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Article

The Effects of Extraction Period Toward Anthocyanin Levels of Blue Pea Vine (*Clitoria ternatea*) Extract Using Maceration Method

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Irna Dwi Destiana[1*], Atika Romalasari[1], Nia Kurnia[1]

[1]Department of Agroindustry, Subang State Polytechnic Dangdeur, Subang, Indonesia

Abstract. Blue Pea Vine (Clitoria Ternatea L.) is an edible flower which is rich in compounds of anthocyanin. The purpose of this research is to determine the best immersion time for anthocyanin extraction of blue pea vine by using maceration method. It also attempts to reveal the anthocyanin content, as well as the color and pH from blue pea vine extraction.Three treatments were treated with two replications, namely the first treatment of soaking is for 24 hours, 48 hours and 72 hours. The best soaking time in the treatment was 48 hours which resulted the highest anthocyanin content of 172,833 mg / 100g. The color result was based on the level of attention to the achromatic color from the extraction of 48 hours of blue connection with an L * value of 25.95, the a* value of -0.4, and b * value of 1.35. The pH value result is pH 4.61

Corresponding Author :
Irna Dwi Destiana
Department of Agroindustry, Subang State Polytechnic,
Dangdeur, Subang, Indonesia
Email : irnadwidestiana@gmail.com

1. Introduction

Blue Pea Vine (Clitoria ternatea L.) is characterized as the tribe of Papilionaceae or Febaceae (legume) which is categorize as a tropical plant that is widely grown in Indonesia [1,2,3,4,5]. Blue Pea Vine have many pharmacological potentials including antioxidants, anticancer, antimicrobial, anti-inflammatory, antidiabetic, analgesic, antipyretic, anticidal as well as for the central nervous system [6,7,8,9,10]. This flower's petals contain phytochemical components such as tannins, plobatin, saponins, phenols, flavonoids, triterpenoids, alkaloids, anthraquinones, anthocyanins, flavonol glycosides, steroids, essential oils, and stigmas -4-ene-3,6-dione [11,12].

Anthocyanin compounds are classified as flavonoid compounds which are the largest group of natural pigments in plants [13,14,15,16,17,18]. It is also able to create blue, purple, red and orange colors in vegetables, fruits, as well as flowers. Anthocyanins are polar color pigments that will dissolve in polar solvents [19,20,21]. Many anthocyanin compounds contained in Blue Pea Vine and have high antioxidant activity compared to other anthocyanins from flower extract. Additionally, the anthocyanin content in Blue Pea Vine was 5.40 or 0.23 mmol / mg [22,23,24,25].

The right maceration time will result in higher anthocyanin levels of Blue Pea Vine extract. The short maceration time will result in not all chemicals dissolving in the solvent used. If the maceration time is too long, the extracted active substance will be damaged [26,27,28,29,30]. Therefore, it is necessary to study the optimal extraction time so that it can produce good quality extracts [31,32,33,34,35]. The purpose of this study was to determine the best extraction time to produce the highest anthocyanin levels.

The removal of anthocyanin compounds can be done by extraction. During the extraction process, the active compound will be dissolved by a pollutant or solvent according to its polarity. There are several factors that affect the extraction rate, namely raw material, extraction time, temperature, and the type of solvent. The method used in this research is maceration. The advantages of the maceration method are low cost, easy to do and and it does not need heated that it will not damage anthocyanin compounds [36,37,38,39]. It is because anthocyanin compounds are easily degraded by heat, therefore the extraction process is carried out using the maceration method. The purpose of this study was to determine the anthocyanin levels in Blue Pea Vine extract using the maceration method, to measure the color and pH of Blue Pea Vine extract.Tips:

2. Experimental Section

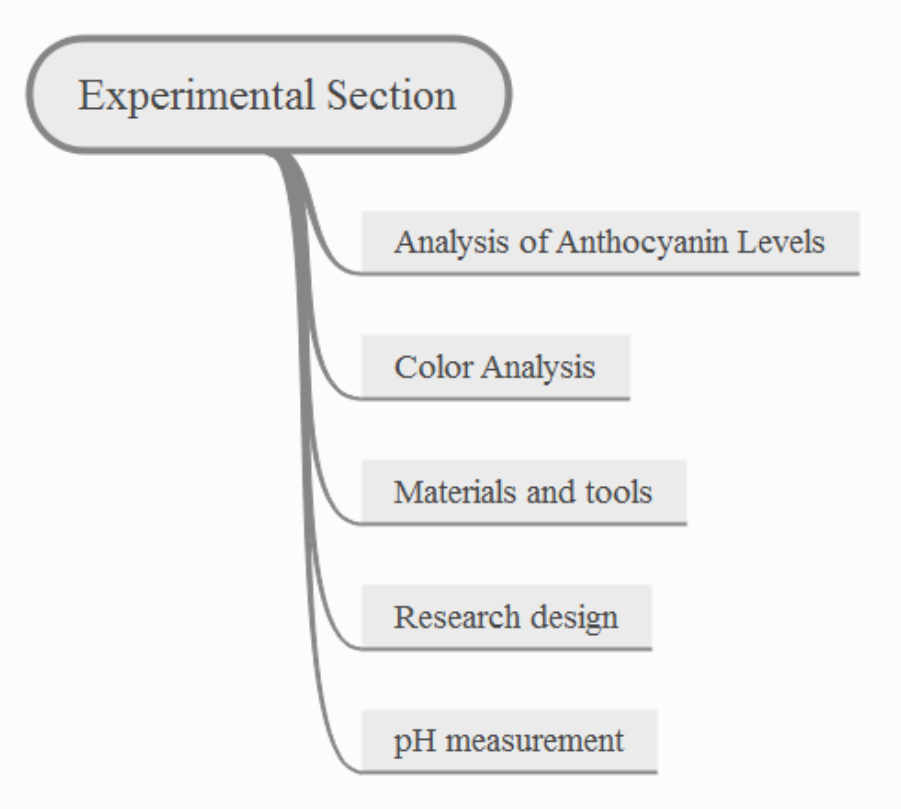

Figure 1. Experimental Section Reserch

2.1. Materials and tools

The materials used in this study were dried Blue Pea Vine from Kediri district, East Java, 95% solvent ethanol (Bratacho), 1% HCl (Merck, 37%), Buffer pH 4.0, 7.0 and 10 (Merck), KCl (Merck), Potassium Acetate (Merck) and aquades. Aditionally, the tools used in this study include the Simadzu UV-Vis Spectrophotometer, Minolta Chromameter, Vortex IKA (Germany), Ohaus pH Meter, 10 ml and 20 ml Iwaki measuring flasks.

2.2 Research design

The research was conducted using a completely randomized design 1 factor with 3 levels and was repeated 3 times. The treatments performed were the differences of anthocyanin extraction time from 95% consisting of 24 hours, 48 hours and 72 hours extraction time. Furthermore, the result of Blue Pea Vine extract will then be measured for anthocyanin levels, color analysis and pH.

2.3 Analysis of Anthocyanin Levels

Testing anthocyanin levels of Blue Pea Vine extract was carried out by taking 1 ml of extracted filtrate using a pipette and placing it in a 20 ml volumetric flask. Next, it is diluted by using buffer pH 1.0 to the limit mark. Then, 1 ml of sample fluid then should be diluted anymore in a 10 ml volumetric flask using a pH 4.5 buffer. The absorbance of the sample was measured at λ540 and λ700 nm. The absorbance can be calculated using the formula:

$$A = (A\ \lambda 540 - A\ \lambda 700\ nm)\ pH\ 1.0 - (A\ \lambda 540 - A\ \lambda 700\ nm)\ pH\ 4.5)$$

$$\text{Anthocyanin Levels (mg)} = \frac{(A \times BM \times FP \times 1000)\ L}{\varepsilon \times 1}$$

2.4 Color Analysis

The measurement of the color of the anthocyanin pigments in Blue Pea Vine was carried out using a chromameter. The measurement procedure is to turn on the chromameter then determine the target reading for L, a *, b * and measure the color. Then, the color scale is read with parameter L * for brightness (lightness) and a *, b * for chromaticity values.

2.5 pH measurement

The measurement is conducted by performing the calibration of the tools used in the pH 4.0 buffer solution then the pH 7.0 buffer, and finally the pH 10 buffer solution. After completing the calibration, it then switches the pH meter tool mode for measurement and dips the pH meter electrode tip in the liquid where pH will be measured.

3. Results and Discussion

3.1 Anthocyanin levels

The determination of the anthocyanin pigments was carried out by the pH difference method, namely pH 1.0 and pH 4.5. Anthocyanin pigments at pH 1.0 are in the form of oxonium compounds. The more acidic conditions approaching pH 1 will cause more anthocyanin pigments to be in the form of colored flavilium or oxonium cations and the absorbance measurements will show the greater number of anthocyanin compounds. Anthocyanin pigments at pH 4.5 are in weak acidic conditions where the flavilium cation changes to a more stable, colorless hemiketal and chalcone form. The difference in absorbance between the two buffer solutions was commensurate with the monomeric anthocyanin pigment.

Based on the measurement of anthocyanin levels at a wavelength of 540 nm, it was carried outusing triplo method or using 3 samples of each sample observed. The average results of measuring the anthocyanin levels of the pH extract can be seen in Table 1.

Table 1. Anthocyanin Levels of Blue Pea Vine

Extraction time	Anthocyanin Levels (mg/100g)
24 Hours	85,443
48 Hours	172,833
72 Hours	58,126

Based on Table 1, it can be concluded that the extraction time has an effect on the anthocyanin levels produced. Extraction time is an important thing that must be considered in the anthocyanin extraction process, because it can affect the quality of the extraction yield [40,41]. The right maceration time will produce high anthocyanin levels as shown in the 48 hours treatment. The short maceration time will cause the active substance contained in the material not to be completely extracted by the solvent used as shown in the 24-hour treatment and if the maceration time is too long, the extracted active substance will be damaged as shown in the 72-hour treatment [39,42]. This is reinforced by the opinion [40,41], the longer the maceration time, the longer the contact between the material and the solvent will be, while each material to be extracted has an optimum limit. If the maceration time exceeds the optimum limit, the extraction will have no effect because the compound will undergo a decomposition or change in chemical structure. Moreover, the results of the optimum anthocyanin levels in the extract of 1% HCl in ethanol 95% of Blue Pea Vine at 48 hours maceration time have resulted in anthocyanin levels of 172.833 mg / 100 g.

The 48 hours treatment is the best treatment when compared to the 24 hours and 72 hours treatment. When compared with the anthocyanin content test of rosella flowers, the highest anthocyanin levels were 4.755mg / 100g with a maceration process of 48 hours [43]. The results of extraction of Blue Pea Vine anthocyanin levels are relatively higher when compared to the results of extraction of rosella flowers that have been tested previously. When compared with testing [44] on anthocyanin levels of other ornamental flower extracts, anthocyanin levels from Blue Pea Vine were higher. Comparison of anthocyanin levels from telang flowers with other ornamental flowers can be seen in Table 2.

Tabel 2. Comparisson Anthocyanin Levels Blue Pea Vine with other flower

Kind of Flower	Anthocyanin Levels (mg/100g)
Telang	172,833
Mawar	3,180 [45]
Bunga mekar pukul 4	3,910 [45]
Rosela	4,755 [46]

This is in accordance with the statement [25,47,48] that many anthocyanin compounds contained in Blue Pea Vine have high antioxidant activity when compared to other ornamental flower extract anthocyanins. Anthocyanin levels of various kinds of flower petals have different levels of anthocyanins contained, several factors that can affect including sunlight, climate and soil [49].

3.2 Color Analysis

The measurement of the color of Blue Pea Vine anthocyanin extract using a chromameter uses a hunter notation system in its measurements. Color measurement with the Hunter notation system is much faster with good precision. This system consists of 3 parameters, namely L, a and b. The color location in this system is determined by the coordinates L *, a * and b *. The results of measuring the color of Blue Pea Vine extract with different extraction times are available in Table 3.

Tabel 3. Color of anthocyanin extract

Treatmen	Average		
	L*	***a****	***b****
24 hours	25,95	-0,4	1,35
48 hours	26,93	-3,48	-0,5
72 hours	23,67	-1,72	5,2

Based on Table 3, it is understood that the highest L * brightness level is in the 48-hour treatment of 26.93, while the L * value in the 24-hour treatment is 25.95. It indicates that the L * value in the 24-hour and 48-hour treatments the resulting color is slightly brighter and closer to black (0). The greater the absorbance value, the greater the brightness of the color. The appearance of color in the extract is also influenced by the pH of the solution, the more the pH value increases, the more colorless carbinol and chalcone bases are formed [50].

The a * value in the 48-hour treatment is -3.48, which means that the resulting color change is close to green. Likewise, in the 24-hour treatment and 72-hour treatment, the resulting color resulted in a color change that was close to green because the resulting value was (-) a *. The value (+) b * shows the change of the color from blue to yellow. The 72 hours treatment showed a value of b * 5.2, meaning that the resulting change in color was closer to blue. When compared with the value of b * in the 24 hour and 48-hour treatment, the position of the color approached the center point of the hunter coloring system.

3.3 pH measurement

The results of the average pH measurement of the anthocyanin extract of Blue Pea Vine increased the pH along with the extraction time by the maceration process. They initial pH of 1% HCl solution is about in 95% ethanol pH range 1. Additionally, the anthocyanin pigments are stable under acidic pH conditions. Anthocyanin pigments will change color from red to less color in weak acids. At low pH, anthocyanins are in the form of flavilium cations which are the most stable form in the pH range 1-3 [28]. The optimum pH of Blue Pea Vine anthocyanin extract is a pH range of 3.5 and a temperature of 50 ° C with a molecular formula of C15H11O, where it can be degraded at temperatures above 70 ° C [51]. The comparison of the average extraction yield can be seen in Figure 2.

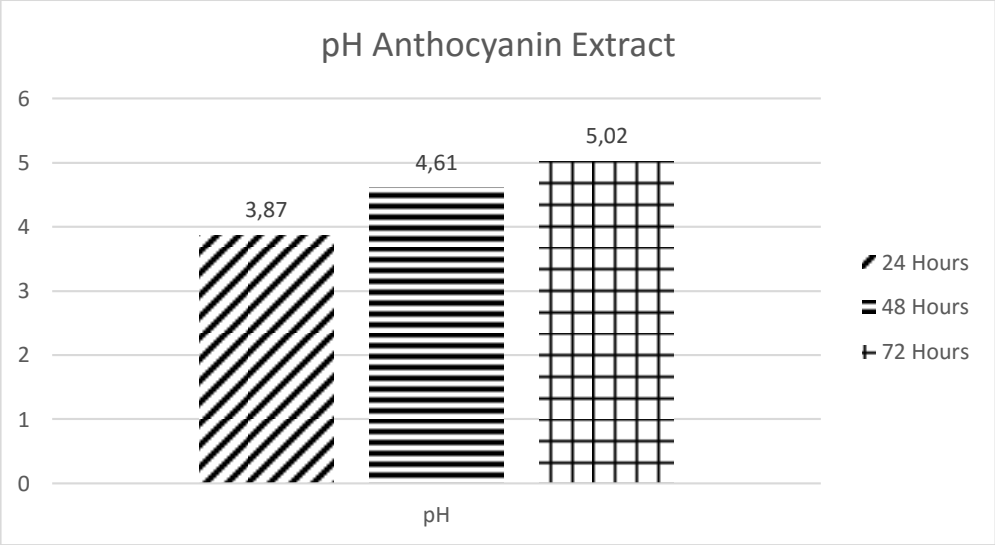

Figure 2. pH of Anthocyanin Extract

The 24-hour treatment resulted in a low pH is pH 3.87. This is due to the short maceration process time. The more acidic conditions approaching pH 1 will cause more anthocyanin pigments to be in the form of colored flavilium or oxonium cations and absorbance measurements will show the greater number of anthocyanin compounds. In the 48 hours treatment, the pH increased to 4.61 because the color of the anthocyanin extract of Blue Pea Vine is blue. According to [52], at pH> 3 (weak acid) the bright red color of the flavilium cation then changes form to a blue quinonoidal base or a colorless carbinol pseudobase as the pH increases to pH 7.

In the 72 hours treatment, there was an increase in the pH increase, namely pH 5.02. This is because the maceration process takes too long, so that the resulting pH increases. The longer the maceration process the structure of the anthocyanin compound will form more OH, causing the resulting anthocyanin pH to approach alkaline. Anthocyanins can degrade between pH increases [53]. The pseudobase formed undergoes tautomeric equilibrium. The balance between the keto form and the enol form produces alpha dicetone which produces a blue color. The decrease in the degree of redness is due to the structural transformation reaction of flavilium cations into chalcones and the higher the pH value will stimulate further hydration to form pseudobasic compounds in the form of keto, anhydrobase and anhydro-alkaline ionized [52].

Relatively, the good pH extraction result is the maceration process with 24 hours treatment because the resulting pH is a pH range of 3.87, where anthocyanins are stable at pH 3.5 or an acidic pH range. When compared with the test [51] related to the extraction of Blue Pea Vine and using a water solvent with a ratio of 1: 500 which was immersed for 75 minutes, it will produce a pH of 3. Although the resulting pH is in accordance with the optimum pH of anthocyanins, the resulting absorbance value is low, namely in the range of 0.167 mg / 100g, while the absorbance value of 1% HCl extract in 95% ethanol with a ratio of 1: 5, the resulting absorbance value is relatively high, namely 1.023 mg / 100g. The pH value resulting from the 48 hours treatment is close to the optimum pH of the anthocyanin pigment (pH 1-3), which is pH 4.61.

4. Conclusion

Based on the tests that have been carried out and the exposure of the discussion, it can be concluded that the best extraction time is at 1% HCl in 95% ethanol, namely the 48 hours treatment. The resulting anthocyanin levels were higher than the 24-hour and 72-hour treatments, namely 172.833 mg / 100g. The color produced is based on the brightness of the achromatic color from the extraction of the 48-hour treatment, which is close to blue with an L * value of 25.95, a * -0.4 value, and a b * value of 1.35. The degree of acidity or the resulting pH is pH 4.61.

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References

[1] Lakshan, S. A. T., Pathirana, C. K., Jayanath, N. Y., Abeysekara, W. P. K. M., & Abeysekara, W. K. S. M. (2020). Antioxidant and selected chemical properties of the flowers of three different varieties of Butterfly Pea (Clitoria ternatea L.). *Ceylon Journal of Science*, *49*(2), 195-201.

[2] Sutakwa, A., & Pangan, P. T. (2021). The Addition of Blue Pea Flower (Clitoria ternatea L.) Extract Increase Antioxidant Activity in Yogurt from Various Types of Milk. *Jurnal Agercolere*.

[3] Suarna, I. W., & Wijaya, I. M. S. (2021). Butterfly Pea (Clitoria ternatea L.: Fabaceae) and Its Morphological Variations in Bali. *Journal of Tropical Biodiversity and Biotechnology*, *6*(2), 63013.

[4] Madukokila, U. A. A. D., Jemziya, M. B. F., Wijewardhane, R. M. N. A., & Rifath, M. R. A. (2021). Development and quality evaluation of blue butterfly pea flower (clitoria tternatea L.) extract incorporated jelly.

[5] Srikanth, N., Mahajon, B., Chincholikar, M., Narayanan, R., Venigalla, S., Sharma, B. S., ... & Ahmad, A. (2020). Basis for Use of Substitutes for Medicinal Flora and Harmonization of Rational Use: A Critical Appraisal Based on "Kitab al-Abdal": A Classical Compendium of Unani Medicine.

[6] Kumar, M., & More, D. (2019). Phytochemical analysis and bioactivity of selected medicinal plant of butterfly-pea (Clitoria ternatea L.) used by Kolam tribe Addjoing region of Telangana and Maharashtra states. *Pharma Innovation*, *8*(1), 417-421.

[7] Chakraborthy, G. S., Kumar, V., Gupta, S., Kumar, A., Gautam, N., & Kumari, L. (2018). Phytochemical and pharmacological aspects of Clitoria ternatea-a review. *Journal of Applied Pharmaceutical Sciences and Research*, 3-9.

[8] Lakshan, S. A. T., Pathirana, C. K., Jayanath, N. Y., Abeysekara, W. P. K. M., & Abeysekara, W. K. S. M. (2020). Antioxidant and selected chemical properties of the flowers of three different varieties of Butterfly Pea (Clitoria ternatea L.). *Ceylon Journal of Science*, *49*(2), 195-201.

[9] Dwiputri, M. C., & Feroniasanti, Y. L. (2019, June). Effect of fermentation to total titrable acids, flavonoid and antioxidant activity of butterfly pea kombucha. In *Journal of Physics: Conference Series* (Vol. 1241, No. 1, p. 012014). IOP Publishing.

[10] Asysyifa, A., Agustiningtyas, A., & Nurgina, A. I. (2020). 63P Butterfly pea (Clitoria ternatea Linn.) flower extract prevents MCF-7 HER2-positive breast cancer cell metastasis in-vitro. *Annals of Oncology*, *31*, S1266.

[11] Jeyaraj, E. J., Lim, Y. Y., & Choo, W. S. (2021). Effect of Organic Solvents and Water Extraction on the Phytochemical Profile and Antioxidant Activity of Clitoria ternatea Flowers. *ACS Food Science & Technology*, *1*(9), 1567-1577.

[12] Lewis, E. R., Nogueira, M., Enfissi, E. M., & Fraser, P. D. (2021). The esterification of xanthophylls in Solanum lycopersicum (tomato) chromoplasts; the role of a non-specific acyltransferase. *Phytochemistry*, *191*, 112912.

[13] Sharif, N., Khoshnoudi-Nia, S., & Jafari, S. M. (2020). Nano/microencapsulation of anthocyanins; a systematic review and meta-analysis. *Food Research International*, *132*, 109077.

[14] Zhuang, H., Lou, Q., Liu, H., Han, H., Wang, Q., Tang, Z., ... & Wang, H. (2019). Differential regulation of anthocyanins in green and purple turnips revealed by combined de novo transcriptome and metabolome analysis. *International journal of molecular sciences*, *20*(18), 4387.

[15] Aliaño-González, M. J., Jarillo, J. A., Carrera, C., Ferreiro-González, M., Álvarez, J. Á., Palma, M., ... & Espada-Bellido, E. (2020). Optimization of a novel method based on ultrasound-assisted extraction for the quantification of anthocyanins and total phenolic compounds in blueberry samples (Vaccinium corymbosum L.). *Foods*, *9*(12), 1763.

[16] Duangpapeng, P., Lertrat, K., Lomthaisong, K., Paul Scott, M., & Suriharn, B. (2019). Variability in anthocyanins, phenolic compounds and antioxidant capacity in the tassels of collected waxy corn germplasm. *Agronomy*, *9*(3), 158.

[17] Stój, A., Kapusta, I., & Domagała, D. (2020). Classification of red wines produced from Zweigelt and Rondo grape varieties based on the analysis of phenolic compounds by UPLC-PDA-MS/MS. *Molecules*, *25*(6), 1342.

[18] Budiene, J., Guclu, G., Oussou, K. F., Kelebek, H., & Selli, S. (2021). Elucidation of volatiles, anthocyanins, antioxidant and sensory properties of cv. Caner pomegranate (Punica granatum L.) juices produced from three juice extraction methods. *Foods*, *10*(7), 1497.

[19] Alappat, B., & Alappat, J. (2020). Anthocyanin pigments: Beyond aesthetics. *Molecules*, *25*(23), 5500.

[20] Yuniati, Y., Elim, P. E., Alfanaar, R., & Kusuma, H. S. (2021). Extraction of anthocyanin pigment from hibiscus sabdariffa l. by ultrasonic-assisted extraction. In *IOP Conference Series: Materials Science and Engineering* (Vol. 1010, No. 1, p. 012032). IOP Publishing.

[21] Kangthin, K., Naratjarunsap, R., Puttaruk, P., Wisidsri, N., Jamkom, K., & Thungmungmee, S. (2021). Pigment stability in Chaba maple (Hibiscus acetosella Welw. Ex Hiern.) petals extract as natural food and cosmetic colorants. *Songklanakarin Journal of Science & Technology*, *43*(2).

[22] Jamil, N., Zairi, M. N. M., Nasim, N. A. I. M., & Pa'ee, F. (2018). Influences of Environmental Conditions to Phytoconstituents in Clitoria ternatea (Butterfly Pea Flower)–A Review. *Journal of Science and Technology*, *10*(2).

[23] Thuy, N. M., Minh, V. Q., Ben, T. C., Thi Nguyen, M. T., Ha, H. T. N., & Tai, N. V. (2021). Identification of Anthocyanin Compounds in Butterfly Pea Flowers (Clitoria ternatea L.) by Ultra Performance Liquid Chromatography/Ultraviolet Coupled to Mass Spectrometry. *Molecules*, *26*(15), 4539.

[24] Pham, T. N., Lam, T. D., Nguyen, M. T., Le, X. T., Vo, D. V. N., Toan, T. Q., & Vo, T. S. (2019, July). Effect of various factors on extraction efficiency of total anthocyanins from Butterfly pea (Clitoria ternatea L. Flowers) in Southern Vietnam. In *IOP Conference Series: Materials Science and Engineering* (Vol. 544, No. 1, p. 012013). IOP Publishing.

[25] Tuan Putra, T. N. M., Zainol, M. K., MohdIsa, N. S., & MohdMaidin, N. (2021). Chemical characterization of ethanolic extract of Butterfly pea flower (Clitoria ternatea). *Food Research*, *5*(4), 127-134.

[26] Vilela, A. (2019). The importance of yeasts on fermentation quality and human health-promoting compounds. *Fermentation*, *5*(2), 46.

[27] Lubis, D. O., & Hendri, M. (2020). Potential Bioactive Compound Seaweed Halimeda micronesica and Halimeda macroloba as Antioxidant and Phytochemical Screening of Active Seaweed Extracts from Maspari Island. *International Journal of Marine Science*, *10*.

[28] Lastriyanto, A., & Kartika, A. A. (2020, October). Innovation Of Propolis Extraction Machine Based On Vacuum Resistive Heating. In *Journal of Physics: Conference Series* (Vol. 1665, No. 1, p. 012012). IOP Publishing.

[29] Isa, A. A., Samsuri, S., & Amran, N. A. (2019, June). Integration of Maceration and Freeze Concentration for Recovery of Vitamin C from Orange Peel Waste. In *IOP Conference Series: Earth and Environmental Science* (Vol. 268, No. 1, p. 012101). IOP Publishing.

[30] Leite, P., Sousa, D., Fernandes, H., Ferreira, M., Costa, A. R., Filipe, D., ... & Salgado, J. M. (2021). Recent advances in production of lignocellulolytic enzymes by solid-state fermentation of agro-industrial wastes. *Current Opinion in Green and Sustainable Chemistry*, *27*, 100407.

[31] Belwal, T., Ezzat, S. M., Rastrelli, L., Bhatt, I. D., Daglia, M., Baldi, A., ... & Atanasov, A. G. (2018). A critical analysis of extraction techniques used for botanicals: Trends, priorities, industrial uses and optimization strategies. *TrAC Trends in Analytical Chemistry*, *100*, 82-102.

[32] Sharayei, P., Azarpazhooh, E., Zomorodi, S., & Ramaswamy, H. S. (2019). Ultrasound assisted extraction of bioactive compounds from pomegranate (Punica granatum L.) peel. *Lwt*, *101*, 342-350.

[33] Ozturk, B., Parkinson, C., & Gonzalez-Miquel, M. (2018). Extraction of polyphenolic antioxidants from orange peel waste using deep eutectic solvents. *Separation and Purification Technology*, *206*, 1-13.

[34] Nadar, S. S., Rao, P., & Rathod, V. K. (2018). Enzyme assisted extraction of biomolecules as an approach to novel extraction technology: A review. *Food Research International*, *108*, 309-330.

[35] Chemat, F., Abert-Vian, M., Fabiano-Tixier, A. S., Strube, J., Uhlenbrock, L., Gunjevic, V., & Cravotto, G. (2019). Green extraction of natural products. Origins, current status, and future challenges. *TrAC Trends in Analytical Chemistry*, *118*, 248-263.

[36] Setford, P. C., Jeffery, D. W., Grbin, P. R., & Muhlack, R. A. (2017). Factors affecting extraction and evolution of phenolic compounds during red wine maceration and the role of process modelling. *Trends in Food Science & Technology*, *69*, 106-117.

[37] Medlej, M. K., Cherri, B., Nasser, G., Zaviska, F., Hijazi, A., Li, S., & Pochat-Bohatier, C. (2020). Optimization of polysaccharides extraction from a wild species of Ornithogalum combining ultrasound and maceration and their anti-oxidant properties. *International Journal of Biological Macromolecules*, *161*, 958-968.

[38] Calvi, L., Pentimalli, D., Panseri, S., Giupponi, L., Gelmini, F., Beretta, G., ... & Giorgi, A. (2018). Comprehensive quality evaluation of medical Cannabis sativa L. inflorescence and macerated oils based on HS-SPME coupled to GC–MS and LC-HRMS (q-exactive orbitrap®) approach. *Journal of pharmaceutical and biomedical analysis*, *150*, 208-219.

[39] Panja, P. (2018). Green extraction methods of food polyphenols from vegetable materials. *Current Opinion in Food Science*, *23*, 173-182.

[40] Pinela, J., Prieto, M. A., Pereira, E., Jabeur, I., Barreiro, M. F., Barros, L., & Ferreira, I. C. (2019). Optimization of heat-and ultrasound-assisted extraction of anthocyanins from Hibiscus sabdariffa calyces for natural food colorants. *Food chemistry*, *275*, 309-321.

[41] Belwal, T., Huang, H., Li, L., Duan, Z., Zhang, X., Aalim, H., & Luo, Z. (2019). Optimization model for ultrasonic-assisted and scale-up extraction of anthocyanins from Pyrus communis 'Starkrimson'fruit peel. *Food chemistry*, *297*, 124993.

[42] Manousi, N., Sarakatsianos, I., & Samanidou, V. (2019). Extraction techniques of phenolic compounds and other bioactive compounds from medicinal and aromatic plants. In *Engineering tools in the beverage industry* (pp. 283-314). Woodhead Publishing.

[43] Mastuti, E., Sari, N. P., & Simangunsong, R. A. (2013). Ekstraksi Zat Warna Alami Kelopak Bunga Rosella dengan Pelarut Aquadest. Universitas Sebelas Maret, 12(2), 43–47.

[44] Sangadji, I., Rijal, M., & Astri, Y. (2017). Kandungan Antosianin di dalam Mahkota Bunga Beberapa Tanaman Hias. Jurnal Biology Science & Education, 6(2), 118–128

[45] Sangadji, I., Rijal, M., & Astri, Y. (2017). Kandungan Antosianin di dalam Mahkota Bunga Beberapa Tanaman Hias. Jurnal Biology Science & Education, 6(2), 118–128

[46] Mastuti, E., Sari, N. P., & Simangunsong, R. A. (2013). Ekstraksi Zat Warna Alami Kelopak Bunga Rosella dengan Pelarut Aquadest. Universitas Sebelas Maret, 12(2), 43–47.

[47] Jeyaraj, E. J., Lim, Y. Y., & Choo, W. S. (2021). Effect of Organic Solvents and Water Extraction on the Phytochemical Profile and Antioxidant Activity of Clitoria ternatea Flowers. *ACS Food Science & Technology*, *1*(9), 1567-1577.

[48] Jeyaraj, E. J., Lim, Y. Y., & Choo, W. S. (2021). Extraction methods of butterfly pea (Clitoria ternatea) flower and biological activities of its phytochemicals. *Journal of food science and technology*, *58*(6), 2054-2067.

[49] Rothenberg, D. O. N., Yang, H., Chen, M., Zhang, W., & Zhang, L. (2019). Metabolome and transcriptome sequencing analysis reveals anthocyanin metabolism in pink flowers of anthocyanin-rich tea (Camellia sinensis). *Molecules*, *24*(6), 1064.

[50] Rakić, V., & Poklar Ulrih, N. (2021). Influence of pH on color variation and stability of cyanidin and cyanidin 3-O-β-glucopyranoside in aqueous solution. *CyTA-Journal of Food*, *19*(1), 174-182.

[51] Zussiva, A., Bertha, K. L., & Budiyati, C. S. (2012). Ekstraksi dan Analisis Zat Warna Biru (Anthosianin Anthosianin) dari Bunga Telang (Clitoria ternatea) Sebagai Pewarna Alami. Jurnal Teknologi Kimia dan Industri, 1(1), 356–365

[52] Ingrath, W., Nugroho, W. A., & Yulianingsih, R. (2015). Ekstraksi Pigmen Antosianin dari Kulit Buah Naga Merah (Hylocereus costaricensis) Sebagai Pewarna Alami Makanan dengan Menggunakan Microwave. Jurnal Bioproses Komoditas Tropis, 3(3), 1–8.

[53] Wu, H. Y., Yang, K. M., & Chiang, P. Y. (2018). Roselle anthocyanins: Antioxidant properties and stability to heat and pH. *Molecules*, *23*(6), 1357.