DONG THAP UNIVERSITY JOURNAL OF SCIENCE
Tạp chí Khoa học Đại học Đồng Tháp
Natural Sciences Issue
ISSN 0866-7675 | e-ISSN 2815-567X

DOI: https://doi.org/10.52714/dthu.ns.3387.2005

EFFECTS OF MIXING RATIO AND PASTEURIZATION ON THE QUALITY OF A BEVERAGE MADE FROM *Pouzolzia zeylanica* EXTRACT AND PASSION FRUIT (*Passiflora edulis* Sims.) JUICE

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Article history

Received: 02/5/2026; Received in revised form: 14/7/2026; Accepted: 17/7/2026

Abstract

This study aimed to develop a beverage from Pouzolzia zeylanica extract mixed with passion fruit juice and identify the most suitable formulation and pasteurization conditions within the tested range in terms of sensory quality, retention of bioactive compounds, and microbiological safety. A completely randomized design was used to evaluate the effects of mixing ratio, sugar addition, citric acid addition, and temperature-time pasteurization. Among the tested formulations, the beverage with an 8:2 (v/v) ratio of P. zeylanica extract to passion fruit juice, 18% sugar, and 0.03% citric acid was selected as the most suitable formulation. This formulation achieved the highest sensory acceptance while maintaining relatively high contents of anthocyanins (1.81 mg/L), total polyphenols (4.15 mg GAE/mL), and vitamin C (8.56 mg/100mL). Pasteurization at 65 °C for 10 min (PU = 18.57 min) ensured microbiological safety for 15 days at 4 ± 2 °C while minimizing the degradation of heat-sensitive compounds. These findings provide a scientific basis for the development of a bioactive-rich beverage from locally available plant materials.

Keywords: Anthocyanins, mixing ratio, passion fruit, pasteurization, polyphenols, *Pouzolzia zeylanica.*

Cite: Doan, P. L., Bui, T. N. T., Huynh, T. N. M., Nguyen, X. H., & Nguyen, T. H. X. (2026). Effects of mixing ratio and pasteurization on the quality of a beverage made from Pouzolzia zeylanica extract and passion fruit (Passiflora edulis Sims.) juice. *Dong Thap University Journal of Science*, *Online First*, 1-14. https://doi.org/10.52714/dthu.ns.3387.2005

ẢNH HƯỞNG CỦA TỶ LỆ PHỐI TRỘN VÀ CHẾ ĐỘ THANH TRÙNG ĐẾN CHẤT LƯỢNG NƯỚC UỐNG TỪ DỊCH CHIẾT CÂY THUỐC DÒI (*Pouzolzia zeylanica* (L.) Benn.) VÀ DỊCH CHANH DÂY (*Passiflora edulis* Sims.)

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Lịch sử bài báo

Ngày nhận: 02/5/2026; Ngày nhận chỉnh sửa: 14/7/2026; Ngày duyệt đăng: 17/7/2026

Tóm tắt

Nghiên cứu này nhằm phát triển đồ uống từ dịch chiết cây thuốc dòi (Pouzolzia zeylanica (L.) Benn.) phối trộn với dịch chanh dây (Passiflora edulis Sims.). Việc lựa chọn công thức phối trộn cũng như chế độ thanh trùng phù hợp nhất trong phạm vi khảo sát dựa trên các khía cạnh cảm quan, khả năng duy trì các hợp chất hoạt tính sinh học và an toàn vi sinh. Thí nghiệm được bố trí hoàn toàn ngẫu nhiên nhằm khảo sát ảnh hưởng của tỷ lệ phối trộn, hàm lượng đường bổ sung, hàm lượng acid citric bổ sung và các tổ hợp nhiệt độ-thời gian thanh trùng. Trong các công thức được khảo sát, công thức có tỷ lệ 8:2 (v/v) giữa dịch chiết cây thuốc dòi và dịch chanh dây, bổ sung 18% đường và 0,03% acid citric, được lựa chọn là phù hợp nhất. Công thức này cho điểm cảm quan cao nhất, đồng thời vẫn duy trì hàm lượng anthocyanin (1.81 mg/L), polyphenol tổng số (4.15 mg GAE/mL) và vitamin C (8.56 mg/100 mL) ở mức tương đối cao. Chế độ thanh trùng ở 65 °C trong 10 phút (PU = 18,57 phút) bảo đảm an toàn vi sinh cho sản phẩm trong 15 ngày bảo quản ở 4 ± 2 °C, đồng thời hạn chế sự suy giảm của các hợp chất nhạy cảm với nhiệt. Kết quả nghiên cứu cung cấp cơ sở khoa học cho việc phát triển đồ uống giàu hợp chất sinh học từ các nguồn nguyên liệu thực vật sẵn có tại địa phương.

Từ khóa: Anthocyanin, cây thuốc dòi, chanh dây, polyphenol, thanh trùng, tỷ lệ phối trộn.

1. Introduction

Addressing the increasing consumer demand for health-promoting foods and beverages, the development of drinks derived from natural raw materials rich in bioactive compounds has attracted considerable attention. Fruits and medicinal plants are important sources of antioxidants, vitamins, minerals, and organic acids that may contribute to health promotion and the prevention of chronic diseases. However, beverages are generally highly perishable and susceptible to quality deterioration during storage due to microbial growth as well as oxidative and hydrolytic reactions, which may lead to losses in nutritional value and sensory quality. Therefore, selecting appropriate blending ratios and heat treatment conditions is an important approach to improving product quality and extending shelf life.

Pouzolzia zeylanica (L.) Benn., commonly known as "thuốc dòi" in Vietnamese, is a medicinal plant widely distributed in Viet Nam, particularly in the Mekong Delta, and is traditionally used to treat respiratory and gastrointestinal disorders (Nguyen & Vo, 2020; Nguyen et al., 2023). The plant contains a variety of bioactive compounds, including anthocyanins, flavonoids, flavones, tannins, polyphenols, and organic acids (Saha et al., 2012). Among these compounds, anthocyanins are of particular interest because they not only possess bioactive properties but also function as natural colorants (Alappat & Alappat, 2020), whereas polyphenols are associated with antioxidant activity. Nevertheless, anthocyanins and polyphenols are sensitive to processing and storage conditions and may degrade under unsuitable pH, temperature, and light exposure.

Passion fruit (*Passiflora edulis* Sims.) is a widely consumed tropical fruit that is rich in organic acids, particularly citric and malic acids, as well as polyphenols, vitamin C, and characteristic aroma compounds (Thokchom & Mandal, 2017). Its high acidity and distinctive flavor make it a promising ingredient for beverage formulation. In addition, its vitamin C content contributes to the nutritional value of the final product and may help protect sensitive bioactive compounds during processing and storage. Therefore, mixing *P. zeylanica* extract with passion fruit juice may improve color expression, flavor balance, and overall acceptability while providing a beverage with desirable nutritional properties.

In addition to their nutritional and functional potential, these two raw materials are of practical interest because they are locally available, support beverage diversification, and may increase the economic value of regional agricultural resources. Although previous studies have reported the bioactive properties of *P. zeylanica* and the nutritional characteristics of passion fruit (Nguyen et al., 2017; Pertuzatti et al., 2015), limited information is available on beverages prepared from the combination of these two materials, particularly regarding formulation selection and pasteurization conditions. Therefore, this study was conducted to evaluate the effects of blending ratio, sugar addition, citric acid addition, and pasteurization conditions on the sensory, physicochemical, and microbiological quality of a beverage made from *P. zeylanica* extract and passion fruit juice, and to identify the most suitable formulation and processing conditions within the tested range.

2. Materials and methods

2.1 Materials and chemicals

Purple *Pouzolzia zeylanica* (L.) Benn. (*P. zeylanica*) plants were obtained from local markets in Can Tho City, Viet Nam. Mature and healthy plants, 20-30 cm in height, with a fresh appearance and free from wilting, yellowing, and pest damage, were selected. Thereafter, raw *P. zeylanica* plants were sorted to remove damaged, yellowed, or defective parts, then washed and drained. The materials were sectioned into 2-3 cm pieces and weighed. Subsequently, the extraction was conducted using an aqueous solvent at a 1:5 (w/v) ratio at 90 °C for 20 min (Tran & Nguyen, 2014), filtered then to obtain the *P. zeylanica* aqueous extract. The *P. zeylanica* extract (PE) was directly used for further experiments.

Purple passion fruits were also purchased in Can Tho City, Viet Nam. Fruits of uniform size and freshness, with smooth and taut peel and purple skin with a slight golden hue, were selected. Wrinkled, shriveled, or overripe fruits were discarded because they could develop off-odors and adversely affect the characteristic quality and flavor of the juice. Passion fruits were washed, cut in half, and the pulp was collected. The mixed pulp was treated with 0.4% enzyme pectinase at 50 °C for 120 min, followed by filtration to obtain clarified juice (Le et al., 2024). The passion fruit juice (PJ) was then used for further experiments.

The additives used in this study included refined sucrose (RE, 99.8% purity; Bien Hoa Sugar Company, Viet Nam) and food-grade citric acid supplied by CEMACO Can Tho Co., Ltd. (Can Tho, Viet Nam). Pectinase enzyme was supplied by the ICIFOOD Company (Ho Chi Minh City, Vietnam).

Plate Count Agar (PCA; India) was used for the enumeration of total aerobic bacteria, whereas Yeast Extract Glucose Chloramphenicol Agar (YGC; India) was used for the determination of yeasts and molds. The chemicals used for analytical determinations, including hydrochloric acid, iodine, starch, phenolphthalein, sodium hydroxide, sodium acetate, ethanol, Folin-Ciocalteu reagent, and sodium carbonate, were supplied by CEMACO Can Tho and originated from China.

2.2. Preparation of the beverage from *Pouzolzia zeylanica* extract and passion fruit juice

The PE and PJ were homogenized upon the investigated ratios, with the sucrose and citric acid levels as per the experimental formulation designs. The resulting mixtures were pre-heated at 90 °C for 5 min, subsequently filled into 250 mL glass bottles, and sealed. Pasteurization was then performed under the investigated temperature-time conditions. The final products were rapidly cooled and stored at 4 ± 2 °C in a refrigerator.

2.3. Experimental design

2.3.1. Effect of the P. zeylanica extract and passion fruit juice ratios on the product quality

A single-factor experiment was conducted using a completely randomized design (CRD) to evaluate the impact of varying mixing ratios between PE and PJ. The investigated volumetric ratios included 9:1, 8:2, 7:3, and 6:4 (v/v). The samples were performed in accordance with the procedure described in Section 2.2, and all the experiment runs were performed in triplicate.

2.3.2. Effect of the added sugar and citric acid levels on the product quality

To optimize the formulation, a two-factor CRD was conducted. The sugar concentrations at 15, 18, and 21% (w/v) and the citric acid levels at 0.01, 0.03, and 0.05% (w/v) were utilized, respectively. The samples were performed in accordance with the procedure described in Section 2.2, and all the experiment runs were performed in triplicate.

2.3.3. Effect of the pasteurization temperature and holding time on the product quality

The pasteurization temperature (60, 65, and 70 °C) and holding time (5, 10, and 15 min) were evaluated through a two-factor CRD. The samples were performed in accordance with the procedure described in Section 2.2. Pasteurization was carried out in a thermostatically controlled water bath. Once the bath reached the target temperature, the bottled products with an initial core temperature of 40 °C were placed into the bath. The core temperature of products were monitored and recorded every minute throughout the heating, holding, and cooling stages. At the end of the holding phase, the bottles were immediately cooled under running water, and the core temperature was continuously recorded during cooling. The samples, thereafter, were stored at 4 ± 2 °C for further analysis. All the experiment runs were performed in triplicate

2.4 Analytical methods

Total anthocyanin content (TAC) was determined using the pH differential method (Ionescu et al., 2025) and expressed as cyanidin-3-glucoside equivalents (mg/L). Total polyphenol content (TPC) was determined using the Folin-Ciocalteu method (Pérez et al., 2023) and expressed as mg gallic acid equivalents per mL (mg GAE/mL). Vitamin C (Vit C) content was determined by iodometric titration (Nhan & Diep, 2014) and expressed as mg/100 mL. Total titratable acidity (TTA) was determined by titration with 0.1 N NaOH and expressed as % citric acid (Diep & Ngo, 2023).

Total aerobic microorganisms, yeasts, and molds were enumerated by the pour-plate method (Wise, 2006) and expressed as CFU/mL.

Sensory evaluation was carried out according to the scoring method described in Vietnamese Standard TCVN 3215:1979, using a 5-point scale based on the attributes of color, odor, taste, and appearance. The sensory panel consisted of 15 trained members to improve the consistency and reliability of the sensory evaluation.

2.5. Data processing method

All data were processed using Minitab 19. Analysis of variance (ANOVA) was conducted, and mean comparisons were performed using the LSD test at a significance level of $P < 0.05$ to determine statistically significant differences among treatment means. Preliminary data processing and visualization in the form of tables and graphs were carried out using Microsoft Excel 2021 to support the presentation and interpretation of the experimental results.

3. Results and discussion

3.1. Proximate composition and bioactive constituents of *P. zeylanica* and passion fruit

The analytical results presented in Table 1 indicate that fresh *P. zeylanica* had a high moisture content (87.49 ± 0.95%) and was particularly rich in bioactive compounds, with TAC and TPC of 26.54 mg CE/g and 4.79 mg GAE/g, respectively. These values are comparable to those reported by Nguyen et al. (2017) for fresh *P. zeylanica* collected from lowland areas. These constituents not only enhance the nutritional value of the raw material but also contribute to the color and potential functional properties of the final beverage.

Passion fruit showed a relatively high Vit C (40.19 mg/100 g), which is consistent with the findings by Phan et al. (2022) (45 mg/100 g) and Pertuzatti et al. (2015) (40 - 65 mg/100 g). In addition, passion fruit contains considerable amounts of organic acids (3.28%), TPC (1.62 mg GAE/g), total sugars (11.01%), and moisture (74.87%), indicating that it is a potential material for the development of natural beverages with desirable nutritional properties.

P. zeylanica was characterized by a high anthocyanin content but relatively low titratable acidity and total sugar content, whereas passion fruit provided high acidity, natural sugars, and vitamin C. This compositional complementarity suggests that combining these two materials is highly suitable for beverage development. However, further investigation is required to identify an appropriate mixing ratio and pasteurization conditions in order to achieve a desirable quality beverage product.

Table 1. Physicochemical properties and bioactive compounds of *P. zeylanica* and passion fruit

Parameter	*P. zeylanica*	Passion fruit
Moisture (%)	87.49 ± 0.95	74.87 ± 0.55
pH	7.96 ± 0.05	2.73 ± 0.06
Total soluble solids (°Brix)	-	14.50 ± 0.50
Titratable acidity (%)	0.12 ± 0.03	3.28 ± 0.05
Reducing sugars (%)	-	7.08 ± 0.01
Total sugars (%)	0.02 ± 0.01	11.01 ± 0.02
Anthocyanins (mg CE/g)	26.54 ± 0.13	-
Polyphenols (mg GAE/g)	4.79 ± 0.01	1.62 ± 0.05
Vitamin C (mg/100g)	-	40.19 ± 0.25

Note: Values are means of three replicates; (-) not determined.

3.2. Effect of the mixing ratio between PE and PJ on product quality

Sensory evaluation of the formulated beverages showed significant differences in color, aroma, and taste, while appearance did not differ significantly among treatments ($P > 0.05$). At the 9:1 ratio, the low proportion of passion fruit juice yielded a dark red beverage with a pronounced herbal odor characteristic of *P. zeylanica*, slight bitterness, and low overall sensory acceptance. In contrast, formulations with higher passion fruit concentrations (7:3 and 6:4 ratios) produced beverages with a less distinctive flavor of *P. zeylanica,* resulting in an excessively sour taste, and received lower sensory scores. Meanwhile, the 8:2 ratio achieved the highest overall sensory scores, exhibiting a bright red hue and a well-balanced aroma and taste, where the characteristic notes of both *P. zeylanica* and passion fruit were effectively preserved (Figure 1).

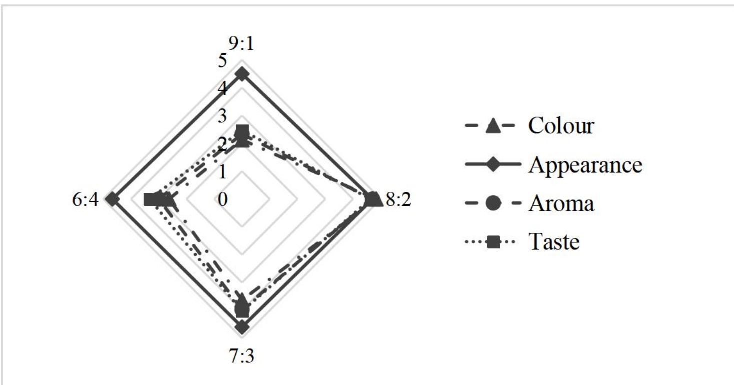

Figure 1. Sensory evaluation of beverages in the different mixing ratios of PE and PJ

The mixing ratio also significantly affected TAC, TPC, titratable acidity, and Vit C (Table 2; $P < 0.001$ for all parameters). As TAC and TPC were mainly derived from *P. zeylanica*, while passion fruit juice provided a major source of vitamin C and organic acids, increasing the proportion of passion fruit juice led to a decline in TAC and TPC alongside a concomitant rise in Vit C and TTA. The 6:4 ratio showed the highest Vit C (13.93 mg/100 mL) and TTA (1.53%). However, this formulation had a pale red color and sour flavor, resulting in low sensory acceptance. In contrast, the 9:1 ratio showed the lowest Vit C content and TTA (6.89 mg/100 mL and 0.37%, respectively), producing a completely dark red beverage dominated by PE with an unbalanced taste profile and low sensory scores.

Table 2. The TAC, TPC, TTA, and Vit C of beverages with different mixing of PE and PJ ratios

Mixing ratio (*PE*: PJ, v/v)	TAC (mg/L)	TPC (mg GAE/mL)	TTA (%)	Vit C (mg/100 mL)
9:1	$2.17^{a} \pm 0.04$	$4.35^{a} \pm 0.02$	$0.37^{d} \pm 0.02$	$6.89^{d} \pm 0.31$
8:2	$1.80^{b} \pm 0.01$	$4.17^{b} \pm 0.03$	$0.73^{c} \pm 0.03$	$8.60^{c} \pm 0.23$
7:3	$1.50^{c} \pm 0.06$	$4.05^{c} \pm 0.03$	$1.05^{b} \pm 0.03$	$11.05^{b} \pm 0.26$
6:4	$1.28^{d} \pm 0.04$	$3.92^{d} \pm 0.02$	$1.53^{a} \pm 0.06$	$13.93^{a} \pm 0.49$

Note: Values are means of three replicates. Different superscript letters in the same column indicate significant differences at $P < 0.05$.

From a product development perspective, the beverage should provide an appropriate balance between sensory quality and nutritional properties. Among the tested formulations, the 8:2 ratio provided the most suitable balance. It achieved the highest sensory scores with a bright red color and a harmonious flavor profile while maintaining relatively high levels of TAC, TPC, TTA, and Vit C. Therefore, the 8:2 ratio was selected as the most suitable formulation for the subsequent experiments.

3.3. Effect of the added sugar and citric acid levels on product quality

The results showed that differences in TAC and TPC among treatments were statistically significant, whereas Vit C was not significantly affected (Figure 2). At the 15% sugar formulation, TAC was highest and decreased progressively as sugar concentrations were increased to 18% and 21%. These findings align with those reported by Doan et al. (2025), anthocyanin losses in litchi juice fortified with roselle calyx, as the increased sugar levels. The decrease in TAC might be associated with sugar degradation products such as furfural and hydroxymethylfurfural, which promote non-enzymatic browning and reduce anthocyanin stability (Song et al., 2023). In contrast, citric acid might contribute to anthocyanin stabilization by maintaining an acidic medium and reducing oxidative deterioration (Nguyen et al., 2016). As of the results, the combination of 15% sugar and 0.05% citric acid yielded the highest TAC (2.21 mg/L), whereas the formulation containing 21% sugar and 0.01% citric acid showed the lowest TAC (1.35 mg/L), reflecting the combined effects of high sugar concentration and low acidity (Figure 2A).

TPC tended to increase with increasing sugar concentration. This trend may be associated with the increase in soluble solids and the resulting protective effect on phenolic compounds. This observation is consistent with the study of Diep and Ngo (2023), who reported progressively higher TPC in jellied Syzygium cumini products supplemented with roselle extract when sugar levels were increased from 40% to 55%. Herein, increasing citric acid concentration from 0.01% to 0.05% contributed to better preservation of TPC across all the same sugar formulations (Figure 2B). These findings are in agreement with the reports of Zhou et al. (2017), who observed improved polyphenol stability in pear juice after citric acid addition, and Özkan (2023), who reported enhanced polyphenol stability in sloe juice supplemented with citric acid.

On the other hand, Vit C had no significant difference among the sugar and citric acid combinations. According to Wang et al. (2017), the degradation of ascorbic acid is largely mediated by the activities of ascorbic acid oxidase and ascorbic acid peroxidase, both of which exhibit peak activity within a pH range of 5 - 7 and are inhibited under more acidic conditions. The beverage prepared from PE and PJ has a low pH (3.02 ± 0.08), which might suppress enzymatic activities, thereby contributing to the relative stability of vitamin C (Figure 2C).

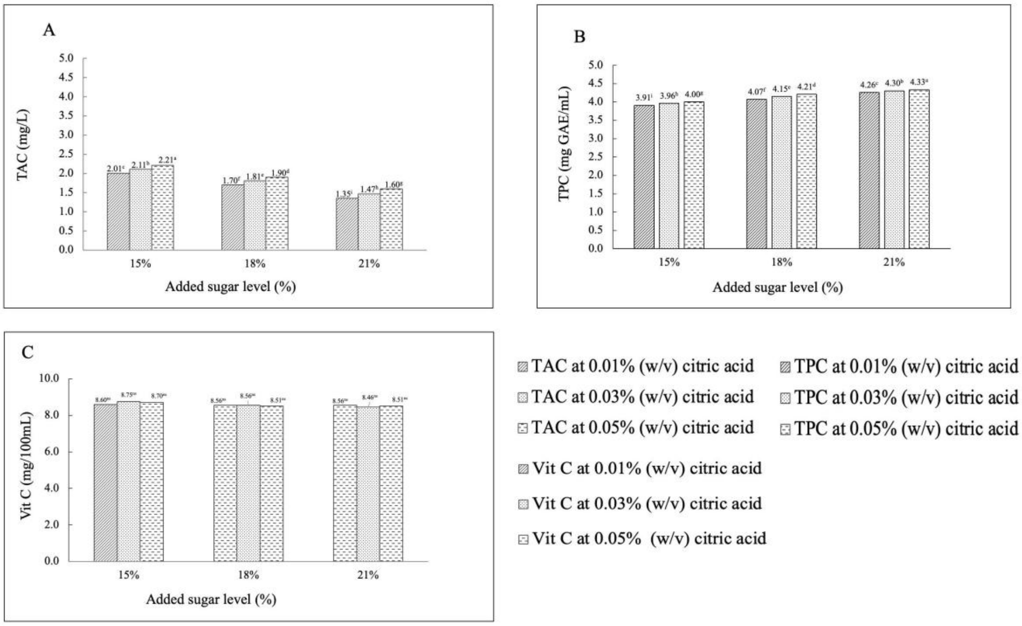

Figure 2. Anthocyanin (A), total polyphenol (B) and vitamin C (C) contents of beverages formulated with different levels of added sugar and citric acid.

Note: Values are means of three replicates. For each parameter, values bearing different superscript letters differ significantly at P < 0.05, whereas ns indicates no significant difference among treatments.

Sensory evaluation results (Figure 3) indicated a clear interaction between sugar and citric acid levels. While color and taste scores were significantly impacted, appearance and aroma remained largely unaffected by variations. At the lowest sugar level (15%), formulations across all citric acid concentrations (0.01 - 0.05%) showed a bright red color but were perceived as excessively sour, resulting in low taste acceptability. Conversely, at the 21% sugar level, the beverages exhibited reduced color intensity and predominantly sweet, weakly acidic taste, resulting in lower sensory scores. The reduction in color intensity at 21% sugar is consistent with the susceptibility of anthocyanins to degradation in high-sugar systems (Diep & Ngo, 2023). Meanwhile, beverages containing 18% sugar retained a vivid red color and exhibited a well-balanced sweet-sour taste, achieving the highest overall sensory scores.

Citric acid addition further influenced both flavor and color stability. In addition to contributing to acidity, citric acid may help improve anthocyanin stability and color retention (Song et al., 2018). At 0.01% citric acid, the beverages had a relatively dull color, suggesting that this level was insufficient to maintain anthocyanin stability effectively. Increasing the citric acid concentration to 0.05% improved color intensity, possibly due to better pigment stabilization. However, the taste became sharply acidic, and the sensory acceptance decreased. The combination of 18% sugar and 0.03% citric acid produced beverages with a bright red color and a well-balanced sweet-sour profile and was therefore considered the most acceptable formulation from a sensory perspective.

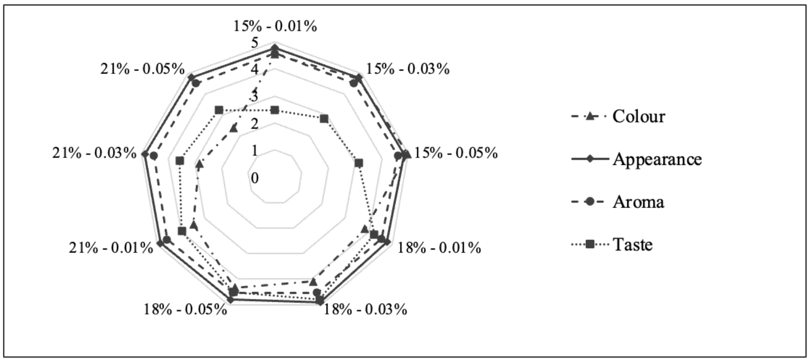

Figure 3. Sensory scores of beverages formulated with different levels of added sugar and citric acid.

3.4. Effect of the pasteurization temperature and holding time on product quality

Due to a pH value of 3.02 ± 0.08, the beverage was classified as a high-acid product. According to Nhan et al. (2017), a reference pasteurization value of 16.7 min at 65 °C with a z value of 5.5 °C can be applied to this type of product. Therefore, in this study, a pasteurization treatment was considered sufficient to meet the microbiological requirement when the calculated pasteurization (PU) value exceeded 16.7 min.

The PU value is an important parameter reflecting the cumulative effect of heat on microbial inactivation in the product. As pasteurization temperature and holding time increase, the PU value also increases, thereby improving microbial control. However, increasing the intensity of heat treatment not only enhances microbiological safety but may also lead to greater losses of heat-sensitive bioactive compounds. The results showed that the treatments at 65 °C (10 and 15 min) and 70 °C yielded PU values greater than 16.7 min, indicating that these treatments met the microbiological safety requirement for the beverage (Table 3). In contrast, the remaining treatments showed PU values below 16.7 min, suggesting that their heat intensity did not reach the reference level for high-acid products.

Table 3. PU of the beverage at different pasteurization temperatures and holding times

Temperature (°C)	Holding time (min)	PU (min)
60	5	1.53
	10	1.97
	15	2.88
65	5	14.33
	10	18.57
	15	22.81
70	5	91.63
	10	147.36
	15	166.01

Note: Values are means of three replicates.

To further evaluate microbiological stability during storage, the pasteurized samples were stored at 4 ± 2 °C for 15 days. Total aerobic microorganisms, yeasts, and molds were periodically determined and compared with the limits specified in QCVN 6-2:2010/BYT for non-alcoholic beverages (total aerobic microorganisms ≤ 10^2 CFU/mL; yeasts and molds ≤ 10^1 CFU/mL).

Table 4. Total aerobic count and total yeast and mold count of the beverage under different pasteurization conditions

Temperature (°C)	Holding time (min)	Total aerobic count (CFU/mL)			Yeasts and molds (CFU/mL)		
		Day 0	Day 8	Day 15	Day 0	Day 8	Day 15
Unpasteurized sample	-	< 1	20.30×10^3	24.65×10^4	< 1	< 1	< 1
60	5	< 1	10.70×10^3	21.25×10^3	< 1	< 1	< 1
	10	< 1	24.45×10^2	14.30×10^3	< 1	< 1	< 1
	15	< 1	9.50×10^1	45.50×10^2	< 1	< 1	< 1
65	5	< 1	2.50×10^1	6.50×10^2	< 1	< 1	< 1
	10	< 1	< 1	< 1	< 1	< 1	< 1
	15	< 1	< 1	< 1	< 1	< 1	< 1
70	5	< 1	< 1	< 1	< 1	< 1	< 1
	10	< 1	< 1	< 1	< 1	< 1	< 1
	15	< 1	< 1	< 1	< 1	< 1	< 1

Note: Values are means of three replicates; < 1 CFU/mL indicates that microbial counts were below the detection limit of the method.

At day 0, no microorganisms were detected in either the unpasteurized or pasteurized samples. By day 8, total aerobic microorganism counts in the unpasteurized sample (20.3×10^3 CFU/mL) and in the samples pasteurized at 60 °C for 5 min (10.70×10^3 CFU/mL) and 60 °C for 10 min (24.45×10^2 CFU/mL) had exceeded the permissible limit of 10^2 CFU/mL (Table 4). By day 15, microbial counts continued to increase, and the treatments at 60 °C for 15 min and 65 °C for 5 min also exceeded the regulatory threshold (Table 4). These results indicate that treatments with low PU values were insufficient to control microbial growth throughout refrigerated storage. In contrast, samples treated at 65 °C for 10 and 15 min and 70 °C for 5-15 min showed no detectable aerobic microorganisms throughout the 15-day storage, aligning with the PU values (>16.7 min) reported in Table 3. This indicates that treatments with higher PU values were more effective in ensuring the microbiological stability of the product. However, high thermal loads may be suboptimal due to the accelerated degradation of heat-labile constituents.

Besides, there was no yeast or mold detected in any sample during the storage period. This result may be associated with the low pH of the beverage (approximately 3.02), which creates unfavorable conditions for its growth. In addition, refrigerated storage at 4 ± 2 °C also suppressed the growth of yeasts and molds during the monitoring period. Thus, the combination of an acidic environment, heat treatment, and low storage temperature likely produced a synergistic effect in controlling microbial growth in the product.

In addition, anthocyanins are heat-sensitive pigments, and increasing temperature may reduce their stability and color retention (Bhalerao & Chakraborty, 2021). The beverage pasteurized at 60 °C for 5 min retained the highest TAC and TPC (2.52 mg/L and 4.49 mg GAE/mL, respectively), while the treatment at 70 °C for 15 min showed the lowest TAC and TPC (1.14 mg/L and 3.72 mg GAE/mL, respectively) (Figure 4A). These findings are consistent with those reported by Nguyen et al. (2021) for *P. zeylanica*-based beverages and by Singh et al. (2022) for grape juice, in which anthocyanin content also decreased as pasteurization temperature increased. Vitamin C is another important nutritional compound, but it is also heat-sensitive and prone to oxidation in liquid systems (Mieszczakowska-Frąc et al., 2021). As shown in Figure 4B, all pasteurization treatments reduced Vit C, and the magnitude of loss increased as temperature and holding time increased. The highest Vit C after pasteurization was observed in the treatment at 60 °C for 5 min (10.46 mg/100 mL), whereas the lowest value was recorded at 70 °C for 15 min (4.45 mg/100 mL). These results are consistent with the findings of Doan et al. (2024) for Hau plum-passion fruit juice and Thanasegaran and Mahror (2025) for red dragon fruit juice, in which increasing pasteurization temperature and time led to progressively greater vitamin C losses.

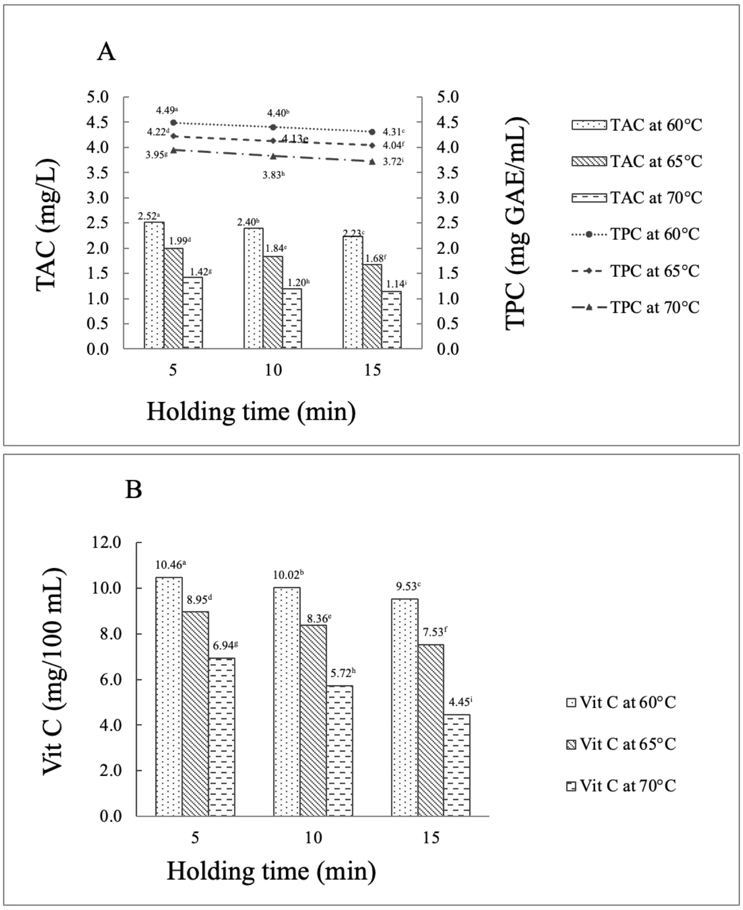

Figure 4. Anthocyanin and total polyphenol (A), and vitamin C (B) contents of beverages at different pasteurization temperatures and holding times.

Note: Values are means of three replicates. For each parameter, values with different superscript letters are significantly different at P < 0.05.

Figure 5. The final *Pouzolzia zeylanica* extract-passion fruit juice product

Overall, pasteurization temperature and holding time had a dual effect on product quality. Pasteurization at 65 °C for 10 min was considered the most suitable treatment within the tested range because it provided a PU value greater than 16.7 min and maintained microbial counts below the detection limit during 15 days of refrigerated storage, while limiting the degradation of heat-sensitive bioactive compounds. The optimized formulation and pasteurization conditions yielded a final beverage characterized by a vibrant, clear red appearance (Figure 5).

4. Conclusion

This study demonstrated that a bioactive-rich beverage can be produced from *Pouzolzia zeylanica* extract and passion fruit juice. Among the experimental formulations, the beverage with an 8:2 (v/v) ratio of PE to PJ, 18% sugar, and 0.03% citric acid had the most suitable balance between sensory quality and retention of bioactive compounds. Pasteurization at 65 °C for 10 min was considered the most suitable condition, as it ensured microbiological stability during refrigerated storage while limiting the losses of anthocyanins, total polyphenols, and vitamin C. These findings support the potential use of *P. zeylanica* and passion fruit as locally available plant resources for the development of value-added functional beverages.

Future work

A limitation of this study is that storage stability was evaluated only for 15 days under refrigerated conditions. Further studies should investigate longer storage periods, consumer acceptance, antioxidant activity during storage, and the stability of color and bioactive compounds under commercial distribution conditions.

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