



Ultrasonic-Assisted Production and Shelf-Life Assessment of Functional Beverage from Purple Rice (*Oryza sativa* L.)

Pornsawan Sombatnan^{1,6}, Yاردfon Tanongkankit^{2*}, Nukrob Narkprasom³, Chanawat Nitatwichit⁴, and Sureewan Rajchasom⁵

¹ Faculty of Engineering and Agro-Industry, Maejo University, Chiang Mai, 50290, Thailand

² Faculty of Engineering and Agro-Industry, Maejo University, Chiang Mai, 50290, Thailand

³ Faculty of Engineering and Agro-Industry, Maejo University, Chiang Mai, 50290, Thailand

⁴ Faculty of Engineering and Agro-Industry, Maejo University, Chiang Mai, 50290, Thailand

⁵ Faculty of Engineering and Agro-Industry, Maejo University, Chiang Mai, 50290, Thailand

⁶ College of Integrated Science and Technology, Rajamangala University of Technology Lanna, Chiang Mai, 50220, Thailand

* Correspondence: yardfon@mju.ac.th

Citation:

Sombatnan, P.; Tanongkankit, Y.; Narkprasom, N.; Nitatwichit, C.; Rajchasom, S. Ultrasonic-assisted production and shelf-life assessment of functional beverage from purple rice (*Oryza sativa* L.). *ASEAN J. Sci. Tech. Report.* **2025**, *28*(1), e255396. <https://doi.org/10.55164/ajstr.v28i1.255396>

Article history:

Received: August 8, 2024

Revised: November 13, 2024

Accepted: November 20, 2024

Available online: February 16, 2025

Publisher's Note:

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Abstract: Finding a new antioxidant-enriched source and understanding a non-thermal technique for functional beverage production is required to expand the range of new functional beverages and enhance their quality. The effects of various parameters of ultrasonic-assisted production (UAP) from purple rice, including the solid-liquid ratio (1:1, 1:2, 1:3, and 1:4.), treatment temperature (30, 40, 50, 60, 70, and 80°C) and treatment time (10, 20, 30, 40, 50 and 60 min) on total anthocyanin content were determined. A comparison between the yield of total anthocyanin obtained by the traditional and ultrasonication methods was performed. Kinetic reaction and coefficient temperature (Q_{10}) were also employed to predict the shelf life of purple rice beverages. Based on our findings, using UAP with the solid-liquid ratio of 1:2 at 60°C for 30 min was the most favorable condition for achieving the highest yield of total anthocyanin with the shortest treatment time. The yield of total anthocyanin in purple rice beverages produced using ultrasonication was 4 times higher than that of traditional methods. The second-order reaction was the best model to describe anthocyanin degradation during storage at temperatures 35–55°C. The predicted half-life of anthocyanin at 5, 15, and 25°C were 397.68, 44.35, and 5.73 days, respectively. The predicted shelf-life calculated using the average Q_{10} value of 1.415 were 12, 9, and 6 days at 5, 15, and 25°C, respectively.

Keywords: Anthocyanin; Half-life time; Kinetic; Ultrasonication; Q_{10}

1. Introduction

The functional beverage market is one of the fastest-growing industries among the functional food market segments. According to the previous report of Gupta et al. [1], the functional beverage market is forecasted to grow at a Compound Annual Growth Rate (CAGR) of 7.5% from 2022–2027, reaching \$208.13 billion. The search for new natural sources of functional beverages and better production techniques to enhance their quality are required for the industry to expand the range of new functional beverages.

Purple rice (*Oryza sativa* L.) is commonly cultivated in North and Northeast Thailand. Its color, from deep purple to black, is exhibited by anthocyanin pigment. Anthocyanin compound is mostly found in the outer grain layers, comprising the pericarp and aleurone layers, accounting for 85% of

the whole grain content [2]. The compound has been reported to be beneficial for human health and protect against free radicals, leading to exceptional properties such as antioxidant and anticancer activity [3]. Purple rice is an interesting option for developing into a functional beverage product because of its benefits. A traditional production process of beverages from purple rice normally begins with rice milling, then boiling it with water for 30 minutes and filtration to obtain clear purple drinks [4]. This traditional method involves thermal treatment that can cause a loss of bioactive compounds, leading to low-quality products. Thus, quality improvement of rice beverages would be concerned with increasing the product's sales and being competitive in national and international markets.

Various non-thermal processing methods, such as pulse electric field, ultrasonication, and high hydrostatic pressure, have been applied in beverage production to intensify bioactive compounds [5, 6]. Among these methods, ultrasonication is an efficient and convenient method. It also requires a relatively low power consumption than other methods. Ultrasonication is performed using ultrasound with a frequency of 20–100 kHz. When ultrasonic waves oscillate through the medium, acoustic cavitation occurs, which is the phenomenon of forming, expanding, and eventual implosive collapse of microbubbles. Consequently, a large amount of energy and shear forces are generated to break cell-wall matrices, thus facilitating the diffusion of target compounds into the solvent [7]. The effect of cavitation on the yield of bioactive compounds in beverages is associated with several factors, such as temperature, frequency, solid and solvent ratio, and ultrasonication duration. As a food product produced by new processing is developed, the shelf life of the food product is investigated to ensure its quality and stability during storage and sales. The indicator of unsatisfactoriness or exceeding the accepted food quality standards can be expressed by microbial spoilage and loss in nutrient content. A kinetic model of nutrient changes and temperature coefficient (Q_{10}) at different temperatures can be evaluated for shelf-life prediction. Therefore, this research aimed to study the effects of ultrasonic-assisted production parameters, including temperature, solid-liquid ratio, and duration of ultrasonication throughout the shelf-life evaluation using kinetic reaction and Q_{10} of this new product.

2. Materials and Methods

2.1 Samples

Purple rice (*Oryza sativa* L.) was provided from the local grinding mill in Doi-Saket, Chiang Mai, Thailand (moisture content was 12% wet basis). Before each experiment, the purple rice was packed in a vacuum plastic bag and stored at room temperature.

2.2 Chemical reagents

Potassium chloride, sodium acetate, and distilled water were purchased from Northern Chemical Co., Ltd. and Union Science Co., Ltd. All chemicals were analytical grade.

2.3 Ultrasonic-assisted processing

Single-factor experiments were conducted in this study. The effects of solid-liquid ratio, temperature, and treatment time on total anthocyanin content were determined by varying the level of one parameter and keeping two parameters constant [8, 9, 10].

2.3.1. effect of solid-liquid ratio on total anthocyanin content

The purple rice (20 g) was added into a 250 mL beaker with 20, 40, 60, and 80 g of distilled water resulting in solid-liquid ratio of 1:1, 1:2, 1:3, and 1:4. The beakers were then placed in ultrasonic bath (Model GT Sonic-D6, Guangzhou, China) with controlled temperature, treatment time, frequency and power of 50°C, 30 min, 40 kHz and 150 W, respectively. After the treatment, the solid was removed, and a transparent purple liquid was kept in an amber glass bottle to determine the total anthocyanin content.

2.3.2. effect of temperature on total anthocyanin content

The beakers containing purple rice and water with a 1:3 ratio were placed in an ultrasonic bath (Model GT Sonic-D6, Guangzhou, China) at 30, 40, 50, 60, 70, and 80°C for 30 min. The constant frequency and power were applied at 40 kHz and 150 W. After the treatment, the solid was removed, and a transparent purple liquid was kept in an amber glass bottle to determine the total anthocyanin content.

2.3.3. effect of treatment time on total anthocyanin content

The beakers containing purple rice and water with a 1:3 ratio were placed in an ultrasonic bath (Model GT Sonic-D6, Guangzhou, China) at 50 °C for 10, 20, 30, 40, 50, and 60 min. The frequency and power were controlled at 40 kHz and 150 W. After the treatment, the solid was removed, and a transparent purple liquid was kept in an amber glass bottle to determine the total anthocyanin content.

2.4 Traditional processing

The purple rice was boiled for 30 min with a 1:3 ratio between purple rice and water, followed by filtration with a cheesecloth. The solid was removed, and a transparent purple liquid was kept in an amber glass bottle for further analysis.

2.5 Total anthocyanin content

The total anthocyanin content of the purple rice extract was determined by the pH differential method [11]. The purple rice extract was diluted tenfold with distilled water and centrifuged at 3,000 rpm for 15 min (Hettich zentrifugen, EBA 20, Baden Württemberg, Germany). Afterward, 200 µL of the supernatant was collected and added with 2 mL of potassium chloride buffer (pH 1.0) and 2 mL of sodium acetate buffer solution (pH 4.5). The mixture solution was incubated in the dark at ambient temperature for 30 min. The absorbance (A) was measured by UV-Vis spectrophotometer (Spectrum Instruments, SP-UV 200 spectrophotometer, Grosshansdorf, Germany). The incubated mixture solution was measured at 510 and 700 nm, respectively. Distilled water was served as a blank. Then, the absorbance of the solution was used to calculate the total anthocyanin content using the following equation (1).

$$\text{Total anthocyanin content (mg/g)} = \frac{A_{\text{diff}} \times M_w \times df \times 1,000}{\epsilon} \quad (1)$$

where A_{diff} is $(A_{510\text{nm}} - A_{700\text{nm}})_{\text{pH 1.0}} - (A_{510\text{nm}} - A_{700\text{nm}})_{\text{pH 4.5}}$, M_w is the molecular weight of cyanidin-3-glucoside (449.2 g/mol), ϵ is the molar absorptivity ($26,900 \text{ M}^{-1}\text{cm}^{-1}$), and df is the dilution factor.

2.6 Shelf-life evaluation

The purple rice beverages produced using ultrasonication with the suggested condition were pasteurized by heating at 60°C for 15 min and contained in an amber glass bottle. The samples were then stored in an incubator (Binder, BD 115, Herrenberg, Germany) with accelerated temperatures at 35, 45 and 55°C temperatures and subjected to total anthocyanin content and pH analysis on days 0, 1, 3, and 7.

2.6.1 Kinetic reaction

The changes in total anthocyanin content during storage could be expressed in zero-, first- and second-order as shown in equations (2), (3), and (4), respectively. The order of the kinetic reaction was investigated by performing regression with the ideal coefficient of determination (R^2) [12].

$$\text{Zero order; } C_A - C_{A0} = -kt \quad (2)$$

$$\text{First order; } -\ln(C_A/C_{A0}) = kt \quad (3)$$

$$\text{Second order; } 1/C_A - 1/C_{A0} = kt \quad (4)$$

where k = the rate constant, C_A = total anthocyanin content (mg/g) at the storage time, C_{A0} = initial value of total anthocyanin content (mg/g), and t is storage time (days)

After the reaction order was determined, the dependence of the degradation rate constant on temperature was determined by applying the Arrhenius equation as shown in equation (5). The activation energy was obtained by plotting a $\ln k$ against the reciprocal of the absolute temperature ($1/T$), and the slope of the linear graph was equivalent to $-E_a/RT$ [13, 14]. The half-life indicating the time needed for 50% degradation of anthocyanins during storage was also calculated.

$$\ln k = \ln k_0 - \frac{E_a}{RT} \quad (5)$$

where k = rate constant, k_0 = Arrhenius constant, R = gas constant (8.314 J/mol.K), T = temperature (K), and E_a = activation energy (J/mol).

2.6.2 Temperature coefficient (Q_{10})

Shef-life evaluation can be determined to estimate the temperature's effect on the reaction rate increase by 10°C, which is called the Q_{10} value. The Q_{10} value and the predicted shelf life were calculated following equation (6) [15]. The shelf-life endpoint of the beverage was defined when the pH value was below 4.5, which was the critical point of pH in beverage products [16].

$$Q^{\Delta T} = \frac{Q_{s(T)}}{Q_{s(T+\Delta T)}} \quad (6)$$

where $Q_{s(T)}$ = shelf life at $T^{\circ}\text{C}$ and $Q_{s(T+\Delta T)}$ = shelf life at $T+\Delta T^{\circ}\text{C}$.

2.7 Statistical analysis

The data were subjected to an analysis of variance (ANOVA) and presented as mean values with standard deviations. Differences between mean values were analyzed using Duncan's multiple-range tests. Values were considered at a confidence level of 95%. All statistical analyses were performed using SPSS® software (version 29) (SPSS Inc., Chicago, IL). All experiments were performed in triplicate. The constant values from the curve-fitting model and the highest coefficients of determination (R^2 values) were determined using Microsoft Excel.

3. Results and Discussion

3.1 Effect of solid-liquid ratio on total anthocyanin content during ultrasonic-assisted processing

Figure 1 shows the total anthocyanin content at different solid-liquid ratios during ultrasonic-assisted processing. It was found that an increase in the ratio of purple rice and water from 1:1 to 1:2 and 1:3 resulted in a slight increase in the total anthocyanin content. However, when the purple rice and water ratio reached 1:4, the total anthocyanin content significantly decreased ($p<0.05$). This might be because, at the very low solid-liquid ratio, the viscosity of the solution was high, leading to difficulty in the cavitation effect. With the higher amount of water, the solution's viscosity was reduced, resulting in a more significant cavitation effect and enhancement of the diffusivity and dissolution of the solute into the solvent. On the other hand, at a very high solid-liquid ratio, the decrease in anthocyanin yield can be caused by the extreme formation of the cavitation effect, which leads to the degradation of the compound itself [17]. This result was agreed with Rittisak et al. [18], who reported that an increase in the yield of anthocyanin extracted from broken riceberry rice occurred as changing solid-liquid ratio from 1:2.5 to 1:5. The further increase in the solid-liquid ratio to 1:7.5 resulted in a significant decrease in the yield of anthocyanin.

3.2 Effect of temperature on total anthocyanin content during ultrasonic-assisted processing

As shown in Figure 2, the results revealed that rising treatment temperature initially increased total anthocyanin content. Then, as the temperature approached 60°C, the total anthocyanin content had no significant difference ($p<0.05$). However, a slight decrease in total anthocyanin content was noted when the temperature exceeded 70°C. This could be because cavitation bubbles formed abundantly at high temperatures. Upon their subsequent collapse, many shear forces were generated to improve the cell wall fragmentation, leading to a higher anthocyanin yield. The further increase in temperature promoted many cavitation bubbles, causing thermal degradation and degradation of the desirable compound. Additionally, a decrease in surface tension of solvent at higher temperatures resulted in the lower intensity of collapse of the cavitation bubble, hence reducing the mass transfer of the component to be extracted [17]. A similar pattern of the changes in the total anthocyanin content at different temperatures was reported by Thuy et al. [19]. Their results showed that the total anthocyanin content of the butterfly pea flower extract using ultrasonication increased when the temperature increased from 60°C to 70°C and tended to decrease when the temperature reached 80°C.

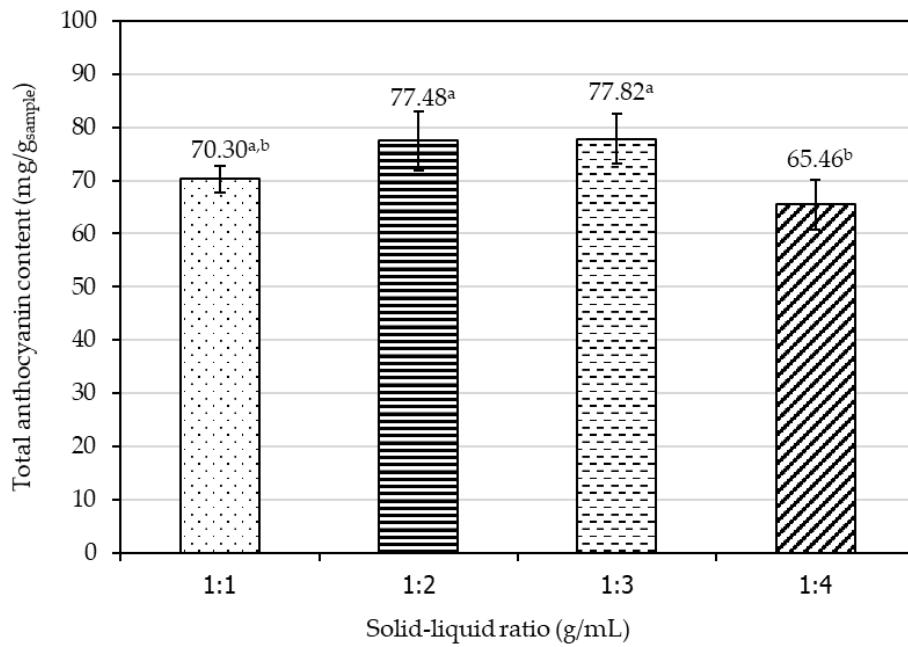


Figure 1. Total anthocyanin content at different solid-liquid ratios. Data are mean values with error bars indicating \pm SD. Different lowercase letters indicate a significant difference ($p<0.05$).

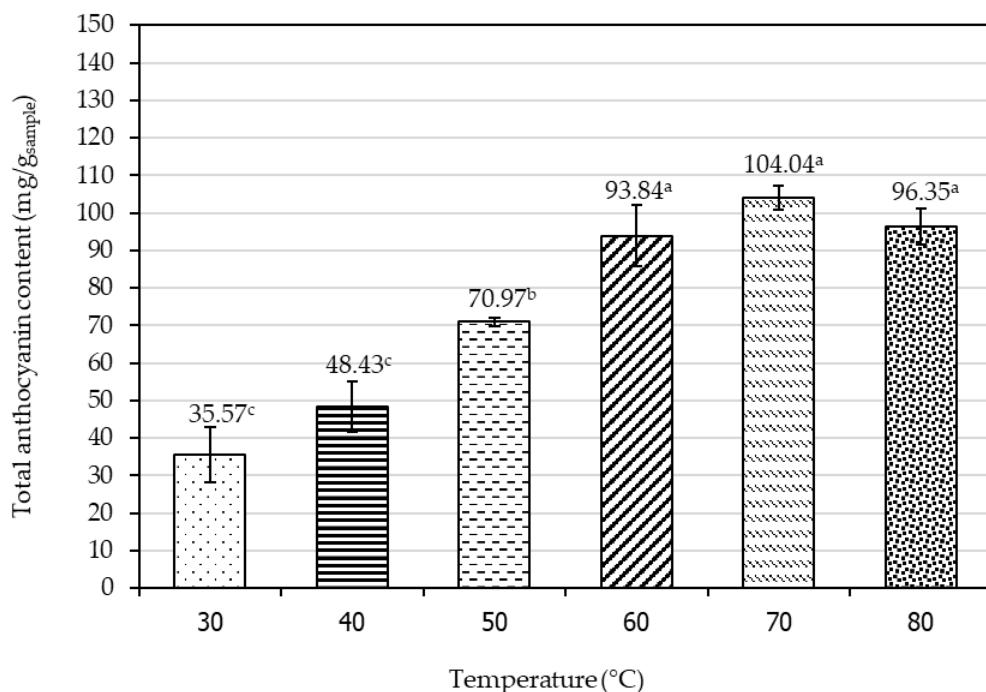


Figure 2. Total anthocyanin content at different treatment temperatures. Data are mean values with error bars indicating \pm SD. Different lowercase letters indicate a significant difference ($p<0.05$).

3.3 Effect of treatment time on total anthocyanin content during ultrasonic-assisted processing

Figure 3 illustrates the effect of treatment time on the yield of total anthocyanin. It was observed that the pattern of change in total anthocyanin content is similar to the effect of temperature. An increase in treatment time initially resulted in a higher yield of total anthocyanin, and thereafter, the yield slightly decreased on prolonged treatment time. The initial increase in treatment time could explain this. The cavitation effect of ultrasound enhanced the swelling, hydration, fragmentation, and pore formation of the plant

tissue matrix, facilitating the diffusion of target compounds into the solvent. Nevertheless, the extended exposure time caused structural damage to the solute, and the yield of total anthocyanin declined. Similarly, Lui et al. [20] reported that the yield of anthocyanins markedly increased with longer extraction time using ultrasonication and reached the peak value at 40 min. However, a decrease in the anthocyanin content was observed when the extraction time was prolonged over 40 min.

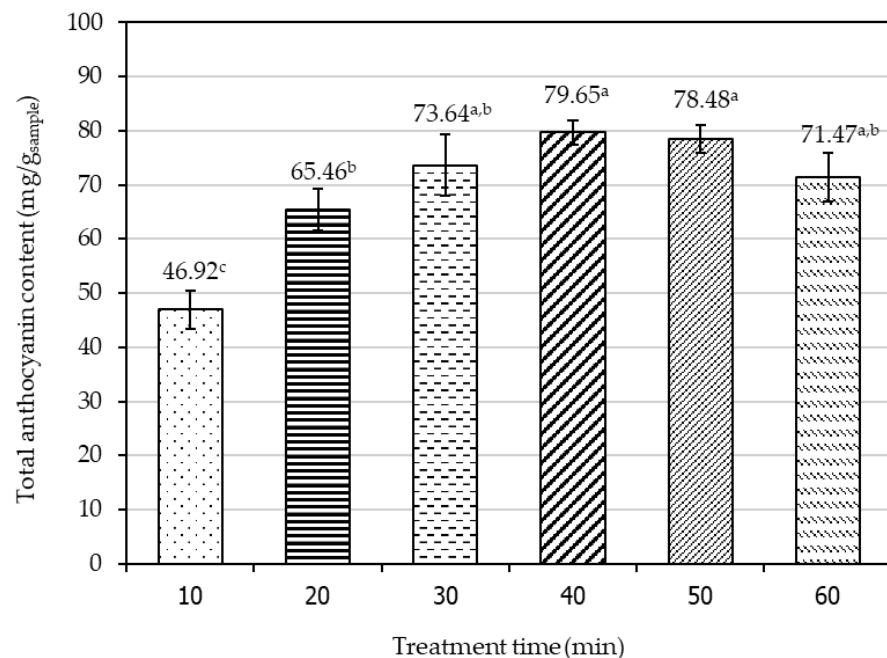


Figure 3. Total anthocyanin content at different treatment times. Data are mean values with error bars indicating \pm SD. Different lowercase letters indicate a significant difference ($p<0.05$).

In summary, the significant factors affecting the total anthocyanin content during ultrasonic-assisted processing were the solid-liquid ratio, treatment temperature, and time. To achieve the maximum total anthocyanin content alongside the lowest operating costs, ultrasonication with a solid-liquid ratio of 1:2 at 60°C for 30 min was suggested to be the most favorable condition. Moreover, a comparison of the total anthocyanin content using ultrasonic-assisted processing with the suggested condition and traditional processing was investigated, as seen in Figure 4. It was observed that ultrasonication provided higher total anthocyanin content than traditional processing. This might be because the thermal degradation of the anthocyanin compound occurred during traditional processing [21]. Hence, ultrasonication with a solid-liquid ratio of 1:2 at 60°C for 30 min was selected for further shelf-life evaluation.

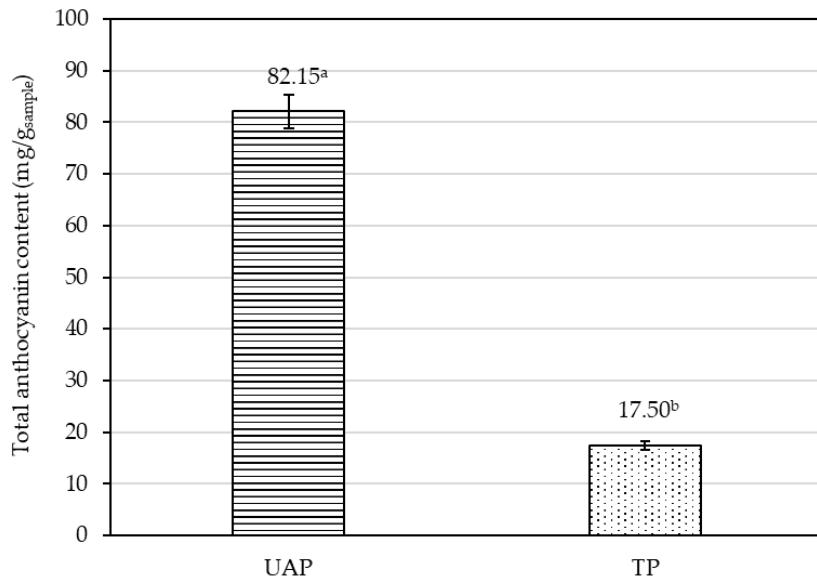


Figure 4. Total anthocyanin content undergoing ultrasonic assisted processing (UAP) and traditional processing (TP). Data are mean values with error bars indicating \pm SD. Different lowercase letters indicate a significant difference ($p<0.05$).

3.4 Shelf-life evaluation

3.4.1 Kinetic reaction

Figure 5 illustrates variations in total anthocyanin content during the storage of purple rice beverages at different temperatures. It was found that the overall trend was a decrease in total anthocyanin content with the extension of storage time. The results also revealed that higher storage temperatures lowered total anthocyanin content as expected. Interestingly, a sharp decrease in total anthocyanin content by 40% and 76% was exhibited at 45 and 55°C, respectively, after 1 day of storage. The results are consistent with those of Muche et al. [22], who found that total anthocyanin content in grape juice decreased considerably with longer storage time. More than 70% loss of total anthocyanin content in the grape juice was also noted at storage temperatures of 25°C and 35°C.

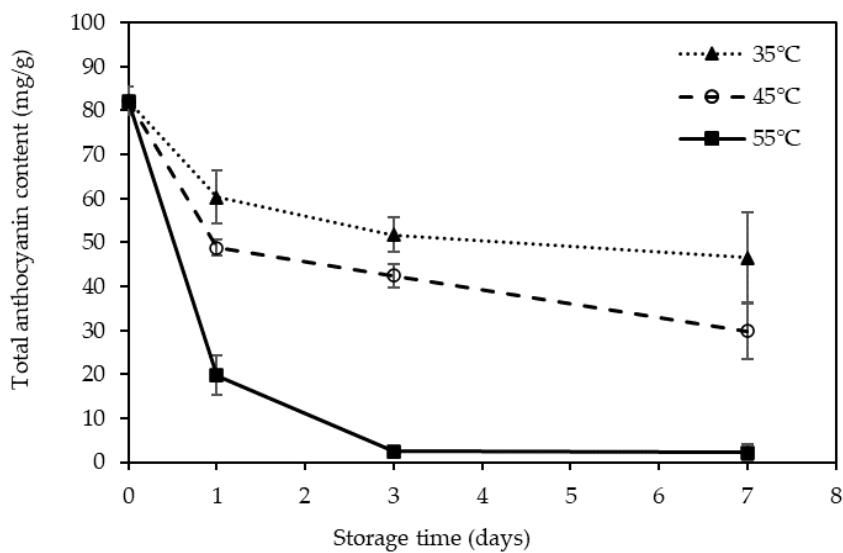


Figure 5. Changes in total anthocyanin content in purple rice beverages during storage at different temperatures.

The kinetic parameters of zero-, first- and second reaction and coefficient determination (R^2) at different storage temperatures were summarized in Table 1. It was clear that higher storage temperatures resulted in greater k values, indicating faster anthocyanin degradation. The reaction order was determined based on the highest determination coefficient representing a better-fitting modeling result. It was found that anthocyanin degradation during storage followed the second-order reaction with the highest R^2 value in the range of 0.992-0.999. The results were similar to a previous study by Ng and Cheok [23], who reported that the order reaction model represented the degradation of total anthocyanins extracted from *Garcinia Mangostana* L. rind with the highest value of coefficient determination in comparison to zero- and first- order.

Table 1. Kinetic parameters and coefficient determination (R^2)

Order	Temperature (°C)	k	R^2
Zero-order	35	4.201	0.685
	45	6.044	0.703
	55	8.702	0.506
First order	35	0.073	0.811
	45	0.074	0.996
	55	0.036	0.580
Second order	35	0.023	0.999
	45	0.038	0.987
	55	0.772	0.992

The second-order kinetic data were then used for determining Arrhenius parameters, including activation energy (E_a) and k_0 value representing the dependence of the degradation rate constant on temperature. Figure 6 shows the Arrhenius plot of the $\ln k$ value with $1/T$. It was found that the activation energy and the k_0 value were 1.46×10^5 J/mol and 9.074×10^{22} d⁻¹. The predicted half-life time of anthocyanins during storage at ambient (25°C) and refrigerated temperatures (5 and 15°C) were subsequently determined. As seen in Table 2, the results indicated that anthocyanins could be preserved for more than a month under storage at refrigerated temperature.

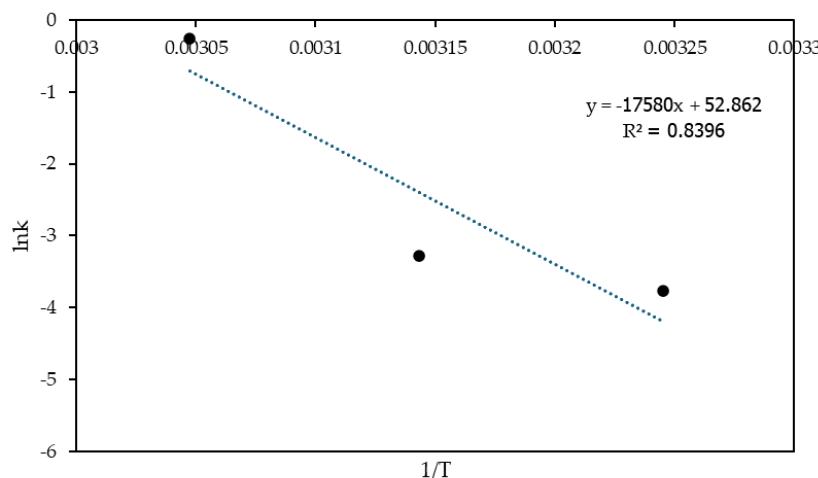


Figure 6. Relationship between the value of $1/T$ to $\ln k$.

Table 2. The predicted half-life time of anthocyanins

Temperature (°C)	K (day ⁻¹)	Half-life time (days)
5	3.226×10^{-5}	397.68
15	2.892×10^{-4}	44.35
25	2.238×10^{-3}	5.73

3.4.2 Temperature coefficient (Q_{10})

Changing the pH value of beverages can be used as an index for microorganism spoilage [24]. The unacceptable point for low-acidity beverages was defined as their pH values were below 4.5 [16, 24]. Figure 7 presents the pH values of purple rice beverages during storage at different temperatures. It can be seen that the pH values of samples were lower than 4.5 on days 4, 3, and 2 of storage at 35, 45, and 55°C, respectively. Hence, the Q_{10} value would be 1.50 and 1.33 when determined at temperatures from 35 to 45°C and 45 to 55°C, respectively. The average Q_{10} value of 1.415 and the storage temperature of 45°C were used to evaluate the predicted shelf life at different temperatures, as shown in Table 3.

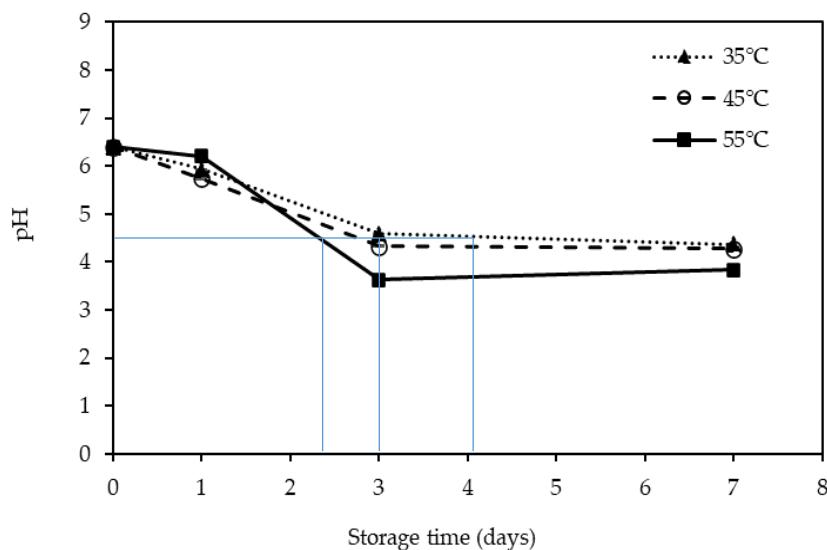


Figure 7. Changes in pH values of purple rice beverages during storage at different temperatures.

Table 3. The predicted shelf-life determined at different temperatures

Temperature (°C)	Predicted shelf life (days)
5	12
15	9
25	6

Overall, the shelf life of the purple rice beverages could be evaluated using the kinetic reaction of anthocyanin degradation and Q_{10} . Notably, the half-life times obtained from the kinetic reaction exhibited a more extended period when compared to the predicted shelf life calculated from Q_{10} . To achieve food security, the shelf life of the purple rice beverages was thus considered based on the Q_{10} since the pH value indicated spoilage of the beverages.

4. Conclusions

The effects of ultrasonic-assisted production parameters, including solid-liquid ratio, treatment temperature and time, were investigated to enhance the quality of purple rice beverages. A comparison of total anthocyanin content in purple rice beverages produced by ultrasonication and traditional methods was also determined. Furthermore, the predicted shelf life using the kinetic reaction of anthocyanin degradation and Q_{10} was evaluated under accelerated temperature. Our finding indicated that higher total anthocyanin content occurred when the solid-liquid ratio increased from 1:1 to 1:2 and reached a constant value at the solid-liquid ratio of 1:3. Once the solid-liquid ratio was 1:4, the total anthocyanin content significantly decreased. The treatment temperature had a significant effect on the total anthocyanin content. Increasing treatment temperatures resulted in higher total anthocyanin content and then gradually dropped when the temperature exceeded 70°C. Similarly, an extended treatment time led to a higher total anthocyanin content before the slight decrease in the total anthocyanin content exhibited at 60 min of treatment time. Based on the findings,

it could be concluded that using ultrasonic-assisted production with the solid-liquid ratio of 1:2 at 60°C for 30 min was suggested. In addition, a higher total anthocyanin content was obtained when employing the ultrasonication method under the suggested condition than when employing a traditional method. For the shelf-life evaluation, the degradation kinetics of total anthocyanin during storage at 35, 45, and 55°C followed the second-order reaction. The predicted half-life values at 5, 15 and 25°C were 397.68, 44.35 and 5.73 days, respectively. The predicted shelf life was also determined using the average Q_{10} value of 1.415. It was found that the shelf life of the purple rice beverages at 5, 15, and 25°C were 12, 9, and 6 days, respectively. Sensory and microbial analysis is recommended for future work to assess shelf life more accurately.

Author Contributions: Conceptualization, P.S. and Y.T.; methodology, P.S., Y.T., N.N. and C.N.; formal analysis, P.S.; investigation, P.S.; resources, S.R.; data curation, P.S.; writing—original draft preparation, P.S. and Y.T.; writing—review and editing, P.S. and Y.T.; visualization, Y.T.; supervision, Y.T.; project administration, Y.T.; funding acquisition, S.R.

Funding: This research was funded by Thailand Science Research and Innovation (TSRI) in 2022.

Conflicts of Interest: The authors declare no conflict of interest.

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