

Development and characterization of cassava starch films incorporated with purple yam (*Dioscorea alata* L.) peel anthocyanins

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Abstract

The packaging industry is now geared towards natural and biodegradable raw materials to reduce packaging wastes. In this study, purple yam (*Dioscorea alata* L.) peels were utilized to extract anthocyanins to be incorporated as a pH indicator in cassava starch films. The extract was analyzed for its total monomeric anthocyanin content (TMAC) using the pH differential method, and antioxidant activity using the DPPH assay. Results showed that the extract contains 155 mg/L cyanidin-3-glucoside and high antioxidant activity of 78.84%. Using the casting technique, thin films were made from cassava starch and glycerol, with varying amounts of the extract (0, 10, 20, 30 and 50%). The films were characterized by their color properties, water activity (A_w) and tensile strength (TS). Results showed decreasing values of L^* , a^* , b^* as the concentration of the extract increased. No significant difference ($p > 0.05$) was observed in the A_w and TS of the control film and the colored films. There was no trend observed in the A_w and TS of the films. The film with 30% extract recorded the lowest A_w (0.214) while the film with 40% extract recorded the highest tensile strength (0.706). The film with the highest TS was subjected to color response analysis by immersing the films in buffer solutions at different pH levels (pH 1.0-12.0) for 10 mins, and the time when the first color change was observed was recorded. The fastest responses were observed at the extreme pH levels (pH 1.0-2.0; 10.0-12.0). This study was able to conclude that purple yam peel extract has the potential as a pH indicator in cassava starch films for application as intelligent packaging.

1. Introduction

With the current trend of biodegradable packaging and zero-waste consumption, new technologies are being developed to improve the functions of packaging materials. An area in the food packaging industry that has gained considerable growth is intelligent food packaging (IFP). An IFP contains an indicator that provides information on product quality and freshness (Lim, 2011). Intelligent packaging technology communicates information regarding a certain quality attribute of a product through visual changes such as that of pH sensors wherein plant pigments are used because they change colors when subjected to different pH levels. Other examples of intelligent packaging technologies are time-temperature indicators (TTI), gas-sensing devices, and microbial growth and spoilage indicators (Pereira *et al.*, 2015). They are currently being studied for application in frozen raw and processed food, peeled fruits and vegetables in the market since these are

susceptible to spoilage and oxidation.

The Philippines is one of the main producers of purple yam (*Dioscorea alata* L.), popularly known as *ubi* or *ube*, in Southeast Asia (FAO, 2006). Purple yam is considered as the third most important tropical root crop, following cassava and sweet potato (Fu *et al.*, 2005). It is a staple food in the Philippines as they can serve as an alternative to rice. Purple yam is also a source of some important industrial products like yam powder, puree, paste, jams, candies, bread, cookies, chips, flavoring, and colorant (Salda *et al.*, 2005). The tuber (peel and flesh) was also reported to have a relatively high antioxidant activity as high as α -tocopherol and butylated hydroxyanisole (BHA) (Lubag *et al.*, 2008). A study by Larief *et al.* (2018) revealed that purple yam has an antioxidant activity of about 79%. The peels were also reported to exhibit antifungal activity (Aderiye *et al.*, 1996). The peels resulting from the processing of purple yam are commonly used in making fodder. Due to its

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anthocyanin content, this study aimed to explore the use of purple yam peels as a source of anthocyanins for application as a pH indicator in intelligent films.

2. Materials and methods

2.1 Extraction of anthocyanins from purple yam (*Dioscorea alata* L.) peels

Purple yam samples were obtained from a local market in Los Baños, Laguna, Philippines. Anthocyanins were extracted using the procedure described by Fuleki and Francis (1968) and Pereira *et al.* (2015) with minor adjustments. An 80 g portion of purple yam peels was macerated in a blender together with 180 mL of ethanol-water (70:30 v/v) extraction solvent. The pH of the mixture was adjusted to pH 2.0 using 1.0 N HCl. The mixture was transferred quantitatively to a beaker using another 50 mL of the extraction solvent. Afterwards, the beaker was completely covered with foil to protect the extract from light and then stored for 24 hrs at 4–5°C in a freezer. After 24 hrs of storage, the mixture was filtered using cheesecloth and the residue was washed with another 20 mL of the extraction solvent. The collected filtrate and washing were transferred to tubes and centrifuged for 10 mins at 2000 rpm. The supernatant was transferred in an amber bottle to protect it from light and then stored in the freezer until used for analysis.

2.2 Characterization of the extract

2.2.1 Total monomeric anthocyanin content

The procedure for the determination of anthocyanin content of purple yam peel extract was obtained from the official method for determination of Total Monomeric Anthocyanin Content (TMAC) of fruit juices, beverages, natural colorants, and wines by AOAC International (AOAC 2005.02). The TMAC expressed in mg/L extract was computed using the formula:

$$\text{Total Monomeric Anthocyanin Content } \left(\frac{\text{mg}}{\text{L}}\right) = \frac{A \times \text{MW} \times \text{DF} \times 1000}{\epsilon \times \ell} \quad (1)$$

Where $A = (A_{520} - A_{700})_{\text{pH } 1.0} - (A_{520} - A_{700})_{\text{pH } 4.5}$, MW = 449.2 g/mol (cyanidin-3-glucoside), DF = dilution factor, ℓ = path length and ϵ = extinction coefficient (26,900 L/mol-cm)

2.2.2 Antioxidant activity

The antioxidant activity of the extract was determined using the 2,2'-diphenyl, 1-picryl-hydrazyl (DPPH) assay according to the procedure of Bhuiyan *et al.* (2009). The extract was diluted with ethanol-water extraction solvent in a 1:100 dilution scheme. In a test tube, a 1.0 mL aliquot of the diluted extract was mixed with 4.0 mL of distilled water and 1.0 mL of 0.004% w/v DPPH-methanol solution (prepared by dissolving 4 mg

DPPH in 100 mL methanol). The tubes were covered with aluminum foil to protect the samples from light and were allowed to stand for 30 mins for the reaction to develop. After 30 mins, the absorbances were read using a UV-vis spectrophotometer at 517 nm against a blank containing only DPPH and ethanol solution (1:1). The % DPPH radical scavenging activity was computed using the formula:

$$\% \text{DPPH Radical Scavenging Activity} = \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}} \times 100 \quad (2)$$

Where A_{blank} = Absorbance of the blank solution and A_{sample} = Absorbance of the sample

2.3 Preparation of the films

Cassava starch (CS) films were prepared using the formulation developed by Golasz *et al.* (2013) with minor modifications. Film-forming solutions were prepared with cassava starch (5% w/w), glycerol (3% w/w) and varying concentrations of purple yam peel anthocyanin extract (CS0 = 0%, CS10 = 10%, CS20 = 20%, CS30 = 30%, CS40 = 40%, CS50 = 50%) in beakers and heated up to 70°C with slow and continuous stirring. Afterwards, approximately 30 mL of the suspensions were poured into polystyrene petri dishes and dried at 40°C for 24 hours in a temperature-controlled chamber.

2.4 Characterization of the films

2.4.1 Determination of water activity

The water activity of the films was determined using the Novasina Water Activity Meter. A portion of the film was placed in the sample slot of the water activity meter and the water activity readings were recorded. The test was done in triplicates.

2.4.2 Color Analysis

The color parameters of the films were evaluated using a hand-held chromameter (Konica Minolta Chroma meter CR-400/410) against a white background. The test was performed in triplicates. Results were expressed as L^* value (lightness; 0 = black to 100 = white), a^* value ($-a$ = green and $+a$ = red), and b^* value ($-b$ = blue and $+a$ = yellow). The color differences between adjacent treatments (ΔE_a) and between the control (CS0) and the treatments (ΔE_b) were calculated using the formula by (Luchese *et al.*, 2018).

$$\Delta E_a = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (3)$$

where $\Delta L^* = L_2^* - L_1^*$ $\Delta a^* = a_2^* - a_1^*$ $\Delta b^* = b_2^* - b_1^*$

$$\Delta E_b = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (4)$$

where $\Delta L^* = L_{\text{control}}^* - L_{\text{sample}}^*$ $\Delta a^* = a_{\text{control}}^* - a_{\text{sample}}^*$

$\Delta b^* = b_{\text{control}}^* - b_{\text{sample}}^*$

2.4.3 Determination of tensile strength

Prior to analysis, test specimens were conditioned at $23\pm 2^{\circ}\text{C}$ and $50\pm 5\%$ relative humidity for 40 hrs. The test specimens were cut to conform to the dimensions described in ASTM D1708-02 method developed by the American Society of Testing and Materials (2002). After preparation of test specimens, these were subjected to tensile test using a universal testing machine (INSTRON 4411, Singapore), following a crosshead speed of 10 mm/min. The maximum load sustained by the test specimen was used to calculate the tensile strength of the film using the formula:

$$\text{Tensile strength, MPa} = \frac{\text{Maximum load sustained by specimen (kN)} \times 1000}{\text{CA (mm}^2\text{)}} \quad (5)$$

where cross-sectional area, CA = (thickness)(width of narrow section)

2.5 Color response analysis of the films

The film with the highest tensile strength was reproduced into 12 films, one for each aqueous buffer solution of a specific pH level (pH 1.0–12.0) to evaluate the changes in the color of the film. Buffer solutions were prepared as follows: KCl (pH 1.0-2.0), sodium citrate-citric acid (pH 3.0-5.0), $\text{K}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$ (pH 6.0-8.0), bicarbonate-carbonate (pH 9.0-11.0), and lastly, KCl-NaOH (pH 12.0). Films were cut into squares with 4 cm^2 surface area and submerged simultaneously in buffer solutions for 10 mins to allow the color to develop. The visual evaluation was performed taking note of the time elapsed for each film to change in color.

2.6 Statistical analysis of data

All data obtained from the analyses were expressed as mean \pm SD. One-way ANOVA was conducted at 5% significance level and post-hoc analysis was conducted using the Tukey's test. SigmaPlot Version 14 was used in the analyses.

3. Results and discussion

3.1 Total monomeric anthocyanin content and antioxidant activity of the extract

Fruits, vegetables, and flowers with colors ranging from red, violet to blue are usually those that contain anthocyanins. The total monomeric anthocyanin content (TMAC) of plant samples can be determined using the pH-differential method, expressed as mg/L cyanidine-3-glucoside, the most abundant monomeric anthocyanin in nature. Aside from the color that anthocyanins give, these compounds were also reported to be strong antioxidants mainly due to their chalcone and quinoidal base structure, which are effective scavengers of free radicals. Studies suggest that anthocyanin-rich foods

such as eggplant, red cabbage, and berries have great potential in preventing certain diseases like diabetes, hypertension, and cancer (Khoo *et al.*, 2017).

In this study, purple yam peels were extracted with anthocyanins to be incorporated in cassava starch films. The TMAC and % DPPH Radical Scavenging Activity (% RSA) of the extract were analyzed spectrophotometrically and the results are summarized in Table 1. While the literature is replete with studies on the anthocyanin content and antioxidant activity of purple yam flesh, no literature was found to report the values for the peels. This study was able to report that purple yam peels contain as much as 155.02 ± 0.48 mg/L cyanidin-3-glucoside TMAC and antioxidant activity of $78.84\pm 0.86\%$. This showed that the peels can be utilized as a source of anthocyanins before being completely disposed to waste.

Table 1. Color parameters (L^* , a^* , b^* values) of cassava starch films incorporated with varying amounts of purple yam peel extract

Treatments	L^*	a^*	b^*	ΔE_a^2	ΔE_b
CS ₀ (control)	54.61 ^a	0.43 ^f	-0.42 ^f	–	–
CS ₁₀	35.25 ^b	18.09 ^c	2.14 ^e	26.33	26.33
CS ₂₀	27.85 ^c	20.50 ^a	5.35 ^a	8.42	33.95
CS ₃₀	25.14 ^d	18.82 ^b	5.03 ^b	3.20	35.17
CS ₄₀	23.87 ^e	15.40 ^d	3.56 ^c	3.93	34.43
CS ₅₀	23.44 ^f	13.21 ^e	2.66 ^d	2.41	33.83

Values with different letter superscript in each column are significantly different evaluated using Tukey's test ($p < 0.05$). ΔE_a , color difference between adjacent treatments, ΔE_b , color difference between control and treatment.

3.2 Water activity of the films

Water Activity (A_w) is an important parameter to determine the stability of foods in terms of microbial, enzymatic, and chemical stability. High A_w foods (> 0.9) are susceptible to microbial growth while foods with low A_w (< 0.5) are generally stable and have longer shelf-life. The A_w of packaging films is an important consideration in selecting the type of food where they will be used. Mold growth is of greatest concern for biodegradable materials because it thrives in a wide A_w range starting from 0.6 (Barbosa-Cánovas *et al.*, 2007).

The films produced from cassava starch and purple yam extract exhibited low water activities ranging from 0.214–0.277. Figure 1 illustrates that there were no differences in the A_w of the films regardless of the amount of extract used. However, it was expected that an increase in the amount of extract added would also increase the water activity of the films due to the plasticizing effect of both the extract and water. According to Sothornvit and Krochta (2005) and Medina

-Jaramillo *et al.* (2016), plasticizers are low molecular weight compounds that improve the mobility of polymer molecules by entering spaces between polymers and disrupting polymer-polymer interaction. They reduce the energy required for the formation of hydrogen bonds between plasticizer and polymer, and since plasticizers are mostly hydrophilic, loss of water by dehydration is lessened thus maintaining a high amount of bound water and water activity. Although both components have the plasticizing effect, the efficiency of these components as plasticizers and the effect on water activity are still determined by several factors such as size and shape of the molecule, concentration and compatibility with the biopolymer (Sothornvit and Krochta, 2005).

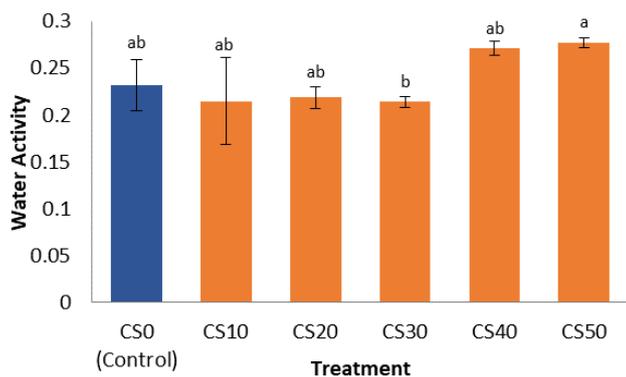


Figure 1. Starch films with increasing amount of extract and their corresponding water activities

3.3 Color analysis of the films

All five treatments including the control were subjected to color analysis and the results were summarized in Table 1. The L^* , a^* , and b^* values represent the color of the films in the 3D color space. Results showed that as the amount of extract is increased, the L^* values also decreased, indicating that the extract gave a darker color to the films. The pH of the extract was adjusted to pH 2.0 during extraction to maintain the stability of the anthocyanins. The dominant structure at acidic condition is the red flavylium cation (Khoo *et al.*, 2017). However, the addition of the extract into the film-forming solution altered the pH of the environment resulting in a shift in the anthocyanin structure from red flavylium cation to the purple quinoidal base usually present at pH 3.0–6.0. The different amounts of extract added caused the differences in the color values (Figure 2).

The color differences (ΔE) were determined to evaluate if the differences are distinguishable to the human eye without subjecting it to sensory analysis. A ΔE value > 3.0 means that the color difference between two samples is distinguishable by the human eye while values greater than 6.0 means there is a very large color difference (Golasz *et al.*, 2013). Results showed that the color differences among adjacent treatments were

distinguishable except between CS40 and CS50. This may imply that increasing the amount of extract to more than 40% will not be distinguishable visually. In addition, the color of the films with added extract was distinguishable from the control.



Figure 2. Cassava starch films incorporated with purple yam peel extract (0, 10, 20, 30, 40 and 50%)

3.4 Tensile strength of the films

Tensile strength (TS) is a parameter used to determine the amount of stress a material can withstand before breaking. For biodegradable films, tensile strength is important as it is one of the determining factors for the efficiency of the polymers used for the production of films as well as the effect of different additives incorporated in the film such as antioxidants and color indicators.

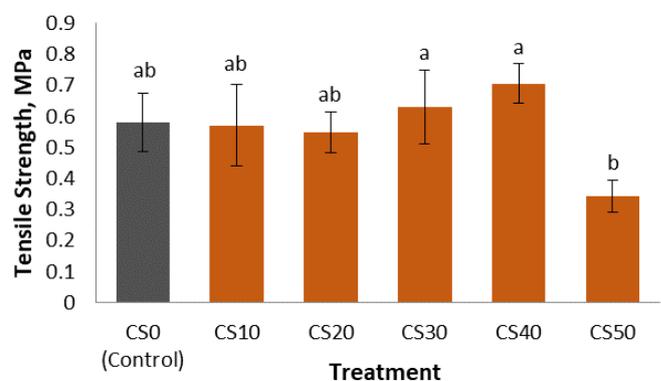


Figure 3. Tensile strength of films with increasing amount of extract

In this study, no trend was observed in the tensile strength values of the films with varying amounts of extract. Figure 3 illustrates that the TS values of the films with extract did not vary significantly from the control, except for CS50 which exhibited the lowest TS (0.343 MPa). However, the TS was expected to decrease as the amount of extract is increased because as mentioned before, natural extracts exhibit a plasticizing effect by weakening the interactions between polymers (Medina-Jaramillo *et al.*, 2016). This may be due to errors committed while conducting the method as it is slightly sensitive in terms of the dimensions and load of the test specimen. This includes slight inconsistency in the dimensions of the test specimen from manually

cutting the films. Peeling off the films from the petri dish may have caused partial stretching, which may have altered the tensile properties.

3.5 Color response analysis of the films

After testing the films for their tensile strength, the treatment with the highest TS (CS40) was chosen for color response analysis. Color changes at different pH levels were easily distinguished as the colors vary from red, purple, blue and, green as seen in Figure 4. Anthocyanins are stable at low pH (Khoo *et al.*, 2017) which may be the reason why film at pH 1.0 had the fastest color response (Figure 5). In addition, anthocyanins become unstable as the pH increases resulting in the most amount of time for a complete change observed at pH 5.0. However, at high pH (9.0–12.0), anthocyanins start to degrade and become green in color as the equilibrium between the blue quinoidal anion and the yellow chalcone occurs. Results of the study showed that extreme values of pH force structural changes in anthocyanins resulting in faster color changes.

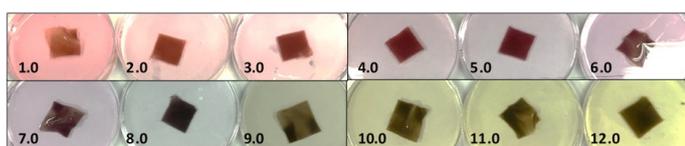


Figure 4. Color response of films at different pH levels after 30 mins

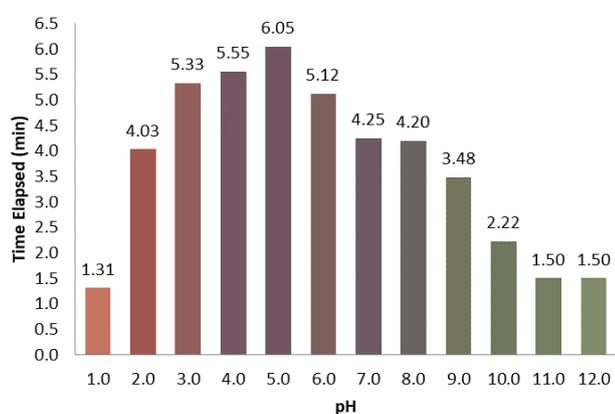


Figure 5. Total time taken by the films to change color at different pH levels

4. Conclusion

The study was able to obtain an extract from purple yam peels with considerable amounts of anthocyanins and antioxidant capacity. Application of the extract to starch films showed that increasing the amount of the extract of up to 50% did not significantly affect the water activity and tensile strength of the films. Color differences among samples were distinguishable by the human eye until 40%. Increasing the amount of extract

from 40% to 50% was no longer distinguishable. Submerging the colored films to different pH levels revealed that the extract was effective in monitoring pH visually where the fastest color change was observed at extreme pH levels. In conclusion, cassava starch films incorporated with anthocyanins from purple yam peels have great potential as an intelligent packaging material. The films were highly sensitive to pH changes which can be used as spoilage indicators in food packaging.

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