Research article

Properties of Gelatin-based Films Incorporated with Anthocyanins and Curcuminoids and Stability of Antioxidant Activity during *In Vitro* Digestion

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FRAP;
DPPH;
in-vitro digestion

Abstract

In this study, gelatin-based films incorporated with two plant pigments; butterfly pea flower anthocyanins (BPA) and turmeric rhizome curcuminoids (TRC) were prepared. Their physical and antioxidant properties were investigated. The results showed that addition of BPA and TRC at different concentrations (10%, 20%, and 30% of gelatin weight) significantly affected the visible color and color values (L*, a*, b*, hue and chroma) of the obtained films. With increasing pigment concentrations, lower % light transmittance was observed ($p<0.05$). The improved light barrier property corresponded with the increased opacity of the two films ($p<0.05$). Compared with the control films, the addition of either BPA or TRC slightly increased thickness of films ($p<0.05$). The moisture content of films increased with increasing BPA concentration but decreased with increasing TRC concentration ($p<0.05$). However, water solubility was not significantly different among films with varying concentrations of BPA or TRC ($p>0.05$). Total anthocyanin content (TAC), total curcuminoid content (TCC), total phenol content, and antioxidant activities by FRAP and DPPH increased with increasing pigment concentration in films ($p<0.05$). Based on *in vitro* digestion, the film forming solution containing 30% (w/w) plant pigment significantly improved the stability of pigment compounds, TPC, and antioxidant activities during the intestinal phase. Compared to the pigment extract alone, film forming solution containing gelatin and glycerol showed protecting effects against biological conditions during intestinal digestion of the two pigments, BPA and TRC, and therefore, offered greater stability of antioxidant activity ($p<0.05$).

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1. Introduction

Edible films are currently made from renewable and natural polymers such as proteins, polysaccharides and lipids that are biodegradable and environmental friendly [1]. Among the proteins used for films, gelatin possesses several advantages over other natural proteins due to its good film forming and gas barrier properties at low to intermediate relative humidity [2]. Addition of active ingredients into films can enhance their uses as antimicrobial and antioxidant agents [3].

Plant pigments as natural antioxidants have been increasingly attracted the interest of researchers and food producers [4]. Butterfly pea flowers (*Clitoria ternatea* L.) are rich sources of anthocyanins of various colors including red, purple and blue. These are classified as water-soluble flavonoid pigments [5] with a strong antioxidant capacities [6]. Anthocyanins are potent nutraceuticals that offer immense health benefits such as cardiovascular protection, antioxidant, anti-ageing, anti-diabetic and vision improvement properties and so on [7]. Turmeric (*Curcuma longa* L.) is a good source of bioactive compounds named curcuminoids, which are mainly yellow-orange color. Curcumin, demethoxycurcumin, and bisdemethoxycurcumin are good examples of curcuminoids [8]. Turmeric is now a popular medicinal plant worldwide as it possesses antioxidant, antibacterial, antifungal, antiparasitic, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties [9].

In vitro digestion models are widely used to study the structural changes, digestibility, and release of food components under simulated gastrointestinal conditions. Anthocyanins were reported to be apparently degraded during digestion [10]. Lang *et al.* [11] reported that blueberry anthocyanins showed minimal or no loss during saliva and gastric digestion but showed a great loss during intestinal digestion. Curcuminoid has a limitation for use in clinical treatment and general healthcare due to its poor aqueous solubility. The low stability of curcuminoids at intestinal pH is a further contributing factor to their poor intestinal absorption and bioavailability [12]. However, Papillo *et al.* [8] reported that curcumins greater decreased in the stomach than in the intestine. The recovery of anthocyanins and curcuminoids during digestion needs to be further investigated.

Many studies incorporated plant extracts into gelatin-based film in order to enhance their antioxidant activities and biological properties of the extracts during digestion. Gelatin films incorporated with green tea extract were highly hydrolyzed during gastrointestinal digestion. However, a high percentage of total polyphenols was still recovered [13]. Wu *et al.* [14] reported that after digestion, low release ratios of anthocyanins and phenolics were obtained from anthocyanin extract encapsulated with gelatin. These reports revealed that gelatin displayed a good protective effect on bioactive compounds. Furthermore, the incorporation of curcumin in gelatin proved to be an effective method of increasing the bioaccessibility of the bioactive compounds [15].

The incorporation of plant pigments into gelatin-based films has captured the keen interest of food technologists and food producers. It has been driven by the potential applications within the food industry. However, there are many aspects of these films that require evaluation. This study aimed to compare the properties of gelatin-based films incorporated with two plant pigments in terms of physicochemical properties, anthocyanins and curcuminoid content, and total phenolic content. The stability of the antioxidant properties of these substances were investigated during simulated *in-vitro* digestion.

2. Materials and Methods

2.1 Plant materials and chemicals

Fresh butterfly pea flowers (*Clitoria ternatea*) and turmeric rhizomes (*Curcuma Longa*) were obtained from local farm in Chiang Rai Province, Thailand. Samples were pre-sorted, washed and cut into small pieces before drying in a hot air oven (Memmert, D-91107 Schwabch) at 60°C to obtain dried samples with less than 10% water content. All chemicals were analytical grade and were obtained from Sigma (St. Louis, MO, USA). Enzymes were obtained from Megazyme (Bray, Ireland) and Sigma (St. Louis, MO, USA).

2.2. Extraction of plant pigment

Based on solubility of the major plant pigment, dried butterfly pea flower was soaked in water whereas dried turmeric was extracted with 50% (v/v) aqueous ethanol. The ultrasonic processor (Sonic & Materials, Inc., VCX500, U.S.A.) was employed to treat a sample mixture comprising 5 g of dried samples and 500 mL of solvent. The processing duration was set at 10 min, and a constant temperature of 40°C was maintained throughout the procedure. Each extract was filtered through Whatman No. 4 filter paper and subsequently evaporated in a rotary evaporator (IKA, RV 10 B, Germany) at 40°C under vacuum and subsequently freeze dried in a freeze dryer (Christ, Delta 2-24 LSC plus, Germany) at -50°C for 24 h. The obtained dried pigments were stored at -18°C for further use.

2.3 Film preparation

Exactly 1.5 g fish gelatin with 120-150 Bloom (McGarrett, Thailand) was dispersed in 50 mL distilled water to obtain 3% (w/v) film-forming solution. The sample was completely dissolved under mechanical stirring. Glycerol at 25% was added as plasticizer. All film forming solution samples were heated to 60°C and stirred continuously for 15 min. After cooling, the two plant pigment powders; anthocyanins from butterfly pea flower (BPF) and curcuminoids from turmeric rhizome (TR), were subsequently added at varying concentrations of 10, 20 and 30% of gelatin weight (w/w). About 10 mL of each film solution was poured into a 90 x 15 mm plastic petri dish and air-blown dried in a fume hood for 24 h to obtain the peelable dried films. The resulting films were then conditioned at 25±0.5°C, 50±5% RH for an additional 48 h before measurement. Film without addition of plant pigments served as the control.

2.4 Color

The film colors were measured in the CIE L*, a*, b* system using a colorimeter (Hunter Lab, ColorQuest XE). The L* chromatic variable ranged from 0 (black) to 100 (white). The a* value is a measure of redness (+a*) and greenness (-a*) while the b* is a measure of yellowness (+b*) and blueness (-b*). The measured a* and b* values were used to estimate hue angle degrees following equation (1)

$$\text{Hue angle} = \arctan[(b^*)/(a^*)] \quad (1)$$

2.5 Thickness

Film thickness was measured by a handheld micrometer (547-401, Mitutoyo, Japan) with 0.001 mm accuracy. Five measurements were taken at random positions.

2.6 Light transmission and opacity of film

Film samples were cut into 2 cm × 4 cm pieces and placed in UV-Vis spectrophotometer (Libra S80, US), and a wavelength range of 250 to 800 nm was used following the method of Fang *et al*. [16]. The opacity was calculated using equation (2)

$$\text{Opacity} = \text{Abs600}/\chi \quad (2)$$

where Abs600 is the value of absorbance at 600 nm and χ is the film thickness (mm).

2.7 Moisture content

Moisture content of film samples was measured and calculated based on weight loss on drying. Film samples were cut into 2 cm squares, dried at 105°C for 24 h and then weighed. Each treatment was measured in triplicate. Moisture content was calculated using equation (3)

$$\text{Moisture content (\%)} = \frac{(\text{Wi-Wf})}{\text{Wi}} \times 100 \quad (3)$$

where Wi and Wf are initial weight of the film and weight of film after drying, respectively.

2.8 Water solubility

Film samples (2 × 2 cm) were first dried at 105°C for 24 h to determine the initial dry matter. A portion of film was then immersed in 15 mL distilled water and then placed in a shaker water bath at 25°C and 60°C for 5 min. Each film solution was then filtered through the pre-dried filter paper. The filter papers with insoluble film were then dried at 105°C for 24 h. All tests were carried out in triplicate. The water solubility (WS) of the film was calculated using equation (4)

$$\text{Water solubility (\%)} = ((\text{Wi-Wf}))/\text{Wi} \times 100 \quad (4)$$

where Wi and Wf are the initial weights of the film and weight of insoluble film, respectively.

2.9 Total phenolic content (TPC)

To determine the bioactive compounds and the corresponding antioxidants, film forming solutions containing 10, 20 and 30% w/w BPA and TRC of gelatin weight were used. A mixture of only gelatin and glycerol (without pigments) served as control. Total polyphenol content (TPC) was determined using the Folin–Ciocalteu method. Each extract solution (500 µL) was mixed with 2.5 mL of 10% (w/v) Folin-Ciocalteu reagent and 2 mL of 7.5% (w/v) sodium carbonate, mixed well, and incubated for 1 h at 25°C. The absorbance was measured spectrophotometrically at 765 nm and expressed as mg Gallic acid equivalents (GAE) per 100 g of dry sample.

2.10 Total anthocyanins content (TAC)

Total anthocyanin content (TAC) in butterfly pea flower samples was measured according to the pH differential absorbance method [4]. Briefly, an aliquot (0.3 mL) of anthocyanin sample was mixed with pH 1.0 (potassium chloride buffer, 9.7 mL) and pH 4.5 (sodium acetate buffer, 9.7 mL) solutions, respectively, and equilibrated for 30 min at room temperature in the dark. A microplate spectrophotometer (Thermo Fisher Scientific, Multiskan GO, USA) was used to measure the absorbance at 525 nm and 700 nm, using water as reference. The total anthocyanin content was calculated as mg cyanidin-3- glucoside equivalent (mgCyE) per 100 g dry sample.

2.11 Total curcuminoid content (TCC)

Total curcuminoid content (TCC) was measured following the method of Martins *et al*. [17]. Tumeric pigment powder was soaked in 95% (v/v) hexane in ether and subsequently centrifuged at 3000x g for 10 min. The supernatant was collected and total curcuminoids were determined spectrophotometrically at 454 nm using a standard curve from analytical grade curcumin ranging from 1.0 to 8.0 µg/mL. The total curcuminoid content was expressed as mg TCC per 100 g dry sample.

2.12 Ferric reducing antioxidant power assay (FRAP)

Ferric reducing antioxidant power was assayed using ferric sulfate as standard [18]. The FRAP reagent was prepared freshly by mixing 300 mM acetate buffer (pH 3.6), 40 mM HCl, 10 mM 2, 4, 6 tripyridyl-s-triazine (TPTZ) and 20 mM FeCl3 in the ratio 10:1:1, respectively. Sample extract (400 µL) was then mixed with 2.6 mL FRAP reagent solution, and the mixture was incubated at 37°C for 30 min. The absorbance at 595 nm was measured against the blank (Thermo Fisher Scientific, Multiskan GO, USA). FRAP value was calculated as mmole FeSO4 per 100g dry sample.

2.13 DPPH radical scavenging activity (DPPH)

The DPPH free radical scavenging activity was determined using Trolox as standard [4]. Sample extract (50µL) was mixed with 1950 µL freshly prepared DPPH 60 mM solution. The sample was then stored in the dark for 30 min and absorbance at 517 nm was measured against the blank. The results were expressed as the equivalent content of Trolox (µmole TE/100 g dry sample).

2.14 Simulated gastrointestinal (GI) digestion

Simulated GI digestion was performed according to the method described by Donlao and Ogawa [19], with some modifications. For the digestion process, 1.53 g of dried BPA and TRC were dissolved in distilled water to prepare 170 mL film forming solution. These solutions served as controls, omitting the inclusion of gelatin and glycerol. In paralell, a film-forming solution was prepared by incorporating the same quantity (0.53 g) of both BPA and TRC, accounting for 30% w/w of gelatin. The total volume of the solution was precisely 170 mL, with the inclusion of gelatin and glycerol. The samples were added into a glass reactor connected to circulating water at 37±1°C (Memmert, D-91126 Schwabach FRG, Germany) and constantly agitated by a stirrer. Pigment solution without gelatin and glycerol served as control. Each sample was first mixed with α-amylase and pH was maintained around 6.0. Aliquots of 1 mL, which represented the oral phase (G0), were collected and rapidly cooled in an ice bath for further analysis. To perform the subsequent gastric digestion process, pH was adjusted to 2.0 by adding 1 M HCl solution. Thereafter, 19 mL pepsin

from porcine gastric mucosa was added to each sample, and the samples were incubated while being continuously stirred for 30 min. The resultant aliquots represented the gastric digested samples (G30). The remaining solution was further submitted to simulate intestinal digestion by adjusttment of pH to 6.8 with 1N NaOH. Then, 23 mL of intestinal enzyme solution containing pancreatin from porcine pancreas, amyloglucosidase, and bile salt were added. The resultant mixture was further incubated for another 2 h and aliquots of each reaction solution were taken at the definite time intervals of 0 (I0), 5 (I5), 10 (I10), 15(I15), 30(I30), 60(I60), 90 (I90) and 120 (I120) min, respectively. The obtained digested samples were analyzed for bioactive compound concentration and antioxidant activity following the methods described earlier. The simulation of the *in vitro* digestion process was performed in triplicate. Furthermore, the digestive recovery of plant pigments, phenolic compounds, and related antioxidant capacities of digested sample at G30 and I120 was calculated based on that of undigested samples at G0 following equation (5) and equation (6).

$$\% \text{ stability of digestive sample after gastic digestion} = ((G30\text{-}G0)/G0)\times 100 \quad (5)$$

$$\% \text{ stavility of digestive sample after intestinal digestion} = ((I120\text{-}G0)/G0\ )\times 100 \quad (6)$$

2.15 Statistical analysis

The results were expressed as means±standard deviation. One way analysis of variance (ANOVA) was used to compare means and Duncan's multiple range test (DMRT) was carried out to test any significant differences between the means. Differences between means at 5% level were considered significant. All statistical analysis was performed using SPSS (PASW Statistics18).

3. Results and Discussion

3.1 Film appearance and color

The photographs of gelatin films incorporated with BPA and TRC at different percentages are shown in Figure 1, and their corresponding color values are shown in Table 1. Overall, with increasing percentage of plant pigments, more intense film color was observed. In films incorporated with BPA, the obtained films appeared purple-blue in color. Anthocyanins have a wide array of color properties, presenting purple-blue color at neutral pH [20]. The films showed lower L* (lightness) but greater a* (redness) and higher b (blueness) values with increase in BPA concentration. Hue values indicating the purple-blue color shade significantly increased from 269.53 to 275.58 ($p<0.05$) as BPA increased from 10 to 30%. The chroma values, indicative of color saturation, also slightly increased from 15.90 to 26.37 ($p<0.05$).

In films incorporated with TRC, there were no significant changes in L* and b* values ($p>0.05$). It was expected that b* (yellowness) would increase as %TRC increased. However, the b* values gradually decreased and film with 30% TRC showed the lowest b* value ($p<0.05$). In addition, hue value decreased from 83.24 to 79.38, indicating a lower degree of yellowness ($p<0.05$). This was in agreement with the reduction in chroma values from 70.32 to 63.31 as %TRC increased ($p<0.05$). Curcuminoids are polyphenol compounds derived from turmeric. Among them, curcumin, which is of a bright yellow color, is the principal component [8]. It was expected that with increasing %TRC in the film, more yellowness would be observed. However, less yellowness and darker film was seen.

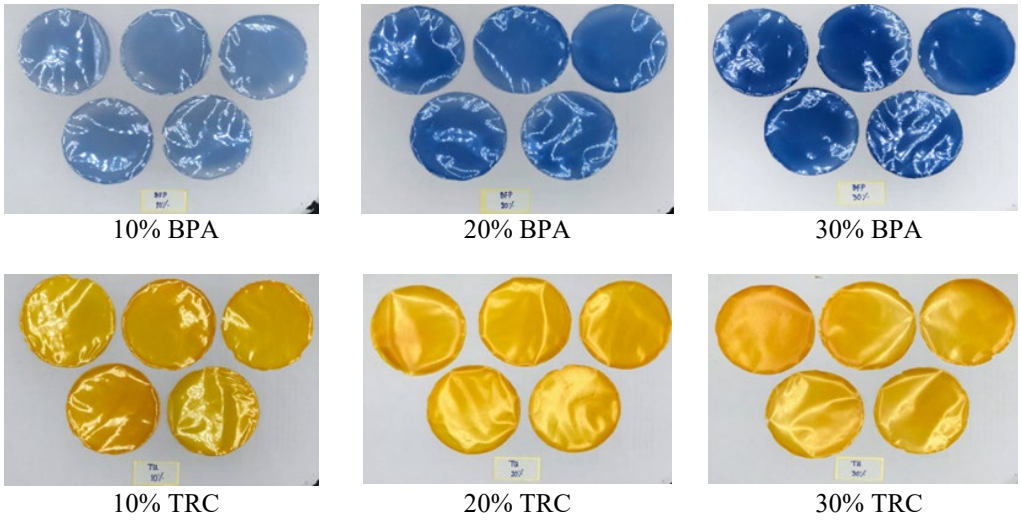


Figure 1. Gelatin-based films containing butterfly pea flower anthocyanins (BPA) and turmeric curcuminoids (TRC)

Table 1. Color values of gelatin-based films containing butterfly pea flower anthocyanins (BPA) and turmeric curcuminoids (TRC)

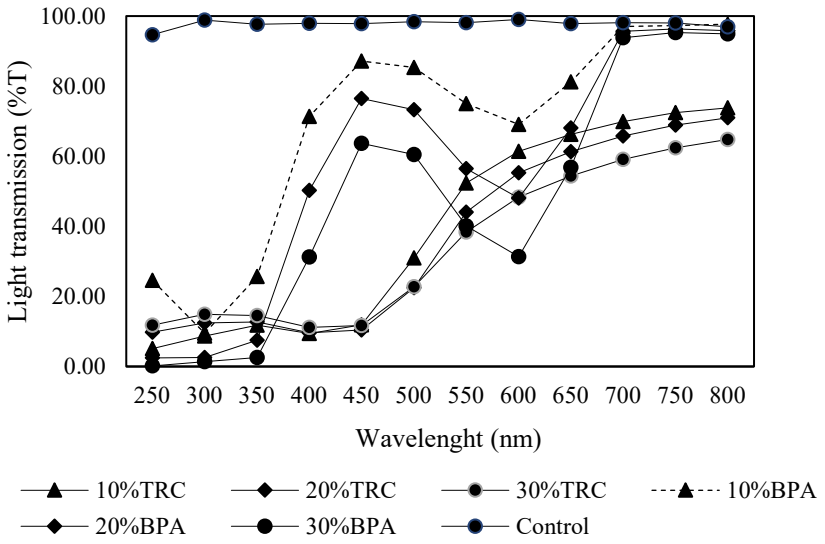
Films	L*	a*	b*	hue	chroma
10% BPA	68.26±0.66^{a}	-0.15±-0.11^{a}	-15.90±-0.52^{a}	269.53±0.60^{a}	15.90±0.53^{a}
20% BPA	55.66±0.78^{b}	0.148±0.08^{b}	-23.89±0.41^{b}	270.32±0.17^{b}	24.02±0.45^{b}
30% BPA	45.98±0.48^{c}	2.54±0.20^{c}	-26.24±0.15^{c}	275.58±0.37^{c}	26.37±0.15^{c}
10% TRC	71.51±1.80^{a}	8.32±3.12^{a}	69.804±0.74^{a}	83.24±2.44^{a}	70.316±1.20^{a}
20% TRC	70.14±0.86^{a}	10.96±3.04^{a}	68.24±2.30^{a}	80.9±1.34^{ab}	69.20±2.04^{a}
30% TRC	69.11±2.57^{a}	11.8±3.96^{a}	62.09±2.19^{b}	79.38±3.07^{b}	63.31±2.92^{b}

Data are mean values±standard deviations. In a column with the same type of film. means followed by the different letters indicate significant differences among treatments at ($p < 0.05$).

3.2 Light transmission and opacity

Figure 2(A) shows the different transmittance patterns of BPA and TRC films as measured over the wavelength range of 250 to 800 nm. Compared to the control film with nearly 100% transmission, films containing either BPA or TRC exhibited an apparent low light transmission. The changes in light transmittance along the varying wavelengths were related to the type of plant pigments which had been incorporated into the films. At wavelengths between 200-400 nm, which were in the UV range, only 5-20%T was observed from the BPA films while about 5%T was seen in the TRC films. The two films exhibited lower light transmission as the concentration of pigment increased. The results indicated the blocking effect of UV light of the two films, and this was conformed with the high opacity results observed for the same films. The control film without plant pigments showed

(A)



(B)

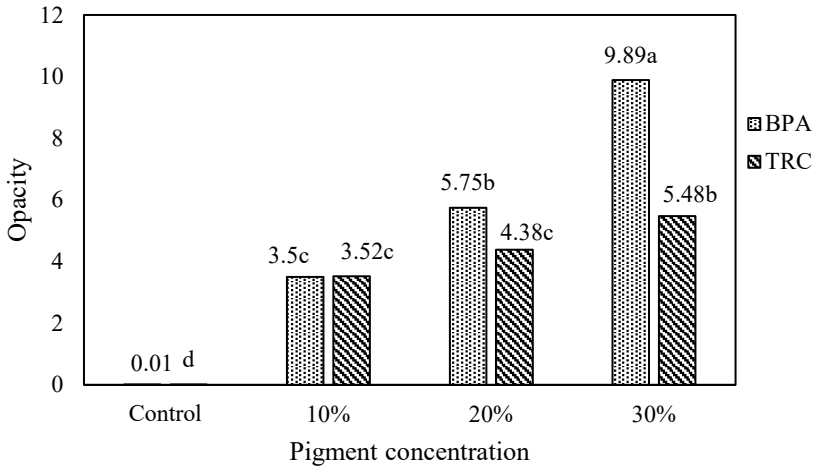


Figure 2. Light transmission (A) and opacity (B) of gelatin-based film containing butterfly pea flower anthocyanins (BPA) and turmeric curcuminoids (TRC)

light transmission of almost 100%T and conversely, nearly zero light absorption at 600 nm and zero opacity. The BPA and TRC films exhibited increased opacity as the percentage of pigments increased ($p<0.05$). At 10% pigments, opacity was comparable ($p>0.05$) between BPA film and TRC film. At 20 and 30% plant pigment, greater opacity was found in BPA films than in TRC films ($p<0.05$). An earlier study by Wu *et al*. [21] stated that gelatin film itself could restrain lipid oxidation induced by UV light in food systems. The addition of green tea extract onto the film could apparently enhance this effect.

3.3 Thickness, moisture content and water solubility

Table 2 shows thickness, moisture content and water solubility of gelatin films. Compared with the control films without pigments, the thickness of films tended to increase with increasing either BPA or TRC concentration. Earlier reports showed that adding plant pigments like anthocyanin [18] and curcumin [22] into films did not exhibit any effect on film thickness. However, film thickness is an important parameter since it has considerable influence on other physical properties such as water solubility, light transmission, and film opacity [23]. In film incorporated with plant pigments, thickness exhibited an inverse relationship with light transmittance. The results of this study showed that the films containing a lower percentage of pigments exhibited lower thickness and higher transmittance (Figure 2(A)).

The moisture content of film incorporated with plant pigments slightly increased with increased % BPA but decreased with %TRC ($p < 0.05$). A slightly higher moisture content in BPA film than in TRC film could be related to the hydrophilicity of anthocyanins [5]. On the contrary, the incorporation of TRC probably increased the hydrophobicity of films [18] and consequently, a lower amount of water presented. In this study, the lower water solubility of TRC compared to BPA film was expected. However, the lower water solubility of TRC films (94.65-97.00%) compared to that of BPA films (97.07-97.71%) were observed only at 25°C ($p<0.05$). At 60°C, the water solubility of the two films was in a similar range of 97.68-99.25%. At higher temperature, the water solubility of all gelatin-based films was greater, and this was probably attributed to gelatin which dissolving better in hot water than in cold water. The effect of pigment concentration was less significant in hot water ($p>0.05$). High water solubility may be beneficial for some applications. However, film with high water solubility can be degraded easily [24].

Table 2. Thickness, moisture content and water solubility of gelatin-based film containing butterfly pea flower anthocyanins (BPA) and turmeric curcuminoids (TRC)

Films	Thickness(mm)	Moisture Content (%)	Water Solubility (%)	
			25°C	60°C
Control	0.044±0.015^{ab}	7.97±0.17^{b}	97.92±0.43^{a}	98.20±0.55^{ab}
10% BPA	0.046±0.003^{ab}	7.33±1.13^{b}	97.71±0.86^{a}	98.76±0.61^{ab}
20% BPA	0.050±0.004^{b}	10.13±1.50^{a}	97.07±0.86^{a}	98.82±0.61^{ab}
30% BPA	0.051±0.003^{bc}	11.51±0.45^{a}	97.48±0.42^{a}	98.90±0.32^{ab}
10% TRC	0.039 ±0.00^{a}	10.23±1.14^{a}	94.65±0.77^{b}	99.25±0.65^{ab}
20% TRC	0.050±0.002^{b}	8.73±1.85^{a}	95.39±0.41^{b}	98.80±0.47^{ab}
30% TRC	0.052±0.002^{b}	5.89±0.77^{b}	97.00±0.21^{a}	97.68±0.07^{a}

Data are mean values±standard deviations. In a column with the same type of films, means followed by the different letters indicate significant differences at ($p<0.05$).

3.4 Pigment compounds, phenolics and antioxidant activities

Table 3 shows total anthocyanin content (TAC), total curcuminoids content (TCC), and total phenolic content (TPC) of the two films incorporated with different concentrations of BPA or TRC, and their corresponding antioxidant activities based on FRAP and DPPH radical scavenging activities. Anthocyanins and curcuminoids are major phenolic compounds presented in butterfly pea flower and turmeric rhizome, respectively [6, 25]. In BPA films, TAC increased with increasing

Table 3. Antioxidant properties of gelatin-based film containing butterfly pea flower anthocyanins (BPA) and turmeric curcuminoids (TRC)

Films	TAC (mg cyanidin-3-glucoside /100g)	TCC (mg TCC/100g)	TPC (mgGAE/100 g)	FRAP (µmol FeSO4/100g)	DPPH (µmol Trolox/100g)
Control	-	-	0.02±0.01^{d}	0.83±0.03^{d}	8.48±0.51^{d}
10% BPA	43.74±2.19^{c}	-	0.36±0.02^{c}	25.13±0.15^{c}	40.52±0.94^{a}
20% BPA	104.04±0.05^{b}	-	0.71±0.01^{b}	40.13±3.15^{b}	38.77±1.01^{b}
30% BPA	124.49±0.04^{a}	-	0.80±0.01^{a}	61.45±0.03^{a}	32.73±0.51^{c}
10% TRC	-	12.63±0.27^{c}	0.68±0.04^{c}	64.56±3.05^{b}	256.69±3.29^{c}
20% TRC	-	14.73±0.47^{b}	0.76±0.01^{b}	67.95±6.39^{b}	269.86±2.44^{b}
30% TRC	-	19.71±0.52^{a}	0.83±0.02^{a}	80.87±10.33^{a}	277.45±11.98^{a}

Data are mean values±standard deviations. In a column with the same type of films, means followed by the different letters indicate significant differences ($p < 0.05$).

%BPA ($p<0.05$). The measured TAC values corresponded well with the film color appearances (Figure 1) and color values including a*(redness), b* (blueness), hue and chroma (Table 1). In TRC films, TCC increased as %TRC increased ($p<0.05$). However, the concentration of TCC was not consistent well with the decreased b* (yellowness), hue and chroma values ($p < 0.05$).

The highest TPC was obtained from 30% BPA and TRC films ($p<0.05$). The presence of phenolic groups contributed to antioxidant activities measured as Ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and reducing power assays. The addition of the two plant pigments significantly increased the antioxidant activities of gelatin film as compared to the control film ($p<0.05$). As the percentage of BPA and TRC increased, an increase in antioxidant activities was expected. The greatest FRAP was obtained from films with 30% BPA and TRC. However, the highest DPPH radical scavenging activities were only obtained from 30% TRC films and not from 30% BPA films. Earlier studied also reported the high antioxidant activities for gelatin film incorporated with anthocyanin [18] and curcuminoid encapsulated with gelatin [15].

3.5 Digestive recovery of plant pigment compounds and antioxidant properties during *in vitro* gastrointestinal digestion

Film forming solutions with 30% BPA or TRC, which exhibited the greatest pigment concentration and the related antioxidant activities, were evaluated upon *in vitro* gastrointestinal digestion. The corresponding 30% BPA or TRC solutions without gelatin and glycerol served as control. Samples were subjected to simulations of the following biological conditions in the different regions of the human GI tract; amylase enzyme at pH 6.0 in the oral phase (G0), pepsin enzyme at pH 2.0 in gastric digestion (G30) and pancreatin enzymes at pH 6.8 in small intestine phase (I0-I120), respectively. The pigment concentrations in terms of TAC and TCC were evaluated in parallel to the antioxidant properties including TPC and antioxidant activity based on FRAP and DPPH radical scavenging activity, and are shown in Figure 3.

Overall, among the digestions, film forming solutions showed a greater pigment concentration (TAC and TCC), TPC, FRAP and DPPH radical scavenging activity than the

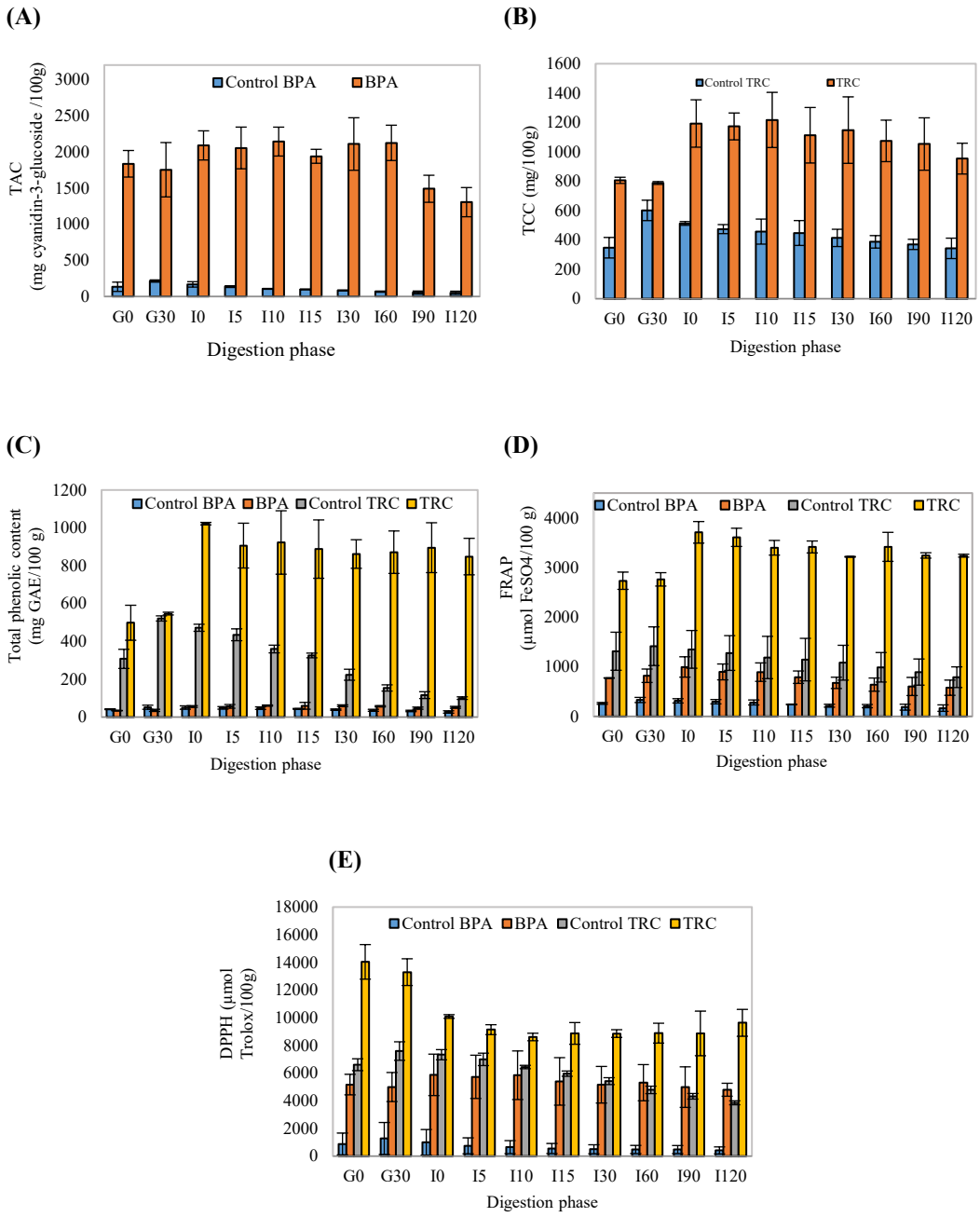


Figure 3. Changes in antioxidant properties of pigment extracts (control BPA and control TRC) and film forming solutions (BPA and TRC) during *in vitro* gastrointestinal digestion; TAC(A), TCC(B), TPC(C), FRAP(D) and DPPH radical scavenging activity (E)

corresponding pigment solutions ($p<0.05$). Trends observed for the pigment solutions were the increase in TAC, TCC, TPC and antioxidant properties from the oral phase (G0) to the gastric phase (G30) and subsequent decrease in the intestinal phase (I0-I120). In BPA solution, the high %stability during gastric digestion (G30) compared to the original values at oral digestion (G0) were 83.03% TAC, 24.77% TPC, 27.73% FRAP and 43.68% DPPH radical scavenging activity. These were in contrast to the low % stability during intestinal digestion (I120) where all values were negative, and were -61.28% TAC, -35.49% TPC, -36.763% FRAP and -42.16% DPPH radical scavenging activity. These findings indicated the high amounts of anthocyanins and phenolics when exposed to acidic conditions in stomach. The low pH value in stomach mainly contributes to the high stability of anthocyanins, which at pH between 1.5-2.0 present as the stable flavylium cation [26]. The transformation of anthocyanins into a more stable structure resulted in greater antioxidant activities. Similar observation was obtained from samples containing TRC. The stability during gastric digestion was 65.86 TCC, 71.44% TPC, 8.01% FRAP and 14.89% DPPH radical scavenging activity. During intestinal digestion, the stability was -5.00% TCC, -67.17% TPC, -39.60% FRAP and -41.46% DPPH. The results indicated the high stability of BPA and TRC upon acidic conditions compared to neutral or alkaline conditions during *in vitro* digestion. According to David *et al*. [26], acid conditions and digestive enzymes stabilized bioactive compounds and antioxidant properties during gastric digestion. In addition, Liang *et al.* [4] reported that total anthocyanins were unstable under intestinal conditions as the flavylium cation was transformed into the colorless chalcone pseudobase at pH levels between 6 and 7. Furthermore, the alkaline conditions destroyed the curcuminoid structure by removing a proton from the phenolic group resulting in the formation of unstable products [27]. The results of this study were in agreement with earlier studies in which BPA, TRC and TPC were substantially degraded in intestinal digestion.

In gelatin film solutions containing either 30%BPA or 30%TRC, digestive behavior differed to that of control samples. The results showed that during gastric digestion, pigment compounds and antioxidant activities were almost unchanged ($p>0.05$) compared to the original values in the oral phase (G0). This was indicated by the low values of %stability of -5.03% TAC, 4.47% TPC, 5.88% FRAP and -3.86% DPPH radical scavenging activity for 30%BPA film solution. Those of the TRC films were -2.20% TCC, 11.47% TPC, 1.07% FRAP and -5.22% DPPH radical scavenging activity, respectively. The results suggested that under the acidic conditions of the gastric phase, the stability of anthocyanins and curcuminoids was less enhanced by the presence of gelatin and glycerol. However, upon intestinal digestion, some positive values were obtained, indicating the greater recovery of pigment compounds and antioxidant activities. For the BPA film solution, the stability during intestinal digestion was 70.48% TAC, 53.06% TPC, -25.23% FRAP and 6.88% DPPH radical scavenging activity. Similar observation was obtained from TRC film solution as the stability was 19.20% TCC, 71.26% TPC, 18.67% FRAP and -30.79% DPPH radical scavenging activity, respectively.

Differences with the corresponding control sample can be contributed to the presence of 3% (w/w) gelatin as film forming agent and 25% (w/w) glycerol as plasticizer in film forming solution. A similar result was also reported for blueberry anthocyanins with added α-casein and β-casein [11]. The addition of α-casein or β-casein did not contribute to the stability of anthocyanins during gastric digestion but apparently enhanced the stability of blueberry anthocyanins during intestinal digestion. The study also highlighted the interaction between protein and anthocyanins in which the protein protected the compounds against biological factors associated with *in vitro* digestion. Another study by Gómez-Estaca [15] also showed a protective effect of gelatin on curcumin during digestion. The curcumin encapsulated with gelatin showed improved antioxidant capacities and bioaccessibility. In this study, gelatin which was a type of protein possibly interacted with the anthocyanins and curcuminoids and consequently improved the stability of the pigment compounds ($p<0.05$), and the TPC and antioxidant activities, during the intestinal phase. The results

indicated the protective effect of the films additives, gelatin, and glycerol, against biological conditions during intestinal digestion of the two pigments, BPA and TRC.

4. Conclusions

Incorporation of butterfly pea anthocyanins and turmeric curcuminoids in different concentrations significantly altered gelatin-based films in terms of their color and moisture content. The light barrier properties and antioxidant activities of the two films were also increased as percentage plant pigments increased. Upon *in vitro* digestion, gelatin films with the two plant pigments showed the lower stability of pigment compounds and antioxidant activities during gastric digestion but greater recovery during intestinal digestion than pigment extracts alone (control). This study demonstrated the viability of improving the bioaccessibility of plant pigments incorporated into gelatin-based films. However, in preparing protective biodegradable film, the mechanical properties of the films such as strength, stretchability, and water vapor and gas permeability must be further investigated.

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