

STUDYING THE DISTRIBUTION OF L-CITRULLINE IN WATERMELON FRUITS UNDER DIFFERENT STORAGE CONDITIONS

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Abstract

Watermelons are a natural and rich source of the non-essential amino acid L-citrulline, including the rind and seeds. Therefore, this study carried out three main tasks: i) Evaluate L-citrulline content in different parts of some watermelon types; ii) Monitor the change of L-citrulline in juice from flesh and rind at -20°C, 0°C, and 4°C at the consecutive intervals of 1 day, 3 days, 6 days, 10 days, 15 days and 21 days; iii) Investigate the effect of fresh cut watermelon (skin and flesh) at storage time on the L-citrulline content. UV-vis absorption spectroscopy method was used to determine the L-citrulline content at 490 nm. As a result, the content of L-citrulline in the rind ranged from 0.764 to 1.277 mg/g, which was greater than that of L-citrulline in watermelon flesh (0.580 to 1.103 mg/g), seeds (0.179 to 0.214 mg/g) (dwt) and similar among three types of watermelons. However, L-citrulline in rind juice was more affected by storage temperature and time than L-citrulline in fruit juice at the same freezing temperature. In contrast, the fresh-cut watermelon rind had less L-citrulline content reduction than the fresh-cut watermelon at -20°C for a longer time. These results indicated that watermelon rind, an agricultural waste rich in natural citrulline, should be exploited. Finally, the low temperature below 4°C influenced the L-citrulline content in watermelon, so watermelon juice and fresh-cut watermelon rind will be suitable for long-term freezing.

Keywords: Distribution, frozen, L-citrulline, watermelon.

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NGHIÊN CỨU SỰ PHÂN BỐ L-CITRULLINE TRONG QUẢ DƯA HẦU VỚI CÁC ĐIỀU KIỆN TỒN TRỮ KHÁC NHAU

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Tóm tắt

Dưa hấu là một nguồn axit amin không thiết yếu L-citrulline tự nhiên và phong phú, bao gồm cả vỏ và hạt. Vì vậy, nghiên cứu này thực hiện ba nhiệm vụ chính: i) Đánh giá hàm lượng L-citrulline trong các bộ phận khác nhau của một số loại dưa hấu; ii) Theo dõi sự thay đổi của L-citrulline trong nước ép từ thịt quả và vỏ ở nhiệt độ -20°C , 0°C , 4°C trong thời gian là 1 ngày, 3 ngày, 6 ngày, 10 ngày, 15 ngày và 21 ngày; iii) Khảo sát ảnh hưởng của dưa hấu tươi cắt miếng (cả vỏ và thịt) trong thời gian bảo quản đến hàm lượng L-citrulline. Phương pháp quang phổ hấp thụ UV-vis được sử dụng để xác định hàm lượng L-citrulline ở bước sóng 490 nm. Kết quả là hàm lượng L-citrulline trong vỏ dao động từ 0,764 đến 1,277 mg/g lớn hơn hàm lượng L-citrulline trong thịt quả (0,580 đến 1,103 mg/g), hạt (0,179 đến 0,214 mg/g) (dwt) và giống nhau giữa ba loại dưa hấu. Tuy nhiên, L-citrulline trong dịch ép vỏ bị ảnh hưởng bởi nhiệt độ và thời gian bảo quản nhiều hơn so với L-citrulline trong dịch ép thịt quả ở cùng nhiệt độ đông lạnh. Ngược lại, vỏ dưa hấu tươi mới cắt làm giảm hàm lượng L-citrulline ít hơn so với dưa hấu tươi cắt ở -20°C trong thời gian dài. Những kết quả này chỉ ra rằng vỏ dưa hấu, một chất thải nông nghiệp sẽ cung cấp một nguồn citrulline tự nhiên dồi dào nên cần được khai thác. Ngoài ra, nhiệt độ thấp dưới 4°C đã ảnh hưởng đến hàm lượng L-citrulline trong dưa hấu nên nước ép thịt quả dưa hấu và vỏ dưa hấu tươi cắt tươi sẽ thích hợp để cấp đông lâu dài.

Từ khóa: Dưa hấu, lạnh đông, L-citrulline, phân bố.

1. Introduction

Watermelon (*Citrullus lanatus* Thumb.) belongs to the family *Cucurbitaceae*, native to the arid and semi-arid regions of Central Africa, the northern Sahara, has a hard flesh and a bland taste, is mainly used as clean drinking water (Paris, 2015). Watermelon is a group of drought-resistant plants, climbing plants with spreading roots. The fruit has a hard, smooth skin, is diverse in shape and color, weighing from 2-15 kg. The main shape is oblong, oval, or round. Its colour varies from black-green, dark green, light green, striped, or non-striped, with some newly bred varieties being yellow (Paris et al., 2017).

Watermelon is a healthy source of nutrients and antioxidants. The quality of watermelons is determined by flesh colour, cells structure, sweetness, amount of seeds, etc. (Ton et al., 2009). Abd-Allahhh (2017) showed that the moisture ranged from 85-95%, carbohydrates ranged from 62.00 - 87.14% (dry basis), and fat content was very low. Potassium was a weak catalyst and fluctuated in the range of 100.50 - 489.24 mg/100 g, vitamin C (2.50 - 8.30 mg/100 g), vitamin B6 (0.060 - 0.150 mg/100 g) and vitamin E (0.01 - 0.04 mg/100 g) (Abu-Hiamed, 2017).

Watermelon areas are concentrated in Northern provinces, South Central provinces, and the Mekong Delta every year, providing large quantities for domestic consumption and export. There are many different varieties of watermelon with different growing times, seasons, and yields, various in color, shape, size, etc. The most commonly used watermelon varieties today are Sugar Baby, Hong Luong hybrid, Hac My Nhan hybrid, An Tiem hybrid, and Happy Sweet seedless variety (Nguyen, 2015).

The fruit can be divided into three main parts: flesh, seeds, and skin (Figure 1). The flesh accounts for more 50% of the total weight (about 68%), the skin is approximately 30%, and the seeds about 2% (Abu-Hiamed, 2017). Flesh colour is red, rose-red, yellow, orange, or white. Watermelon seeds are also quite diverse from white, black, and brown in sizes (large, medium, and small). There are also popular seedless watermelons. For watermelons, the waste consisting of white skin and discarded outer skin accounts for about 35% and 15% of the total weight of the watermelon. The peel contains many vitamins

and mineral salts, especially with the most abundant amount of L - citrulline in nature (Barón et al., 2021; Rimando & Perkins-Veazie, 2005).

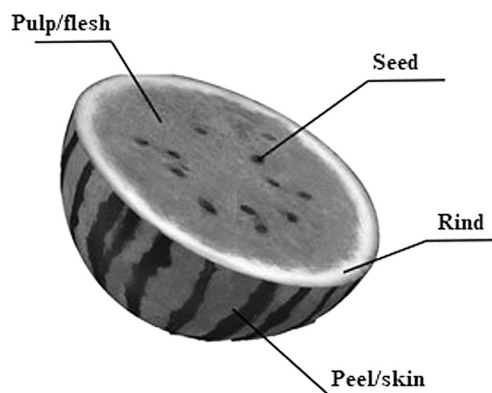


Figure 1. The various parts of watermelon fruit (Neglo et al., 2021)

There have been studies worldwide related to the distribution of L-citrulline in watermelon and watermelon varieties. Rimando and Perkins-Veazie (2005) determined the citrulline content among varieties, flesh color, and tissue of watermelon. The citrulline content ranged from 3.9 to 28.5 mg/g dry weight (dwt) and was similar between seed and seedless varieties (16.6 and 20.3 mg/g dwt, respectively). Red flesh watermelon has slightly less citrulline than yellow or orange watermelon (7.4, 28.5, and 14.2 mg/g dwt, respectively). The citrulline content in the white rind of watermelon was relatively higher than that of the flesh on a dry basis (for all watermelon colors), but this concentration was lower than that of the flesh on a wet basis (Rimando & Perkins-Veazie, 2005). However, studies on the distribution of L-citrulline in watermelon fruit are affected by preservation and storage conditions in the food industry for frozen watermelon products to export such as watermelon juice, fresh-cut watermelon that has not been mentioned yet. Therefore, this study, in addition to analyzing the distribution of L-citrulline in the parts of different watermelons in Can Tho city, investigated the effect of temperature and storage time of juice from watermelon flesh and rind, storage time of fresh-cut watermelon on the distribution of L-citrulline in watermelon were also investigated.

2. Materials and methods

2.1. Chemicals and materials

Red-flesh seeded watermelon, red-flesh seedless watermelon, and yellow-flesh seeded watermelon were purchased at a local market in Can Tho City, Vietnam.

L-citrulline (purity 98%, Germany), sodium hydroxide (Vietnam), diacetyl monoxime (DAMO) (Germany), hydrochloric Acid (China), phosphoric acid (China), sulfuric acid (Vietnam), activated carbon (Vietnam), methanol (China).

2.2. Methods

Material handling: After purchasing, watermelons were washed with 10% NaCl solution and rinsed under running water to remove soil, dust, and microorganisms clinging to the watermelon skin. Then, use a stainless steel knife to remove the green skin and seeds. Finally, separate the white rind, the flesh, and the seeds of each type of watermelon into zip bags and kept at -20°C until use (Fan et al., 2021).

+ The flesh: was pressed by a juicer at room temperature. Then, the juice was filtered through a filter cloth with a pore size of $1\ \mu\text{m}$ to remove the residues. The crude filtrate was further filtered through filter paper by a vacuum filter device to remove the suspended components of the flesh. The filtrate was analyzed for L-citrulline content.

+ The white rind: was treated similarly to the process of the flesh.

+ The seeds: were ground by blender into powder and extracted 3 times with 80% methanol (ratio of ingredient: solvent was 1:3), shaken by vortex for 30 seconds, followed by sonication for 30 minutes in chill water. The mixture was centrifuged for 10 minutes at 4°C , 4500 rpm (Fan et al., 2021). The supernatant would be collected and evaporatively rotated to recover the solvent. The extract was added to 10 mL of distilled water before being analyzed for L-citrulline content.

Physico-chemical properties of watermelon: The randomly chosen watermelons were used for analysing the physical properties such as weight and outer diameter. Their colour and shape were observed from its physical or visual appearance. After separating three parts of watermelons, the digital balance was used to weigh them; the average was then calculated and expressed in gram. Watermelon diameter was measured for half-cutting by digital vernier calipers. At three different locations, the outer diameter of the fruit was recorded at the positions as shown in Figure 2 (D1, D2, D3) (Yau et al., 2010).

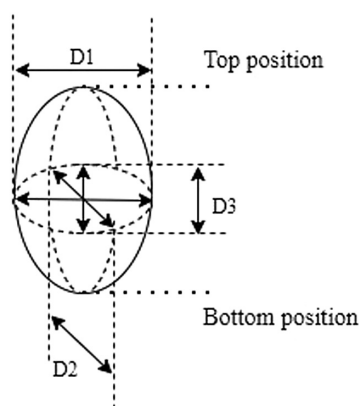


Figure 2. The positions of watermelon fruit to determine outer diameter

Determination of colour: After half-cutting fruits, watermelons were cut into a 5 cm slice to measure colour of flesh, $L^*a^*b^*$ scale was recorded by ColorLite sph870 (Germany). The result was the average value of the three different positions from the heart to the rind of the fruit.

Determination Brix and pH of watermelon flesh and rind juice

The watermelon juice was analysed for soluble solids, reported at $^{\circ}\text{Brix}$ values by the ATAGO MASTER-53M Refractometer (0.0 – 33.0% Brix) (Japan) at 25°C .

A desktop pH meter (Hanna HI 2550-02, USA) was used to measure pH value of samples. Placing the probe in 10 mL of watermelon juice and the pH was recorded at 25°C . The pH meter was calibrated with pH 4, 7, and 10 buffers prior to usage.

Determination of Citrulline: Extracts of each part of the watermelon (fruit flesh, white skin, seeds) were decolorized with 10 g activated carbon, then 1 mL of the filtered solution was diluted with 7 mL distilled water. After that, 1 mL of the diluted solution was added to 4 mL distilled water, 2 mL of sulphuric acid: phosphoric acid (3:1 in volume), and 0.25 mL 30 g/L diacetyl monoxime in order. Then, the solution was then heated in a 100°C water bath for 30 min. The final samples were measured with UV spectrophotometer at 490 nm (Wenge et al., 2010). The citrulline content was calculated upon a calibration curve of external standard citrulline with 100 - 1000 mg/L concentration range of L-citrulline ($y = 302.67x - 26.738$; $R^2 = 0.99$).

2.3. Experimental layout

2.3.1. Effect of watermelon varieties on L-citrulline distribution in watermelons

Three kinds of watermelons were experimented, namely red-flesh seeded, red-flesh seedless, and yellow-flesh seeded. Three fruits of each type were chosen randomly. Selection criteria were based on green humus, hard skin, not bugs, average weight of one fruit was 1.5 - 2 kg. Watermelons were managed at room temperature and analyzed for some physico-chemical and organoleptic parameters. The raw watermelons were processed to obtain extracts from the fruit flesh, white skin, and seed for analysis of L-citrulline content.

2.3.2. Effect of storage time and temperature of juices from watermelon flesh and rind on L-citrulline content

Selecting one type of watermelon from experiment 1, the white skin and fruit flesh were separated. Then, a juicer was used to squeeze the white skin and flesh of the fruit. The juice would be put in a plastic box and refrigerated at -20°C, 0°C, 4°C for 1, 3, 6, 10, 15 and 21 days, respectively. After the survey time points, samples at each temperature would be thawed and analyzed for L-citrulline content.

2.3.3. Effect of storage time of fresh-cut watermelon on the distribution of L-citrulline in watermelon fruit

One watermelon type in the experiment 1 was selected to cut into 8 equal pieces and then separated the white skin and fruit flesh. After being cut into pieces, the watermelon rind and flesh would be placed in a zip bag and stored at -20°C for 1, 4, and 8 weeks, respectively. After the survey timelines, the samples were thawed and analyzed for L-citrulline content.

2.4. Data analysis

Data analysis was done by ANOVA using Statgraphics 18; all experiments were triplicated and mean values were compared by LSD (Least Significant Difference) with a confident coefficient $p \leq 0.05$.

3. Results and Discussion

3.1. Effect of watermelon varieties on L-citrulline distribution in watermelons

The watermelon types used for the study were red-flesh seeded, red-flesh seedless, and yellow-flesh seeded (Figure 3). Watermelons were analyzed for sensory description, L, a, b value and physicochemical criteria (Table 1).

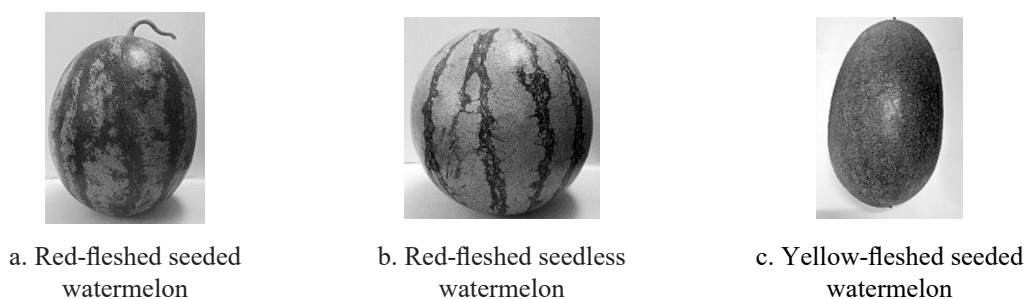


Figure 3. The various parts of watermelon fruit

Table 1 showed that each type has different shapes, sizes, and thickness characteristics of the rind, specific to each type. However, all three types had Brix, and the pH of rind juice was 2.5 and pH 4 ÷ 5, respectively. The weight of the rind accounted for an average of 30% of the total weight of the watermelon. For the flesh of all three types, the pH of the juice was slightly acidic at 4 ÷ 6; the Brix was 2 to 4 times greater than the Brix of the rind juice. For two types with seeds, the seed weight was also different; the yellow-flesh watermelon had more seeds than the red-flesh, and the L, a, b value of the yellow watermelon was different

from that of the red-flesh. Red-flesh watermelons had almost the same L value for both seeded and seedless of 30.5 and 30.55 but a smaller L-value for yellow-flesh watermelons, which indicates that yellow-flesh watermelons were brighter than the red-flesh. The value of the colour system was from green to red (values from -a to +a, respectively), so the red flesh had a positive a value and was larger than the a value of yellow-fleshe. In contrast, the b value of the colour system was from blue to yellow (values from -b to +b, respectively), so the yellow-flesh had a positive b value and was larger than that of the red-flesh.

Table 1. The sensory description and some physicochemical parameters of different watermelons

Criteria	Types			
	Red-flesh seeded	Red-flesh seedless	Yellow-flesh seeded	
Shape	Round, a bit long	Round	Oval	
Color	Dark green skin, many stripes, red flesh, black seeds	Light green skin, few stripes, red flesh, seedless	Dark green skin, dark green veins, yellow flesh, black seeds	
Outer diameter (cm)	68.00 ± 0.100 ^c	48.17 ± 0.289 ^b	42.07 ± 0.929 ^a	
Rind	Thickness (cm)	0.80 ± 0.200 ^a	0.50 ± 0.265 ^a	0.73 ± 0.252 ^a
	Weight (g)	250.35 ± 0.302 ^a	200.43 ± 0.666 ^a	379.17 ± 0.153 ^c
	Brix of juice	2.47 ± 0.058 ^a	2.50 ± 0.050 ^a	2.47 ± 0.058 ^a
	pH of juice	4.85 ± 0.020 ^a	5.23 ± 0.025 ^b	5.47 ± 0.092 ^c
Flesh	Weight (g)	900.82 ± 0.761 ^c	750.13 ± 0.153 ^a	899.5 ± 0.500 ^b
	Brix of juice	9.47 ± 0.058 ^c	4.83 ± 0.289 ^a	7.53 ± 0.058 ^b
	pH of juice	4.73 ± 0.020 ^a	5.13 ± 0.017 ^b	5.57 ± 0.059 ^c
Seed	Weight (g)	3.10 ± 0.242 ^b	0.00 ± 0.000 ^a	13.27 ± 0.252 ^c
	L* value	30.50 ± 7.623 ^a	30.55 ± 4.351 ^a	46.31 ± 0.755 ^a
	a* value	17.00 ± 5.201 ^b	16.11 ± 1.893 ^b	6.20 ± 0.900 ^a
	b* value	6.48 ± 4.405 ^a	4.78 ± 1.343 ^a	19.30 ± 3.531 ^b

Note: Different letters in the same row represented statistically significant differences at the 5% level of significance.

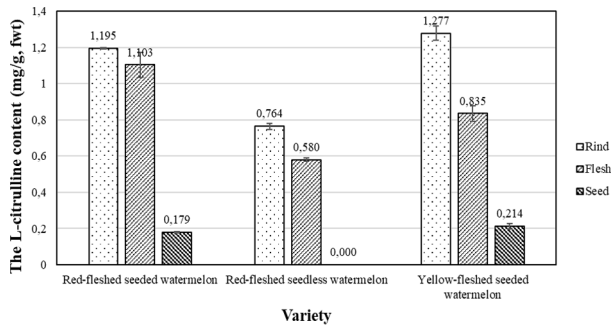


Figure 4. The distribution of L-citrulline in different types of watermelons

Figure 4 displayed the distribution of L-citrulline in three parts (fruit flesh, white skin, and seeds). It showed that the type of watermelon had influenced L-citrulline content. In general, L-citrulline was highly concentrated in the white skin, whereas the least in the seeds. When comparing two types with the same red flesh colour, the seeded watermelon had a higher concentration of L-citrulline in the rind and flesh than the seedless. For two types of the seeded but different flesh colours, the yellow-flesh had a higher content of L-citrulline in the rind but lower in the flesh than the red-flesh. It was demonstrated

that the hybridization of new types also affects the distribution of L-citrulline in watermelon fruit.

Some related research results also showed that the distribution of L-citrulline in watermelon seeds was the least but the most in watermelon rind (Gu et al., 2023; Ridwan et al., 2018; Rimando & Perkins-Veazie, 2005). The results of comparison of L-citrulline content in the rind and flesh were different since the calculation methods and the methods were conducted in different ways. The rind would contain more L-citrulline by fresh weight than the flesh but give the opposite result when calculated by dry weight (Ridwan et al., 2018; Rimando & Perkins-Veazie, 2005). Rimando and Perkins-Veazie explained that the difference had been due to the moisture content of the two parts, namely 95% moisture in the rind and 90% moisture in the flesh. However, the results of Rimando & Perkins-Veazie (2005) showed that the L-citrulline content in both the fruit flesh and the white rind of red-flesh seedless was higher than that of red-fleshed seeded.

Gu et al. (2023) also analyzed the flesh with the highest citrulline content using a human intestinal

epithelial Caco-2 cell monolayer model *in vitro* (Gu et al., 2023). On the other hand, Gu et al. proved this difference was due to the types in food intake of different watermelon parts; the citrulline bioavailability results showed a higher transport rate in the watermelon rind than that of flesh. In addition, the results in Figure 4 revealed the L-citrulline content in yellow-fleshed watermelon was higher than the red watermelon flesh. However, Ridwan et al. (2018) gave the opposite result. Joshi et al. found that there was no difference in L-citrulline content in both rind and flesh (Joshi et al., 2019). The different results in the L-citrulline distribution of different watermelons could be influenced by variety, genes, growing conditions, weather, temperature, and water availability in each region (Volino et al., 2021). Davis et al. (2011) demonstrated that L-citrulline concentrations were affected by the environment, and L-citrulline concentrations varied significantly in different cultures (Davis et al., 2011). Therefore, in order to limit the scope of research for the following experiments, a red-flesh seeded with morphological characteristics described in Table 1 was selected as the research object and fixed condition for further experiments. Besides, because this watermelon had relatively L-citrulline content, neither high nor low, it is easier to find and buy than the other two types. In addition, with the amount of L-citrulline in watermelon seed being too low, the next experiments only monitored the change of L-citrulline content in the white skin and fruit flesh under the investigated conditions.

3.2. Storage time and temperature effects on L-citrulline content of juice from watermelon flesh and rind on

Fruit juice spoilage is mainly due to a rapid increase in acid tolerance and osmotic microflora. There is also a risk of microbial contamination from food, so to reduce those risks, fruit juices are preserved using a variety of techniques. For fruit juice to be used for a longer time, factories often heatedly pasteurize the juices, but this will lead to the loss of essential nutrients and changes in the physico-chemical properties as well as the organoleptic products. Therefore, preserving the juice at a low temperature is an effective method to overcome heat treatment methods. The storage temperature and time significantly changed the L-citrulline content in rind juice and flesh juice (Figure 5).

The results in Figure 5 showed that the longer

the time, the lower the L-citrulline content compared to the original L-citrulline content. However, the content of L-citrulline had a slight increase during the initial three days for the rind at -20°C and 4°C , the L-citrulline in the watermelon rind at 0°C gradually decreased with time and had more stability in the L-citrulline content. This could be explained that the watermelon rind contained a large amount of water (greater than 90%), making the water crystalline at 0°C faster, and the L-citrulline molecules in the water were frozen faster, so L-citrulline content was more stable. For the heart of the product could reach to -20°C , the freezing process of L-citrulline molecules would be slower than the temperature of 0°C . On the other hand, after 21 days of freezing, the amount of L-citrulline at -20°C and 0°C was statistically similar, but the amount of L-citrulline in the rind decreased at 0°C (77.06%) more than at -20°C (75.65%) (Data not shown). Therefore, to avoid losing L-citrulline content in watermelon rind juice, the juice should be used best within one week.

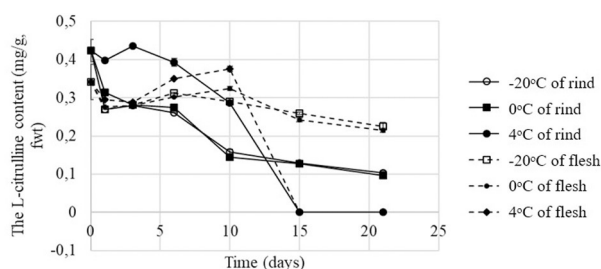


Figure 5. The L-citrulline content in watermelon rind and flesh juice changed with time at different temperatures

In addition, at 4°C , the measured L-citrulline content in the rind juice spiked after three days and was higher than the initial measured values; however the flesh juice had the same irregularity in the 6th day. It was proved that the temperature of 4°C was confirmed as a good condition for the enzyme polyphenol oxidase (PPO) maintained activity (Tran et al., 2014) with total polyphenols in watermelon white rind and red flesh were 63.33 ± 1.455 mg TAE/g and 47.3 ± 0.888 mg TAE/g dry extract, respectively) (Dieng et al., 2017). Therefore, the juice from the rind and flesh of watermelon fruit after the pressing process would be easily browned (Liu et al., 2013). Similarly, previous studies demonstrated that internal browning occurs when exposed to oxidative stress, temperature, and prolonged storage time (Mohamad Salin et al., 2022).

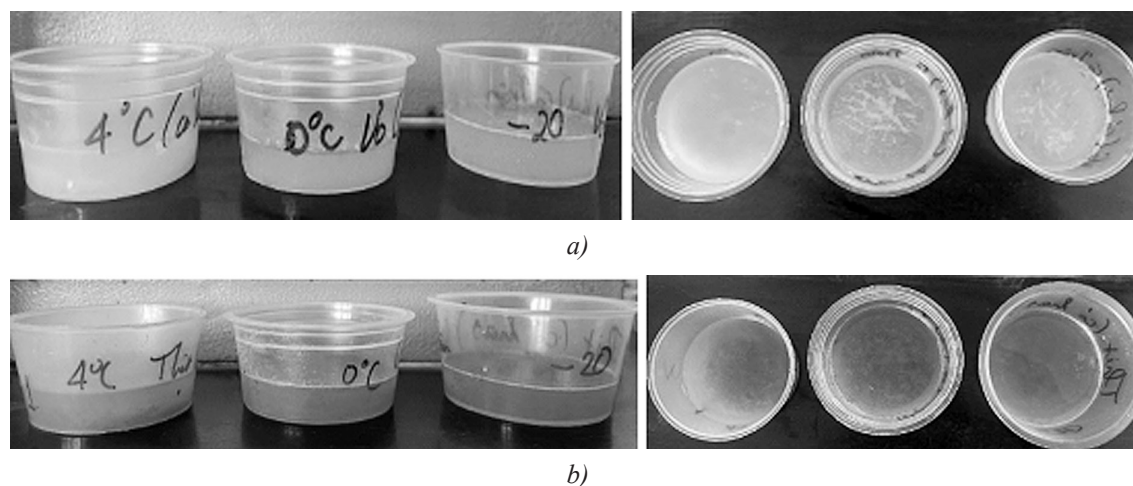


Figure 6. Images of watermelon rind juice (a) and watermelon flesh juice (b) at temperatures of 4°C, 0°C and -20°C after 15 days

The influence of PPO enzyme on L-citrulline in watermelon rind juice occurred faster on the 3rd day than the fruit flesh juice at the same temperature of 4°C and had the same microbial infection at the 15th day onwards. The juice had an odor and discoloration (Figure 6). Thus, through the analytical results, the stability of L-citrulline in the juice of the fruit flesh was more stable than in the rind; indeed, after 21 days, the amount of L-citrulline decreased in the fruit flesh less than in the watermelon rind at 0°C and -20°C. At 4°C, preserving the rind and the flesh of the watermelon for a long time was impossible because of the negative effects of organoleptic and microbiological criteria.

3.3. Effect of storage time of fresh-cut watermelon flesh and rind on L-citrulline content

Watermelon flesh has increased at an annual rate of 20-30% (Ebadi et al., 2013). Fresh-cut watermelon fruit is often sold as quarters and halves with the skin on or as cubes without the skin. However, the deterioration of the fresh-cut watermelon results in a loss of texture, colour, and sweetness (Curis et al., 2005). Moreover, exporting frozen fruit is considered a potential market for Vietnam's economy. However, the freezing time of watermelon with both skin and flesh affected the L-citrulline content (Figure 7).

Bekele and Ramaswamy (2013) optimized the confectionery handling and shipping temperature of 10°C with a market shelf life of 21 days. Storage

below 10°C resulted in chilling injury, and with increasing storage temperature, market age was reduced with significant damage in lycopene and sweetness (ibid). Thus, the freezing process of fresh-cut watermelon flesh and rind at -20°C stabilized L-citrulline more than in juice, the amount of L-citrulline was also reduced very little, and the storage time was also longer than watermelon juice containing L-citrulline.

According to Nogales-Delgado (2021), the main factors affecting PPO activity are temperature and cell integrity. Therefore, in order to reduce PPO activity in fresh-cut produce, the experiment 3 was performed in freezing condition (-20°C) and fruits were cut in block form. However, both L-citrulline in flesh and rind were not stable over the 8-week period. It proved that the method for quantifying L-citrulline was affected by enzymes involved in the catabolism of the watermelon (Volino et al., 2021). Moreover, in many studies, the PPO activity determination in some fresh-cut fruits was carried out in the absorption wavelength range of 400 ÷ 500 nm (Nogales-Delgado, 2021) whereas, the colour complex of L-citrulline and DAMO also absorbed on 490 nm. Thus, the L-citrulline content after a freezed week was higher than the initial day. It could be explained that the results of the L-citrulline determination could be included a part of the quantity of PPO in the frozen samples.

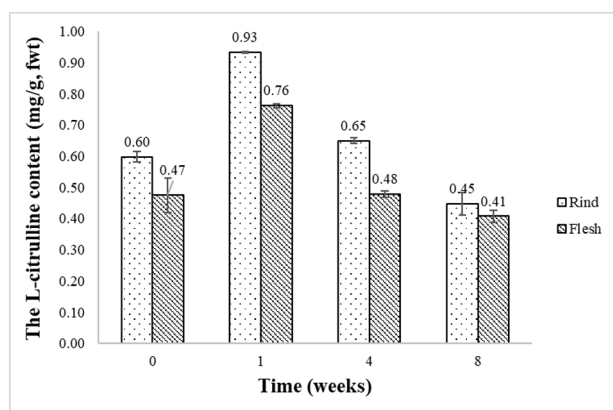


Figure 7. The L-citrulline content in fresh-cut watermelon flesh and rind during frozen storage time

4. Conclusions

Watermelon contains a different distribution of L-citrulline its body parts (skin, flesh, and seeds), in which watermelon rind is a source of by-products often released into the environment but not rarely utilized in Vietnam. However, it is the largest source of L-citrulline in watermelon. The result contributed an excellent way to increase the L-citrulline in watermelons by preserving, and storing watermelons at low temperatures for a long time, which is necessary to pre-treat watermelons before freezing them to prevent the browning phenomenon in watermelon because the temperature of 4°C is a suitable condition for the enzyme polyphenol oxidase (PPO) actives. As a result, the L-citrulline content in both of watermelon rind and flesh juice suddenly increased within a week (0.44 and 0.35 mg/g dwt, respectively) compared to the initial L-citrulline content in watermelon rind and flesh (0.42 and 0.34 mg/g dwt, respectively). Herein, the watermelon rind should fully be utilized for L-citrulline supply for the functional food industry in the future.

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