The preventing method of browning and γ-aminobutyric acid (GABA) contained in *Luffa cylindrica* Roem. cultivated in Okinawa

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Abstract

Luffa (*Luffa cylindrica* Roem.) is a popular vegetable in Okinawa, and it has abundant nutrients, including γ-aminobutyric acid (GABA). We focused on GABA content in luffa, taking into consideration registering it as foods with functional claims in Japan. Besides, when selling cut luffa and frozen cut luffa at supermarkets, they are supposed to get browned due to air exposure and other causes. In the present study, we developed the prevention method of browning cut luffa and frozen cut luffa using 0.5, 1.0, 2.0, and 4.0 % ascorbic acid solution. It was found that 55 L of 4.0 % ascorbic acid solution could be used for soaking of 70 kg cut luffa to prevent browning, but GABA content decreased in food processing of luffa in the factory. Besides, GABA content in luffa fruits was found not to change during storage for seven days at room temperature after harvest.

Keywords: *Luffa (Luffa cylindrica* Roem.*), cut luffa, frozen cut luffa, GABA, browning.*

1. INTRODUCTION

"Nabera" is a word in the Okinawan dialect, which means luffa or towel gourd (*Luffa cylindrica* Roem.), belongs to the family Cucurbitaceae. Luffa is commonly cultivated to eat in Okinawa, including Miyako Island and Ishigaki Island, and a popular recipe is "Nabera n'bushi", which simmers the vegetable in miso. It is also good in soups and stir-fries. Luffa can be eaten to some extent at once because of its soft texture. Accordingly, it is possible to ingest a certain quantity of nutritive components because luffa has abundant nutrients [1], including γ-aminobutyric acid (GABA). GABA is contained in eggplant with 24 mg/100 g FW [2], tomato with 30.4 mg/100g FW [3], and vegetable soybean with 79.6 mg/100g DW [4] and widely in other vegetables [5]. Indeed, GABA is also expected to be rich in luffa. GABA was reported to improve blood pressure [6] and to reduce psychological stress [7]. However, there is little information about GABA contained in luffa and its change after harvesting. Thus, we focused on GABA content in luffa, taking into consideration registering it as foods with functional claims in Japan. For processed food products, cut luffa and frozen cut luffa are going to be sold at supermark, etc, but most of the varieties in Okinawa quickly got browned owing to air exposure, being thawed, or being cooked. Luffa contains phenylpropanoids [8], which are known as substrates for oxidative enzymes responsible for the browning reactions [9]. By the way, ascorbic acid can act as an antioxidant to protect cellular components from free radical damage [10] and is frequently utilized as an anti-browning agent for many foods such as fresh-cut salad [11]. In order to prevent the browning of cut luffa and frozen cut luffa, the test of soaking them in ascorbic acid solution was tried in the present study. Besides, keeping GABA in cut luffa and frozen cut luffa after food processing is very important. We developed the prevention method of browning of cut luffa and frozen cut luffa, along with analyzing the change of GABA content in the food process of the factory. Besides, the change of GABA content when luffa fruits were stored at room temperature after being harvested was analyzed in the present study. Besides, keeping GABA in cut luffa and frozen cut luffa after food processing is very

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important. We developed the prevention method of browning of cut luffa and frozen cut luffa, along with analyzing the change of GABA content in the food process of the factory. Besides, the change of GABA content when luffa fruits were stored at room temperature after being harvested was analyzed in the present study.

2. MATERIALS AND METHODS

2.1. The effect of ascorbic acid on browning of cut luffa

2.1.1. Preparing luffa samples

Three luffa fruits cultivated in the farmer in Itoman (Figure 1) were obtained on May 27, 2019. After being slightly peeled, three cuts sliced approximately 1 cm from the upper, middle, and lower parts of one fruit were sampled as one set with three repetitions using three different luffa fruits. One set with three cuts was packed together with poly-nylon bag made of nylon and polyethylene, in which cut luffas were soaked in 0, 0.5, 1.0, 2.0, and 4.0 % ascorbic acid solution, respectively. Those packed samples with each ascorbic acid solution were stored in the refrigerator at 4°C for one day.

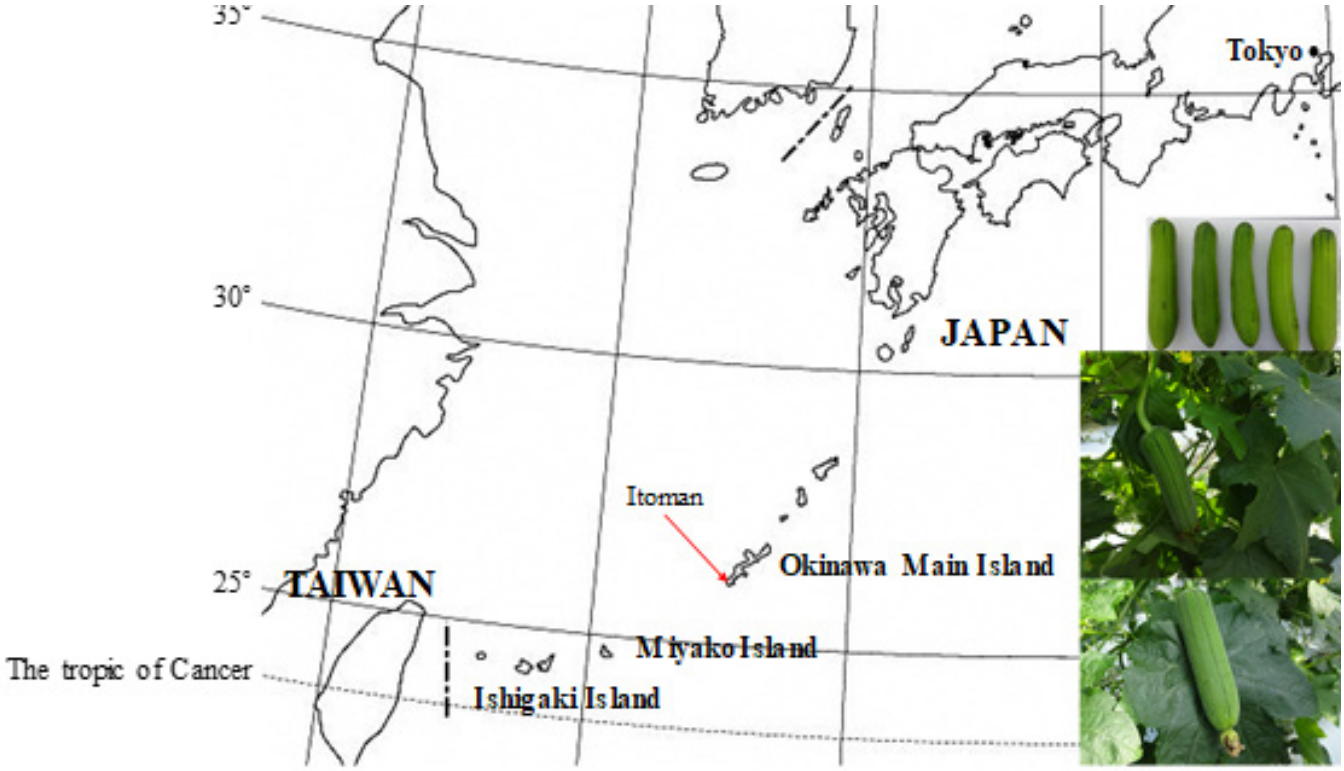

Figure 1. *Map of Okinawa indicating where luffas were cultivated*

2.1.2. The degree of browning

After storage in the refrigerator, samples were unpacked and were kept in the laboratory with an air conditioner set at 25°C for three hours or were boiled at approximately 100°C for one minute. After that, we investigated the degree of browning of cut luffas and color of the cut luffa peel, compared with control (0 % ascorbic acid solution) judged by their appearances.

2.2. The degree of browning and the change of GABA in the food process of the factory

2.2.1. Preparing luffa samples and investigating the degree of browning

Luffa fruits were obtained from Orion Shouji Co., Ltd. and were processed in the factory of Green Field Ltd. Luffa fruits were soaked in cold water after being peeled by workers in the factory, and they were manufactured in the process as shown in Figure 2. For the prevention of browning, approximately 55 L

of 4 % ascorbic acid solution was used. Approximately 7 kg cut luffas were soaked in the solution at once, which repeated 30 times for the process. In each 10th time, 15th time, and 30th time of the process, cut luffas were sampled after being frozen to investigate the degree of browning. The degree of browning was evaluated as none, slight, small, normal, and intense.

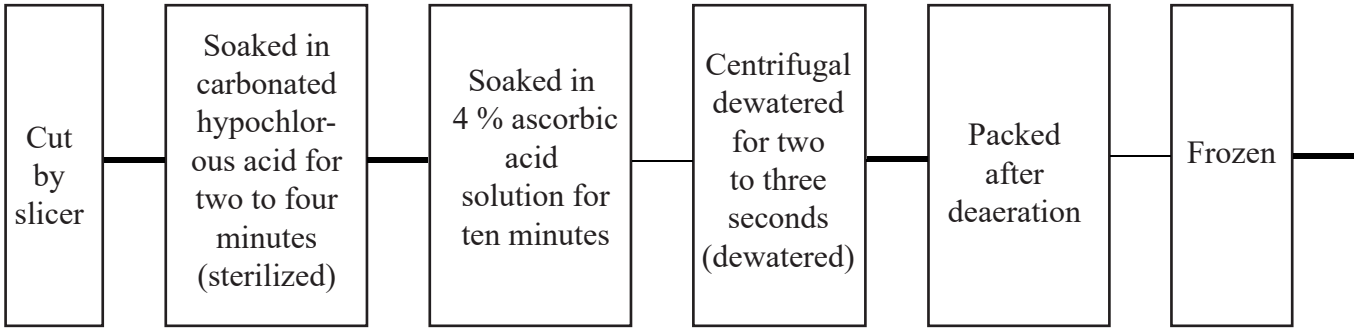

Figure 2. *The process of producing frozen cut luffa in the factory*

Note: Bold lines indicate sampling for GABA analysis.

2.2.2. Analysis of GABA

In each 10th time, 15th time, and 30th time of the process as shown in Figure 2, cut luffas were sampled after being cut by slicer, being soaked in carbonated electrolytic hypochlorous acid solution (Hereinafter, carbonated hypochlorous acid), being centrifugal dewatered, and being frozen at -30°C. 10 g from the part of each three cut luffa were sampled for GABA analysis with three repetitions. Extraction was achieved to homogenize with the same volume of ethanol twice. The concentration of GABA in extracts was analyzed by using the ultra-fast amino acid analysis system (Nexera X2, Shimadzu, Kyoto, Japan) [12] equipped with a C18 HPLC column (YMC-Triart C18, 1.9 µm, 75 mm × 3.0 mm, YMC, Kyoto, Japan), according to pre-column derivatization method. The mobile phase used was 20 mol/L phosphate potassium buffer (pH 6.9) and 45/40/15 acetonitrile/methanol/water. The eluents were monitored by a fluorescence detector ((RF-10AXL, Shimadzu) at 350 nm and at 450 nm of excitation and emission wavelength, respectively using o-phthalaldehyde as a fluorescent reagent. Three measurements of duplicate data were expressed as average % compared to samples after being cut by the slicer.

2.3. The change of GABA during storage after harvest

2.3.1. Preparing luffa samples

The variety of luffa, "SouthernHetima" was harvested as sample 10 or 11 days after pollination in Okinawa Agricultural Research Center on July 1, 2019. Harvested luffas were stored in the laboratory with an air conditioner set at 25°C (24.4°C ± 0.4°C). As for luffa harvested 11 days after pollination, they were sampled zero or four days after storage. For ones harvested ten days after pollination were sampled zero or seven days after storage.

2.3.2. Quantitation of GABA

Luffa samples were cut in round slices by 1 cm after peeling and cutting the fruits' edge parts. They were then freeze-dried using an FD-550 freeze-drier (Tokyo Rikakikai co. ltd., Tokyo, Japan) and were crushed by a mill for GABA analysis. Moisture content was calculated by weighing the difference between before and after freeze-drying. 0.2 g of freeze-dried powder sample were extracted with 10 mL of 75 % ethanol at 80°C for 30 minutes. The supernatant was obtained after centrifugation at 2,000 g for ten minutes. The insoluble constituent was again extracted with 10 mL of 75 % ethanol and centrifuged in the same way. The supernatant was filled up to 50 mL with 75 % ethanol as an extract. The concentration of GABA in the extract was analyzed as described above [12]. Three measurements of duplicate data were expressed as mg of GABA per 100 g of fresh weight (FW).

3. RESULTS AND DISCUSSIONS

3.1. The effect of ascorbic acid on cut luffas kept at room temperature

When cut luffas are sold at supermarkets, browning cut luffa is definitely avoided. Accordingly, we have to develop a method to preve king in each ascorbic acid solution for one day, an investigation of the browning degree and the color change was conducted three hours later. Pulp of cut luffas had soaked in 0 % ascorbic acid solution (Control) was normally browned (Table 1). However, the pulp of cut luffas had soaked in 0.5, 1.0, 2.0, and 4.0 % ascorbic acid solution was not browned (Table 1 and Figure 3). As the concentration of the ascorbic acid solution was higher, the peel of cut luffa became closer to yellow (Table 1 and Figure 3). In detail, samples with 0 and 0.5 % ascorbic acid solution were clear lime green, one with 1.0 % ascorbic acid solution was lime green, ones with 2.0, and 4.0 % ascorbic acid solution were lime green but close to yellow (Table 1 and Figure 3). Browning in cut luffas occurs as a result of the polymerization of quinone [9] oxidated by polyphenol oxidase from phenylpropanoids such as caffeic acid [8]. Ala et al. (2019) [13] reported that ascorbic acid inhibits polyphenol oxidase. Therefore, we elucidated that ascorbic acid prevents browning reactions in luffa.

Table 1. *The degree of browning on luffa pulp and color of luffa peel*

The concentration of ascorbic acid (%)		***0***	***0.5***	***1***	***2***	***4***
Kept at room temperature	Degree of browning on pulp	Normal	None	None	None	None
	Color of peel	Lime green (clear)	Lime green (clear)	Lime green	Lime green (close to yellow)	Lime green (close to yellow)
Boiled for one minute	Degree of browning on pulp	Intense	Nore	Nore	Nore	Nore
	Color of peel	Lime green	Brown-green	Brown-green	Brown-green	Brown-green

Figure 3. *The appearance of cut luffas kept at room temperature for three hours (from left to right, samples soaked in 0, 0.5, 1.0, 2.0, and 4.0 % ascorbic acid solution)*

3.2. The effect of ascorbic acid on cut luffas boiled for one minute

Pulp of cut luffas had soaked in 0 % ascorbic acid solution (Control) was intensely browned (Table 1 and Figure 4). However, ones had soaked in 0.5, 1.0, 2.0, and 4.0 % ascorbic acid solution were not browned (Table 1 and Figure 4). Besides, as the concentration of the ascorbic acid solution was higher, the peel of cut luffa became closer to brown (Table 1 and Figure 4). In detail, samples had soaked in 0 % ascorbic acid solution were lime green, but samples had soaked in 0.5, 1.0, 2.0, and 4.0 % ascorbic acid solution were brown-green. Thus, the pulp of cut luffas soaked in ≥ 0.5 % ascorbic acid solution did not get browned, but its peels were closer to brown as the concentration was higher, not the lime green of the original color. By the way, using ascorbic acid costs money, and accordingly, 4.0 % of the ascorbic acid

solution is considered to be limit to turn a profit. Being brown-green in peel by heating is not thought to be a problem because the common dish "Nabera n'bushi" requires the condiment "miso" (fermented soybean paste) that has a brown color. In the present study, we presume that cut luffas are soaked for one day and directly packed for sale. Therefore, we found an effective way that cut luffas packed with an ascorbic acid solution can be sold at supermarkets without browning. Moreover, it is not browning even after cooking.

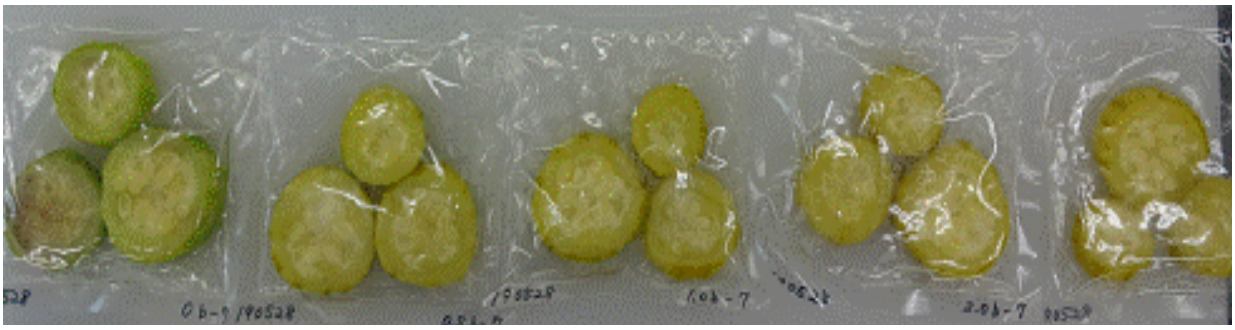

Figure 4. *Appearance of cut luffas boiled for one minute*
(From left to right, samples soaked in 0, 0.5, 1.0, 2.0, and 4.0 % ascorbic acid solution)

3.3. The degree of browning depending on the number of using 4 % ascorbic acid solution

It is better that ascorbic acid was used as many times as possible from the point of cost reduction. Indeed, the ascorbic acid solution was more diluted, and the effect was lower as the number of using the ascorbic acid solution increased (Table 2). In fact, 55 L of 4 % ascorbic acid solution did not make approximately 70 kg of frozen cut luffas brown but using 55 L of 4 % ascorbic acid solution 15 times made ones slight brown (Total processing amount of cut luffa was approximately 105 kg) and using it 30 times made ones small brown (It was approximately 210 kg). Thus, in order to prevent the browning of cut luffa, 55 L of 4 % ascorbic acid solution can be used for 70 kg of cut luffas.

Table 2. *The degree of browning depending on the using number of 55 L of 4 % ascorbic acid solution*

The number of using 55 L of 4 % ascorbic acid solution (times)	*Processing amount of cut luffa (kg)*	*The degree of browning on pulp*
10th	70	None
15th	105	Slight
30th	210	Small

3.4. The change of GABA in the food process of the factory

GABA contents in cut luffas tended to decrease in the food process of the factory (Figure 5). It finally became approximately 50 % of the initial content in which samples after being cut by the slicer. One reason that GABA decreases in the food process is thought to be that GABA changes in a chemical structure by being oxidized by carbonated hypochlorous acid [14]. For another reason, GABA was probably eluted during the entire process, especially in being sterilized or frozen. In fact, there is peeling work by workers before the process, and cut luffas after being peeled is considered to be fresh and clean. If there is no problem with bacteriological examination on those cut luffas, the process of soaking in carbonated hypochlorous acid can be removed to keep as much GABA as possible.

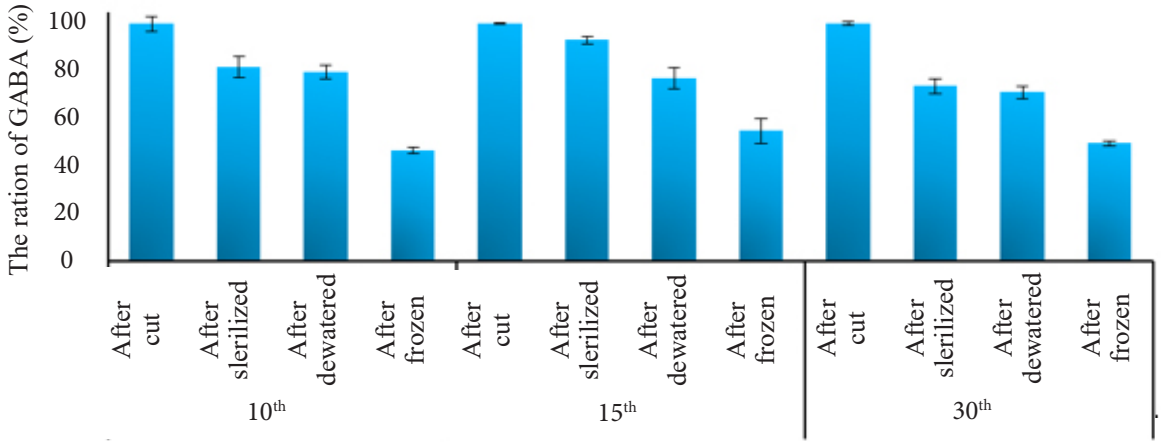

Figure 5. *The change of GABA in the food process of the factory*
Mean ± SD (n = 3); samples of cut luffas were sampled in the food process (Figure 2)

3.5. The change of GABA content during storage

Luffa fruit weight decreased as the number of storage days increased (Figure 6). The ratio of it against initial weight was 89.6 % after seven days of storage. However, the moisture content of the edible part did not increase even though these fruit weights decreased. The moisture content value became 95.0 % from 94.4 % during storage for four days and 94.8 % from 94.5 % during storage for seven days. As for luffa fruits pollinated and harvested on the same day, GABA content did not change during storage for four days or seven days. The GABA content became 10.6 ± 0.5 mg/100 g FW from 10.8 ± 0.1 mg/100 g FW during storage for four days and 10.8 ± 0.2 mg/100 g FW from 11.6 ± 0.8 mg/100 g FW during storage for seven days. Thus, GABA content and moisture content of luffa did not change during storage even though the weight of the fruits decreased. In this context, there is no possibility of storage at room temperature to increase GABA content in luffa fruits, considering that over seven days storage is not good for luffa fruits themselves. The next step was to research methods to increase GABA content in luffa.

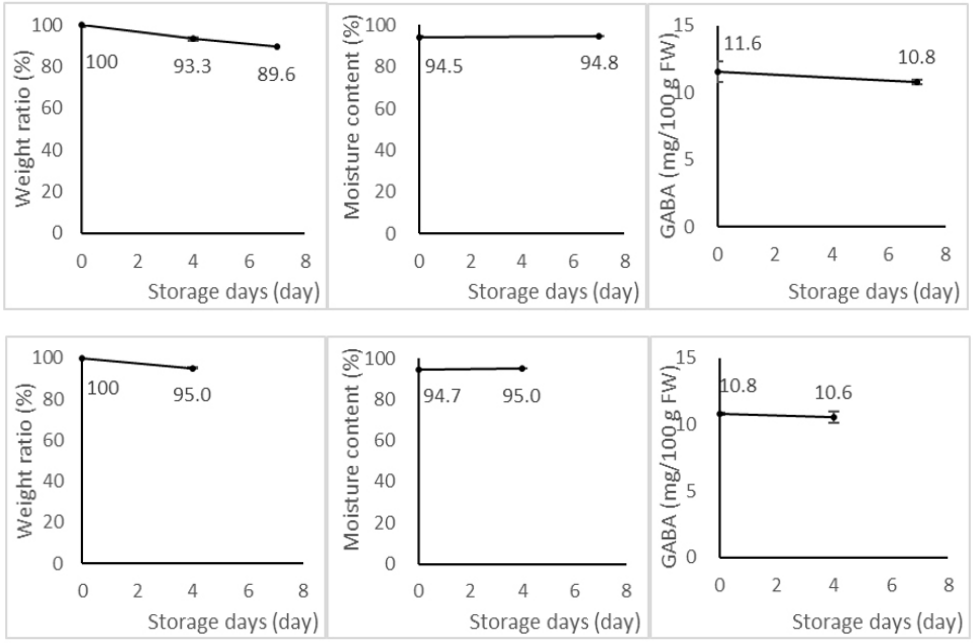

Figure 6. *The change of the weight ratio, moisture content, and GABA content in luffa fruits during storage for four and seven days*

(Upper graphs represent the data from luffa samples 11 days after pollination. Mean ± SD (n = 2); samples stored for zero day, (n = 3); samples stored for four days. Lower graphs represent the data from luffa samples ten days after pollination. Mean ± SD (n = 3); samples stored for zero day, (n = 3); samples stored for seven days).

4. CONCLUSION

We found the effective way that cut luffas packed with an ascorbic acid solution can be sold at supermarkets without browning; even after cooking, it is not browning. The higher concentration of ascorbic acid caused the peel of cut luffa to become closer to brown. 4 % ascorbic acid solution is considered a limit to turn a profit. From the result, soaking in 55 L of 4 % ascorbic acid solution could prevent 70 kg of cut luffas from browning in the food process of the factory. However, GABA contents in cut luffas tended to decrease in food processing. Besides, GABA and luffa fruits' moisture content did not change during storage for seven days even though the fruits' weight decreased.

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Phương pháp ngăn ngừa hóa nâu và hàm lượng γ-aminobutyric acid (GABA) trong mướp hương (*Luffa cylindrica* Roem.) được trồng tại Okinawa

Takashi Hanagasaki

Trung tâm nghiên cứu nông nghiệp Okinawa, Itoman, Okinawa, Nhật Bản

Tóm tắt

Mướp hương (*Luffa cylindrica* Roem.) là một loại rau quả phổ biến ở Okinawa và có nhiều chất dinh dưỡng bao gồm cả γ-aminobutyric acid (GABA). Nghiên cứu tập trung vào xác định hàm lượng GABA trong mướp hương được đăng ký là thực phẩm chức năng ở Nhật Bản. Ngoài ra, sản phẩm mướp hương cắt nhỏ và mướp hương cắt nhỏ đông lạnh được bán ở siêu thị, thường bị chuyển sang màu nâu do tiếp xúc với không khí và các nguyên nhân khác. Trong nghiên cứu này, chúng tôi đã phát triển phương pháp ngăn ngừa sự hóa nâu của mướp hương cắt nhỏ và mướp hương cắt nhỏ đông lạnh bằng cách sử dụng dung dịch acid ascorbic 0,5, 1,0, 2,0 và 4,0 %. Kết quả nghiên cứu cho thấy, với 55 L dung dịch acid ascorbic 4,0 % được sử dụng để ngâm 70 kg mướp đã cắt để tránh bị hóa nâu, nhưng hàm lượng GABA giảm trong quá trình chế biến mướp trong nhà máy. Bên cạnh đó, hàm lượng GABA trong quả mướp không thay đổi trong thời gian bảo quản trong 07 ngày ở nhiệt độ phòng sau khi thu hoạch.

Từ khóa: *Mướp hương (Luffa cylindrica), mướp hương cắt nhỏ, mướp hương cắt nhỏ đông lạnh, GABA, hóa nâu.*

Note: *Tiêu đề và tóm tắt tiếng Việt do Ban Biên tập biên dịch với sự đồng ý của tác giả / The Vietnamese title and abstract is translated by the Editorial Board with the agreement of the Author.*