Smart food packaging from sago starch incorporated with anthocyanin from Brassica oleracea

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Abstract. Smart packaging is a general term that refers to any material or system employed to detect, perceive, and record any changes during the life cycle of packaged foods or their surroundings. In this study, the pH indicator film developed from sago starch and natural colourants or anthocyanin extracted from red cabbage (Brassica oleracea). Incorporating anthocyanin into food package matrices applies the bio-switch concept, which aids automatic response to changes (external stimuli) in the environment. Consumers are extra alert to the freshness of the food based on the colour changes of the bio-switch system. The formulation of sago starch film incorporated with different concentrations of anthocyanin (0, 10, 20, 30, 40 and 50 %) was investigated. The sago starch-based film with anthocyanin showed colour variations in response to different pH. Scanning Electron Microscopy (SEM) showed the compatibility of the starch with the anthocyanin extract and film morphology. Chemical characterization of the synthesized films by Fourier Transform Infrared Spectroscopy Analysis (FTIR) showed no chemical changes when compared to the control film. However, the films showed a decreasing lightness trend when a higher concentration of anthocyanin was incorporated. Smart food packaging is a promising response to consumers’ demand for packaging that accommodates a hectic way of life and offers greater economic potential.

1 Introduction

Smart packaging can be defined as any material or system employed to detect, perceive, and record any changes during the life cycle of packaged foods or their surroundings. Such systems can communicate the information related to the quality or safety of food and warn about potential problems during transport and storage. Controlling the state of food content and alerting the consumer when the food begins to deteriorate or is no longer fit for consumption has the potential to eliminate the waste caused by conventional expiry date labels. Besides overcoming the waste problems caused by food spoilage, the food packaging must be sustainable since plastic pollution is currently the biggest global threat to the environment. Studies on the use of biodegradable plastics or bioplastics derived from renewable resources such as starch have been reported by many researchers [1, 2].

Sago (Metroxylon sagu) is a palm species indigenous to the Southeast Asia region, specifically Malaysia, Indonesia, Papua New Guinea, and the Philippines. Sago palm is mainly cultivated as a
smallholder crop in Southeast Asia. With the establishment of sago estate plantations, Malaysia (particularly Sarawak) has the potential to become the largest global exporter despite having only an estimated planted area of around 60,000 hectares [3].

The sago waste locally known as sago hampas is a starchy (contains 60–80 wt.% of sago starch) lignocellulosic by-product generated from the pith of sago palm (M. sagu) after the starch extraction process [4, 5]. It has been reported that approximately one ton of sago waste is generated from every ton of sago starch (on a dry weight basis) [6]. Therefore, approximately 60,000 tons (dry weight) of sago hampas is available annually from the eight sago mills in Mukah, Sarawak [7].

Currently, the waste is simply discharged into the river thereby decreasing the water quality along with potential risk to aquatic life. This is due to the microbiological degradation of the sago waste, which depletes the dissolved oxygen in water required to support higher forms of life [6].

Therefore, the utilization of sago pith waste for bioplastics formulation could prove to be an alternative to solid waste management, while ensuring better food quality for consumption. In addition, the incorporation of anthocyanin as a natural colour indicator in the bioplastics as food packaging will alert consumers to the quality of the food. Red cabbage (Brassica oleracea) is one of the sources of anthocyanin used for food colouration. The extracted anthocyanin is unique and exhibits various colours over a very broad pH range [8]. In addition, anthocyanin is a water-soluble pigment that changes colour when mixed with an acid or base. Typically, the pH of food changes over time due to the presence of bacteria or fungus. Since spoiled food has a different pH from fresh produce, smart packaging can be used to detect and inform consumers about food quality and safety using the colour changes. Thus, food wastage could be avoided, while preserving the freshness of the food.

Nowadays, consumers are exploring biodegradable and environmental-friendly packaging that also ensure longer-lasting nutrients in food. In addition, the increasing incidence of foodborne diseases is increasing consumer consciousness to the packaging materials for foods such as meat, vegetables and milk. Thus, this paper aims to investigate the development of sago starch films incorporated with anthocyanin from Brassica oleracea as an alternative to petroleum-based synthetic food packaging. The product will serve as a pH indicator film because the biosensor properties of the anthocyanin will alert the consumers when the food has spoiled based on the pH changes on the food surface attached to the film.

2. Materials and Methods
The materials used in this study are red cabbage (Brassica oleracea), sago starch flour, and glycerine which acts as a plasticizer. Firstly, the red cabbage was fractionated and soaked in 20% ethanol for 24 hours. Next, it was boiled for 5-10 minutes until the purple colour appeared.

2.1. Film formation
The sago starch-based film was synthesised using the petri dish casting method. The sago starch, distilled water, glycerine and anthocyanin were mixed and heated at different concentrations (0, 10, 20, 30, 40 and 50) grams, with constant stirring until each mixture was gelatinized. Each film was then labelled: S0, S1, S2, S3, S4 and S5 respectively since the concentration added to the starch film formation ranged from 0g, 10g, 20g, 30g, 40g, and 50g. The gel solution was subsequently cast onto the petri dish (about 1 mm thickness) before drying at room temperature.

2.2 Physicochemical analyses using scanning electron microscopy (SEM)
The morphology and molecular structure of the sago starch-based films were examined by Scanning Electron Microscopy (SEM) with a Focused Ion Beam (FIB) Zeiss Crossbeam 340. The SEM micrographs of the control film were visually compared to each indicator film to detect any differences on the surface and or cross-section.
2.3 Moisture content

The moisture content (MC) of the different film concentrations was determined using the standard method of the International Association of Official Analytical Chemistry (AOAC 1995). The films were dried in an oven at 100°C for 24 hours. Water content was calculated as Equation (1):

\[ MC = \frac{m_f - m_i}{m_i} \times 100\% \]

Where \( m_i \) is the initial mass and \( m_f \) is the final mass of each sample.

2.4 Analysis of pH – colour changes in the indicator film

The colour analysis of the film was performed using the Colour reader Hunter L, a, and b. Each prepared film was cut into a circular disc of 2 cm in diameter and placed on a clean petri dish that contained distilled water. After 8 hours, each film was observed using a colour reader to determine the colour release of the anthocyanin from the film into the water. The red cabbage-based anthocyanin is originally dark purple. The anthocyanin colour released from the film was observed every 8 hours. The colour release was determined by analysing the colour changes measured in lightness (L), red and green (a) and yellow and blue (b) values using the Color Reader (Model: CR-10, Japan). The colour parameters were determined using white patterns. The total differences (\( \Delta E \)) were calculated based on equation (2).

\[ \Delta E = \sqrt{((\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2)} \]

Where:

\( \Delta L = (L^*_{\text{standard}} - L^*_{\text{sample}}) \) = difference in lightness and darkness (+ = lighter, - = darker)

\( \Delta a = (a^*_{\text{standard}} - a^*_{\text{sample}}) \) = difference in red and green (+ = redder, - = greener)

\( \Delta b = (b^*_{\text{standard}} - b^*_{\text{sample}}) \) = difference in yellow and blue (+ = yellower, - = bluer)

2.5 Chemical characterization using fourier transform infrared spectroscopy (FTIR)

The FTIR analysis was performed using the Recorder Spectra (Model: FTIR Nicolet MAGNA-IR 860 spectrometer; Thermo Fisher Scientific, Inc, USA). The spectra were recorded at 4 cm\(^{-1}\) resolution with a total of 6 scans.

3. Results and Discussion

3.1 Anthocyanin extraction and buffer solution

![Anthocyanin colour changes at different pH of the buffer solution](image)

**Figure 1.** Anthocyanin colour changes at different pH of the buffer solution

The anthocyanin (natural colourant) extracted from red cabbage was added and mixed with a buffer solution ranging from pH 1 to 13 to observe the colour changes. The original colour of anthocyanin from red cabbage is purple. However, the anthocyanin changed its colour from purple to red when placed in a highly acidic solution. As the pH approached neutral (pH 7), the purple colour remained constant.
However, the colour changed to brown when added to the solution with pH 10 and 11 before changing to green at pH 12 and yellow at pH 13, as shown in Figure 1.

Typically, the colours of anthocyanin vary from red at low pH to blue and green at high pH [11]. The colours of anthocyanin are unstable when there are any changes in its pH values [12]. Thus, this broad colour change makes it attractive for application as natural pH indicators.

### 3.2 Scanning electron microscopy (SEM)

The sample films were analysed by SEM to obtain the surface and cross-section micrographs (circle). From Figure 2, the micrograph of the control film is observably smooth with whitish granules on the surface. This observation reveals the characteristic patterns that correspond to the withered ghost granules of starch [13].

![Figure 2. Micrographs of the surfaces and the cross-sections of the starch films without anthocyanin (control), S0 and films incorporated with anthocyanin (S1, S2, S3, S4, S5).](image)

Samples S1, S2, and S4 show a relatively smooth and continuous cross-section without pores or cracks, which confirms a dense and homogeneous structure. However, samples S3 and S5 indicate a rougher surface structure compared to other films. The uniform distribution of anthocyanin throughout the film matrix is indicated by the homogenous appearance of the film. The absence of pores and cracks indicate the film can be easily detached from the cast plate. The creases visible in the film might be due to the concentration gradient of different constituents in the material. The SEM images proved that the
incorporation of different concentrations of anthocyanin influence the morphology of the film and affect the compatibility of the starch with the anthocyanin extract.

3.3 Moisture content
The incorporation of anthocyanin resulted in a decreasing trend of moisture content in the film incorporated with anthocyanin. This shows the ability of the pH indicator film to absorb and retain moisture reduced after 24 hours. Sample S0 with zero concentration of anthocyanin (control film) showed the highest moisture content at 28.12%. However, sample S5 which has the highest concentration of anthocyanin recorded the lowest moisture content at 18.50% due to the higher hydrophobicity of the film. Therefore, the film is a hydrophobic material as there is a trend towards greater hydrophobicity in the film containing the anthocyanin. The increasing hydrophobicity of materials with natural extracts is expected [14]. Food packaging films must have a good moisture barrier to extend shelf life. The reduction in the moisture content of the pH indicator film indicates a relationship between anthocyanin and water molecules in the diffusion mechanism throughout the film matrices. The determination of moisture content was performed on three replicates.

![Figure 3. The moisture content of control film and pH indicator films.](image)

The previous study suggested that the decreasing moisture content will significantly increase the crystalline phase of a semi-crystalline material [15]. The changes may be caused by the increase in the crystalline fraction on adding antimicrobial materials. Therefore, the percentage of moisture content decreased in the film due to the incorporation of anthocyanin.

3.4 Colour analysis of the pH film indicator
The colour changes in the film indicator were observed every 8 hours until it became constant after 32 hours. The colour changes show that the anthocyanin was released from the film. Each sample from S0 to S5 displayed different colours on release. Hence, each vertical bar in the graph demonstrates the colour release every 8 hours. Based on the results, there is an increasing trend in colour intensity. From Figure 4, the anthocyanin colour release increased drastically after 8 hours and the release (colour reading) became slightly constant after the next 8-hour cycle.

The colour release from the films demonstrates the stability of anthocyanin. If the anthocyanin cannot be held longer in the film matrix, the packaging will have difficulty functioning as smart packaging, which also acts as a pH indicator film.
Figure 4. Release colour of anthocyanin from each film after 32 hours.

3.5 Chemical Characterization using Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is widely used to characterize the chemical structure and structural interactions of different substances. The functional groups observed in the control film were compared with the synthesised films that contain anthocyanin, based on the spectrum of absorption intensity plotted against wavenumber. From the FTIR profiles, there is a strong and broad absorption peak within the wavenumber range 3250 to 3330 cm\(^{-1}\), which is the characteristic absorption peak of the stretching vibration of the O-H functional group.

Besides, the bands from 990 cm\(^{-1}\) to 1000 cm\(^{-1}\) are attributed to the stretching vibration of the C=O functional group. There are not many changes in the physical and chemical characteristics of the film with the incorporation of anthocyanin. The results imply that the chemical composition and structure of the pH indicator films and the control film is consistent. This indicates that the addition of the anthocyanin does not influence the chemical changes in the film. The bands detected on the starch-based film are in agreement with a previous study of the starch-based film [13].

3.6 Analysis of changes in indicator film towards pH changes

Sample S3 was further analysed to determine the colour changes after soaking in a different buffer solution that has different pH values, as shown in Figure 6. It is clearly seen that S3 shows a distinct
change especially when the film is in contact with the buffer solution of pH 9 and above. This result is supported by the colour changes of anthocyanin in Figure 6, which illustrate explicit colour changes between pH 4 and pH 9 until pH 13. This remarkable colour change shows that the sensitivity of anthocyanin during chemical reactions at different pH values. The left circle in the figure highlighted the significant decrease before the values increase again in the right circle, pH is considered as one of the important factors affecting the growth of microorganisms in food. This is because pH affects the metabolic energy of microbes, which is required to build the hydrogen ion gradient across membranes and promote microbial enzyme activity and the stability of cellular macromolecules. Fish and meat have a neutral pH i.e. 7. Bacteria can only grow at a minimum pH of 4.5 and a maximum of 8–9 with an optimum of 6.5–7.5. correspondingly, fish and meat are highly susceptible to spoilage. Foods such as tomatoes, apple and citrus fruits contain natural acidity with varying degrees of preservative power [14].

Figure 6. Changes in Hunter colour values (L, a and b) of sample S3.

4 Conclusions
In conclusion, the pH indicator film incorporated with anthocyanin extracted from red cabbage showed colour changes toward pH. Thus, the synthesised film can notify consumers when the food is spoiled. Although the purple colour of the anthocyanin is released after a period of time, the S3 film retained its colour for up to 32 hours. Hence, S3 can be incorporated in the sago starch smart film, since it shows the best retention of anthocyanin. Therefore, a smart film with such colour changes can act as a simple indicator or visual method for detecting quality changes of food product, since the pH values of food change upon spoilage. Further research is needed to explore the potential of such films as effective packaging for food applications.

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