ISSN: 1410-8917
e-ISSN: 2597-9914

Jurnal Kimia Sains dan Aplikasi 27 (11) (2024): 549-559

Jurnal Kimia Sains dan Aplikasi
Journal of Scientific and Applied Chemistry

Journal homepage: http://ejournal.undip.ac.id/index.php/ksa

Extraction of Natural Colorant from *Clitoria ternatea* Flowers Using Conventional Solvent Extraction (CSE) and Ultrasound-Assisted Extraction (UAE) Techniques: Kinetic Modeling and Compound Stability

Pra Cipta Buana Wahyu Mustika [1], Hadiyatni Rita Priyantini [1], Eryan Dwi Krisna [1], Firman Aldani [1], Fikrah Dian Indrawati Sawali [2], Moh. Azhar Afandy [2], Mega Mustikaningrum [3,*]

[1] Chemical Engineering Department, Faculty of Engineering, Universitas Surabaya, Jl. Raya Kalirungkut, Surabaya, 60293, Indonesia
[2] Minerals Chemical Engineering, Politeknik Industri Logam Morowali, Morowali, 94974, Indonesia
[3] Chemical Engineering Department, Faculty of Engineering, Universitas Muhammadiyah Gresik, Gresik, 61121, Indonesia

* Corresponding author: megamustikaningrum@umg.ac.id

https://doi.org/10.14710/jksa.27.11.549-559

Article Info

Article history:

Received: 19th June 2024
Revised: 29th November 2024
Accepted: 29th November 2024
Online: 30th November 2024

Keywords:
Anthocyanin; ultrasonic extraction; extraction rate constant

Abstract

The *Clitoria ternatea* flowers, a prevalent local commodity in Indonesia, are extensively found, particularly in the East Java region. One approach to enhance the utility and economic significance of these flowers involves isolating their active component, specifically anthocyanin. Anthocyanins have several health benefits, especially in preventing cardiovascular disease, improving vision, and being anti-diabetic, anti-inflammatory, and anti-cancer. The isolation methods used in this research were conventional solvent extraction (CSE) and ultrasonic-assisted extraction (UEA) methods. The use of UAE has been experimentally proven to accelerate the extraction rate of bioactive compounds. This dal is caused by a driving force in the form of energy produced from bubble cavitation resulting from ultrasonic energy. The specific aim of this study was to compare the effectiveness of the two methods in anthocyanin isolation. The extraction stages were carried out at 30, 40, 50, 60, and 70°C operating temperatures, with an S/L ratio of 1:10, 1:15, 1:20:1:25, and 1:30 with samples conditioned in dry and wet conditions. The optimal conditions for ultrasound-assisted extraction (UEA) involve dry samples with a S/L ratio of 1:30 at a temperature of 70°C with the resulting final concentration of 16.5234 g/L. This configuration ensures an efficient extraction process, completed in less than 30 minutes, thereby preventing the degradation of anthocyanins. Analysis indicates that the extraction process adheres to a second-order kinetic model with a constant (k) of 0.1039. Stability testing revealed that the first–order kinetic model accurately represents the impact of temperature on anthocyanin degradation.

1. Introduction

Food colorants are crucial in modifying or imparting hues to food to enhance its appeal to consumers [1]. Food coloring is categorized into natural and artificial types based on its source of origin. Artificial colorant are typically synthesized from derivatives of coal tar and predominantly comprise azo groups, whereas natural colorant are obtained through extraction from plants, animals, and microorganisms [2, 3, 4]. Presently, the utilization of artificial colorant in the food industry is subject to extensive deliberation, particularly concerning the potential hazards to both short-term and long-term human health. These risks encompass metabolic disturbances, induction of cellular mutations leading to carcinogenesis, gastrointestinal toxicity, respiratory ailments, and various additional adverse effects [5]. Beneath these circumstances, natural colorant are purported to exhibit a diminished level of risk and offer potential health advantages. Natural food colorant

frequently encompass pigments such as anthocyanins, carotenoids, chlorophyll, and various other bioactive compounds [6]. A multitude of scientific studies have consistently reported the potential health benefits associated with anthocyanins, in addition to their role as natural colorants [7, 8, 9]. As a result, the acquisition of natural colorant for utilization as natural colorants has emerged as a growing field of research [10].

Nevertheless, at an industrial scale, the extraction of natural colorant encounters numerous challenges, primarily arising from their inherent instability under elevated temperatures, pH variations, bacterial presence, oxidative processes, and other factors [11, 12]. Furthermore, challenges emerge from the limited accessibility of relatively expensive natural raw materials, necessitating the prioritization of appropriate extraction techniques to minimize production costs. Several established techniques employed in the extraction process encompass conventional solvent extraction (CSE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and various others [13]. From a technical perspective, conventional solvent extraction (CRE) encounters challenges such as elevated solvent consumption and lengthy processing times. Moreover, these prolonged processing times can lead to detrimental degradation of the targeted bioactive components intended for extraction. Given these limitations, the constraints have driven the exploration of alternative techniques, leading to the development of innovative approaches that serve as substitutes for conventional solvent extraction (CRE).

Among these alternatives, ultrasound-assisted extraction (UAE) is a particularly promising approach. The application of ultrasound induces enhanced solvent penetration into the material matrix, concomitant with the disruption of the biological cell wall. Additionally, ultrasound contributes to reduced energy consumption during the process, owing to the mechanical momentum facilitating matrix breakdown more efficiently than thermal effects. Notwithstanding the inherent advantages of ultrasound-assisted extraction (UEA), optimizing this process necessitates meticulous consideration of critical parameters, including time, temperature, solid-liquid ratio, and ultrasound power. Moreover, it is important to consider the control factors that influence the raw materials' physical properties. Several previous studies have tried to explore various applications of extraction technology, especially for natural colorant, which in this research are natural anthocyanin compounds (Table 1).

This research aims to conduct a comprehensive investigation into the recovery of color compounds, specifically focusing on anthocyanins, from *Clitoria ternatea* flowers using two distinct techniques: conventional solvent extraction (CSE) and ultrasound-assisted extraction (UAE). Several independent parameters are used in research, such as solid-liquid ratio, temperature, and raw material conditions. These parameters are anticipated to offer insights to address the limitations inherent in yield considerations and the maximum achieved concentrations outlined in Table 1.

2. Experimental

2.1. Raw Material and Chemicals

The raw materials, *Clitoria ternatea* flowers, were procured from a local market in Surabaya, East Java, Indonesia. The floral crowns were isolated from the rest of the flower material and then further divided into wet and dry portions for subsequent processing. The floral crowns that were previously separated underwent a drying process using a circulating oven at a temperature of 60°C for 24 hours (Memmert GmbH, UF55). Subsequently, the dry ingredients were subjected to grinding (Philips HR1393/90 Chopper Daily) and sieving (Retsch, Mesh 40). Conversely, the remaining portion underwent direct grinding and sieving without drying. Moreover, dry and wet materials were stored in hermetically sealed containers, ensuring protection from direct sunlight exposure.

High-purity water, serving as the solvent, is obtained through the purification of raw water using a reverse osmosis (RO) system, resulting in the attainment of high-purity water with a conductivity of approximately ±15 µS. The reagents employed for measuring the total anthocyanin content comprised potassium chloride (KCl), hydrochloric acid (HCl), and sodium acetate (CH_3COONa), all of which were sourced from Sigma-Aldrich (Singapore). All reagents used in this research were of analytical grade, eliminating the need for additional purification steps.

2.2. Extraction Experiments

2.2.1. Conventional Solvent Extraction (CSE)

The conventional solvent extraction (CSE) research incorporated two methods: (1) maceration and (2) Soxhlet extraction. Maceration was conducted using a constant solvent volume of 200 mL, with the solid/liquid ratio varied across five levels: 1:10, 1:15, 1:20, 1:25, and 1:30 (For example, 1 g of solid in 10 mL of solvent, and so on for all variations). The maceration process lasted 24 hours with continuous stirring at 300 rpm at room temperature (28±3°C). The Soxhlet method employed the same solid/liquid ratios as the maceration method, with a duration of 2 hours. However, the evaporation process in the Soxhlet extraction featured temperature variations in the cycle, specifically at 40, 50, 60, and 70°C. Following the extraction methods, both techniques involved the separation of solid and liquid phases using vacuum filtration (Hawach, BIO-1-090). The liquid phase was subsequently concentrated using a rotary evaporator (B-One, RE-2000A) at 40°C with a rotation speed of 40 rpm for 2 hours.

Table 1. Several research studies are on extracting natural colorant (anthocyanins) using various methods

Extraction method	Optimum operating conditions		Amount of anthocyanin	Anthocyanin content (mg cyanidin-3-glucoside/g)	Ref.
Maceration-assisted by heat (CRE)	Solvent = Water S/L ratio = 1:20	Temperature = 60°C Time = 60 min	56.1%	4.0	[14]
Maceration-assisted by heat (CRE)	Solvent = Water S/L ratio = 0.125:25	Temperature = 40°C Time = 30 min	2%	-	[15]
Microwave-assisted extraction (MAE)	Solvent = Water S/L ratio = 1:20	Microwave power = 770 W Time = 1 min	-	30.9	[16]
Ultrasound-assisted extraction (UEA)	Solvent = Water S/L ratio = 1:20	Temperature = room temperature Time = 60 min Ultrasonic power = 560 W	36.1%	4.2	[14]
Maceration (CRE)	Solvent = Water S/L ratio = 1:16.67	Time = 24 hours		4,841	[17]
Maceration-assisted by heat (CRE)	Solvent = Water S/L ratio = 1:20	Temperature = 60°C Time = 20 min		0.058	[18]
Maceration-assisted by heat (CRE)	Solvent = Ethanol S/L ratio = 1:23	Temperature = 60.6°C Time = 46 min	132.756 mg/L	-	[19]
Maceration-assisted by heat (CRE)	Solvent = Water S/L ratio = 1:37	Temperature = 54°C Time = 74 min	45.5%	-	[20]
Microwave-assisted extraction (MAE)	Solvent = Ethanol S/L ratio = 1:15	Temperature = 60°C Time = 15 min	49.97%	-	[21]
Maceration-assisted by heat (CRE)	Solvent = Water S/L ratio = 1:33.33	Temperature = 60°C	11.6 g/mL	-	[22]
Maceration-assisted by heat (CRE)	Solvent = Water S/L ratio = 1:20	Temperature = 70°C Time = 45 min	34.98 mg/L	-	[23]
Ultrasound-assisted extraction (UEA)	Solvent = Water	Temperature = 74°C Time = 56.88 min S/L ratio = 1:20 Ultrasonic power = 490 W	15.01 % 39.90 mg/L	-	[23]
Microwave-assisted extraction (MAE)	Solvent = Water S/L ratio = 1:20	Microwave power = 400 W Time = 5 minutes	14.11 % 39.34 mg/L	-	[23]
Maceration-assisted by heat (CRE)	Solvent = Water S/L ratio = 1: 20	Time = 60 min Temperature = 60°C	71.1 %	4.88	[24]
Pectinase Extraction	Solvent = Water Pectinase 3% (w/v) S/L ratio = 1: 15	Time = 30 min Temperature = 50°C	27.8 %	0.62	[24]
Ultrasound-assisted extraction (UEA)	Solvent = Water S/L ratio = 1: 15	Time = 30 min Temperature = 50°C	40.3 %	7.00	[24]
Microwave-assisted extraction (MAE)	Solvent = Water S/L ratio = 1: 15	Time = 30 min Temperature = 50°C	37.2 %	9.61	[24]

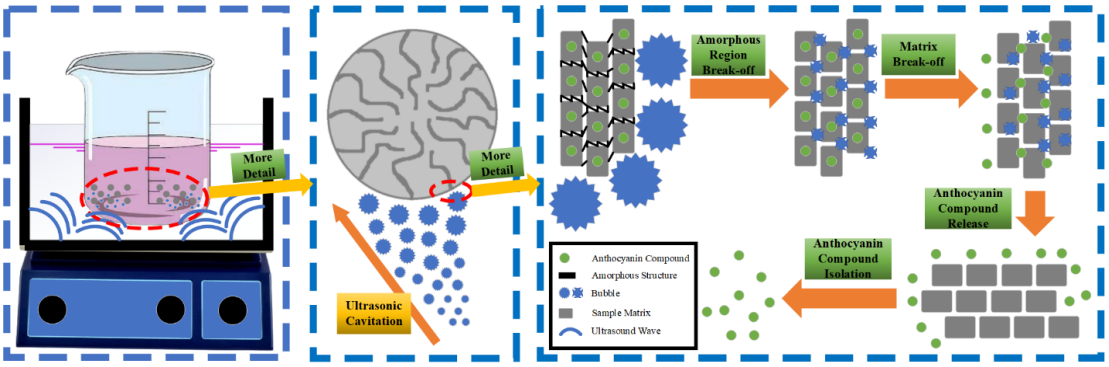

Figure 1. Detailed mechanistic phenomena of ultrasound-assisted anthocyanin extraction

2.2.2. Ultrasound-assisted Extraction (UAE)

Ultrasound-assisted extraction (UAE) is conducted using an ultrasonic apparatus (Elma Schmidbauer GmbH, Elmasonic S30H [Ultrasonic frequency = 37 kHz; Ultrasonic power effective = 80 W]), with operating temperatures varied similarly to those employed in Soxhlet extraction. However, in UEA, external heat energy is added during the process, necessitating careful consideration of the operating temperature. In this case, the set operating temperature aligns precisely with CSE. Nonetheless, the UEA process is shortened to 30 minutes. Specifically, a longer extraction process of 4 hours was used for kinetic studies. Following the extraction, the subsequent steps involve solid-liquid separation, which is performed like CSE procedures.

2.3. Total Monomeric Anthocyanins

The total anthocyanin content was determined using the classical pH differential method, and absorbance readings were taken with high-purity water as the control for baseline comparison [25]. The total monomeric anthocyanin (MA) content was determined by measuring the extract solution's UV – Vis absorption using a Thermo Scientific Genesys 10S spectrophotometer. The MA species is specifically represented by cyanidin-3-glucoside, characterized by a molar extinction coefficient (26900 $L.cm^{-1}.mol^{-1}$) and a molecular weight (449.2 $g.mol^{-1}$). The total MA content was determined by measuring the extract solution's UV-Vis absorption (A). Sample analysis was conducted at a wavelength (λ) of 510 nm under two distinct pH conditions, pH 1.0 and pH 4.5. Additionally, standard measurements were performed at λ = 700 nm, and the final values were determined using Equations (1) and (2).

$$R_a = \frac{m_{a/L}}{m_{a/S}} = \frac{C_{a/L} m_L}{C_{a/S} m_S} \quad (1)$$

Where, $m_{a/L}$ and $m_{a/s}$ denote the overall mass of monomeric anthocyanins, while $C_{a/L}$ and $C_{a/S}$ refer to the concentrations of anthocyanins in liquid and solid samples, respectively. Furthermore, m_L and m_S represent the mass of a liquid and a solid, respectively.

2.4. Natural Colorant Stability

2.4.1. Thermal Stability

A set of concentrated natural colorant samples with previously determined concentrations was stored and distributed into four test jars. Stability tests were conducted at temperatures of 10, 40, 50, and room temperature (28±3°C). Moreover, each test jar was covered with aluminum foil to shield it from light exposure. Sampling was carried out at certain time intervals, and total MA was measured.

2.4.2. UV-C Light Exposure Stability

A set of concentrated natural colorant samples with previously determined concentrations were stored and distributed into a single jar. Subsequently, the jar was placed inside an ultraviolet light (UV-C; 254 nm) exposure chamber (Philips, TUV6W), incorporating an air circulation system to maintain a controlled temperature. The chamber was also equipped with a lux meter (Fisherbrand™, light meter) for measuring light intensity. Sampling was conducted at certain time intervals, and total MA was measured.

2.5. Extraction Kinetic Model

This research's modeling approach was exclusively centered on the UAE, considering multiple potential phenomena and their associated mass transfer mechanisms. Furthermore, the model developed by Bonfigli *et al.* [13] was also applied with several minor modifications. Kinetic studies were conducted to determine the rate at which adsorption occurs to reach equilibrium.

2.5.1. Development of a Mechanistic Ultrasound-assisted Extraction (UAE) Model

The model's development assumes that the particles within the *Clitoria ternatea* flower sample (particle matrix) are extremely small. Consequently, the active anthocyanin component can readily diffuse out of the matrix when subjected to high-pressure bubbles generated by ultrasonic cavitation. This facilitates the rapid release of the active anthocyanin components into the solvent. Furthermore, the consistently resonating acoustic waves effectively position the resulting bubbles, promoting the creation of a uniform solvent flow pattern. Additionally, the transfer of bubbles carrying the active anthocyanin components to the liquid body does not compromise the integrity of the anthocyanin structure. Lastly, equilibrium becomes pertinent when a saturated fluid body can no longer accommodate additional active anthocyanin components. An illustration of the UAE mechanism can be seen in Figure 1. Based on macroscopic observations, the mass balance in liquid bodies and solid matrix particles can be written in Equations (2) and (3).

The mass balance in liquid bodies (Equation (2)).

$$\frac{dC_a}{dt} = -\frac{k\,a\,m}{V}(C_a - C_a^*) \quad (2)$$

The mass balance in solid matrix bodies (Equation (3)).

$$\frac{dX_a}{dt} = k\,a\,(C_a - C_a^*) \quad (3)$$

Where, m is the mass of *Clitoria ternatea* flower samples, k is the extraction speed constant (m/min), a is the surface area of samples (m^2), C_a is the anthocyanin concentration (g/L), dC_a/dt is the anthocyanin concentration in the liquid, and dX_a/dt refers to the rate of change of anthocyanin concentration within the particle matrix. C_a^* is the anthocyanin concentration at the film layer, which cannot be directly measured. Instead, C_a^* is estimated using the partition coefficient described in Equation (4).

$$X_a = K\,C_a^* \quad (4)$$

Although not the primary focus of this research, several other kinetic models, including the second-order rate model and Peleg's model, were also examined. This exploration highlights the diverse mechanistic approaches under investigation.

2.5.2. The Second-order Rate Model

The second-order rate model is extensively employed in solid-liquid extraction processes due to its ability to effectively represent data and underlying mechanisms. In its differential form, it expresses concentration as a function of time, as shown in Equation (5) [26].

$$\frac{dC_t}{dt} = k(C_s - C_t)^2 \quad (5)$$

Where, C_t represents the concentration of anthocyanin in the liquid phase at a specific extraction time (t), C_S denotes the saturation concentration of anthocyanin in the liquid ($g.L^{-1}$), and k is the second-order extraction rate constant (min^{-1}). To ascertain the kinetic parameters, Equation (5) is integrated concerning the boundary conditions, from C_t = 0 to C_t and t = 0 to t, resulting in its expression as Equation (6).

$$\frac{t}{C_t} = \frac{1}{kC_s^2} + \frac{t}{C_s} = \frac{1}{h} + \frac{t}{C_s} \quad (6)$$

Where, h represents the initial extraction rate as t and C_t approach 0. The rearranged expression for the concentration of extractable substances at any given time is represented in the final Equation (7).

$$C_t = \frac{1}{(1/h)+(t/C_s)} \quad (7)$$

The second-order extraction rate constant (k) can be experimentally determined by plotting t/C_t against t as in Equation (6) and analyzing the resulting graph's slope and intercept.

2.5.3. Peleg's model

Peleg's empirical model was applied to extract anthocyanins from solid matrices in a solid-liquid process, as represented in Equation (8).

$$C_t = C_0 + \frac{t}{K_1 + K_2 t} \quad (8)$$

Where, K_1 is Peleg's rate constant ($min.L.mg^{-1}$) and K_2 is Peleg's capacity constant ($L.mg^{-1}$). C_0 represents the initial anthocyanin concentration at the start of the process, with fresh solvents consistently used in this research. Equation (8) is then transformed into the final form, as shown in Equation (9).

$$C_t = \frac{t}{K_1 + K_2 t} \quad (9)$$

The constants K_1 and K_2 are determined by plotting and analyzing the experimental data using Equation 9.

2.6. Colorant Degradation Kinetic Model

In this research, the degradation of monomeric anthocyanins was studied using two kinetic models: (1) first-order kinetics and (2) the Weibull model. The first-order kinetics can be described by Equation (10).

$$C_t = C_0 \exp(kt) \quad (10)$$

Where, C_0 is the monomeric anthocyanin concentration ($mg.L^{-1}$) at time t = 0; C_t is the monomeric anthocyanin concentration at time t; k is the degradation rate constant (min^{-1}); and t is the degradation time (min). The first-order kinetics model was chosen because this model can describe the value of the velocity rate of an equilibrium phenomenon, which can be adjusted to trends in research data. The decrease in data resulting from degradation can be calculated by linearizing the data using Equation 10. The Weibull model (Equation 11) incorporates scale parameters (α) and shape parameters (β) of the monomeric anthocyanins into the kinetics of degradation. The Weibull model was chosen to compare kinetic parameters based on regression results.

$$C_t = C_0 \exp\left[-\left(\frac{t}{\alpha}\right)^{\beta}\right] \quad (11)$$

The curve exhibits a concave upward shape when β<1, a concave downward shape when β<1, and a linear shape when β=1. This implies the Weibull model equation resembles the first-order kinetic equation for β = 1.

3. Results and Discussion

Successful research has been conducted on the extraction of anthocyanins from *Clitoria ternatea* flowers. The findings from CSE methods, specifically maceration and Soxhlet extraction, are presented initially. Subsequently, the discussion extends to UAE, incorporating the formulation of an extraction model to facilitate simultaneous extraction and degradation of anthocyanins. Finally, the study investigates the stability of anthocyanins concerning temperature and UV-C light exposure.

3.1. Maceration and Soxhlet Extraction

In this section, we compare the anthocyanin concentrations obtained from wet and dry butterfly pea flower samples using the maceration technique. The optimal results from the maceration stage are followed by a comparison of the effect of temperature on anthocyanin extraction based on Soxhlet extraction.

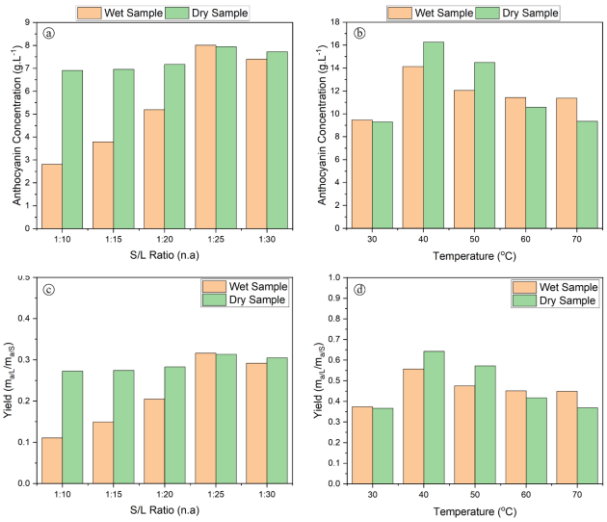

Figure 2. Anthocyanin extraction using CSE: (a) and (c) maceration, (b) and (d) Soxhlet extraction (fixed ratio of S/L = 1:25)

In Figure 2a, the extraction of anthocyanins through maceration techniques is depicted at varying S/L ratios. Generally, an increase in solvent volume corresponds to a higher anthocyanin extraction. However, this study reveals that the peak anthocyanin concentration occurs at S/L=1:25. This phenomenon is attributed to reaching equilibrium at this ratio, wherein further solvent increment diminishes the anthocyanin concentration [19, 23, 24]. Emphasizing the reliance of maceration extraction on the diffusion process, it operates effectively when the solvent diffusion penetrates the solid matrix at the optimal S/L ratio, ensuring complete extraction of anthocyanins from the matrix [27]. In addition, Figure 2a highlights that dry samples exhibit a superior ability to release anthocyanins compared to wet samples. This observation is closely linked to the presence of water trapped in the sample matrix, which impedes the ongoing extraction process. This consistent pattern is evident across all S/L ratios employed [28].

Hence, when employing the Soxhlet extraction method, the impact of S/L variations can be disregarded by adopting a fixed ratio of S/L = 1:25. The primary focus of this method is on the effect of extraction temperature, as detailed in Figure 2b. As depicted in Figure 2b, the anthocyanin concentration in Soxhlet extraction at 30°C closely mirrors that of the maceration method conducted at room temperature. However, with a temperature rise of 10°C, specifically at 40°C, the highest anthocyanin concentration was observed, gradually diminishing at temperatures of 50, 60, and 70°C. This trend is intricately linked to the optimal extraction temperature, where the solvent can extract the anthocyanin without inducing structural damage due to excessive heat exposure [29, 30].

Table 2. Calculated variable quantities and units

Parameter	Unit	Value
Mass (m)	gr	20 ± 0.15
Volume (V)	L	0.2 ± 0.01
Surface area (a)	$m^2.gr^{-1}$	0.75 ± 0.03

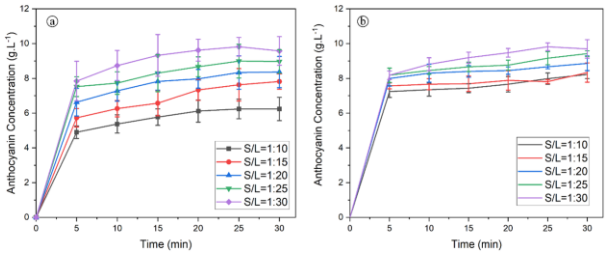

Figure 3. Effect of S/L ratios on anthocyanin extraction via UAE at 30°C: (a) wet sample and (b) dry sample

3.2. UAE Extraction

Anthocyanin extraction through UAE was conducted at 30°C on both dry and wet samples, employing various S/L ratios, as depicted in Figures 3a and 3b. Across all S/L ratios, a consistent pattern was observed, displaying a classic behavior wherein an augmentation in the S/L ratio corresponds to an increase in the extraction rate. Anthocyanin yields exhibit an asymptotic increase, approaching equilibrium concentrations [29]. This differs from the outcomes of the maceration technique, where the solvent optimization is evident only at a specific S/L ratio [31]. Nevertheless, a discernible distinction emerged between wet and dry samples: in wet samples, higher S/L ratios significantly improved the extraction rate due to the solvent's ability to displace anthocyanins from the particle matrix. In dry samples, however, extraction occurred more rapidly, diminishing the impact of increased S/L ratios. These findings align with prior studies on the influence of S/L ratios in UAE across different materials [32, 33].

Enhancing the rate of anthocyanin extraction can be achieved by elevating the extraction process temperature. A temperature range of 30-70°C was systematically applied to assess its impact on the extraction rate. The outcomes for both wet and dry samples are depicted in Figures 4a and 4b, respectively. The findings indicate a notable acceleration in the extraction rate with increasing temperature. Nevertheless, a disparity in the efficacy of temperature elevation exists between the two sample types, revealing a fundamental limitation attributed to the water content composition within the particle matrix. This tendency is more consistently observed in wet samples and tends to be less apparent in dry samples.

A notable decrease in anthocyanin concentration was observed after 25 minutes of processing, suggesting potential decomposition or damage to the anthocyanins due to excessive heat exposure. A more comprehensive understanding of this phenomenon becomes apparent after a 4-hour extraction process, as illustrated in Figure 5. In Figure 5, it is evident that heat exposure at temperatures of 30°C and 40°C exhibits a milder pattern compared to 50-70°C, allowing for a comparative analysis of the rate of anthocyanin degradation over time. This observation is consistent with the findings of Nhut Pham *et al.* [19], who demonstrated that anthocyanin degradation accelerates at higher temperatures. Given the complexity of anthocyanin degradation, heat processing, particularly above 50°C, may trigger unforeseen and potentially undesirable chemical reactions.

Table 3. Tabulation of extraction modeling results of anthocyanin extraction for 30 minutes

Kinetic model	Parameter	Temperature (°C)				
		30	40	50	60	70
The second-order rate model	k (min^{-1})	0.0901	0.1440	0.1223	0.0977	0.1039
	r^2	0.9955	0.9981	0.9976	0.9984	0.9988
	SSE	0.0194	0.0067	0.0060	0.0031	0.0018
The Peleg's model	K_1 (min^{-1}.L. g^{-1})	0.1487	0.0995	0.1058	0.0604	0.0468
	K_2 ($L.g^{-1}$)	0.1011	0.0903	0.0758	0.0668	0.0588
	r^2	0.8707	0.9475	0.9759	0.8954	0.8943
	SSE	0.3223	0.0990	0.0915	0.2765	0.2933
Proposed UAE kinetics model	k ($m.min^{-1}$)	0.0055	0.0058	0.0064	0.0070	0.0078
	r^2	0.9789	0.9722	0.9798	0.9693	0.9679
	SSE	0.0211	0.0228	0.0202	0.0307	0.0321

*MATLAB R2023b software was employed to perform calculations, utilizing the Curve Fitter APPS available within the software. The chosen solving algorithm for Nonlinear Least Squares is Trust-Region.

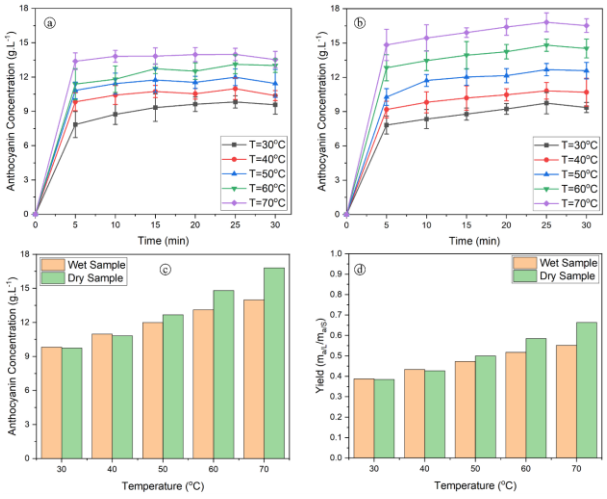

Figure 4. Effect of temperature on anthocyanin extraction via UAE at S/L=1:30; (a) wet sample, (b) dry sample, (c) anthocyanin concentration, and (d) extraction yield

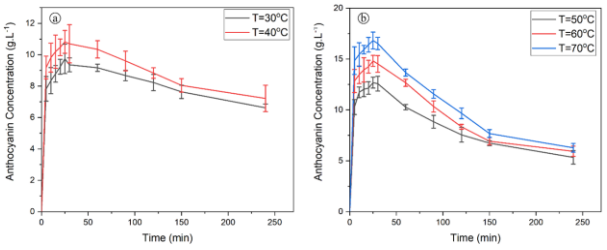

Figure 5. Effect of temperature on anthocyanin extraction via UAE after 4 hours (S/L = 1:30, dry sample): (a) for 30-40°C, (b) for 50-70°C

3.3. Kinetics of Ultrasound-Assisted Extraction (UEA)

The kinetics study of colorant extraction, represented by anthocyanins, from the *Clitoria ternatea* flower matrix was conducted on dry samples with an S/L ratio of 30. As shown in Figure 6, the kinetic model described by Equations (2–4) does not provide a fairly good approximation. The details about the extraction process conditions are outlined in Table 2, and the estimated data align closely with the actual experimental results. The solution to the simultaneous model of Equations (2–4) was obtained using the *ode15s* and *lsqnonlin* toolboxes in MATLAB R2023b. Based on the calculated results, the influence of the temperature variable used during extraction affects the extraction speed. The greater the operating temperature value used in this case, the faster the extraction speed is constant, and the extraction results are processed at the end of the process.

Various mathematical models were tested to validate the results further, as detailed in Table 3. Based on the coefficient of determination and sum of squared error values presented in Table 3, it is evident that the model proposed in this research is inadequate for predicting the data related to UAE. In contrast, the second-order rate model consistently produces favorable results, effectively representing and predicting the data. However, Peleg's model demonstrates consistency in only a limited number of experiments, suggesting that the methodology employed in formulating the current model may be insufficient to encapsulate the intricacies of the actual extraction phenomenon despite the mechanical approach being regarded as closer to reality.

3.4. Natural Colorant Stability

Anthocyanins' stability can be affected by several elements, such as pH, temperature, light, and the existence of metal ions. This study investigates the effects of temperature and light intensity on the rate of anthocyanin degradation. Figure 7 illustrates the impact of temperature on anthocyanin stability. The temperature range used to assess anthocyanin stability is between 10 and 50°C. Temperatures above 50°C can cause partial or complete degradation of anthocyanins, resulting in a decrease in color intensity [34]. The results indicate that temperature conditions during the anthocyanin storage process significantly affect the degradation rate. Higher storage temperatures accelerate the degradation rate, with temperatures above 28°C leading to faster anthocyanin breakdown. In contrast, the degradation rate slows at temperatures below 28°C.

Table 4. Tabulation of degradation modeling results of anthocyanin extraction for 30 minutes

Kinetic model	Parameter	Temperature (°C)				UV–C @28°C
		10	28	40	50	
The first-order rate model	k (min^{-1})	$6.9035.10^{-4}$	0.0013	0.8865	0.0066	0.0037
	r^2	0.9093	0.9069	0.9198	0.9662	0.9290
	SSE	0.5972	0.8865	4.7966	2.2898	2.6184
The Weibull model	α (min^{-1})	$2.6048.10^{-4}$	$1.7367.10^{-3}$	200.2905	153.3127	323.2798
	β (min^{-1})	0.3988	0.6052	0.6985	0.7926	0.6953
	r^2	0.9912	0.9755	0.9750	0.9854	0.9780
	SSE	0.0199	0.6052	1.4970	0.9925	0.8123

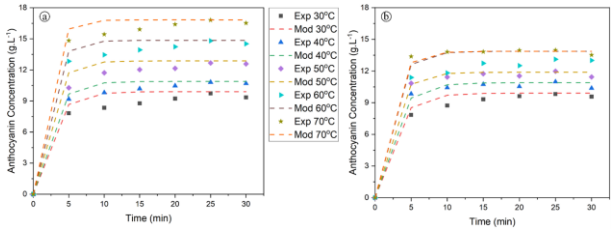

Figure 6. Comparison of experiment and model for (a) dry sample and (b) wet sample

This study shows that the concentration of anthocyanins decreases over time, with a storage temperature of 10°C demonstrating higher stability than the other conditions, exhibiting a degradation rate of 6.9035×10^{-4} min^{-1} based on the first-order rate model. Anthocyanin degradation accelerates at temperatures above 10°C. A lower storage temperature is beneficial in reducing anthocyanin degradation during storage. This finding aligns with studies by Yusoff *et al.* [35], which suggests that the ideal storage temperature for anthocyanins is around 4°C and Liu *et al.* [36], which also reported that increasing temperatures accelerate anthocyanin degradation. In conclusion, high temperatures contribute to the degradation of anthocyanins in *Clitoria ternatea* in this study.

In addition, this research also explains the effect of UV-C light on the rate of anthocyanin degradation at the same operating temperature and reveals that the influence of UV-C light can accelerate the rate of anthocyanin degradation. Anthocyanin compounds tend to have poor stability when exposed to UV-C radiation, resulting in photodegradation. UV-C light can cause severe and permanent metabolic and physiological damage, followed by the breakdown of secondary plant components [37]. A study by Mahmad and Taha [38] reports that anthocyanins from *Clitoria ternatea* are less stable under light exposure than at elevated temperatures. The degradation of anthocyanins when exposed to UV-C light occurs rapidly due to the hydrolysis of glycosidic bonds, resulting in the formation of colorless carbinol and chalcone groups from the aglycones, which tend to be unstable. Março *et al.* [39] also reveals that UV-C light promotes the instability of flavylium cations in anthocyanins, accelerating their transformation into deprotonated forms.

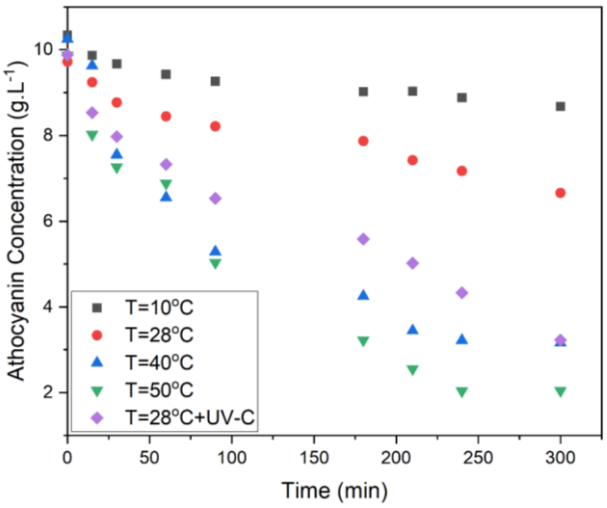

Figure 7. The outcome of assessing the stability of natural colorant across diverse conditions

The degradation of the total monomeric anthocyanin in *Clitoria ternatea* flowers was analyzed for 300 minutes at various temperatures (10, 28, 40, and 50°C) using kinetic models. Table 4 presents the kinetic parameters for the first-order kinetic and the Weibull models (k, α, and β). According to the first-order kinetic model, the degradation rate increases with temperature and UV-C radiation intensity. The degradation rate follows a first-order kinetic model, showing a slower rate at 10°C and a rapid increase above 28°C. The effect of UV-C light further accelerates the anthocyanin breakdown rate when maintained at the same temperature. This study supports this observation, with the first-order rate model demonstrating that the rate of anthocyanin degradation in *Clitoria ternatea* extract exposed to UV-C light is faster, with a rate constant of k = 0.0037 min^{-1}, compared to the slower degradation rate of k = 0.0013 min^{-1} at 28°C without UV-C radiation.

Based on the Weibull kinetic model, two significant factors are utilized as a reference: the scale parameter (α) and the shape parameter (β). The α value represents the typical life or the point when the degradation rate is at the highest level. A higher α suggests longer stability before major degradation occurs. Meanwhile, the β value determines the degradation pattern. When β > 1, the distribution shows a decreasing degradation rate (early-life degradation). Conversely, when β < 1, the distribution

exhibits a rising degradation rate (wear-out degradation). Based on the α and β values obtained in Table 4, it reveals that when the temperature is increased, there would be considerable degradation of anthocyanins in *Clitoria ternatea*.

Based on the Weibull kinetic model, at temperatures below 28°C, the α value tends to be relatively small. However, as the temperature exceeds 28°C, the α value increases significantly, indicating a substantial rise in the degradation rate with temperature. Additionally, exposure to UV-C radiation considerably influences the degradation rate, as demonstrated by the Weibull kinetic model. This can be observed at 28°C, where the α value is 1.7367×10^{-3} min^{-1}, but when exposed to UV-C radiation, the α value increases dramatically to 323.2798 min^{-1}. The Weibull kinetic model also shows that the degradation rate tends to increase, supported by the β value being less than 1 under all conditions.

4. Conclusion

Ultrasound-assisted extraction (UEA) proves significantly more effective in obtaining natural colorant, specifically anthocyanins, than conventional solvent extraction (CSE), resulting in enhanced extract yields. Beyond emphasizing yield, the upward trajectory in extraction temperature consistently corresponds to an increased extraction rate. The mechanistic second-order rate model aptly characterizes the extraction kinetics of this process. However, it is crucial to carefully consider the duration of the extraction process, with optimal results observed in shorter processes (less than 30 minutes). This aligns with the degradation pattern of the anthocyanin extract, which steadily diminishes due to prolonged exposure to elevated temperatures.

Acknowledgment

The author is grateful for research funding support from the University of Surabaya (UBAYA).

References

[1] Wei-Sheng Lin, Pei Hua He, Chi-Fai Chau, Bo-Kang Liou, Shiming Li, Min-Hsiung Pan, The feasibility study of natural pigments as food colorants and seasonings pigments safety on dried tofu coloring, *Food Science and Human Wellness*, 7, 3, (2018), 220-228 https://doi.org/10.1016/j.fshw.2018.09.002

[2] P. G. T. Dilrukshi, Helani Munasinghe, A. Buddhika G. Silva, P. G. S. M. De Silva, Identification of Synthetic Food Colours in Selected Confectioneries and Beverages in Jaffna District, Sri Lanka, *Journal of Food Quality*, 2019, 1, (2019), 7453169 https://doi.org/10.1155/2019/7453169

[3] Tanuka Sen, Colin J. Barrow, Sunil Kumar Deshmukh, Microbial Pigments in the Food Industry—Challenges and the Way Forward, *Frontiers in Nutrition*, 6, 7, (2019), https://doi.org/10.3389/fnut.2019.00007

[4] Nannan Li, Qirou Wang, Jingna Zhou, Shuqin Li, Junyu Liu, Haixia Chen, Insight into the Progress on Natural Dyes: Sources, Structural Features, Health Effects, Challenges, and Potential, *Molecules*, 27, 10, (2022), 3291 https://doi.org/10.3390/molecules27103291

[5] Maria Manuela Silva, Fernando Henrique Reboredo, Fernando Cebola Lidon, Food Colour Additives: A Synoptical Overview on Their Chemical Properties, Applications in Food Products, and Health Side Effects, *Foods*, 11, 3, (2022), 379 https://doi.org/10.3390/foods11030379

[6] Delia B. Rodriguez-Amaya, Natural food pigments and colorants, *Current Opinion in Food Science*, 7, (2016), 20-26 https://doi.org/10.1016/j.cofs.2015.08.004

[7] Hui Yi Leong, Pau Loke Show, Miang Hoong Lim, Chien Wei Ooi, Tau Chuan Ling, Natural red pigments from plants and their health benefits: A review, *Food Reviews International*, 34, 5, (2018), 463-482 https://doi.org/10.1080/87559129.2017.1326935

[8] Yilin You, Xue Han, Jielong Guo, Yu Guo, Manwen Yin, Guojie Liu, Weidong Huang, Jicheng Zhan, Cyanidin-3-glucoside attenuates high-fat and high-fructose diet-induced obesity by promoting the thermogenic capacity of brown adipose tissue, *Journal of Functional Foods*, 41, (2018), 62-71 https://doi.org/10.1016/j.jff.2017.12.025

[9] Daotong Li, Pengpu Wang, Yinghua Luo, Mengyao Zhao, Fang Chen, Health benefits of anthocyanins and molecular mechanisms: Update from recent decade, *Critical Reviews in Food Science and Nutrition*, 57, 8, (2017), 1729-1741 https://doi.org/10.1080/10408398.2015.1030064

[10] Bianca R. Albuquerque, José Pinela, Lillian Barros, M. Beatriz P. P. Oliveira, Isabel C. F. R. Ferreira, Anthocyanin-rich extract of jabuticaba epicarp as a natural colorant: Optimization of heat- and ultrasound-assisted extractions and application in a bakery product, *Food Chemistry*, 316, (2020), 126364 https://doi.org/10.1016/j.foodchem.2020.126364

[11] Luxsika Ngamwonglumlert, Sakamon Devahastin, Naphaporn Chiewchan, Natural colorants: Pigment stability and extraction yield enhancement via utilization of appropriate pretreatment and extraction methods, *Critical Reviews in Food Science and Nutrition*, 57, 15, (2017), 3243-3259 https://doi.org/10.1080/10408398.2015.1109498

[12] José Pinela, M. A. Prieto, Eliana Pereira, Inès Jabeur, Maria Filomena Barreiro, Lillian Barros, Isabel C. F. R. Ferreira, Optimization of heat- and ultrasound-assisted extraction of anthocyanins from *Hibiscus sabdariffa* calyces for natural food colorants, *Food Chemistry*, 275, (2019), 309-321 https://doi.org/10.1016/j.foodchem.2018.09.118

[13] M. Bonfigli, E. Godoy, M. A. Reinheimer, N. J. Scenna, Comparison between conventional and ultrasound-assisted techniques for extraction of anthocyanins from grape pomace. Experimental results and mathematical modeling, *Journal of Food Engineering*, 207, (2017), 56-72 https://doi.org/10.1016/j.jfoodeng.2017.03.011

[14] Ethel Jeyaseela Jeyaraj, Yau Yan Lim, Wee Sim Choo, Extraction methods of butterfly pea (*Clitoria ternatea*) flower and biological activities of its phytochemicals, *Journal of Food Science and Technology*, 58, 6, (2021), 2054-2067 https://doi.org/10.1007/s13197-020-04745-3

[15] Graziela Bragueto Escher, Mariza Boscacci Marques, Mariana Araújo Vieira do Carmo, Luciana Azevedo,

Marianna Miranda Furtado, Anderson S. Sant'Ana, Marcia Cristina da Silva, Maria Inês Genovese, Mingchun Wen, Liang Zhang, Won Young Oh, Fereidoon Shahidi, Neiva Deliberali Rosso, Daniel Granato, *Clitoria ternatea* L. petal bioactive compounds display antioxidant, antihemolytic and antihypertensive effects, inhibit α-amylase and α-glucosidase activities and reduce human LDL cholesterol and DNA induced oxidation, *Food Research International*, 128, (2020), 108763 https://doi.org/10.1016/j.foodres.2019.108763

[16] Aishah Mohd Marsin, Yanti Maslina Mohd Jusoh, Dayang Norulfairuz Abang, Zanariah Hashim Zaidel, Abdul Halim Mohd Yusof, Ida Idayu Muhamad, Microwave-assisted encapsulation of blue pea flower (*Clitoria ternatea*) colourant: maltodextrin concentration, power, and time, *Chemical Engineering Transactions*, 78, (2020), 199-204 https://doi.org/10.3303/CET2078034

[17] Amin Abdillah Harahap, Kelvin Letare S., Nuniek Hendrianie, The effect of solvents and extraction time on anthocyanin extraction from butterfly pea (*Clitoria ternatea* L.), *AIP Conference Proceedings*, 2667, 1, (2023), 020003 https://doi.org/10.1063/5.0112770

[18] Adriana S. López Prado, Yixiao Shen, Ryan Ardoin, Luis F. Osorio, Jorge Cardona, Zhimin Xu, Witoon Prinyawiwatkul, Effects of different solvents on total phenolic and total anthocyanin contents of *Clitoria ternatea* L. petal and their anti-cholesterol oxidation capabilities, *International Journal of Food Science & Technology*, 54, 2, (2019), 424-431 https://doi.org/10.1111/ijfs.13953

[19] Tri Nhut Pham, Duy Chinh Nguyen, Tri Duc Lam, Pham Van Thinh, Xuan Tien Le, Dai Viet Vo Nguyen, Hieu Vu Quang, Trinh Duy Nguyen, Long Giang Bach, Extraction of anthocyanins from Butterfly pea (*Clitoria ternatea* L. Flowers) in Southern Vietnam: Response surface modeling for optimization of the operation conditions, *IOP Conference Series: Materials Science and Engineering*, 542, (2019), 012032 https://doi.org/10.1088/1757-899X/542/1/012032

[20] A. Baskaran, S. K. A. Mudalib, I. Izirwan, Optimization of aqueous extraction of blue dye from butterfly pea flower, *Journal of Physics: Conference Series*, 1358, (2019), 012001 https://doi.org/10.1088/1742-6596/1358/1/012001

[21] I. Izirwan, T. D. Munusamy, N. H. Hamidi, S. Z. Sulaiman, Optimization of microwave-assisted extraction of anthocyanin from *Clitoria ternatea* flowers, *International Journal of Mechanical Engineering Robotics Research*, 9, 9, (2020), 1246-1252 https://doi.org/10.18178/ijmerr.9.9.1246-1252

[22] Bambang Sugiarto, Yustin Rirung, Anita Nur Ardiyani, Zubaidi Achmad, Extraction of natural color of butterfly pea (*Clitoria ternatea* L) with variables pH-temperature and concentration for food coloring, *Journal Techno*, 8, 1, (2022),

[23] Nguyen Minh Thuy, Tran Chi Ben, Vo Quang Minh, Ngo Van Tai, Effect of extraction techniques on anthocyanin from butterfly pea flowers (*Clitoria ternatea* L.) cultivated in Vietnam, *Journal of Applied Biology & Biotechnology*, 9, 6, (2021), 173-180 https://dx.doi.org/10.7324/JABB.2021.96022

[24] Gayan Chandrajith Vidana Gamage, Wee Sim Choo, Hot water extraction, ultrasound, microwave and pectinase-assisted extraction of anthocyanins from blue pea flower, *Food Chemistry Advances*, 2, (2023), 100209 https://doi.org/10.1016/j.focha.2023.100209

[25] Jungmin Lee, Robert W. Durst, Ronald E. Wrolstad, Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method: Collaborative Study, *Journal of AOAC INTERNATIONAL*, 88, 5, (2019), 1269-1278 https://doi.org/10.1093/jaoac/88.5.1269

[26] Carla Da Porto, Andrea Natolino, Extraction kinetic modelling of total polyphenols and total anthocyanins from saffron floral bio-residues: Comparison of extraction methods, *Food Chemistry*, 258, (2018), 137-143 https://doi.org/10.1016/j.foodchem.2018.03.059

[27] Gayan Chandrajith Vidana Gamage, Yau Yan Lim, Wee Sim Choo, Anthocyanins From *Clitoria ternatea* Flower: Biosynthesis, Extraction, Stability, Antioxidant Activity, and Applications, *Frontiers in Plant Science*, 12, (2021), 792303 https://doi.org/10.3389/fpls.2021.792303

[28] Toktam Taghavi, Hiral Patel, Reza Rafie, Anthocyanin Extraction Method and Sample Preparation Affect Anthocyanin Yield of Strawberries, *Natural Product Communications*, 17, 5, (2022), https://doi.org/10.1177/1934578X221099970

[29] Tri Nhut Pham, Thuong Nhan Phu Nguyen, Tri Duc Lam, Thien Hien Tran, Duy Chinh Nguyen, Dai-Viet N. Vo, Xuan Tien Le, Sy Trung Do, Long Giang Bach, Effects of various solvent concentration, liquid-solid ratio, temperatures and time values on the extraction yield of anthocyanin from Vietnam *Hibiscus sabdariffa* L. (*Roselle*), *IOP Conference Series: Materials Science and Engineering*, 542, (2019), 012033 https://doi.org/10.1088/1757-899X/542/1/012033

[30] Fernanda Rodríguez-Aguilar, Ana Eugenia Ortega-Regules, Milena M. Ramírez-Rodrigues, Influence of time-temperature in the antioxidant activity, anthocyanin and polyphenols profile, and color of *Ardisia compressa* K. extracts, with the addition of sucrose or citric acid, *Food Chemistry*, 440, (2024), 138181 https://doi.org/10.1016/j.foodchem.2023.138181

[31] Vanessa Barbieri Bombana, Lucas Henrique do Nascimento, Diane Rigo, Bruno Fischer, Rosicler Colet, Natalia Paroul, Rogério Marcos Dallago, Alexander Junges, Rogério Luis Cansian, Geciane Toniazzo Backes, Extraction by maceration, ultrasound, and pressurized liquid methods for the recovery of anthocyanins present in the peel of guabiju (*Myrcianthes pungens*): Maximizing the extraction of anthocyanins from the peel of guabiju, *Sustainable Chemistry and Pharmacy*, 36, (2023), 101264 https://doi.org/10.1016/j.scp.2023.101264

[32] Guillermo Linares, Meliza Lindsay Rojas, Ultrasound-Assisted Extraction of Natural Pigments From Food Processing By-Products: A Review, *Frontiers in Nutrition*, 9, (2022), https://doi.org/10.3389/fnut.2022.891462

[33] Kshitiz Kumar, Shivmurti Srivastav, Vijay Singh Sharanagat, Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review, *Ultrasonics*

Sonochemistry, 70, (2021), 105325
https://doi.org/10.1016/j.ultsonch.2020.105325

[34] Graziela Bragueto Escher, Mingchun Wen, Liang Zhang, Neiva Deliberali Rosso, Daniel Granato, Phenolic composition by UHPLC-Q-TOF-MS/MS and stability of anthocyanins from *Clitoria ternatea* L. (butterfly pea) blue petals, *Food Chemistry*, 331, (2020), 127341
https://doi.org/10.1016/j.foodchem.2020.127341

[35] Aiman Yusoff, N. T. R. N. Kumara, Andery Lim, Piyasiri Ekanayake, Kushan U. Tennakoon, Impacts of Temperature on the Stability of Tropical Plant Pigments as Sensitizers for Dye Sensitized Solar Cells, *Journal of Biophysics*, 2014, 1, (2014), 739514
https://doi.org/10.1155/2014/739514

[36] Ya Liu, Yongxiao Liu, Cui Tao, Mei Liu, Yue Pan, Zhaolin Lv, Effect of temperature and pH on stability of anthocyanin obtained from blueberry, *Journal of Food Measurement and Characterization*, 12, 3, (2018), 1744-1753 https://doi.org/10.1007/s11694-018-9789-1

[37] S. Huyskens-Keil, I. Eichholz-Dündar, K. Hassenberg, W. B. Herppich, Impact of light quality (white, red, blue light and UV-C irradiation) on changes in anthocyanin content and dynamics of PAL and POD activities in apical and basal spear sections of white asparagus after harvest, *Postharvest Biology and Technology*, 161, (2020), 111069
https://doi.org/10.1016/j.postharvbio.2019.111069

[38] Noraini Mahmad, R. M. Taha, Effects of pH, UV-B radiation and NaCl on anthocyanin stability from vivid blue petals of *Clitoria ternatea* L., a potential natural colourant from legume crop, *Pigment & Resin Technology*, 47, 6, (2018), 507-510
https://doi.org/10.1108/PRT-11-2016-0106

[39] Paulo Henrique Março, Ronei Jesus Poppi, Ieda Spacino Scarminio, Romà Tauler, Investigation of the pH effect and UV radiation on kinetic degradation of anthocyanin mixtures extracted from *Hibiscus acetosella*, *Food Chemistry*, 125, 3, (2011), 1020-1027
https://doi.org/10.1016/j.foodchem.2010.10.005