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Effects of extraction, fermentation, and storage processes on the levels of choline derived from calabash fruit (*Crescentia cujete* L.)

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ABSTRACT: Calabash fruit (*Crescentia cujete* L.) is one of the herbs cultivated in Indonesia. Calabash fruit has nutrients, including choline, required for neurotransmitter synthesis in neuronal cells. However, the choline levels in fresh fruits decrease during storage. Therefore, there is a need to isolate choline and maintain its level during storage. This study aimed to analyze the effects of extraction, fermentation, and storage processes on the level of choline derived from the calabashh fruit. Calabash fruits were classified into three groups: group I (fresh fruit), group II (extracted), and group III (fermented). All groups were stored in the refrigerator at 4 ℃. Their choline content was measured using liquid chromatography-tandem mass spectrometry on days 0, 5, and 10 after storage. The data were analyzed using the Statistical Package for Social Sciences version 26. The result showed that the calibration curve equation of choline was y = 3.15e + 5x + 2.0e+4, with *R2* = 0.998 and a linear regression of concentration range of 0–24 ng/mL. The processing of calabash fruits using extraction or fermentation decreased the choline level ($p \leq 0.05$). However, the product produced during extraction and fermentation of the calabash fruit had better stability regarding its choline levels after 10 days of storage compared with that of the fresh calabash fruit ($p \leq 0.05$). Therefore, the calabash fruit extraction and fermentation processes can bind choline and maintain its stability after storage. The extraction and fermentation produce products containing pure choline with minimum interfering compounds that affect its stability.

KEYWORDS: calabash; choline; liquid chromatography-tandem mass spectrometry; processing; storage.

1. INTRODUCTION

Calabash fruit (*Crescentia cujete* L.) is a tropical fruit in the *Bignoniaceae* family distributed in Asia, Africa, and South America [1]. Furthermore, the calabash fruit has been cultivated in Indonesia. In Indonesia, the fruit is known as Buah Maja, Bila, or Berenuk [2]. Calabash fruit is popular in Indonesia and is associated with the legendary Majapahit kingdom. Locals in Indonesia use the calabash fruit as an unregistered herb or "*jamu*" against circulatory disturbances and as an anticancer, anthelmintic, antidiabetic, anti-inflammatory agent, wound healing promoter, and a source of antioxidants [3].

The potential benefits of the calabash are due to its biochemical contents, such as flavonoid, saponin, tannin, hydrogen cyanide, phenols, cardenolide, tartaric acid, and phytosterols [4]. Antioxidant compounds from calabash have been used as antibacterial agents in *Litopenaeus vannamei* against *Vibrio alginolyticus*, *Vibrio harveyi*, and *Vibrio parahaemolyticus* due to their mechanism as immune-protectants in activating hyaline, semi-granular, granular, and hemocyte cells of the vanamei shrimp [5]. A previous study conducted by Anitha and Nazeema [6] reported that calabash fruit prevents neurotoxicity in neuroblastoma cells. The effectiveness of the calabash fruit in protecting neuronal cells is probably due to it containing choline [6]. However, no studies have explored the choline content of the calabash fruit. Therefore, it is essential to determine the choline content of the calabash fruit to determine its neuroprotective ability.

Choline (2-hydroxyethyl-trimethyl-ammonium salt) is essential for muscle, liver, and brain functions [7]. Choline is required for neurotransmitter synthesis in neuronal cells. Choline can be found in salmon, meat,

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milk, mushrooms, fresh vegetables, and fruits, including calabash [8]. However, choline from herbs is degraded during preservation and storage [9]. The decrease in nutrition occurs because of the catalytic activities of different enzymes and the transduction of ethylene-related processes within vegetables and fruits [10]. Based on the illustration above, this study aimed to analyze the effects of extraction, fermentation, and storage processes on the levels of choline derived from calabash fruits.

2. RESULTS

2.1. Chromatogram and calibration curve of choline using liquid chromatography-tandem mass spectrometry (LC-MS/MS)

A chromatogram of choline obtained using the LC-MS/MS method is shown in Figure 1. The retention time observed using this method was 2.35 min. The calibration curve equation used in liquid chromatography for the separation of choline was y = 3.15e + 5x + 2.0e+4, with $R2$ = 0.998 and linear regression of the concentration ranging between 0–24 ng/mL (Figure 2). The chromatogram of the analyte peak area (count area) for fresh, extracted, and fermented calabash fruits was 4.02e + 005, 9.61e + 004, and 2.92e + 005, respectively. Furthermore, the analyte peak heights for fresh, extracted, and fermented calabash fruits were 1.64e + 004, 3.90e + 003, and 1.12e + 004, respectively. The chromatograms of choline from the extracted and fermented calabash fruits are shown in Figures 3A and B.

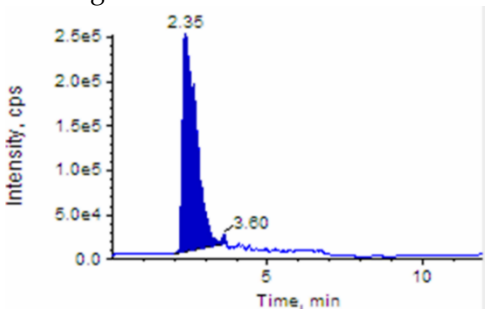

Figure 1. Chromatogram of choline.

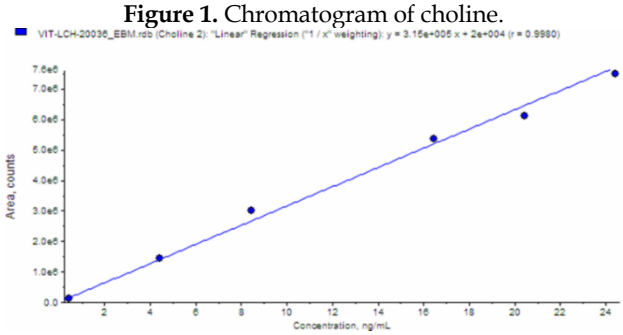

Figure 2. Calibration curve of choline.

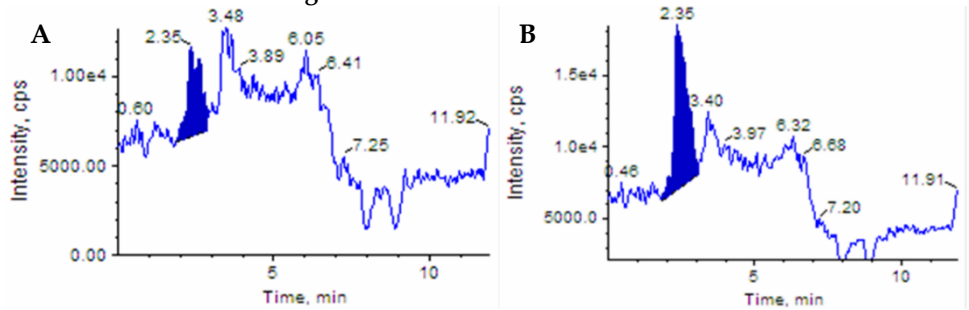

Figure 3. Chromatogram of choline from the extracted (A), and fermented (B) calabash fruit.

2.2. Choline levels from fresh, extracted, and fermented calabash fruits after storage

The present study showed that fresh calabash fruits had the highest choline level (149.33 ± 3.21 mg/kg) compared with those of extracted and fermented fruits (Table 1), indicating that fresh calabash fruits can be used as a source of natural choline for humans. Processing through extraction and fermentation of the calabash fruit decreases the choline content. However, the fermented calabash fruit had 110.33 ± 5.03 mg/kg choline, which was almost similar to that in fresh calabash fruits, indicating that the fermentation process can still be used to isolate choline from calabash. However, the extraction group had the lowest choline levels (Table 1)

compared with those of other groups. Additionally, storing in the refrigerator at 4 ℃ significantly decreased the fresh calabash fruit choline level ($p \leq 0.05$), but had no significant effect on extracted and fermented calabash ($p \geq 0.05$ [Table 1]). The decrease in choline levels in fresh calabash fruits occurred on day 5 of storage and worsened on day 10. Contrastingly, there was no significant decrease in choline levels in the extracted and fermented calabash on days 5 and 10 of storage, indicating that the extraction and fermentation processes maintained the stability of the isolated choline.

Table 1. Choline levels in fresh, extracted, and fermented calabash fruit after 10 days of storage in a refrigerator at 4 ℃.

Group	Choline level (mg/kg) ± SD		
	Day – 0	Day – 5	Day – 10
I	149.33 ± 3.21$^{a, a}$	142.33 ± 2.51$^{b, a}$	138.00 ± 1.73$^{c, a}$
II	3.42 ± 0.13$^{a, b}$	3.42 ± 0.13$^{a, b}$	3.38 ± 0.17$^{a, b}$
III	110.33 ± 5.03$^{a, c}$	110.33 ± 5.03$^{a, c}$	109.00 ± 3.60$^{a, c}$

SD Standard of deviation.
I Fresh calabash fruit.
II Extracted calabash fruit.
III Fermented calabash fruit.
$^{a, b, c}$ Different superscripts in the same row and column indicate significant differences.

The residual choline percentage in fresh calabash fruits decreased significantly ($p \leq 0.05$) after storage. Contrastingly, the extracted and fermented calabash fruits showed no significant difference in the percentage of residual choline after storage ($p \geq 0.05$). Storing in a refrigerator at 4 ℃ reduced choline levels in the fresh calabash fruit, which reached 7.57% after 10 days (Table 2). However, the choline percentage in extracted and fermented calabash fruits ranged from 0.99–1.16% on day 10.

Table 2. Percentage of residual and reduction in choline level from fresh, extracted, and fermented calabash fruits after 10 days of storage in a refrigerator at 4 ℃.

Group	Percentage of residual choline level after storage (%) ± SD			Percentage reduction of choline level after storage (%) ± SD		
	Day – 0	Day – 5	Day – 10	Day – 0	Day – 5	Day – 10
I	100.00 ± 0.00^{a,a}	95.31 ± 0.59^{b,a}	92.42 ± 0.85^{c,a}	0.00 ± 0.00^{a,a}	4.68 ± 0.59^{b,a}	7.57 ± 0.85^{c,a}
II	100.00 ± 0.00^{a,a}	100.00 ± 0.00^{a,b}	99.00 ± 1.72^{a,b}	0.00 ± 0.00^{a,a}	0.00 ± 0.00^{a,b}	0.99 ± 1.72^{a,b}
III	100.00 ± 0.00^{a,a}	100.00 ± 0.00^{a,b}	98.83 ± 1.32^{a,b}	0.00 ± 0.00^{a,a}	0.00 ± 0.00^{a,b}	1.16 ± 1.32^{a,b}

SD Standard of deviation.
I Fresh calabash fruit.
II Extracted calabash fruit.
III Fermented calabash fruit.
$^{a, b, c}$ Different superscripts in the same row and column indicate significant differences.

3. DISCUSSION

Choline is an essential nutrient for human metabolism, particularly for activiting the neuronal system. Choline can be synthesized by the body and is supplied through food. Calabash contains high choline levels, which generally decrease after storage. A preservation process is required to stabilize choline in the calabash fruit. Extraction or fermentation are the main processes for isolating and stabilizing choline.

Extraction is a process that separates components from a mixture based on their solubility. In the present study, solvent extraction was performed by maceration. This process depends on the extraction procedure, polarity, and particle charge of the organic component [11] and the properties of the herbs. The decrease in choline levels in calabash fruits after the extraction process is due to the drying and Soxhletation process of the calabash before maceration. The drying process causes evaporation and component damage, particularly for choline, consistent with a previous study that showed that thermal conductivity reduces choline levels in water-soluble specimens [12]. Therefore, when conducting the heating process for isolating choline from calabash or other fruits, future researchers should not make the same mistakes.

Furthermore, the choline level from water-soluble ingredients decreases during intermittent heating, including pasteurization [13]. Pasteurization is generally conducted at ≥72 ℃ for 15 s, whereas Soxhletation is conducted at 69 ℃ for several hours. Therefore, Soxhletation is suspected of causing choline loss and degradation. Another factor contributing to the decrease in choline levels during extraction is its solubility. Choline is a water-soluble nutrient that can be easily degraded by heating [14]. Therefore, the decrease in choline levels can be attributed to the evaporation of choline along with water and other water-soluble components.

In the present study, the choline lost during the extraction process was similar to that lost during fermentation. However, the choline loss during fermentaion was not as high as that during the extraction process. During fermentation, several compounds are partially oxidized, and energy from the natural elements is released [15]. Moreover, vegetables and fruits are perishable; therefore, fermentation becomes an essential food processing technique in food preservation processes. After fermentation, phenolic compounds can be separated [16]. The fermentation of calabash fruits can allow binding of higher amounts of choline than extraction process because the fermentation process does not involve the heating process like extraction.

Furthermore, the present study proved that storage decreases choline levels in fresh fruits, consistent with a previous study conducted by Wang et al. [17] which showed that choline level in various plants is reduced to 20% during storage at 25 ℃. Furthermore, the choline level in fresh calabash fruits in the present study decreased significantly on days 5 and 10 after storage at 4 ℃, consistent with a previous study that showed a slight decrease in the nutritional contents of fresh vegetables and fruits during storage in the refrigerator at 4 ℃ [18]. Another report exhibited that the freezing procedure considerably decreased the nutrient content of fresh vegetables [19].

Contrastingly, extraction and fermentation processes did not considerably decrease choline levels. We suspected that the decreased choline levels could be correlated with the purity of the product produced through extraction and fermentation. A previous study showed that pure caffeoylgluconic acid has better quality and stability than its mixture product [20].

The purity of the choline product derived from the extraction and fermentation of calabash is influenced by the interfering compounds that impair and react with choline. Choline is degraded by cholinesterase, which is naturally contained in fruits [21]. The occurrence of cholinesterase in fresh fruit products may decrease choline levels. Kiczorowski et al. [22] found that the fermentation process in some fruits causes a relative decrease in some nutrients and biochemical compounds, but increases vitamins and fat, maintains their stability, and extends shelf life. Moreover, the fermentation process decreases the pH and heavy metals in fruits, preventing the oxidation of some nutrients, including vitamins, consistent with results from the present study. Processing fresh vegetables and fruits is important in maintaining the integrity and stability of their nutritional value. However, studies exploring the choline level in calabash after fermentation and extraction are still limited, and further studies are required.

4. CONCLUSION

The extraction and fermentation processes of calabash fruits can be used to bind choline and maintain its stability after storage at 4 ℃. This is because extraction and fermentation produce products containing purer choline with minimum interfering compounds that impair its stability. The stability of choline in fermentation and extraction products could be due to a decrease in the pH and heavy metals that prevent oxidation. Fermentation can be used as an alternative preservation method for separating and stabilizing choline from calabash fruits.

5. MATERIALS AND METHODS

5.1. Species authentication of the Calabash fruit

The calabash species were determined at the Herbarium Bogoriense, Direktorat Pengelolaan Koleksi Ilmiah BRIN Cibinong, West Java, Indonesia. The calabash fruit was identified as *Crescentia cujete* L and authenticated with registration number: B-1446/IV/DI.05.07/5/2022.

5.2. Calabash fruit collection and processing

Calabash was obtained from the botanical garden of the University of Wijaya Kusuma Surabaya, Indonesia. Fresh and green calabash fruits with a diameter of 44.98 ± 2.34 cm were harvested between 08.00–09.00 a.m. (Surabaya time: UTC+7), and transported to the laboratory for subsequent measurements.

5.3. Research design

The collected calabash fruits were divided into three groups: group I, fresh; group II, extracted; group III, fermented fruits. Calabash fruits in group I were washed with tap water and stored in the refrigerator at 4 ℃. The calabash fruits in group II were extracted using alcohol, and those in group III were fermented. All the extraction and fermentation products were stored in the refrigerator at 4 ℃ for 10 days. Choline levels in all groups were measured using LC-MS/MS. The test was conducted on days 0, 5, and 10 of storage.

5.4. Fresh calabash fruit treatment

Harvested fresh calabash fruits were transported to the Laboratory of Pharmacology, Faculty of Veterinary Medicine, University of Wijaya Kusuma Surabaya, East Java, Indonesia, washed with tap water, dried using tissue paper, and stored in a refrigerator at 4 ℃.

5.5. Calabash fruit extraction

Before extraction, calabash fruits were washed with tap water, peeled, and dried at 25 ℃. The calabash fruit was macerated with 70% alcohol (1:4). The maceration was conducted thrice, and the macerate was evaporated at 69 ℃ using a Soxhlet evaporator (Buchi R-100; Soxhlet, Buchi, Indonesia). Evaporation was performed until a thick calabash-fruit extract was obtained [23]. The thick extract obtained during the extraction process was 126.66 ± 7.84 g/kg and was stored at 4 ℃.

5.6. Calabash fruit fermentation

The calabash fruits were washed with tap water and peeled. The fruit pulp was then fermented using the following composition: water: pulp: sugar: pectinase (Pectinex Ultra AF-P, Novozymes, London, UK) at a weight ratio of 1,000:400:40:40. The mixture was stored in a bottle at 25 ℃ for 30 days.A gauze was used as a seal on the mouth of the bottle. The mixture was manually stirred every 24 h. On the final day of the experiment, the supernatant of fermented calabash was collected, placed in a sterile glass bottle, and stored in a refrigerator at 4 ℃.

5.7. LC-MS/MS

LC-MS/MS (SCIEX Triple Quad 5500+, SCIEX, Framingham, MA, USA) was used to determine the choline content of fresh, extracted, and fermented calabash fruits. The LC-MS/MS procedure was modified based on a previous study [24]. Each specimen measured 10 mg, and 200 µL of 50 mM hydrochloric acid was added to it. The samples were vortexed for 5 min for homogenization. The extraction procedure was repeated thrice for each specimen. The supernatant was diluted 100-fold with the mobile phase and filtered through a 0.45 µm pore-syringe filter (Biochem Life Sciences, New Delhi, India). Liquid chromatography separation was conducted using YMC Triart-PFP (YMC America, St Devens, MA, America), and the column was set at 40 ℃. The mobile phase was made at 50% concentration (v/v), consisting of methanol containing 0.01% formic acid. The flow rate of this procedure was 0.5 mL/min, and the injection volume was 50 µL. Furthermore, mass spectrometry was performed in the positive mode with electrospray ionization. The choline transition mode within the mass/charge ratio was 104.2 to 60.2. The amount of choline in the samples is presented as mg/kg. LC-MS/MS was performed in triplicate for each specimen per period.

5.8. Validation

The choline standard solution was diluted using the mobile phase and adjusted to 0.00, 4.00, 8.00, 12.00, 16.00, 20.00, and 24.00 mg/kg. Each standard solution was analyzed using LC-MS/MS. The generated peak area and concentration of each standard were transformed into a regression line, and linearity was evaluated using *R2* (Rental software, Santa Monica, CA, USA). Data precision was analyzed using relative standard deviation.

5.9. Data analysis

All data are presented as mean ± standard deviation. Statistical analysis was performed using two-way analysis of variance at $p \leq 0.05$ significance level. If the data showed significant differences, they were analyzed using Duncan's test. All statistical procedures were conducted using Statistical Package for Social Sciences version 26 (IBM Corp, NY, USA).

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REFERENCES

[1] Correa AMD, Galdames C, Stapf MS, Catalogo de las Plantas Vasculares de Panama, Smithsonian Tropical Research Institute, Panama, 2004.

[2] Sagrin MS, Lasano NF, Shukri R, Ramli NS. Antioxidant properties and toxicity assessment of the *Crescentia cujete* extracts in Brine shrimp (*Artemia salina*). Sains Malaysiana. 2019; 48(4): 831–840. [CrossRef]

[3] Balogun FO, Sabiu S. A review of the phytochemistry, ethnobotany, toxicology, and pharmacological potentials of *Crescentia cujete* L. (Bignoniaceae). Evid Based Complement Alternat Med. 2021; 2021: 6683708. [CrossRef]

[4] Das N, Islam ME, Jahan N, Islam MS, Khan A, Islam MR, Parvin MS. Antioxidant activities of ethanol extracts and fractions of *Crescentia cujete* leaves and stem bark and the involvement of phenolic compounds. BMC Complement Altern Med. 2014;14:45. [CrossRef]

[5] Rahmaningsih S, Andriani R, Pujiastutik H. Effect of Majapahit (*Crescentia cujete* L.) fruit powder on the immune profile of *Litopenaeus vannamei* after infection with *Vibrio* spp. Vet World. 2021; 14(6): 1480-1486. [CrossRef]

[6] Anitha P, Nazeema TH. In vitro neuroprotective potential of *Crescentia cujete* leaves against mptp-induced toxicity in SHSY5Y neuroblastoma cells. World J Pharmaceutic Res. 2020; 9(13): 898–909.

[7] Wallace TC, Blusztajn JK, Caudill MA, Klatt KC, Natker E, Zeisel SH, Zelman KM. Choline: the underconsumed and underappreciated essential nutrient. Nutr Today. 2018; 53(6): 240-253. [CrossRef]

[8] Wiedeman AM, Barr SI, Green TJ, Xu Z, Innis SM, Kitts DD. Dietary choline ıntake: current state of knowledge across the life cycle. Nutrients. 2018; 10(10): 1513. [CrossRef]

[9] Horiuchi Y, Kimura R, Kato N, Fujii T, Seki M, Endo T, Kato T, Kawashima K. Evolutional study on acetylcholine expression. Life Sci. 2003; 72(15): 1745-1756. [CrossRef]

[10] Al-Juhaimi F, Ghafoor K, Özcan MM, Jahurul MHA, Babiker AE, Jinap S, Sahena F, Sharifudin MS, Zaidul ISM. Effect of various food processing and handling methods on preservation of natural antioxidants in fruits and vegetables. *J Food Sci Technol*. 2018; 55(10): 3872-3880. [CrossRef]

[11] Abubakar AR, Haque M. Preparation of medicinal plants: basic extraction and fractionation procedures for experimental purposes. J Pharm Bioallied Sci. 2020; 12(1): 1-10. [CrossRef]

[12] Ibrahim TH, Sabri MA, Abdel Jabbar N, Nancarrow P, Mjalli FS, AlNashef I. Thermal Conductivities of Choline Chloride-Based Deep Eutectic Solvents and Their Mixtures with Water: Measurement and Estimation. Molecules. 2020; 25(17): 3816. [CrossRef]

[13] Nessel I, Khashu M, Dyall SC. The effects of storage conditions on long-chain polyunsaturated fatty acids, lipid mediators, and antioxidants in donor human milk - A review. Prostaglandins Leukot Essent Fatty Acids. 2019; 149:8-17. [CrossRef]

[14] Moretti A, Paoletta M, Liguori S, Bertone M, Toro G, Iolascon G. Choline: an essential nutrient for skeletal muscle. Nutrients. 2020;12(7):2144. [CrossRef]

[15] Marco ML, Heeney D, Binda S, Cifelli C, Cotter PD, Foligne B, Ganzle M, Kort M, Pasin G, Pihlanto A, Smid EJ, Hutkins R. Health benefits of fermented foods: microbiota and beyond. Curr Opin Biotechnol. 2017; 44: 94-102. [CrossRef]

[16] Shabbir U, Tyagi A, Ham HJ, Elahi F, Oh DH. Effect of fermentation on the bioactive compounds of the black soybean and their anti-alzheimer's activity. Front Nutr. 2022;9:880361. Published 2022 May 13. [CrossRef]

[17] Wang W, Yamaguchi S, Koyama M, et al. LC-MS/MS Analysis of Choline Compounds in Japanese-Cultivated Vegetables and Fruits [published correction appears in Foods. 2022 Jul 29;11(15):]. *Foods*. 2020; 9(8): 1029. [CrossRef]

[18] Moukarzel S, Wiedeman AM, Soberanes LS, Dyer RA, Innis SM, Lamers Y. Variability of water-soluble forms of choline concentrations in human milk during storage, after pasteurization, and among women. Nutrients. 2019; 11(12): 3024. [CrossRef]

[19] Bouzari A, Holstege D, Barrett DM. Vitamin retention in eight fruits and vegetables: a comparison of refrigerated and frozen storage. J Agric Food Chem. 2015; 63(3): 957-962. [CrossRef]

[20] Yu H, Yang J, Ding J, He Y, Jiang Z, Chai X, Wang Y. Stability study and ıdentification of degradation products of caffeoylgluconic acid derivatives from fructus euodiae. Molecules. 2018; 23(8): 1975. [CrossRef]

[21] Hwang H, Kim YJ, Shin Y. Assessment of physicochemical quality, antioxidant content and activity, and ınhibition of cholinesterase between unripe and ripe blueberry fruit. Foods. 2020; 9(6): 690. [CrossRef]

[22] Kiczorowski P, Kiczorowska B, Samolińska W, Szmigielski M, Winiarska-Mieczan A. Effect of fermentation of chosen vegetables on the nutrient, mineral, and biocomponent profile in human and animal nutrition. Sci Rep. 2022; 12(1):13422. [CrossRef]

[23] Prakoso YA, Kurniasih. The Effects of *Aloe vera* Cream on the Expression of $CD4^+$ and $CD8^+$ Lymphocytes in Skin Wound Healing. *J Trop Med*. 2018; 2018: 6218303. [CrossRef]

[24] Yamaguchi S, Matsumoto K, Koyama M, Tian S, Watanabe M, Takahashi A, Miyatake K, Nakamura K. Corrigendum to "Antihypertensive effects of orally administered eggplant (Solanum melongena) rich in acetylcholine on spontaneously hypertensive rats" [Food Chem. 276 (2019) 376-382]. Food Chem. 2022;381:132228. [CrossRef]