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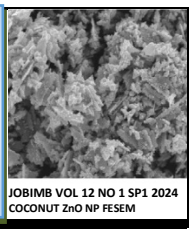

Utilization of Butterfly Pea Flower as Shelf-Life Colour Indicator for Keropok Lekor Smart Packaging

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ABSTRACT

Studying the feasibility of using *Clitoria ternatea* or butterfly pea extract (BPE) as a colorimetric indicator to determine the shelf life of Keropok Lekor would aid in monitoring storage conditions, emphasizing its potential contribution to the smart packaging industry. The objective of this research is to develop pH-sensitive packaging using gelatine film infused with butterfly pea extract (BPE) along with ultrasonic-assisted extraction methods. The investigation involves quantifying the physicochemical attributes of the extracted butterfly pea and ascertaining the colour alterations during the storage of Keropok Lekor over a duration of six days. The determination of total anthocyanin content in BPE, conducted through a pH differential assay, yielded a value of 1.08 mg/g. Texture analysis revealed that the film with 10% BPE exhibited elevated tensile strength (0.11±0.01) and toughness (0.03±0.01). Total phenolic compound analysis indicated that the film containing 30% BPE possessed the highest value at 0.16±0.02. Notably, the film with 30% BPE demonstrated the highest colour change in Keropok Lekor storage from dark blue (-22.5±80.95) to dark green (6.34±1.85) over the course of six days. The film incorporated with 30% BPE stands as a viable colour indicator, demonstrating the potential for monitoring the freshness and quality of Keropok Lekor.

INTRODUCTION

Clitoria ternatea or commonly known as 'Butterfly pea', is a flowering plant that belongs to the family of Fabaceae/Leguminosae [1]. It is native to tropical regions of Asia, including countries such as Thailand, Malaysia, and Indonesia. It is known for its vivid blue petals that contain a natural pigment called anthocyanin, which is pH-sensitive and changes colour depending on the acidity of its environment. It is used as a natural food antioxidant and colour staining [2]. Butterfly pea has gained popularity as a natural pH indicator for food packaging due to its anthocyanin content, which is highly sensitive to changes in pH [3]. When the petals are steeped in water, they release the blue pigment, which can be used to dye food products. The colour of the dye changes from blue to purple or even pink depending on the pH of the solution. For example, in acidic environments with a pH of 4 or lower, the dye turns purple or pink, while in basic environments with a pH of 8 or higher, the dye turns blue or green.

MATERIALS AND METHODS

Raw materials

C. ternatea or butterfly pea used in this study was obtained from Kuala Besut, Terengganu. All the samples were harvested and then chosen part which was the petals were isolate and stored at -18°C in freezer.

Ultrasonic-assisted extraction method

The samples were cleaned to remove any dirt and then dried in the oven and placed in an ultrasonic bath, where high-frequency sound waves created cavitation bubbles in the solution [4]. These bubbles helped break down plant cell walls and release phenolic

chemicals. After sonication, the liquid extract was filtered (Whatman filter paper 41) to remove any insoluble particles.

Determination of Total Anthocyanin Content

The total anthocyanin content of Butterfly Pea Flower was assessed utilizing the pH differential method [5]. Initially, the Butterfly Pea Flower extract test fraction was diluted in buffers of pH 1.0 and pH 4.5. After a preparation period of 50 min, the absorbance at wavelengths of 520 nm and 700 nm was measured.

Preparation of pH-Sensitive Film

Two milliliters of glycerol, and 5 g of maize starch were mixed in 100 ml of distilled water. The solution was then heated to at 120°C (1 h) and, was cooled to 40°C [6]. Then 10%, 20%, and 30% butterfly pea extract (BPE) then added and mixed for 30 min. The finished mixture was the poured into a cleaned mold and left to set for 48 h.

Physicochemical and microbial Analysis

Moisture, ash, crude fibre, crude protein, crude fat, and carbohydrate content were measured using AOAC (2023) methods [7]. The texture of the samples using a TA. XT Plus Texture Analyzer equipped with a 36 mm cylinder probe with radius (P/36R) and a 5kg load cell [8]. Colour analysis was carried using a colorimeter (Konica Minolta Chroma metre CR-400) to assess colour value [9]. Fourier Transform analytical analysis was carried out to determine the functional group present in the samples [10]. The total phenolic content (TPC) was executed employing the Folin-Ciocalteu method [3].

Application of film for keropok lekor

The pH-sensitive films were cut into 2 x 2 cm^2 pieces and attached to the tops of the petri dishes. Colour changes were observed from days 1 to 6, and a Minolta CT-310 Colorimeter (Konica Minolta) was used to measure the values of L*, a*, and b*.

Statistical analysis

Statistical analysis was performed through one-way analysis of variance (ANOVA) utilizing Minitab 21 statistical software. The mean comparisons were executed using Fisher's least significant difference (LSD) method, with results presented in the format of mean ± standard deviation.

RESULTS AND DISCUSSION

Determination of total anthocyanin content

The total anthocyanin content in BPE was determined 1.08 mg/g^{-1} by pH different assay. The blue pea flower anthocyanin extract's total anthocyanin concentration (TAC) can be impacted by the pH of the extraction water [11]. The extraction efficiency of anthocyanins can be affected by the acidity or alkalinity of the extraction solution, which can cause changes in the observed anthocyanin concentration. Similarly, Hasanah et al. [4] state that utilizing Ultrasonic Assisted Extraction (UAE) resulted in a 246.48% higher anthocyanin production with a total anthocyanin content of 1.13 mg/g. Anthocyanin is soluble in both warm and cold water and stable in the presence of citric acid. However, because it is a protein, it is very unstable in the presence of light and tends to get denatured at high temperatures and low pH levels [13].

Colour profile analysis

Table 1 shows that the control sample exhibited the lightest colour with a value of 91.92±0.51[a]. There is a significant difference ($p<0.05$) between all samples, with sample A and B having mean values of 79.72±0.47[b] and 71.65±0.41[c], respectively. Sample C showed the lowest brightness values, with a significant difference ($p>0.05$), and a mean value of 51.01±1.23[d], respectively. That means sample C was the darkness between all samples. A significant aspect of the topic is the relationship between the brightness of the film and the concentration of BPE. A higher concentration of BPE may lead to a darker pigmentation, as indicated by the inverse connection between 'L*' values and BPE concentration. This is consistent with the observed colour characteristics of butterfly pea blooms, which are colour purple, blue, and red by the phytochemicals called anthocyanins [4]. The overall hue of the film is affected by the concentration of these chemicals, which leads to lower 'L*' values and a darker look. The results suggest that the brightness of the film is influenced by the concentration of BPE.

Table 1. L* value of Film Butterfly Pea Extract.

Butterfly pea extract	L*	a*	b*
Control (0%)	91.92±0.51[a]	0.51±0.03[c]	-1.53±0.24[a]
A (10%)	79.72±0.47[b]	0.98±0.04[c]	-5.96±0.01[b]
B (20%)	71.65±0.41[c]	3.11±0.19[b]	-13.25±0.18[c]
C (30%)	51.01±1.23[d]	9.97±1.15[a]	-22.58±0.95[d]

Each value is presented as mean ± standard deviation (n=3). Mean value followed with different alphabet within column are significantly different *($p< 0.05$)*

Determination of Total Phenolic Compound (TPC)

Fig. 1 depicts that the concentration of BPE extract was influenced by the amount of phenolic compound. There are significant differences ($p<0.05$) between sample C compared to all samples which have the higher mean value, 0.16±0.02[a], followed by sample B, 0.10±0.10[b,] and the lowest mean value, which is sample A, 0.06±0.01[c], respectively. This result show that the TPC were increased as the ratio of BPE concentration increased. This indicating that concentration of BPE have a potential role as ingredients rich in antioxidant in food or nutraceutical formulation. Based on previous study state that [13] the phenolic compounds are naturally occurring water-soluble antioxidants that typically include an aromatic ring with one or more hydroxyl substituents, which explains the high level of total phenolic.

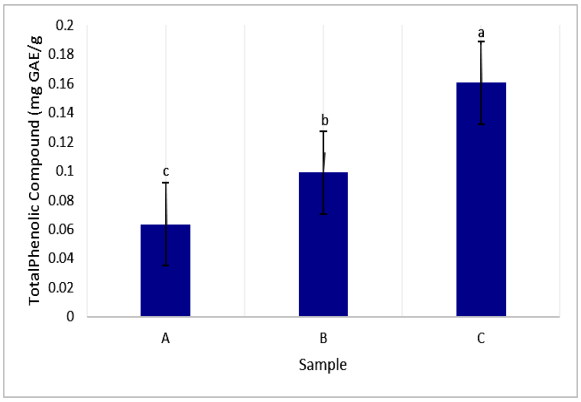


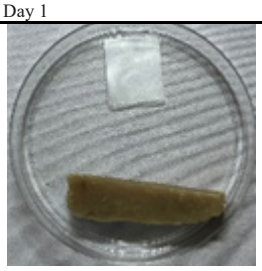
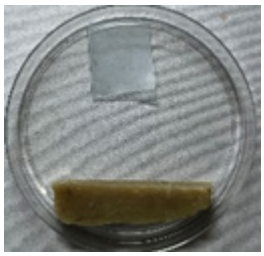
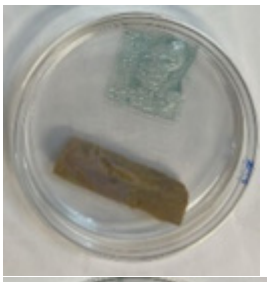
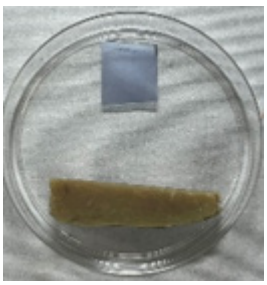
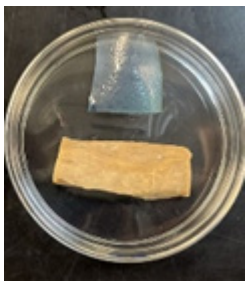
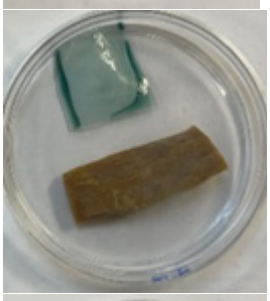
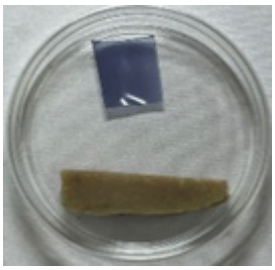
Fig. 1. Total Phenolic Compound of different concentrations of BPE. Each value is presented as mean ± standard deviation (n=2). Mean value followed with different alphabet within column are significantly different ($p< 0.05$).

Application of film for Keropok Lekor spoilage observation

Table 2 compares the colour changing of film BPE for 6 days. There are significant differences ($p<0.05$) between sample C in terms of 'a*' that have the higher mean value, 9.97±1.15[a], for the first days but have the lowest mean value, -5.38±0.65[c] for the sixth days respectively. There are no significant differences ($p<0.05$) between sample C and sample B in terms of 'b*' for day 6 that have the higher mean value 6.34±1.85[a] but have the lowest

mean value, -22.58±0.95[d] respectively, for day 1. This showed that sample C had a higher colour change from dark blue to dark green. In this experiment, a sample of *Keropok Lekor* was subjected to storage at room temperature under different conditions, leading to observable changes in the colour of the film used for freshness indication. Initially, no discernible colour alteration was noted on the freshness indicator film after the second day of storage. However, on the third day, a noticeable shift in colour to light blue occurred, suggesting a potential increase in pH and signaling the onset of early deterioration in the *Keropok Lekor*. As the storage period extended to the sixth day, a significant transformation in colour was observed, with the film turning entirely dark green. This indicates the complete decomposition of the *Keropok Lekor*, pointing towards the advanced stages of deterioration. The enzymatic activities responsible for the deterioration of seafood and poultry were implicated in this process. These enzymatic activities release volatile alkaline chemicals, including ammonia, dimethylamine (DMA), and trimethylamine (TMA), which impart a disagreeable odour and colour to the decomposing food product [4].

Table 2. Comparison between colour changing of film BPE for 6 days.

Sample	Day 1	Day 3	Day 6
Control (0%)			
A (10%)			
B (20%)			
C (30%)			

CONCLUSION

The sample containing 30% of Butterfly Pea Extract (BPE) demonstrated significant effects on the film's texture. Analysis of the total phenolic content in BPE revealed an increase in phenolic compounds, ranging from 0.06±0.01 to 0.16±0.02 mg GAE/g dried sample, with higher concentrations of BPE. Over a storage period of one to six days, noticeable colour changes in *Keropok Lekor* were observed. The colour of the butterfly pea flower extract shifted from dark blue to dark green, offering valuable insights into the freshness and quality of *Keropok Lekor* throughout its storage.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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