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Release Behavior of Butterfly Pea Anthocyanins from Sago Starch Micro-composite Films Under Food Stimulants

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Abstract

This study investigates the release behaviour of Butterfly Pea (BP) anthocyanins from Sago starch micro-composite (SMC) films in the presence of food stimulants. To comprehensively describe the release profiles of BP from SMC, six well-established kinetic release models were employed which includes Korsmeyer-Peppas, zero-order, first-order, second-order, Higuchi, and Hixson-Crowell models. These models played a crucial role in examining how the release fraction changes over time, facilitating a comprehensive understanding of the release kinetics of BP anthocyanins. Our data analysis revealed a strong conformity with the Korsmeyer-Peppas model, suggesting its applicability in describing the release of BP anthocyanins from SMC. The predominant release mechanism observed in this study was consistent with Fickian diffusion, as indicated by values of $n < 0.50$. This investigation provides valuable insights into the release dynamics of BP anthocyanins from SMC films when subjected to food stimulants, contributing to the scientific understanding of controlled re-lease systems in food and pharmaceutical applications.

Keywords: Release kinetics, Anthocyanin, Butterfly pea, Sago starch, Fickian diffusion

1. Introduction

Anthocyanins, a class of natural pigments responsible for the vibrant red, blue, and purple hues in various fruits, vegetables, and flowers, have garnered substantial attention in recent years due to their multifaceted roles in food science, nutrition, and health. Among the numerous aspects of anthocyanin research, understanding their release kinetics from diverse matrices has emerged as a pivotal area of investigation. The release kinetics of anthocyanins play a fundamental role in determining their bioavailability, stability, and functionality in various applications, particularly in the fields of food science and pharmaceuticals. Release kinetics, addresses the processes that lead to anthocyanins being released from their host matrices, are influenced by a multitude of factors, including the physicochemical properties of both the anthocyanins and the matrices in which they are integrated. To comprehend and predict the release behavior of anthocyanins, researchers have employed various kinetic models that offer valuable insights into the underlying mechanisms (Wang et al., 2017).
Clitoria ternatea L., commonly referred to as butterfly pea or blue pea (BP), is a perennial leguminous herb belonging to the Fabaceae family (Gamage et al., 2021). This versatile plant holds numerous agricultural and medical applications (Ogus et al., 2019). In Southeast Asia, BP flower is even consumed as a vegetable (Leong et al., 2017), and its extract finds its way into desserts and beverages, particularly in countries like Malaysia and Thailand (Pasukamonset et al., 2017). BP also owns a broad spectrum of colour changes when exposed to various pH buffers (Ahmad et al., 2020). This pH-sensitive colour-changing feature holds significant importance in smart packaging applications, where it can serve as a real-time indicator of product freshness and quality. In this investigation, the immobilization of BP anthocyanin was achieved by strategically employing a micro-composite composed of sago starch (Metroxylon sagu) as the designated polymer matrix. Undeveloped and underutilized sago starch emerges as a strong competitor in the field of packaging applications, potentially outperforming conventional corn, or maize starch. The growing importance of sago starch is particularly highlighted by its expanding applications (Tan et al., 2015; Oladzadabbasadi et al., 2016), where its adaptable characteristics have been harnessed to create innovative solutions. Furthermore, sago starch has made new beginnings into the areas of pharmaceutical and medical applications (Tan et al., 2015; Oladzadabbasadi et al., 2017) demonstrating its capacity to go beyond traditional boundaries. In summary, the trajectory of sago starch research has transitioned from relative obscurity to prominence, driven by its flexible attributes and wide-ranging applicability.

In this study, several kinetic models have been utilized to describe and quantify the release of anthocyanins from sago starch micro-composite (SMC). These models encompass a range of mathematical expressions, including zero-order, first-order, second-order, Higuchi, Korsmeyer-Peppas, and Hixson-Crowell models, among others. Each of these models serves a unique purpose in interpreting different sides of anthocyanin release behavior. Studying anthocyanin release kinetics has impacts that go far beyond just scientific interest. It holds practical use for industries involved in food product development, drug delivery systems, and functional food formulation. By comprehending the release kinetics of anthocyanins, researchers and industries can fine-tune formulations to optimize colour stability, bioavailability, and sensory attributes, thus enhancing the overall quality and efficacy of products. To the best of our knowledge, no prior studies have assessed the release kinetics from sago starch micro-composite. This distinctive approach to using sago starch as a matrix for release kinetics study adds a new and unexplored perspective to our pursuit of knowledge.

2. Materials and methods

2.1. Materials

Sago starch and butterfly pea (Clitoria ternatea) were purchased from local market. Ethanol and Kurroman Chloride were obtained from Sigma-Aldrich (USA). All reagents employed in this study were of analytical grade and were used without further purification.

2.2. Anthocyanin Extraction

Fresh BP flowers were collected and subjected to a 24 h drying period to eliminate excess moisture. The resulting dried BP remnants were then ground for 2 min using a mixer (Phillips HL7756/00). Anthocyanin extraction was carried out using an aqueous method, wherein 1 mg/mL extracts were prepared by combining BP powder with distilled water. This extraction process occurred at room temperature (25 ± 1 °C), employing a magnetic stirrer (CB162 Stirrer Hotplate, Keison Products, UK) for continuous agitation over the course of 1 h to ensure thorough homogenization. Subsequently, the extract solution underwent vacuum filtration (GM-0.33II Diaphragm Vacuum Pump, Oriner Hightech Sdn. Bhd., Malaysia) to eliminate any undissolved solids. The resulting extract was stored in the dark at 4 ± 1 °C until further analysis.

2.3. Preparation of Films

The experimental design consisted of two stages and the preparation and characterization of micro-particles employing sago starch was described in our previous study (Ahmad et al., 2023). For the preparation of micro-composite films, only 3-SMPs films were selected and utilized based on the results of its optimal physical-mechanical properties (Ahmad et al., 2023). Sago micro-composite films were prepared by preparing films containing 0, 1, 2, 3, 4, and 5 wt% of extract into the cooled film forming solutions (wt% on suspension basis). The nomenclature of the films was as follows: films devoid of extracts were termed “SMC0” (0% w/w), while the designations “SMC1,” “SMC2,” “SMC3”...
“SMC4” and “SMC5” were attributed to films containing varying concentrations of BP extracts. The solutions were stirred and shaken for 30 min at 25 ± 1°C. Subsequently, aliquots (40 mL) of the final film-forming solutions were poured onto Teflon plates of equal dimensions and dried in an oven at 38 ± 1°C for 15 h. The films were conditioned in a desiccator at 24°C with a 50% relative humidity for 24 h until further analysis.

2.4. Determination of Anthocyanin Content

The anthocyanin content was determined by the pH differential method according to AOAC, Official Method 2005.02 (Lee et al., 2005). The absorbance was measured with a UV–Vis Spectrophotometer (Jenway 6850 UV/Vis Spectrophotometer, UK). Anthocyanin content was expressed as cyanidin-3-glucoside equivalent according to Eq. (1):

\[ C \left( \frac{mg}{L} \right) = \frac{A \times M_w \times DF \times 1000}{\varepsilon_m \times d} \]  

Where,

\[ A = (A_{\lambda_{\text{vis-max}}} \text{ pH 1.0} - (A_{\lambda_{\text{vis-max}}} \text{ pH 4.5}) \] is the molecular weight (g/mol) = 449.2 g/mol for Cy-3-glc, \( DF \) is the dilution factor, \( \varepsilon \) is the extinction coefficient (26,900 for Cy-3-gluc), and \( d \) is path length (1 cm in this analysis).

2.5. Release Behavior of Anthocyanin

Using food simulant solution (95% ethanol), the release of BP anthocyanin from the films was determined following the method of (Razavi et al., 2020) with minor modifications. The films (20 mm × 20 mm) were immersed into 100 mL aqueous ethanol in an Erlenmeyer flask and shaken at 100 rpm at 25°C under dark conditions. During the release process, 10 mL aliquots of fluids were periodically taken (5, 10, 15, 20, 40, 80, 100, 120 and 240 min) and measured at 525 nm using spectrophotometer. It should be noted that the solutions were returned right away to maintain the same amount of original solution. The amount of BP anthocyanin released from films was determined on the basis of regression equation. The mean values of cumulative release (%) of anthocyanin was then calculated as Eq. (2) as follows:

\[ \text{Cumulative release (\%)} = \frac{M_t}{M_0} \times 100 \]  

Where \( M_t \) (mg) is the amount of BP released from the films at time \( t \) and \( M_0 \) (mg) is the total amount of BP combined in the films. The cumulative release data have been fitted using a well-known Korsmeyer and Peppas semi-empirical model (Ritger & Peppas, 1987) to describe the release mechanism of BP from the films, as shown in Eq. (3):

\[ \frac{M_t}{M_\infty} = k \times t^n \]  

Where \( M_t/M_\infty \) is the fractional release of BP at time \( t \), \( k \) is the release rate constant of the macromolecular network system; \( n \) is the release exponent characterizing the BP release mechanism.

Other five classic models of kinetic release were also employed as comparison to fit the experimental data as follows (Eq. (4) – Eq. (8)).

(a) Zero-order kinetic model

\[ \frac{M_t}{M_\infty} = k \times t \]  

(b) First-order kinetic model

\[ 1 - \frac{M_t}{M_\infty} = e^{-kt} \]  

(c) Second-order kinetic model

\[ \frac{M_t}{M_\infty} = \frac{kt}{1 + kt} \]  

d) Higuchi kinetic model

\[ \frac{M_t}{M_\infty} = kt^{1/2} \]  

(e) Hixson-Crowell kinetic model

\[ \left( 1 - \frac{M_t}{M_\infty} \right)^{1/3} = -kt \]  

2.6. Statistical Analysis

All data in this study were performed in triplicates and expressed as means ± standard deviation. The statistical significance of differences between mean values was assigned by Analysis of Variance (ANOVA) with Tukey’s test using statistical software R-4.3.1 and OriginPro® 2021 (OriginLab Corporation, USA). The significance level used in this study was 0.05.
3. Results and discussion

3.1. Total Anthocyanin Content of BP

The assessment of total anthocyanin content in the samples revealed the total anthocyanin content extracted aqueously was 30.2 mg/g (expressed as cyanidin-3-glucoside equivalent), indicating high extraction efficiency in water.

3.2. Cumulative Release of BP Anthocyanin

The standard curve for anthocyanin quantification was constructed using known concentrations of Kuromanin Chloride as standards as seen in Fig. 1(a). The resulting equation, \( y = 0.7966x + 0.1415 \), demonstrated a strong correlation coefficient \( (R^2 = 0.9912) \), indicating the suitability of this standard curve for accurate anthocyanin quantification in the subsequent experiments. The release characteristics of anthocyanin from SMC1 to SMC5 in a 95% ethanol medium were investigated over a specified duration, as depicted in Fig. 1(b). The cumulative release data, expressed as a percentage relative to the total anthocyanin content, displayed unique patterns for each formulation. In the case of SMC1, the cumulative anthocyanin release after 240 min was 40.21%, whereas SMC5 exhibited a notably higher cumulative release of 78.94% within the same timeframe. These distinct release profiles highlight the varying abilities of the BP content formulations to control the release of anthocyanin in 95% ethanol.

The rapid initial release of BP, primarily from the surface or its immediate area within the SMC films, resulted in a minor burst release phenomenon within the first 10 min for all curves. Subsequently, the cumulative release percentage rose progressively before reaching a plateau. This plateau formation was attributed to the additional time required for BP anthocyanin, confined within the central part of the matrix, to be released through a longer release pathway (Wang et al., 2017). This observation suggests a commendable controlled release characteristic exhibited by the SMC films. Additionally, it was noted that higher BP content correlated with greater cumulative release at the same release time, indicating that BP release was influenced by changes in the diffusion driving force associated with BP content variations. A similar trend has been documented in other studies involving biopolymers incorporated with extracts high in anthocyanin (Liu et al., 2018; Wang et al., 2017).

3.3. Release Kinetics of BP anthocyanin from SMC Films

The release kinetics of BP anthocyanin from SMC films were investigated utilizing five well-known kinetic models, including zero-order, first-order, second-order, Korsmeyer-Peppas, Higuchi, and Hixson-Crowell models by analysing the release fraction \( M_t/M_{\infty} \) as a function of time \( t \). The release data exhibited distinctive trends for each model, revealing insights into the mechanisms of anthocyanin release (Fig. 2). Meanwhile, Table 1 shows the correlation coefficients and the release exponent (n). Among the kinetic models examined, the Korsmeyer-Peppas \( (R^2 = 0.9820 \text{ to } 0.9920) \) and Hixson-Crowell \( (R^2 = 0.9908 \text{ to } 0.9888) \) models emerged as the most suitable fits for describing the release behavior of BP anthocyanin from the SMC films. These models, which are widely applied in drug delivery and release studies, offered valuable insights into the release mechanism. The results showed that Fickian diffusion \( (n < 0.50) \) was the
primary mechanism for the release of BP from SMC films (0.22 for SMC1, 0.21 for SMC2, 0.12 for SMC3, 0.15 for SMC4 and 0.18 for SMC5). The release of BP from SMC films was partly ascribed to diffusive or permeative processes occurring within the swollen SMC films matrix. Additionally, it involved the traversal of aqueous pores and channels embedded in the matrix’s structural framework (Neo et al., 2013). Further, this diffusion process was driven by alterations in the BP content, contributing to the overall release dynamics.

Similarly, the Hixon-Crowell model, which describes changes in the surface area, demonstrated its applicability by revealing that the release of BP anthocyanin was influenced by changes in the SMC film structure. This suggests that alterations in the
4. Conclusions

In conclusion, our comprehensive investigation into the release kinetics of BP anthocyanin from SMC films has shed light on the intricate dynamics in relation to anthocyanin release. Employing a range of kinetic models, distinct release patterns were determined, with the Korsmeyer-Peppas and Hixson-Crowell models emerging as the most fitting representations for describing the release behavior. Our findings pointed to a Fickian diffusion mechanism as the predominant mode of BP anthocyanin release from the SMC films. This diffusion was not only influenced by the swollen sago starch matrix but also by the aqueous channels within it, with the diffusion driving force derived from changes in BP content. The applicability of the Hixson-Crowell model reinforced the influence of structural alterations within the film on anthocyanin release, documented by high $R^2$ values. The complexity revealed in our study illustrated the relationship between diffusion and structural changes within the SMC matrix. These insights hold significant promise for the development of controlled-release systems in diverse applications, particularly in the creation of active (e.g., antimicrobial) and intelligent (e.g., pH-sensing) films.

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Authors’ contributions

Afiqah Nabihah Ahmad played a role in conceptualization, original draft preparation, methodology design, software development, and formal analysis. Eng Tong Phuah engaged in meticulous reviewing and contributing to the methodology and visualization aspects. Namasivayam Navaranjan provided essential supervision and ensured the validation of the work. Syazana Abdullah Lim made significant contributions through reviewing and supervision, collectively shaping the project’s success. All authors approved the final version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

References


