JOURNAL OF AGROBIOTECHNOLOGY 2024, VOL 15(S1):34-39.
e-ISSN: 2180-1983
http://dx.doi.org/10.37231/jab.2024.15.S1.371
https://journal.unisza.edu.my/agrobiotechnology/index.php/agrobiotechnology/index

Rheological Properties of Butterfly Pea- derived anthocyanins extracted using Ultrasonic Assisted Extraction method

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Received: 05/07/2023, Accepted: 06/01/2024, Available Online: 30/01/2024

ABSTRACT

Anthocyanin from butterfly pea extract is used for the skin brightening, rejuvenating, and hydration of serum product as the phenolic compound have the antioxidants effect. Its stability generally influenced by pH, temperature, light, metal ions in media, and other factors. It is important to monitor the temperature fluctuations because rising temperatures will lead to the partial or complete degradation of natural anthocyanins, which will reduce the intensity of the colour. However, the rheology involving density and viscosity of anthocyanin derived from butterfly pea flower are yet to be verified. In this study, we investigate the rheological characteristics of synthetic skin infused with anthocyanin from butterfly pea. Two sources of anthocyanin were used in this study: liquid anthocyanin from Bionutricia Malaysia, and raw extracted anthocyanin from dried butterfly pea flower. The dried butterfly peas are extracted using the Ultrasonic Assisted Extraction (UAE) process in an ultrasonic water bath. Total anthocyanin content (TAC) and physicochemical analysis consist of measurement of density, viscosity, and shear stress of extract have been investigate throughout the process. The total anthocyanin content (TAC) of raw and liquid butterfly pea extract was determined to be 261.28 mg/L and 32.272 mg/L respectively from the obtained absorbance at wavelength 510 nm and 700 nm using UV-Visible Spectroscopy. It is discovered that the extracted anthocyanin indicates non-Newtonian behaviour and pseudoplastic rheology behaviour for both samples.

Keywords: Anthocyanin, Butterfly Pea, Rheology, Ultrasonic Assisted Extraction

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INTRODUCTION

The natural pigments known as anthocyanins are commonly present in plants, that provides flowers, fruits, and vegetables their blue, purple, and red hues (Khoo et al, 2017). It has a variety of biological functions and are potential natural antioxidants and colourants for the food sector (Lourenço et al., 2019). A tropical plant known as the butterfly pea is rich in anthocyanins, and linked to a number of health advantages, including anti-inflammatory, anti-diabetic, and antihypertensive effects (Ashraf et al., 2023).

The extraction of anthocyanins from plant materials is a crucial step in their utilization as natural colorants and antioxidants. Various extraction methods have been developed to isolate anthocyanins, including conventional solvent extraction, microwave-assisted extraction, and ultrasonic-assisted extraction (UAE) (Rodsamran and Sothornvit, (2019). Among them, UAE has gained considerable attention due to its advantages, such as high extraction efficiency, short extraction time, and low energy consumption (Shen et al., 2023).

Rheology is the study of the flow and deformation of matter. It is an important tool for characterizing the physical properties of materials and is widely used in the food and pharmaceutical industries. Rheological measurements can provide information about the texture, viscosity, and flow behavior of materials, which are important for their processing and quality control.

In this study, we aim to extract anthocyanin derived from Butterfly Pea using the ultrasonic-assisted extraction method and to evaluate the rheological properties of the extracted anthocyanin. The rheological properties of the extracted anthocyanin were investigated using a rheometer. The results of this study provide important information on the rheological properties of anthocyanin extracted from Butterfly Pea and contribute to the development of natural colorants and antioxidants for the food industry.

MATERIALS AND METHODS

Preparation of butterfly pea extract from dried flowers

Liquid butterfly pea flowers were bought from Bionutricia Malaysia. 3kg of raw butterfly pea flowers was prepared for sorting, cutting, and cleaning process. The flowers were dried in drying oven (Memmert, UN30, USA) at 60°C for 2 days to remove moisture. Then, the acidified water extraction solvent was prepared by mixing 1M acetic acid and distilled water until reach pH 4.0. The dried flowers were soaked in acidified water with ratio 1:4 (w/v) in a 250ml beaker. The mixture of soaked flowers with acidified water was placed into ultrasonic water bath at 30°C for 30 minutes (Syafa'Atullah et al., 2020). The extract was filtered and stored at 4 ℃ in the refrigerator for subsequent analysis (Erna et al., 2022).

Determination of total anthocyanin content (TAC)

TAC was analyzed using the differential pH method described by Guisti and Wrolstad (2001). The pH-difference method is based on the reaction at pH 1 and pH4.5, allowing accurate and rapid measurement of total anthocyanins. The total anthocyanin content (TAC) was determined as delphinidin 3-glucoside using the pH-differential approach (Ayu et al., 2018) using UV-Visible Spectroscopy. For 15 minutes, anthocyanin extracts were liquefied in a 0.025 M KCl buffer at pH 1 and 0.4 M $C_2H_3NaO_2$ at pH 4.5 with an extract-to-buffer ratio of 1:33 (v/v). The absorbance value and TAC was calculated using **Eqn. 1** respectively (Erna et al., 2022).

$$TA = \frac{A_{530-}\ 0.3_{657} \times V}{M} \qquad \textbf{Eqn. 1}$$

TA = total anthocyanin, *A* = absorbance at 530 and 657 nm, *V* = volume of extract (mL) and *M* = fresh mass of the sample (g).

Rheological Analysis

The rheological properties of both anthocyanins were evaluated using a Modular compact rheometer (Anton Parr, MCR 302) throughout a temperature range of 25 to 85 °C. The samples were distributed abundantly to fill the space between gap at 0.5mm with 50mm flat spindle.

RESULTS AND DISCUSSION

Total Anthocyanin Content (TAC)

The total anthocyanin content (TAC) of raw extracted and liquid butterfly pea extract was determined to be 261.28 mg/L and 32.272 mg/L respectively from the obtained absorbance at wavelength 510 nm and 700 nm. Butterfly pea contains a high concentration of ternatins, which are blue in colour and acylated on the basis of delphinidin. These structures were identified as delphinidin-3-glycosides, with a total of 15 (poly) acylated delphinidin glucosides, namely ternatins, and numerous more delphinidin derivatives identified in various research (Jeyaraj et al., 2021). The overall anthocyanin concentration in crude extracts containing other phenolic compounds was calculated by measuring the solution's absorptivity at a single wavelength (Mónica Giusti & Wrolstad, 2005).

The anthocyanin absorption spectrum and pH differential for raw and liquid are depicted in **Fig. 1** respectively. Anthocyanin absorbs less light at pH 4.5 due to hydration of the anthocyanin structure's C-ring, and the positive charge is neutralised. When exposed to pH 4.5 or higher, it will also change into the more stable colourless carbinol. At pH less than 2, the anthocyanin structure is found to be stable in blue ternatins. The total anthocyanin content can change due to differences in colour strength, type, and ambient conditions (Erna et al., 2022).

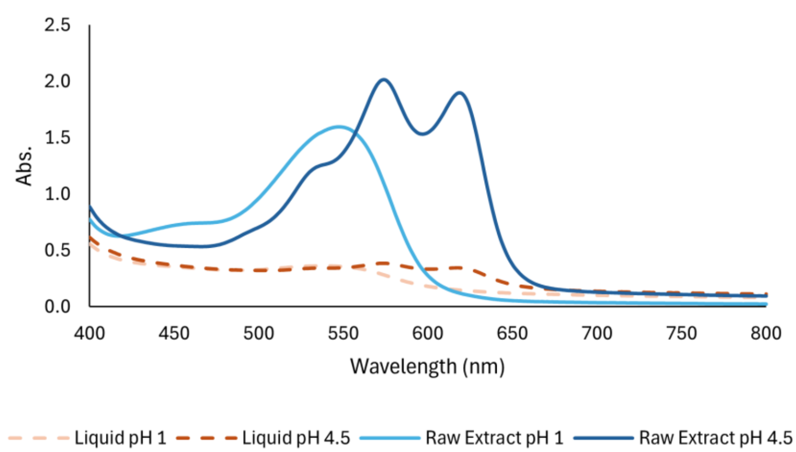

Fig. 1 Spectral characteristics of two sources anthocyanin from butterfly pea anthocyanins (Delphinidin-3-glucoside) in pH 1.0 KCl and pH 4.5 NaOAc buffers.

Temperature has a strong influence on the viscosity of butterfly pea extract, and viscosity influences the shear stress (Song et al., 2015). This experiment primarily characterises the rheological physico-chemical properties of butterfly pea extract with relative of temperature changes from 25°C to 85°C (Smith, 2021). The gap distance between the geometry and plate of rheometer comprising each extract sample tested is constant at 0.05 mm. **Fig. 2** shows as the temperature increase, the shear stress of raw extract escalates higher than liquid extract. However, the shear stress value starts to decline after exceeding 75°C. Shear stress of liquid extract increases relatively during the temperature increments.

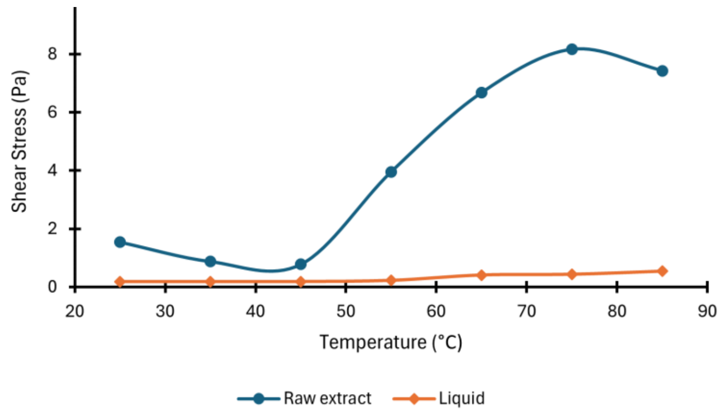

Fig. 2 Corelation of shear stress on temperature changes for both anthocyanin sources.

The liquid state of the substance is composed of an enormous number of random motion molecules, and its viscosity performance is highly dependent on the molecule state. The viscosity of liquid is the total manifestation of intermolecular attraction and momentum transfer; intermolecular attraction decreases significantly as the space between molecules increases, and molecular momentum relies on the rate of movement.

Due to the small space between liquid molecules and the average velocity of random motion is low, intermolecular force is the primary determinant of viscidity performance. When the temperature rises, the average velocity of butterfly pea extract molecular motion increases, as does the spacing between molecules. As a result, as the temperature rises, the viscosity of the liquid lowers (Song et al., 2015).

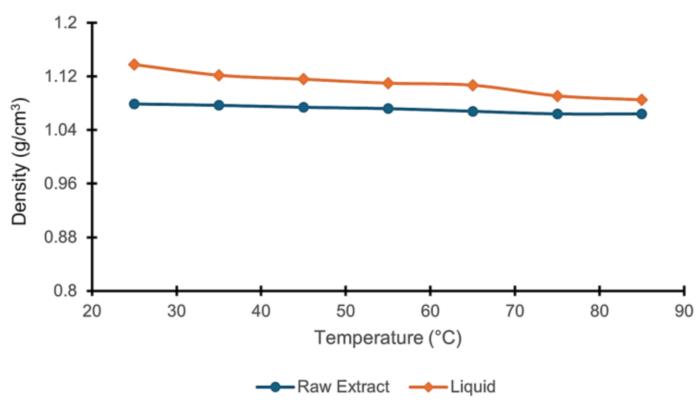

Fig. 3 Corelation of density on temperature changes for both anthocyanin sources.

Based on **Fig. 3**, the density of raw and liquid anthocyanin extract decrease relatively as the temperature rises from 25°C to 85°C. This is due to the molecules of anthocyanin extract are in continual motion. A high temperature is inextricably linked the liquid's lower density. Temperature influences particle motion in the form of thermal and kinetic energy, which dictates particle velocity. As the temperature rises, so does the molecular kinetic energy, and hence the velocity of the particles. Density decreases as temperature rises because faster particles have shorter contacts with molecules, reducing internal friction. Hence, decreasing density. It was proven that the liquid anthocyanin extract is denser than the raw anthocyanin extract.

CONCLUSION

In conclusion, this study demonstrated that UAE is an effective method for the extraction of anthocyanin from butterfly pea flowers as it produced high TAC content at 261.28 mg/L in comparison with liquid anthocyanin manufactured by Bionutricia with TAC value of 32.272 mg/L. The extracted anthocyanin exhibited pseudoplastic behavior with high viscosity and yield stress. The rheological properties of the anthocyanin were dependent on the solvent used for extraction. The use of acidified distilled water as the solvent and an extraction time of 30 minutes yielded the high anthocyanin content with desirable rheological properties in comparison liquid anthocyanin extracted by 50% ethanol. The findings of this study could be useful for the development of functional foods and nutraceuticals enriched with anthocyanins derived from butterfly pea flowers.

ACKNOWLEDGMENTS

The author would like to thank Ministry of Higher Education under the Fundamental Research Grant Scheme (FRGS) number: FRGS/1/2021/STG01/UNIKL/02/3 for funding this project. Thank you too, Universiti Kuala Lumpur Kampus Cawangan Malaysia Institute of Chemical and Bioengineering Technology (Unikl-MICET) for the equipmemts provided throughout the process of achieving the objective of the research.

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How to cite this paper:
Hamdan. S.H., Asri, M.A.A, Halid, I , Mohamad, S.N, Ahmad Hamidi F.W, and William C. W. F (2024). Rheological Synthesis of Anthocyanin derived Butterfly Pea using Ultrasonic Assisted Extraction method, Journal of Agrobiotechnology, *15*(S1), 34-39.