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THE EFFECT OF PASTEURIZATION AND PRESERVATION ON THE QUALITY OF A BEVERAGE FROM PERILLA LEAF (*Perilla frutescens* L. Britton) EXTRACT - PINEAPPLE

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Abstract

This study evaluated the impact of pasteurization and storage conditions on the quality of a beverage produced from perilla leaf extract combined with pineapple. Pasteurization was carried out at temperatures of 80°C, 85°C and 90°C held for 1, 3 and 5 minutes, respectively, to ensure both sensory quality and microbiological safety. The product was then stored at a low temperature (4 ± 2°C) under two different light conditions (light-blocking and non-light-blocking) to assess the effect of light on product quality over 8 weeks. The results showed that pasteurization at 85°C for 3 minutes achieved a pasteurization value (pasteurization units: PU) of 7.64 minutes, effectively eliminating microorganisms while retaining higher anthocyanin and vitamin C contents and favorable sensory properties. During storage, light significantly influenced quality deterioration, with anthocyanin and vitamin C contents decreasing sharply under non-light-blocking conditions (by 57.5% and 58.6%, respectively), whereas light-blocking conditions mitigated the losses (19.7% and 44.7%). Under light-blocking conditions, the product maintained its color, flavor, desirable sensory attributes, and microbiological safety throughout the storage period.

Keywords: *Ascorbic acid, Anthocyanin, Ananas comosus, Perilla frutescens, Pasteurization.*

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ẢNH HƯỞNG CỦA QUÁ TRÌNH THANH TRÙNG VÀ BẢO QUẢN ĐẾN CHẤT LƯỢNG NƯỚC UỐNG TỪ DỊCH CHIẾT LÁ TÍA TÔ (*Perilla frutescens* L. Britton) - KHÓM

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Tóm tắt

Nghiên cứu này đánh giá tác động của quá trình thanh trùng và điều kiện bảo quản đến chất lượng nước uống từ dịch chiết lá tía tô kết hợp với khóm. Quá trình thanh trùng được thực hiện ở các mức nhiệt độ 80°C, 85°C và 90°C với thời gian giữ nhiệt 1, 3 và 5 phút, nhằm đảm bảo chất lượng cảm quan và an toàn vi sinh cho sản phẩm. Sản phẩm được bảo quản ở nhiệt độ thấp (4 ± 2°C) trong hai điều kiện ánh sáng khác nhau (ngăn sáng và không ngăn sáng) để đánh giá tác động của ánh sáng đến chất lượng sản phẩm trong 8 tuần. Kết quả cho thấy, chế độ thanh trùng ở 85°C trong 3 phút đạt giá trị thanh trùng (pasteurization units: PU) là 7,64 phút, đảm bảo tiêu diệt vi sinh vật, đồng thời duy trì hàm lượng anthocyanin và vitamin C cao, cùng giá trị cảm quan tốt. Trong thời gian bảo quản, ánh sáng có ảnh hưởng lớn đến sự suy giảm chất lượng, với hàm lượng anthocyanin và vitamin C giảm mạnh ở điều kiện không ngăn sáng (giảm 57,5% và 58,6%), trong khi điều kiện ngăn sáng giúp hạn chế sự suy giảm (giảm 19,7% và 44,7%). Sản phẩm bảo quản trong điều kiện ngăn sáng duy trì được màu sắc, hương vị, giá trị cảm quan tốt hơn và đảm bảo an toàn vi sinh trong suốt thời gian bảo quản.

Từ khóa: *Acid ascorbic, Anthocyanin, Khóm, Lá tía tô, Thanh trùng.*

1. Introduction

Perilla (*Perilla frutescens* L. Britton), also known as purple mint, is a plant species belonging to the Lamiaceae family and is widely cultivated in many Asian countries such as India, Korea, Japan, Thailand, China and Vietnam (Vo & Nguyen, 2019). Perilla leaves contain anthocyanin, a bioactive compound with strong antioxidant capacity that can neutralize free radicals and reduce the risk of cancer. In addition to its medical applications, anthocyanin is used as a natural colorant in food products (Hiroyo & Tomohiko, 2002). In Japan, perilla leaf beverages are popular, especially during summer, owing to their refreshing properties and health benefits (Nguyen et al., 2021).

However, in Vietnam, research and production of perilla leaf extract-based beverages remain limited. One major challenge is the often less appealing color of the final products after processing, which affects sensory value and consumer acceptance. To address this problem, incorporating pineapple (*Ananas comosus* (L.) Merr.) into the product is considered a promising solution. Pineapple, a member of the Bromeliaceae family, contains high levels of ascorbic acid (vitamin C) and citric acid helping reduce the product's pH, impart a distinctive bright red color, and enhance sensory properties. Moreover, pineapple offers multiple health benefits, including immune system support, improved iron absorption, and protection against vascular damage (Lauren et al., 2021).

Developing a beverage combining perilla leaf extract and pineapple can increase nutritional value and diversify natural beverage products. In addition, it efficiently utilizes readily available ingredients, thereby contributing to the economic value of local agriculture and the food processing industry. This study investigated pasteurization conditions and storage under both light-blocking and non-light-blocking conditions to evaluate how these factors affect the sensory and nutritional quality of a beverage made from perilla leaf extract and pineapple.

2. Materials and Methods

2.1. Materials and chemicals

Perilla leaves (*Perilla frutescens* L. Britton var. frutescens) and pineapples were purchased from local markets in Can Tho City. The perilla leaves selected are the common variety, ensuring consistent quality and availability. The leaves must be fresh, intact, and free from bruises or pest damage. Preliminary processing includes removing roots, stems, young leaves, and any substandard parts. After washing with water, the leaves were drained in preparation for subsequent research steps.

The pineapples used were the Queen ("Hoàng hậu") variety with uniform size and ripeness level 4 (100% bright yellow peel and open "eyes"). The pineapple flesh had a deep yellow color, a crisp texture, a characteristic aroma, and showed a Brix of approximately 12 ± 0.1 and a pH of about 3.80 ± 0.1, meeting the sensory requirements for this study.

Chemicals and additives included enzymes, culture media, and auxiliary materials. Pectinase enzyme (provided by ICIFOOD Company) was used to break down pectin in the fruit pulp and optimize juice recovery, showing optimal efficiency at pH 5.0 and 50°C (Sharma et al., 2013). Plate Count Agar (PCA) culture media, sourced from India, met the necessary technical standards for microbiological analysis. Additives included RE-grade saccharose sugar (99.8% purity, provided by Bien Hoa Sugar Company) and citric acid produced in Vietnam.

2.2. Method for processing the beverage from perilla leaf extract and pineapple

Perilla leaves were washed with water to remove any adhered impurities, then drained. The extraction process was carried out using water at a ratio of 1:3 (w/v), at 100°C for 5 minutes (Nguyen et al., 2021). After filtration, the extracted solution was used for subsequent

steps. Pineapples were washed to eliminate debris, peeled, and cored to remove inedible parts. The pineapple flesh was blended to obtain a pineapple juice mixture. This mixture was treated with 0.03% pectinase at 50°C for 120 minutes (Do et al., 2024) to degrade pectin, then filtered to collect a clarified juice.

The perilla leaf extract and pineapple juice were blended at a 7:3 (v/v) ratio. The mixture was supplemented with 17% sugar (w/v) and 0.15% citric acid (w/v) and then heated to 90°C for 5 minutes (Doan et al., 2023). The product was poured into bottles, sealed, and subjected to pasteurization at the investigated temperatures and holding times. After pasteurization, it was cooled and stored at low temperature (4 ± 2°C) under two different conditions (light-blocking vs. non-light-blocking) to assess the stability of product quality during storage.

2.3. Experimental design

2.3.1. Investigating the effect of pasteurization temperature and holding time on product quality

This experiment aimed to determine the optimal pasteurization regime for inhibiting microbial growth and prolonging shelf life while maintaining the product's sensory and nutritional values. A completely randomized design with two main factors - pasteurization temperature and holding time - was conducted in triplicate. The beverage production process was carried out following Section 2.2. mentioned above. After blending, the juice mixture was heated to 90°C for 5 minutes, then poured into bottles, sealed, and pasteurized at 80°C, 85°C, and 90°C for 1, 3, and 5 minutes, respectively. The products were cooled, and then subjected to sensory evaluation and analysis of vitamin C (mg%) and anthocyanin (mg/L) content. Additionally, the pasteurization value (minutes) was calculated, and total aerobic plate count as well as yeast and mold counts (CFU/mL) were checked to assess pasteurization efficacy and product quality.

2.3.2. Investigating the effect of light conditions on product quality during storage

This experiment aimed to evaluate how light conditions affect the quality of the beverage made from perilla leaf extract and pineapple during storage. A completely randomized design with two factors - light conditions and storage time - was performed in triplicate. After optimal pasteurization based on the previous experiment, the products were stored at a low temperature (4 ± 2°C) under non-light-blocking (with light exposure) and light-blocking (no light exposure) conditions for 8 weeks. Every week, product samples were collected to assess quality parameters, including sensory evaluation (color, aroma, taste, and texture), anthocyanin content (mg/L), vitamin C content (mg%), and total counts of aerobic plate count, yeast, and mold (CFU/mL).

2.4. Analysis Methods

High-accuracy scientific methods were employed to analyze quality indices. Anthocyanin content was determined using the pH-differential method (Lee et al., 2005). Vitamin C content was measured by titration with an iodine solution (Nhan & Dịep, 2014). Sensory evaluation was conducted following the scoring procedure outlined in TCVN 3215-79, with a panel of 15 assessors evaluating parameters including color, aroma, taste, and texture. The total counts of aerobic microorganisms and spores of yeast and mold were determined using the plate-pour technique (Wise, 2006).

2.4.1 Determination of Anthocyanin Content

The anthocyanin content was determined using the differential pH method as described by Lee et al. (2005). Perilla leaf-pineapple beverage samples were diluted in buffer solutions (pH 1.0 and pH 4.5) at a ratio of 1:3. The maximum absorption wavelength was determined at pH 1.0 (each anthocyanin-containing material exhibits a unique maximum absorption wavelength). The beverage sample showed maximum absorption at the wavelength of $\lambda = 515$

nm. Subsequently, the optical density (OD) of the beverage sample was measured at wavelengths of 515 nm and 700 nm. The anthocyanin content in the analyzed sample was calculated using the following formula:

$$a = \frac{A \,.\, M \,.\, V \,.\, K}{\varepsilon \,.\, l} \; (mg/L)$$

Where:

A (absorbance) is calculated using the following formula:

$$A = \left(A_{\lambda max.pH=1} - A_{700nm.pH=1}\right) - \left(A_{\lambda max.pH=4.5} - A_{700nm.pH=4.5}\right)$$

$A_{\lambda max}$, $A_{\lambda 700}$: Absorbance at maximum wavelength and 700 nm, at pH = 1.0 and pH = 4.5.

a: Anthocyanin content (mg/L).

M: Molecular weight of anthocyanin (g/mol).

l: Cuvette path length (cm).

K: Dilution factor.

V: Sample volume (mL).

ε = 26,900 mol^{-1} cm^{-1}.

2.4.2 Determination of Vitamin C Content

The vitamin C content was determined by titration using iodine solution. In this procedure, 5 mL of HCl acid solution and 20 mL of the beverage sample were pipetted into each of the 50 mL Erlenmeyer flasks. The samples were then titrated with iodine solution (0.01 N) and 1% starch solution until the solution turned blue, indicating the endpoint. The initial and final readings were recorded, and the difference was calculated to determine the amount of dye used for each titration. The experiment was conducted in triplicate (Nhan & Diep, 2014)

2.4.3 Determination of Pasteurization Unit (PU) Value

Pasteurization units (PU) were calculated according to the following equation:

$$PU = \int_0^\infty 10^{\frac{T-Tref}{z}} . dt$$

Where: T_{ref} is the "reference" temperature for the thermal treatment (°C)

T is the actual processing temperature (°C)

z is the temperature range required for the "decimal reduction time" curve to complete one logarithmic cycle

2.5. Data Analysis

Experimental data were processed and presented in charts using Microsoft Excel 2010 (Microsoft Corporation, USA). Statistical analyses were performed with Statgraphics Centurion XVIII to ensure accuracy and reliability of results.

3. Results and discussion

3.1. Effect of pasteurization temperature and holding time on product quality

The perilla leaf–pineapple beverage had a pH of 3.9 ± 0.1 aligned with the heat treatment regime $PU_{85}^{8,3}$ =5 minutes, applicable for products with a pH ranging from 3.7 to 4.2. This regime corresponds to pasteurization at 85°C, a thermal resistance constant (z) of 8.3°C, and a required pasteurization value (PU_0) of 5 minutes, ensuring effective microbial inactivation (Ly & Nguyen, 2011). Accordingly, pasteurization experiments were conducted at 80°C, 85°C, and 90°C, combined with corresponding holding times of 1, 3 and 5 minutes.

Table 1. Pasteurization values (pu) of the product at different temperatures and holding times

Temperature (°C)	Holding Time (min)	PU (min) (*)
80	1	1.42
	3	1.72
	5	2.31
85	1	4.88
	3	7.64
	5	10.15
90	1	20.01
	3	24.12
	5	29.41

Note: (*) Data are the mean values of three replicates

Table 1 shows the pasteurization values (PU) at various pasteurization temperatures and holding times. Pasteurization conditions at 85°C for 3 and 5 minutes, as well as at 90°C for 1, 3, and 5 minutes, all achieved PU > PU_o. This indicates that these conditions meet the requirement for complete microbial destruction, ensuring microbiological safety for the product.

After pasteurization, the product was stored at low temperature (4 ± 2°C) for two weeks to maintain microbiological stability. During this period, the total aerobic count and yeast and mold spores were periodically assessed to evaluate the effectiveness of pasteurization and storage conditions.

As shown in Table 2, at Week 0 (initial sampling), no microorganisms were detected in either the non-pasteurized sample or the pasteurized samples at 80°C, 85°C, and 90°C for all holding times (1, 3, and 5 minutes). This can be attributed to the preheating of the mixture at 90°C for 5 minutes to dissolve sugar and citric acid, inactivate enzymes, and remove dissolved gases, effectively destroying microorganisms present in the product.

Table 2. Total aerobic count and total yeast and mold count of the product at different pasteurization temperatures and holding times

Temperature (°C)	Holding time (min)	Total Aerobic Count (CFU/mL) (*)			Total yeast and mold (CFU/mL) (*)		
		Week 0	Week 1	Week 2	Week 0	Week 1	Week 2
No pasteurization	-	< 1	$32.5x10^3$	$16.12x10^4$	< 1	< 1	< 1
80	1	< 1	$17.3x10^3$	$94.4x10^3$	< 1	< 1	< 1
	3	< 1	$21.4x10^2$	$49.5x10^3$	< 1	< 1	< 1
	5	< 1	$18.3x10^1$	$56.0x10^2$	< 1	< 1	< 1
85	1	< 1	$2.8x10^1$	$7.6x10^2$	< 1	< 1	< 1
	3	< 1	< 1	< 1	< 1	< 1	< 1
	5	< 1	< 1	< 1	< 1	< 1	< 1
90	1	< 1	< 1	< 1	< 1	< 1	< 1
	3	< 1	< 1	< 1	< 1	< 1	< 1
	5	< 1	< 1	< 1	< 1	< 1	< 1

Note: (*) Data are the mean values of three replicates.

A pasteurization regime is considered suitable if total microbial counts remain below

legal limits (QCVN 6-2:2010/BYT). By Week 1, total aerobic counts appeared in the non-pasteurized sample (32.5×10^3 CFU/mL) and the sample pasteurized at 80°C for 1 minute (17.3×10^3 CFU/mL) and 3 minutes (21.4×10^2 CFU/mL) - all of which exceeded the allowable limit of 10^2 CFU/mL. By Week 2, microbial counts continued to rise, and samples pasteurized at 80°C for 5 minutes and at 85°C for 1 minute failed to meet microbiological safety standards.

In contrast, samples pasteurized at 85°C for 3 and 5 minutes and at 90°C for 1, 3, and 5 minutes showed no detectable aerobic microorganisms after two weeks of storage, indicating microbiological safety. Regarding yeast and mold, none of the samples exhibited their presence at any time point. This could be due to the product's low pH (3.9 ± 0.1), where spore-forming and non-spore-forming bacteria, as well as yeast and mold, generally display lower heat tolerance in acidic environments. Heat treatment effectively inactivated these microorganisms (Nguyen & Nguyen, 2009).

Although pasteurization aims to eliminate microorganisms, it is also crucial to maintain the product's nutritional value. Therefore, alongside ensuring food safety, changes in nutritional components such as anthocyanin and vitamin C significantly impact product quality.

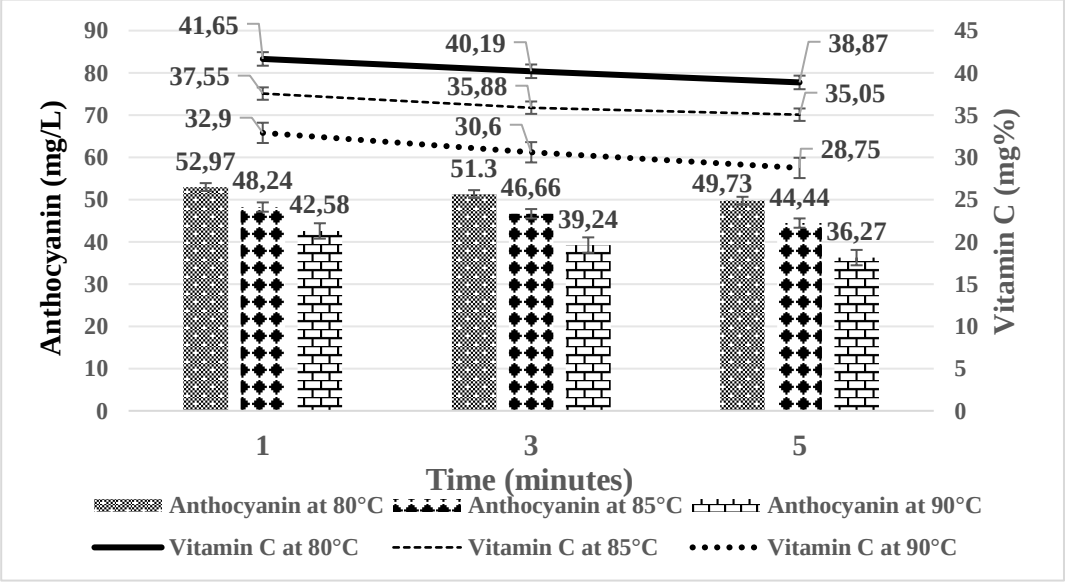

Figure 1. Anthocyanin and vitamin C contents of the product at different pasteurization temperatures and holding times

Pasteurization temperature and holding time interact and significantly affect anthocyanin and vitamin C contents (Figure 1). According to Nguyen et al. (2022), higher pasteurization temperature and prolonged treatment accelerate anthocyanin degradation, leading to pigment loss and reduced anthocyanin stability. Specifically, pasteurizing at 80°C for 1 minute retained the highest anthocyanin content, preserving the characteristic bright red color due to the shorter heating duration. Conversely, at 90°C for 5 minutes, anthocyanin content dropped sharply to 36.27 mg/L (about 1.5 times lower than at 80°C for 1 minute), causing a marked color loss. These findings align with previous studies by Duong et al. (2023) and Bhalerao and Chakraborty (2021), which reported significant anthocyanin degradation at high temperatures in herbal beverages and mixed fruit juices.

Vitamin C, a water-soluble and heat-sensitive compound, also suffered considerable losses under more severe pasteurization conditions. The analysis showed that the product pasteurized at 80°C for 1 minute had the highest vitamin C content (41.65 mg%), whereas

pasteurizing at 90°C for 5 minutes yielded the lowest content (28.75 mg%). This decrease reflects the susceptibility of vitamin C to breakdown under elevated temperature and prolonged heating. Similar results were also noted previously by Dang (2022), where vitamin C losses increased at higher temperatures and longer pasteurization times, particularly in bottled beetroot juice processed at 85°C and 90°C.

Hence, pasteurization is pivotal for controlling food safety risks, yet temperature and holding time notably affect product quality and sensory attributes.

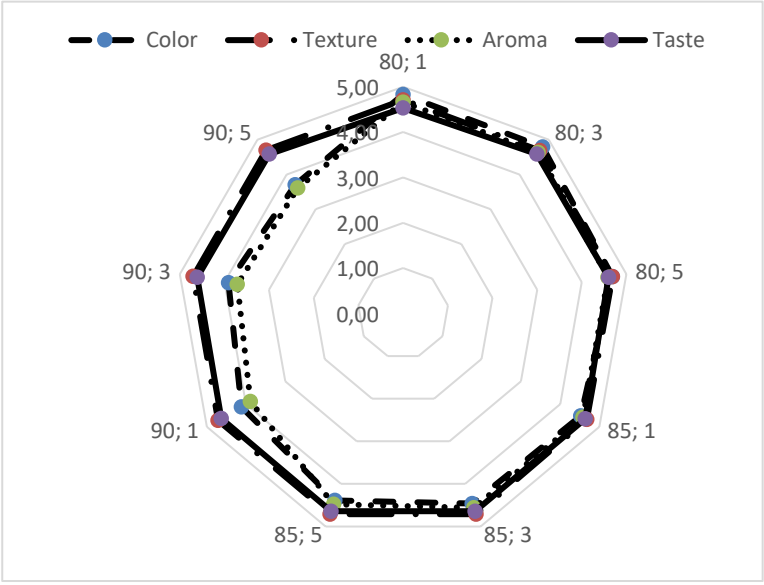

Figure 2. Sensory scores of the product at different pasteurization temperatures and holding times

Pasteurization temperature and holding time have a marked effect on product color and aroma. According to Le et al. (2009), thermal treatment alters chemical components, causing nutrient losses and modifying compound properties - especially anthocyanin - leading to deterioration in color and characteristic aroma. For instance, products pasteurized at 90°C for 1, 3 and 5 minutes had the lowest color scores, dropping most sharply at 5 minutes due to accelerated anthocyanin breakdown, causing darker, less appealing color. Tran et al. (2022) also showed that prolonged heat treatment at high temperatures can produce a “cooked” odor, diminishing the distinctive aroma and reducing sensory quality.

Although pasteurization at 80°C yielded the highest color scores, it did not meet the microbiological safety requirements. In contrast, at 85°C for 1 and 3 minutes, the product retained a bright red color and a harmonious flavor. However, extending the holding time to 5 minutes led to noticeable color changes due to anthocyanin degradation. Pasteurization at 85°C for 3 minutes struck the best balance between microbiological safety and desirable sensory traits, preserving anthocyanin content and thus maintaining the product’s characteristic color and aroma.

In summary, based on the investigated temperatures and times, pasteurization at 85°C for 3 minutes resulted in favorable sensory quality and minimal loss of anthocyanin and vitamin C. Furthermore, after 15 days of storage, no aerobic microorganisms, yeast, or mold appeared, confirming a pasteurization value PU > 5 minutes and ensuring the elimination of microorganisms.

3.2. Effect of light conditions on product quality during storage

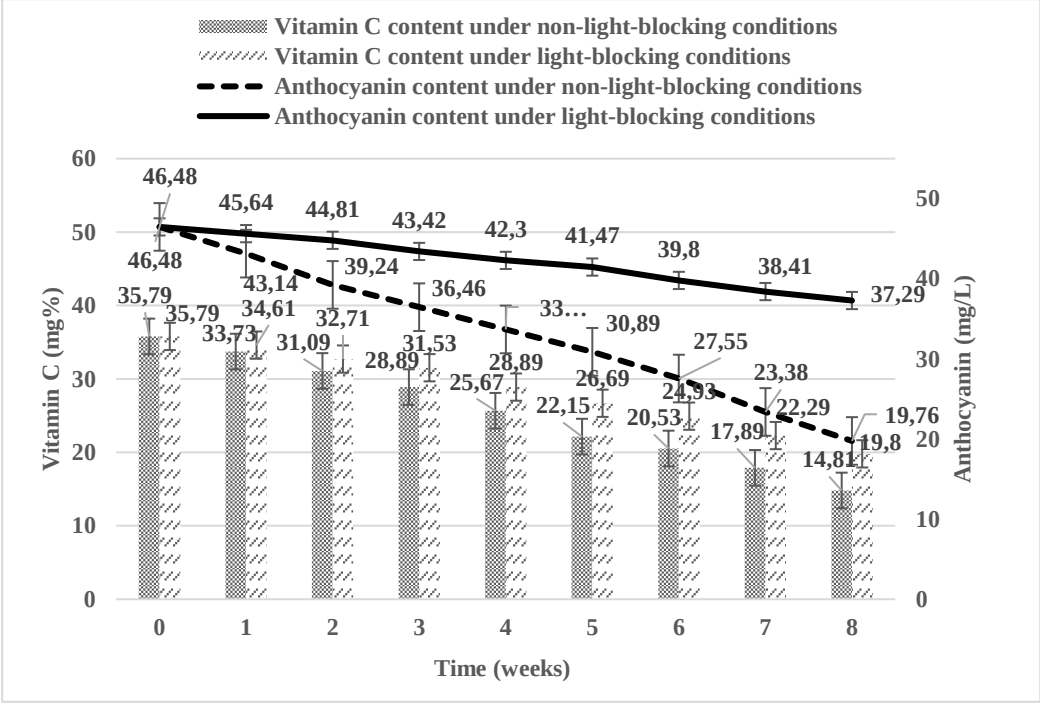

Figure 3. Vitamin C and anthocyanin contents of the product during 8 weeks of storage under two different conditions

Figure 3 depicts the changes in vitamin C and anthocyanin contents after 8 weeks of storage at low temperature (4 ± 2°C) under two different conditions: non-light-blocking (with light) vs. light-blocking (without light).

After 8 weeks of storage, vitamin C content decreased substantially under both conditions. Under non-light-blocking conditions, vitamin C content dropped by 58.6%, from 35.79 mg% down to 14.81 mg%. In contrast, under light-blocking conditions, the decrease was only 44.7%. This reduction can be attributed to the ease of vitamin C oxidation - it is a strong antioxidant but sensitive to oxygen, light, and temperature. These findings are consistent with Çakmakçı and Turgut (2005), who reported significantly higher vitamin C retention in milk samples stored without light exposure, and also observed that vitamin C content decreases over storage time, especially under light.

Similarly, anthocyanin breakdown was strongly influenced by storage conditions. Specifically, under non-light-blocking conditions, anthocyanin content fell by 57.5% after 8 weeks, while under light-blocking conditions, it dropped by only 19.7%. This likely stems from anthocyanin's sensitivity to light, oxygen, and temperature (Jan & Masih, 2012). Prolonged light exposure accelerates the degradation of these color compounds, causing noticeable fading (Mishra et al., 2012). Woo et al. (2011) reported that red-fleshed dragon fruit juice under direct light exposure lost up to 50% of its pigments after just one week of storage. Hence, light significantly increases anthocyanin degradation, leading to a substantial reduction of this compound in non-light-blocking conditions. This highlights the importance of light-blocking storage to maintain color quality and anthocyanin stability.

As presented in Table 4, the product stored at low temperature (4 ± 2°C) under both non-light-blocking and light-blocking conditions remained safe throughout 8 weeks, showing no detectable aerobic microorganisms, yeast, or mold.

Table 4. Total aerobic count and yeast and mold counts during storage under two different conditions

Time (weeks)	Total aerobic count (cfu/mL) (*)		Total yeast and mold (cfu/mL) (*)	
	Non-Light-Blocking	Light-Blocking	Non-Light-Blocking	Light-Blocking
0	< 1	< 1	< 1	< 1
1	< 1	< 1	< 1	< 1
2	< 1	< 1	< 1	< 1
3	< 1	< 1	< 1	< 1
4	< 1	< 1	< 1	< 1
5	< 1	< 1	< 1	< 1
6	< 1	< 1	< 1	< 1
7	< 1	< 1	< 1	< 1
8	< 1	< 1	< 1	< 1

Note: (*) Data are the mean values of three replicates.

Table 5. Sensory descriptions of the product during storage under two different conditions

Time (weeks)	Non-Light-Blocking	Light-Blocking	Comparison
0 - 2	Bright red color, stable sensory attributes.	Same as "Non-Light-Blocking."	No difference.
3 - 4	Light red color, stable sensory attributes.	Same as "Non-Light-Blocking."	Color in "Non-Light-Blocking" starts to fade.
5 - 6	Yellowish-red color, stable sensory attributes.	Same as "Non-Light-Blocking."	Noticeable fading in "Non-Light-Blocking."
7 - 8	Brownish yellow color, stable sensory attributes.	Same as "Non-Light-Blocking."	Significant fading in "Non-Light-Blocking."

1.1 Before storage ***1.2 After 8 weeks***

Figure 4. Beverage product from perilla leaf extract - pineapple under two different light exposure conditions during storage

Note: (a) Non-Light-Blocking*, (b)* Light-Blocking

The visual changes are further illustrated in Figure 4. Image (a) shows the beverage stored under non-light-blocking conditions, displaying visible discoloration after 8 weeks, whereas Image (b) demonstrates the product under light-blocking conditions, which appeared to better retain its original hue. These findings align with previous studies (Wojdyło et al., 2019) indicating that prolonged exposure to light accelerates anthocyanin degradation due to photooxidation and pigment breakdown.

In conclusion, while refrigeration effectively maintains the microbiological safety of the perilla leaf - pineapple beverage, light-blocking storage is crucial for preserving its sensory attributes, particularly color stability. These results highlight the importance of packaging choices in extending product quality and consumer appeal.

4. Conclusion

The research findings indicate that pasteurizing the perilla leaf - pineapple beverage at 85°C for 3 minutes best balances microbiological safety and quality preservation, retaining higher anthocyanin and vitamin C contents and maintaining favorable sensory attributes. After 8 weeks of cold storage (4 ± 2°C), samples stored under light-blocking conditions showed significantly lower degradation of anthocyanin and vitamin C compared to those exposed to light. Hence, combining a carefully controlled pasteurization regime with proper light-blocking storage conditions is key to maintaining the nutritional and sensory quality of this naturally sourced functional beverage.

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