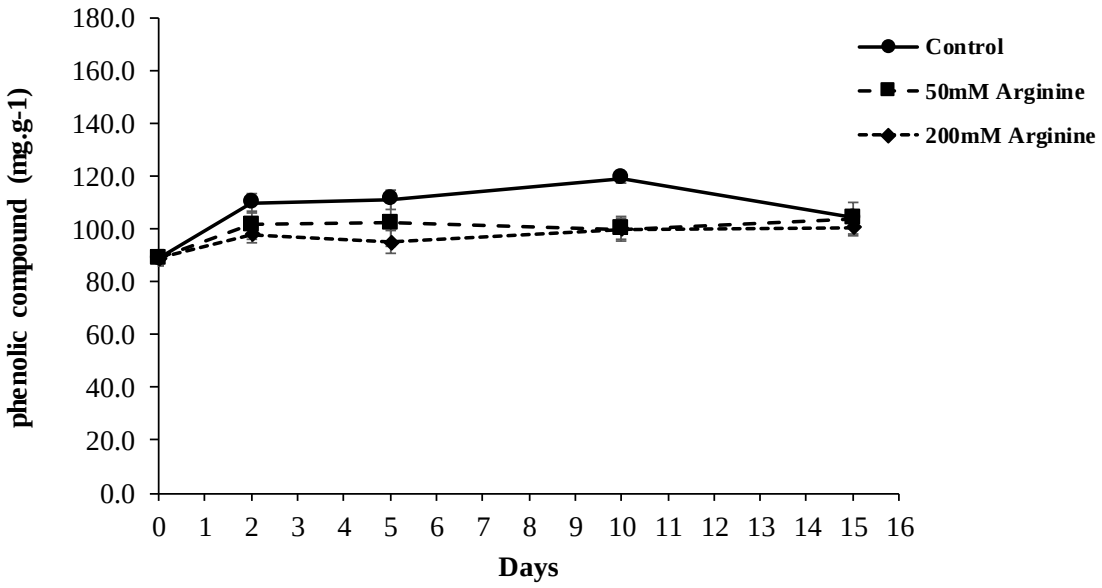

Figure 3 Effect of different concentrations of arginine treatment on the phenolic compound (mg/100 g) of fresh-cut red cabbage stored at 6 ˚C. Each data point is the mean ± SE of three replicates.

The browning symptom in fresh-cut red cabbage can be studied on the basis of changes in PPO and PAL activities (Figure 4). The PPO activity in the control treatment group was stable, whereas that in the arginine treatment group increased at 2 d of storage. Then, PPO activity in the control treatment increased at 5 d, decreased at 10 d and stabilised. Comparison between the control and arginine treatment groups showed that the arginine treatment decreased the PPO activity compared with the control treatment.

PAL activity plays a crucial role in the synthesis of phenolic compounds, and the increase in PAL activity causes an accumulation in phenolic compounds [21,22]. PAL is a key enzyme in the synthesis of phenolic compounds, which are synthesised primarily from products of the shikimic pathway. The shikimic acid pathway participates in the biosynthesis of most plant phenolic compounds. Phenolic compounds are substrates for oxidative enzymes, such as PPO, which catalyses browning reaction [23]. This research demonstrated that PAL activity in 50 mm arginine treatment decreased rapidly until 5 d and then increased slightly until the final d of storage. Hence, the decrease in PAL activity is directly correlated with the decrease in browning symptom. Wang et al. [19] proposed that arginine plays an important role in decreasing PAL activity on green asparagus (*Asparagus officinalis* L.). In addition, the decrease in browning symptom of fresh-cut red cabbage treated with arginine compared with the control is due to the association of enzymatic browning with the decrease in phenolic compound and PPO activity.

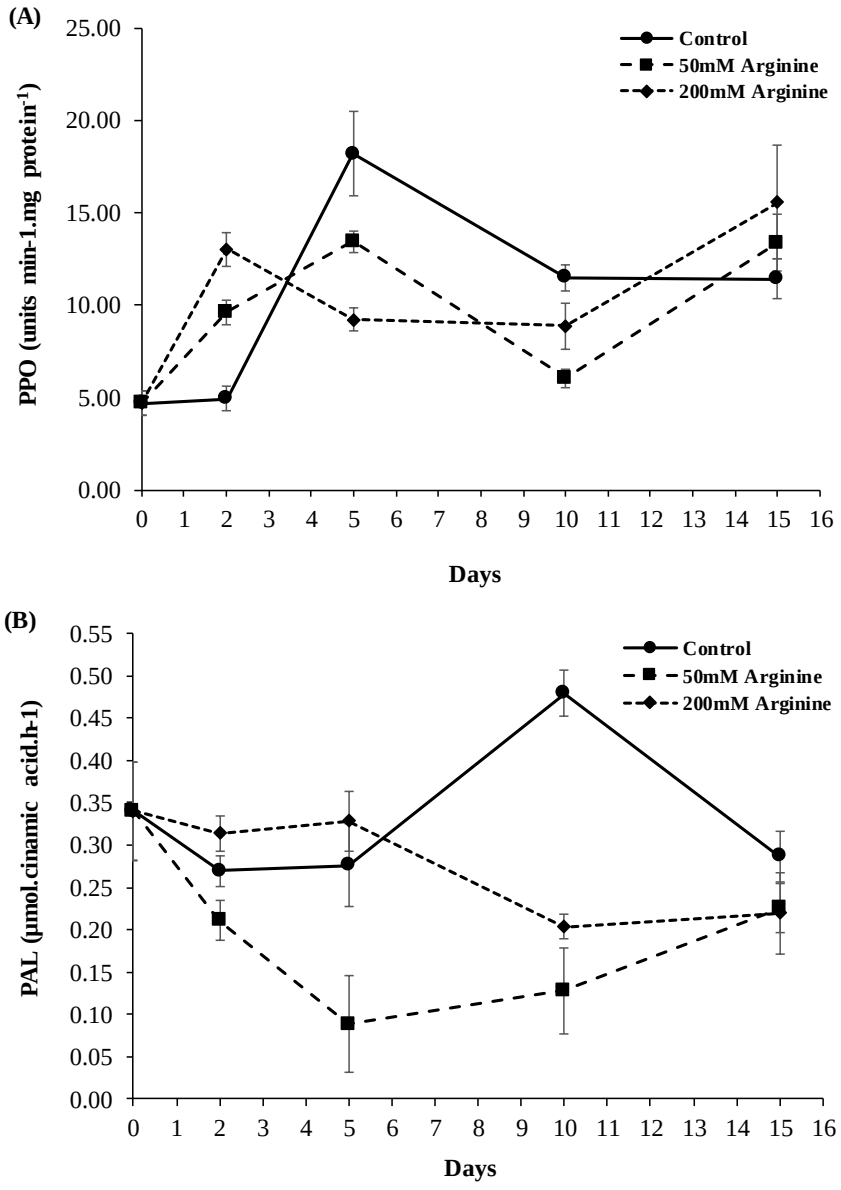

Figure 4 Effect of different concentrations of arginine treatment on the (A) polyphenol oxidase (PPO) and (B) phenylalanine ammonia lyase (PAL) activities of fresh-cut red cabbage stored at 6 ˚C. Each data point is the mean ± SE of three replicates.

3.2 Influence of arginine to improve quality

The quality fresh-cut red cabbage can be measured by changing the weight loss, leaf colour and anthocyanin content. An important factor in consumer acceptance is visual appearance, which is associated with weight loss (Figure 5). Weight loss constantly increased in the control and arginine treatment groups. The lowest weight loss was recorded in the 50 mm arginine, control and 200 mm arginine treatment groups in the final d of storage. The weight loss in the 200 mm arginine treatment was the highest, approximately 10.46% at 15 d, leading to consumer refusal. In these results, the 50 mm arginine treatment showed better efficiency in retarding weight loss than the 200 mm arginine treatment during storage. Evidence of how arginine retards weight loss is lacking, but a previous study showed that that arginine affects indirectly the retardation of senescence. Moreover, a previous report showed that arginine is the precursor of PAs, which implicates a role of retarding plant senescence under stress conditions [24].

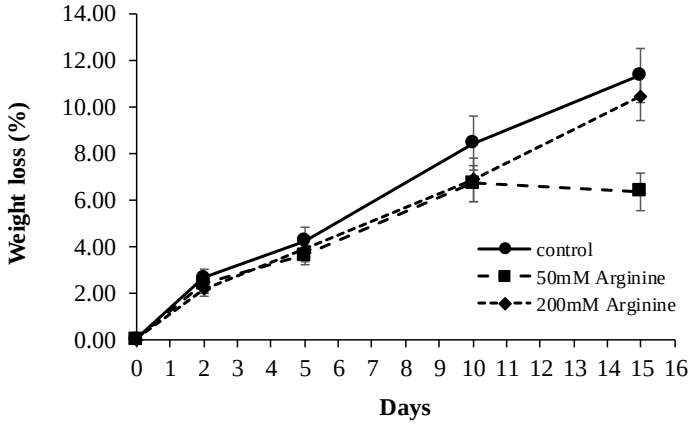

Figure 5 Effect of different concentrations of arginine treatment on the weight loss (%) of fresh-cut red cabbage stored at 6 ˚C. Each data point is the mean ± SE of three replicates.

Another factor that may have contributed to quality is the change in leaf colour as assessed by hue, a* and b* values (Figure 6). The a* value ranges from negative to positive values; negative value is green, and positive value is red. The a* value in 50 mm arginine was significantly higher than the other treatments at 2 and 5 d, slightly decreased at 10 d and then decreased rapidly in the final d of storage. No significant difference in a* value was found between treatments at 10 d. The b* value is the yellow/blue coordinate, and it increased rapidly at 2 d and then stabilised. The fresh-cut red cabbage treated with 200 mm arginine showed the highest increase in b* value. The increase in b* value indicated a change in blue tone into yellow tone.

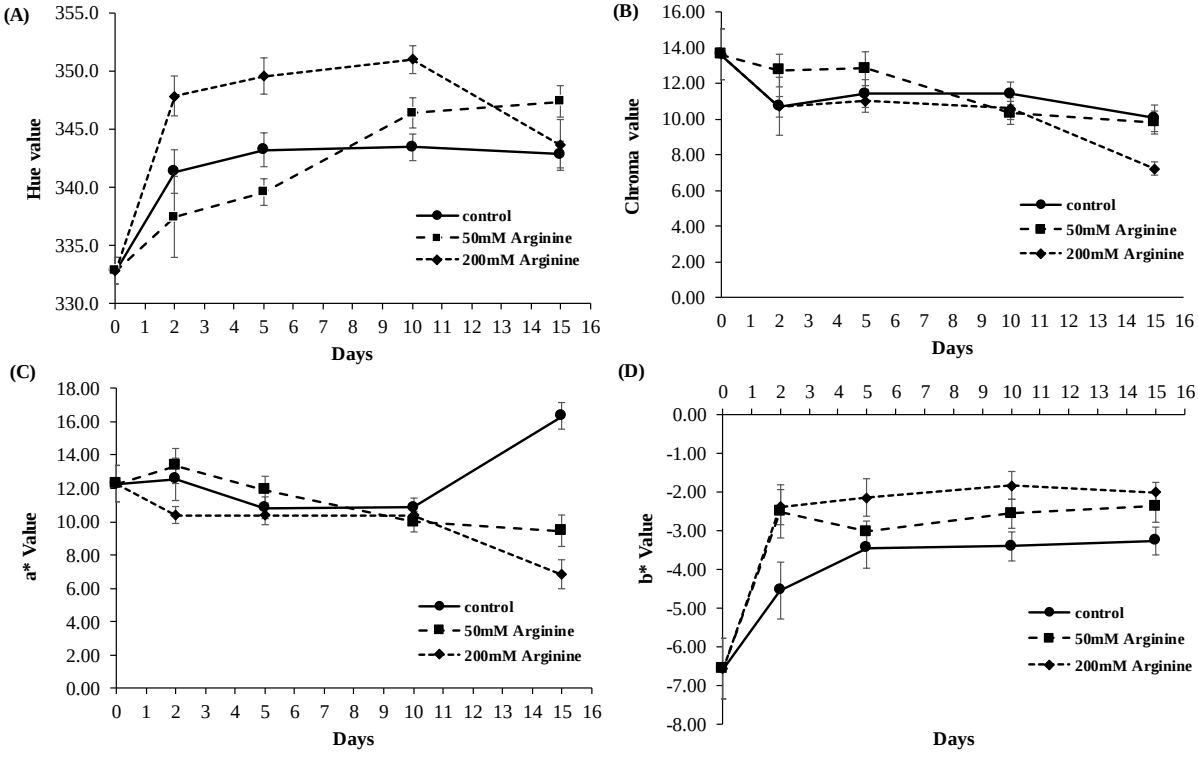

Figure 6 Effect of different concentrations of arginine treatment on the leaf colour of fresh-cut red cabbage stored at 6 ˚C. (A) hue value; (B) chroma value; (C) a* value; (D) b* value. Each data point is the mean ± SE of three replicates.

Hue value is the colour degree followed as 0 degree = red, 90 degrees = yellow, 180 degrees = bluish-green and 270 degrees = blue. Consequently, the hue value of leaf colour in red cabbage ranges from 330-360 degrees, indicating that the leaf colour ranges from blue to red. The fresh-cut red cabbage treated with 200 mm arginine had higher hue value than the cabbage treated with control and 50 mm arginine until 5 d of storage. The hue value in the 50 mm arginine treatment group increased continuously throughout the experiment, decreased at 2 and 5 d and then increased at 10 and 15 d compared with that in the control treatment group. Therefore, arginine triggered leaf colour development from red-purple (magenta) to red, which is possibly dependent on arginine concentration and storage period. The possible factor contributing to the change in leaf colour must deal with the change in pH in cells, which is caused by the acidity of NO.

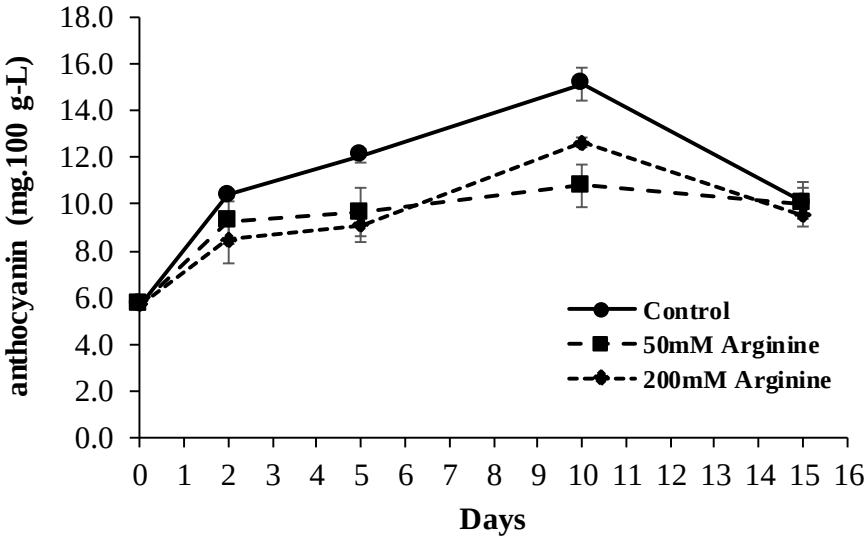

Figure 7 Effect of different concentrations of arginine treatment on the anthocyanin content (mg/100 g) of fresh-cut red cabbage stored at 6 ˚C. Each data point is the mean ± SE of three replicates.

Furthermore, the change in leaf colour may be correlated to anthocyanin content. The effect of arginine on anthocyanin content is presented in Figure 7. Anthocyanin content increased in the control and arginine treatment groups at 2 d of storage, and it was higher in the control treatment group than in the arginine treatment group. The significant increase in anthocyanin was distinct at 10 d of storage. Data showed that anthocyanin content was the lowest in the 50 mm arginine treatment group, followed by the 200 mm arginine treatment group and then the control group. Arginine treatment increased the content of anthocyanin, which is associated with a change in leaf colour. The colour stability of anthocyanin on food depends on different pH levels in cell; in specific, anthocyanin is red under acidic conditions and purple and blue under basic conditions [25]. Excessive arginine concentration possibly contributes to the increase in cell acidity, leading to cell damage [26].

4. Conclusion

Arginine treatment can inhibit the browning symptom and improve the quality of fresh-cut red cabbage. The 50 mm arginine treatment reduced phenolic compound content and PPO and PAL activities. Moreover, arginine can retard weight loss, change leaf colour and decrease anthocyanin content. Conversely, excessive arginine concentration negatively affected the quality of fresh-cut red cabbage.

5. Acknowledgements

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