L-Arginine to inhibit browning on fresh-cut salacca (*Salacca edulis* Reinw)

I Prabasari1*, N A Utama1, E P Wijayanti1, N A U Hasanah1, S Riyadi2 and T K Hariadi2

1Faculty of Agriculture Universitas Muhammadiyah Yogyakarta, Indonesia
2 Faculty of Engineering Universitas Muhammadiyah Yogyakarta, Indonesia

*E-mail: i.prabasari@umy.ac.id

**Abstract.** The research aimed to study the effect of various concentration of L-arginine and time of immersion in inhibiting enzymatic browning on fresh-cut Salacca. The experiment was conducted in Single Factor Completely Randomized Design with treatments as follows: (P0) Control, (P1) L-arginine 50 mM and immersion time 5 min, (P2) L-arginine 50 mM and immersion time 10 min, (P3) L-arginine 50 mM and immersion time 15 min, (P4) L-arginine 100 mM and immersion time 5 min, (P5) L-arginine 100 mM and immersion time 10 min, (P6) L-arginine 100 mM and immersion time 15 min, (P7) L-arginine 150 mM and immersion time 5 min, (P8) L-arginine 150 mM and immersion time 10 min, (P9) L-arginine 150 mM and immersion time 15 min. The result showed that L-arginine prevented synthesis of phenolic compound that promotes browning development. However color measurement of fresh-cut Salacca indicated that L-Arginine was able to prevent browning only up to 9 days. Meanwhile various immersion times did not give significant difference in preventing browning. Based on weight loss and hardness analysis, L-Arginine was able to prolong the quality of fresh-cut Salacca. The result in this study will be elaborated and used as a reference to develop non-destructive method using SVM (Support Vector Machine) to analyze the degree of browning on fresh-cut Salacca.

1. Introduction

*Fresh-cut product* or *minimally processed* of fruits and vegetables is a way to enhance quality and convenience, to expand food distribution and to reduce waste. To produce *fresh-cut product* steps of treatments are performed involving washing, stripping, slicing, packaging in low temperatures and modified atmospheres to maintain freshness and nutrient content in fruits and vegetables [1, 2]. However, the process of cutting increase respiration and thus speed up degradation process, i.e. hardness loss, enzymatic browning, damage, losing moisture and off flavors. In addition, process of cutting creates open wound in fruits and make them more vulnerable to microbial attacks [3].

In fruit, browning occurs when substrate in the form of phenolic compounds found in vacuoles mixed with polyphenol oxidase (PPO) enzymes and peroxidase (POD) enzymes found in the cytoplasm and assisted by oxygen as a co-substrate [4]. This reaction produces quinone which will react spontaneously to produce melanin and substances those trigger brown colour appearance in fruit flesh [5]. Prevention of browning can be done in some ways: (1) removing oxygen on the surface of fresh-cut products, for example by immersing in water, (2) removing copper contained in the PPO enzyme prosthetic group using a chelating agent such as EDTA, organic acids, and phosphate thus PPO enzymes cannot carry out browning reactions, (3) inactivation of PPO enzymes, i.e. with
blanching method, (4) storing in cold temperatures, (5) adding antioxidants and (6) using edible coating [4].

Research conducted by Zhang et al. [6] with L-arginine by dipping tomatoes in 0.2 mM L-Arginine solution at sub atmospheric pressure (35 kpa) in 2°C storage for 2-4 weeks resulted in 20% reduction in chilling injury. L-arginine produces an increase in nitric oxide (NO) in the tissues after several days of storage and thus increase the activity of NOS (nitric oxide system) in tomatoes. It is assumed that environmental pressure and room temperature simultaneously promote increased absorption of arginine in the fruit.

To understand the effect of L-Arginine in preventing the browning of fresh-cut Salacca, this experiment was conducted by immersing fresh-cut Salacca in various concentration of L-Arginine and different time of immersion.

2. Materials and Methods

The research was conducted from March to April 2018 in Faculty of Agriculture, Universitas Muhammadiyah Yogyakarta and Faculty of Agricultural Technology Gadjah Mada University, Indonesia. Salacca fruits (Salacca edulis) used in the study was harvested with same maturity index from Sleman, Yogyakarta, Indonesia. Experiment was carried out using a single factor treatment arranged in a Completely Randomized Design (CRD). The treatments given as follows P0: control, P1: L-Arginine 50 mM and immersion time of 5 min, P2: L-Arginine 50 mM and immersion time 10 min, P3: L-Arginine 50 mM and immersion time 15 min, P4: L-Arginine 100 mM and immersion time 5 min, P5: L-Arginine 100 mM and immersion time 10 min, P6: L-Arginine 100 mM and immersion time 15 min, P7: L-Arginine 150 mM and immersion time 5 min, P8: L-Arginine 150 mM and immersion time 10 min, P9: L-Arginine 150 mM and immersion time 15 min. Analysis of weight loss, hardness, phenolic compound, colour were performed every 3 days, starting from day 0 to 15. Data obtained were analyzed using Analysis of Variance (ANOVA) with significance level of α = 5%. Further tests will be carried out using Duncan's Multiple Range Test (DMRT) at a significance level of α = 5% for data with significant differences between treatments.

Weight loss (%) - Percentage of weight loss of fresh-cut Salacca was performed using gravimetric method (AOAC, 2012) every three days for 15 days.

Hardness (N/mm²) - hardness of fresh-cut Salacca was measured using hand penetrometer fruit (Lutron, FR-520, USA) every three days for 15 days. The surface of each replicate sample of fresh-cut Salacca was pierced by a cone probe with a diameter of 3 mm in 3 areas, i.e. top, middle and bottom of the fruits. The depth of holes caused by stabbing indicated the hardness of fresh-cut Salacca.

Phenolic compound (ppm) - Measurement of phenolic compound in fresh-cut Salacca was conducted based on the modification of method from Folin-Ciocalteu [7]. Each replicate sample was mashed then weighed 1 g to be dissolved in 10 mL of distilled water. Solution of 0.5 mL was taken into the test tube, then added by 5 mL of distilled water. After 5 min, 1.5 mL of 15% sodium carbonate and 1.5 ml Folin-Ciocalteu reagent was added and shaken. Phenol compound measurements were carried out by inserting the solution into a spectrophotometer with a wavelength of 765 nm. Phenol content was expressed based on the equation of the standard of gallic acid curve made at concentrations of 5, 10, 20, 30, 40 and 50 ppm [8].

Color - Color changes were measured using a chromameter CR-400. L*(lightness), a* (red-green), b* (yellow-blue) of each replicate of samples were analysed. L showed brightness with a value of 0 (dark / black) to 100 (bright /white), while a and b are chroma coordinates, where a for green (a negative) to red (a positive) and b for blue (b negative) to yellow (b positive). Change of color then calculated using the formula:

\[ \Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2} \]

with \( \Delta L = L_{\text{sample}} - L_{\text{standard}}, \Delta a = a_{\text{sample}} - a_{\text{standard}}, \Delta b = b_{\text{sample}} - b_{\text{standard}} \).
3. Results and Discussion

3.1. Weight loss

During producing fresh-cut products, cutting process damage protective layer on the fruit so that the tissue is directly exposed to environment and therefore increases water loss and results in loss of freshness and appearance. That including weight loss which promotes sloughing and damage to fresh-cut products [9]. Weight loss occurs in fruit and vegetables due to the presence of respiration and transpiration during storage. Transpiration is the process of water loss in the form of moisture through evaporation process while respiration is a metabolic process to break complex compounds such as starch, sugar, protein, fat and organic acids to produce simpler molecules i.e. CO₂ and H₂O and produce energy used by cells for synthesis reactions [10].

Salacca has a water content of 78% [11] and it can be partially lost due to transpiration process. The results showed that there was an increase of weight loss during storage for 15 days (Table 1; Figure 1). The weight loss of fresh-cut Salacca represented the water loss evaporated from the fruit by turgor pressure in the cell. Water loss not only increase weight loss but also decrease the quality of fresh-cut Salacca. Furthermore, there was significant difference between treatments, and it showed that treatment with 150 mM L-Arginine for 10 min (P8) had the lowest weight loss (Table 1; Figure 1). Fresh-cut Salacca in this experiment had wound caused by cutting process and the tissue was exposed to the environment. This had an impact on increasing the speed of respiration and evaporation thus increased weight loss. In addition, study from [12] shows that weight loss in stored fruits is mainly due to water loss as a result of evaporation process and loss of carbon (CO₂) during transpiration. Water is released in the process of transpiration through stomata and other parts of plant tissue that are related to epidermal cells.

Table 1. Weight loss of fresh-cut Salacca treated with various L-Arginine concentrations and immersion times.

| Treatments                  | Weight loss (%) |
|-----------------------------|-----------------|
|                             | Day-            |                |
|                             | 3   | 6   | 9   | 12  | 15  |
| Control                     | 0.69bcd | 1.94cd | 3.31cd | 4.22a | 5.30abc |
| L-Arginine 50 mM for 5'     | 0.94a | 2.35ab | 3.78abc | 4.55a | 5.15abc |
| L-Arginine 50 mM for 10'    | 0.82abc | 2.32ab | 4.00ab | 4.95a | 6.12ab |
| L-Arginine 50 mM for 15'    | 0.72bcd | 1.78de | 3.20cd | 4.01ab | 5.70ab |
| L-Arginine 100 mM for 5'    | 0.92a | 2.28abc | 4.06ab | 4.83a | 5.78ab |
| L-Arginine 100 mM for 10'   | 0.81abc | 2.47a | 4.34a | 5.03a | 6.53a |
| L-Arginine 100 mM for 15'   | 0.84ab | 2.01bcd | 3.65bc | 4.36a | 5.69ab |
| L-Arginine 150 mM for 5'    | 0.63d | 1.74de | 2.84de | 3.08bc | 4.23cd |
| L-Arginine 150 mM for 10'   | 0.64d | 1.51e | 2.48e | 2.83c | 3.60d |
| L-Arginine 150 mM for 15'   | 0.68cd | 1.74de | 3.25cd | 3.92ab | 4.92bcd |

The average number with the same letter at the same column are not significant at (α = 5%).

3.2. Hardness

Hardness is an important parameter in terms of consumer acceptance where level of hardness inS fruits and vegetables during ripening process affects its quality and shelf life. The results showed that immersion of L-Arginine was able to maintain the hardness of fresh-cut Salacca (Table 2, Day 12 and Day15). It represented the ability of L-Arginine in inhibiting softening and senescense process in fresh-cut Salacca. L-Arginine which belongs to polyamines strongly bonded to pectin compounds in
the middle lamella and therefore strengthen the cell wall, a phenomenon that also found in study from [13, 14].

![Figure 1](image)

**Figure 1.** Weight loss of *fresh-cut* Salacca treated with various L-Arginine concentrations and immersion times. P0: control, P1: L-Arginine 50 mM for 5', P2: L-Arginine 50 mM for 10', P3: L-Arginine 50 mM for 15', P4: L-Arginine 100 mM for 5', P5: L-Arginine 100 mM for 10', P6: L-Arginine 100 mM for 15', P7: L-Arginine 150 mM for 5', P8: L-Arginine 150 mM for 10', P9: L-Arginine 150 mM for 15'.

| Treatments                        | Day-0 | Day-3 | Day-6 | Day-9 | Day-12 | Day-15 |
|----------------------------------|-------|-------|-------|-------|--------|--------|
| Control                          | 1.22d | 1.33a | 1.07b | 0.98b | 0.59c  | 0.18c  |
| L-Arginine 50 mM for 5'          | 1.56bc| 1.57a | 1.39a | 0.92b | 0.92bc | 0.85b  |
| L-Arginine 50 mM for 10'         | 1.45bc| 1.20a | 1.12b | 1.19ab| 0.80c  | 1.71a  |
| L-Arginine 50 mM for 15'         | 1.40cd| 1.17a | 1.17b | 1.20ab| 1.39ab | 1.28ab |
| L-Arginine 100 mM for 5'         | 1.54bc| 1.61a | 1.47a | 1.15ab| 1.34ab | 1.60a  |
| L-Arginine 100 mM for 10'        | 1.49bc| 1.40a | 1.53a | 1.02ab| 1.70a  | 1.54a  |
| L-Arginine 100 mM for 15'        | 1.39cd| 1.37a | 1.47a | 0.98b | 1.62a  | 1.57a  |
| L-Arginine 150 mM for 5'         | 1.53bc| 1.40a | 1.41a | 0.92b | 1.50a  | 1.75a  |
| L-Arginine 150 mM for 10'        | 1.68b | 1.50a | 1.58a | 1.53a | 1.44a  | 1.54a  |
| L-Arginine 150 mM for 15'        | 1.89a | 1.85a | 1.63a | 1.55a | 1.49a  | 1.73a  |

The average number with the same letter at the same column are not significant at (α = 5%).

3.3. Phenolic compound

Phenolic compounds are a large group of plant secondary metabolites with at least one aromatic ring (C6) containing one or more hydroxyl groups (-OH). Phenol components in plants are classified based on the number of the atom in the structure, starting from simple phenol, phenolic acid (hydroxybenzoate and hydroxysinamic), flavonoids and tannins [15]. Synthesis of phenolic compounds begins after the presence of wound on the fruit resulting in an increase in phenolic
compounds as a sign of defense mechanism. To prevent browning enzymatic that affected by phenolic compound L-arginine is added in post-harvest treatment to inhibit synthesis of phenolic compound by surpressing activity of chitinase, glucanase, phenylalanin ammonia-lyase (PAL), and polyphenol oxidase (PPO) in fruit [6].

The result found in this experiment on fresh-cut Salacca (Table 3, Day 12 and Day 15) was similar with the study from [6] on tomatoes that immersion of L-arginine was able to prevent enzymatic browning. The phenomenon indicated that L-Arginine prevented synthesis of phenolic compound in fresh-cut Salacca thus slowed down browning development. Furthermore, another study revealed that L-arginine can promote NO (nitric oxide) in fruit tissue and enhance NOS (nitric oxide system) activity during storage [16]. Although the connection between NO in inhibiting enzymatic browning on fruit and vegetable is still unclear, study of lettuce from [17] showed that the oxidative properties in NO was able to bind free radicals involved in the browning process thus surpressed browning development reactions on lettuce.

Table 3. Phenolic compound of fresh-cut Salacca treated with various L-Arginine concentrations and immersion times.

| Treatments                  | Phenolic compound (%) | Day- |
|-----------------------------|-----------------------|------|
|                            |                       | 0    | 3    | 6    | 9    | 12   | 15   |
| Control                     | 0.014c                | 0.029b| 0.025a| 0.039a| 0.044a| 0.021ab|
| L-Arginine 50 mM for 5'     | 0.028a                | 0.027b| 0.028a| 0.046a| 0.038ab| 0.025a|
| L-Arginine 50 mM for 10'    | 0.028a                | 0.028b| 0.022a| 0.039a| 0.030bc| 0.016bc|
| L-Arginine 50 mM for 15'    | 0.024ab               | 0.026b| 0.019a| 0.043a| 0.022cd| 0.016bc|
| L-Arginine 100 mM for 5'    | 0.028a                | 0.025b| 0.023a| 0.036a| 0.019d | 0.015bc|
| L-Arginine 100 mM for 10'   | 0.024ab               | 0.025b| 0.023a| 0.043a| 0.019d | 0.017bc|
| L-Arginine 100 mM for 15'   | 0.018bc               | 0.032ba| 0.020a| 0.036a| 0.016d | 0.019abc|
| L-Arginine 150 mM for 5'    | 0.015c                | 0.023b| 0.023a| 0.034a| 0.019d | 0.014bc|
| L-Arginine 150 mM for 10'   | 0.023ab               | 0.039a| 0.033a| 0.034a| 0.017d | 0.013c |
| L-Arginine 150 mM for 15'   | 0.020bc               | 0.023b| 0.023a| 0.042a| 0.019d | 0.016bc|

The average number with the same letter at the same column are not significant at (α = 5%).

3.4. Color

Color measurement is one of the physical indicators that relates to the level of preference and subjective perception of food product. Furthermore color measurement is used as an interpretation of enzymatic browning which have been previously quantified through biochemical index. Color indicators measured in L* a* b* are the most widely used color reference because they have a uniform color distribution and are close to reflect human perception [18]. Calculations based on L* a* b* produce a value of hue or a combination of red, green and blue (RGB). Fruit or vegetable with a high color percentage shows an increasingly bright color. The darker or brighter the surface of fruit or vegetable depends on the enzymatic browning reaction that occurs. Browning begins with monophenol enzymatic oxidation to o-diphenol and then o-diphenol to quinone, which will then undergo non-enzymatic polymerization to form brown / melanin pigments [19].

Color measurement correlated with the state of fresh-cut Salacca was examined from day 9 to day 15 and showed in Tabel 4 and Figure 4. The data revealed that various concentration of L-Arginine and immersion time gave different response in browning development where L-Arginine with concentration 100 mM gave the brightest colour value which indicated the lowest development for browning with the number of 87.27, 87.01 and 87.41, respectively (Table 4, Day 9, P4, P5 and P6). Meanwhile immersion time from 5 min to 15 min did not give different result. L-Arginine was able to prevent browning only up to 9 days and after 9 days, L-Arginine was not able to inhibit browning.
development (Table 2, Day 12 and Day 15), indicated by no significant differences between control and treatments with L-Arginine in Day 12 and Day 15.

However, the data of color measurement and phenolic compound was not in line. Phenolic compound in Day 9 was not affected by L-Arginine (Table 3, Day 9), but in Day 12 and 15 phenolic compound of samples treated with L-Arginine was significantly different with control (Table 3, Day 12, Day 15). On the contrary, treatments with L-Arginine in Day 9 on color measurement gave significant difference with control but after 9 days there was no significant difference between control and treatments (Table 4, Day 12, Day 15). The phenomenon explained that browning development did not only depend on phenolic compound as substrate but also depend on the role of enzyme, i.e. polyphenol oxidase (PPO) and phenilalanin ammonia-lyase (PAL). Unfortunately, we did not analyse the activity of PPO and PAL in this experiment.

**Table 4.** Colour measurement of fresh-cut Salacca treated with various L-Arginine concentrations and immersion times.

| Treatments                  | Color       |
|-----------------------------|-------------|
|                             | Day -       |
|                             | 9 12 15     |
| Control                     | 84.09d 85.61abc 86.01ab |
| L-Arginine 50 mM for 5'     | 86.87ab 85.44abcd 85.27ab |
| L-Arginine 50 mM for 10'    | 87.28a 86.19ab 85.48ab |
| L-Arginine 50 mM for 15'    | 85.67bc 84.22d 83.50b |
| L-Arginine 100 mM for 5'    | 87.27a 85.95abc 84.82ab |
| L-Arginine 100 mM for 10'   | 87.01a 85.87abc 79.85c |
| L-Arginine 100 mM for 15'   | 87.41a 85.69abc 79.50c |
| L-Arginine 150 mM for 5'    | 85.44c 84.82cd 86.09ab |
| L-Arginine 150 mM for 10'   | 86.43abc 86.32ab 86.92a |
| L-Arginine 150 mM for 15'   | 86.52abc 85.02bcd 83.49b |

The average number with the same letter at the same column are not significant at (α = 5%)

4. Conclusion

L-Arginine was able to inhibit browning by preventing synthesis of phenolic compound that promotes browning development. However color measurement indicated another role in browning development, possibly the role of enzyme i.e. PPO and POD, something we need to examine further. Immersion times did not give significant difference in preventing browning whether 5 min, 10 min or 15 min. In addition, based on weight loss and hardness, L-Arginine prolonged the quality of fresh-cut Salacca. The result in this study will be elaborated and used as a reference to develop non-destructive method using SVM (Support Vector Machine) to analyze the degree of browning on fresh-cut Salacca.

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