

## STABILITY OF ANTHOCYANIN DURING PROCESSING, STORAGE AND SIMULATED DIGESTION OF PURPLE SWEET POTATO PASTA

### *Stabilitas Antosianin Selama Proses, Penyimpanan, dan Simulasi Pencernaan Pasta Ubi Jalar Ungu*

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#### ABSTRACT

Purple sweet potato is rich in anthocyanin giving a potential application in food product development. However, anthocyanin is relatively unstable and easily degraded during processing and storage. Understanding the stability and bio-accessibility of anthocyanin during processing, storage and simulated digestion is very important. The study aimed to investigate changes in anthocyanin degradation during processing, storage and simulated digestion of purple sweet potato pasta. The pasta was prepared through several processing steps, i.e. steaming the tuber, steaming the dough formula, extrusion, drying and boiling. Anthocyanin was analyzed at every stages of processing and storage of the pasta. The durability of the pasta during storage was analysed using an accelerated shelf-life testing method at 30, 40 and 50 °C for 28 days. The study showed that anthocyanin content decreased during the whole stages of processing and storage, but slightly increased during steaming. The highest loss of the anthocyanin occurred in the boiling process. Based on resistance to stomach and intestinal conditions, the bio-accessibility of anthocyanin was better in the digestive system in the stomach than that in the intestines. The increased anthocyanin appeared again in the colon. This study provides useful information for designing an effective method to minimize an extensive loss of anthocyanin of purple sweet potato for food product development.

[**Keywords:** anthocyanin, purple sweet potato pasta, stability]

#### ABSTRAK

Ubi jalar ungu kaya dengan antosianin yang memberikan potensi untuk diaplikasikan dalam pengembangan produk pangan. Namun, antosianin relatif tidak stabil dan mudah terdegradasi selama pengolahan dan penyimpanan. Memahami stabilitas dan bioaksesibilitas antosianin selama pengolahan, penyimpanan, dan simulasi pencernaan sangat penting. Penelitian ini bertujuan untuk mengetahui degradasi antosianin pasta ubi jalar ungu selama pengolahan, penyimpanan, dan dalam simulasi sistem pencernaan. Pasta disiapkan melalui beberapa langkah pengolahan, yaitu pengukusan umbi, pengukusan adonan, ekstrusi, pengeringan, dan perebusan. Analisis antosianin dilakukan pada setiap tahap pengolahan pasta ubi jalar ungu dan selama penyimpanan. Analisis lama penyimpanan pasta dilakukan

dengan menggunakan metode ASLT pada suhu 30, 40, dan 50 °C selama 28 hari. Hasil penelitian menunjukkan bahwa kandungan antosianin sedikit meningkat pada tahap pengukusan, namun pada tahap lainnya terjadi penurunan. Kehilangan antosianin tertinggi terjadi pada proses perebusan. Berdasarkan ketahanan terhadap kondisi lambung dan usus, bioaksesibilitas antosianin lebih baik dalam sistem pencernaan di lambung dibandingkan dengan di usus. Peningkatan antosianin muncul kembali di usus besar. Penelitian ini memberikan informasi yang berguna untuk merancang metode yang efektif untuk mengendalikan kehilangan antosianin ubi jalar ungu untuk pengembangan produk makanan.

[**Kata kunci:** antosianin, pasta ubi jalar ungu, stabilitas]

#### INTRODUCTION

Purple sweet potato (PSP) is a nutritive tuber with a high content of anthocyanin providing a good source of functional food. Anthocyanin is a pigment that gives color to PSP and acts as an antioxidant. Anthocyanin of PSP primarily exists as an acylated form of phenolic acids having some advantages in pH and heat resistance, light sensitivity, and overall sensitivity (Xu et al. 2015). The stability of anthocyanin and its color are correlated with anthocyanin structure and affected by heat, pH, light, and the presence of enzymes, phenolic acids, oxygen, sugar, sulfur dioxide and a metal ion (Askar et al. 2015). Besides, color stability depends on the concentration of anthocyanin (Durge et al. 2013).

PSP is potentially processed into several food products, such as beverages, cake and chips, as well as extrusion products such as noodle and pasta. Pasta is a staple food in many countries. It has an excellent nutritional profile, being a good source of complex carbohydrates and a moderate source of proteins and vitamins. Besides easy to prepare and a very versatile food, pasta has a relatively long shelf life when it is stored appropriately. It is also considered an

adequate vehicle for food supplementation with minerals, proteins and many other valuable healthy components (Borneo and Aguirre 2008; Boroski et al. 2011).

PSP pasta is a food product made from mixtures of PSP, mungbean flour and cassava starch. PSP pasta has functional properties because of its high anthocyanin content and free from gluten. Processing steps of PSP into pasta include steaming, pulping, mixing, extrusion, drying and cooking involving heat and light exposure that can affect the survival of anthocyanin. Research on extrusion of bilberry anthocyanin in starch-based food demonstrated that total anthocyanin retention decreased at a higher temperature and a lower moisture content. Lowest degradation was found in lowest processing temperature and highest moisture content (Hirth et al. 2014). The same result was found in the processing of strawberry jams. This research explained that anthocyanin change was affected by storage conditions (Holzwarth et al. 2013).

Since the stability of anthocyanin is affected by several process using stages and storage conditions, and the color is one of the most quality attributes of the product and also correlated with anthocyanin content and structure, numerous studies have been conducted to investigate the changes in anthocyanin content and color during processing and storage. Besides, understanding the potential health benefit of anthocyanin and the low stability and bio-accessibility was important to evaluate the stability of anthocyanin in the simulated gastric and intestinal conditions. The changes in anthocyanin content and color in processing, storage and simulated digestion of PSP pasta have not been studied extensively. This study is important to predict anthocyanin losses after going through several stages of processing, storage and simulated digestion.

## MATERIALS AND METHODS

### Materials

Purple sweet potato of Ayamurasaki variety from Malang, East Java-Indonesia was used for the study. Flour of mungbean and cassava starch were purchased from a local market.

### Preparation of PSP Pasta

Preparation of PSP pasta included steaming, pulping, mixing, extrusion, drying and cooking. Fresh PSP was steamed for 45 minutes using a steamer with boiling water. Steamed PSP was then peeled and pulped using a food processor to make puree. The PSP puree was then

mixed with mungbean flour and cassava starch. The dough was steamed for 15 minutes and extruded using multifunction noodle machine at low temperature (45 °C). The extruded product was dried using a tray dryer at a temperature of 50 °C for 10 hours and then the dried pasta was boiled in water for 8 minutes (optimal cooking time). PSP pasta was prepared three times as replications.

### Storage of PSP Pasta

The PSP pasta was packed in the aluminum foil bag, sealed and stored in incubators at three different temperatures, i.e. 30, 40 and 50 °C for 28 days.

### Anthocyanin Extraction

Anthocyanin of PSP pasta was extracted using distilled water (pH 2) in a ratio of 1 : 5 (pasta : distilled water). The solution was shaken for 6 hours followed by maceration for 12 hours. The solution was then filtered through a Whatman 42 filter paper.

### Anthocyanin Quantification

Monomeric anthocyanin contents were determined using the pH-differential method (Truong et al. 2012) based on the structural changes of anthocyanin between pH 1.0 and 4.5. Potassium chloride buffer (0.025 M, pH 1) and sodium acetate buffer (0.4 M, pH 4.5) was added into anthocyanin extract. The absorbance of each equilibrated sample solution was measured at  $\lambda$  520 and 700 nm using a UV 6500 Spectrophotometer (Kruss Optronic, Germany). Pigment content was calculated based on cyaniding-3-glucoside using the following equation:

$$C = (A \times MW \times DF \times 1000) / (\epsilon \times l) \quad (1)$$

where C is the anthocyanin concentration, A is the absorbance value, DF is the dilution factor, MW is the molecular weight of cyanidin-3-glucoside amounted (448,8 g mol<sup>-1</sup>) and  $\epsilon$  is the molar extinction coefficient (29600 l mol<sup>-1</sup> cm).

### Color Analysis

Color was analyzed for their CIE-Lab L\*, a\* and b\* values using a Minolta Chromameter (Model CR-300, Osaka, Japan). Evaluation of color changes of PSP pasta was done by calculating X and Y parameters and plotting the values into a chromaticity diagram.

### Determination of Degradation Kinetics of Anthocyanin and Color

Degradation kinetics of anthocyanin and color were determined at different temperature conditions (30, 40 and 50 °C) during storage (28 days). Data were collected every 4 days of storage along 28 days. All assays were performed in duplicate. The arithmetic means standard deviations and correlation coefficients were calculated using Excel (Microsoft Inc., Washington, USA).

Degradation rate constants of anthocyanins and color changes were calculated by multiplying the values of the slopes of regression line. The slope describes the rate of anthocyanin change as storage time changes. The regression lines were obtained by plotting the data of anthocyanin concentration as a function of storage time. The kinetic models for zero and first-order anthocyanin degradation are as follow:

$$\text{Zero order kinetic equation: } C_t = C_0 - K_t \quad (2)$$

$$\text{First order kinetic equation: } \ln(C_t) = \ln(C_0) - K_t \quad (3)$$

where  $C$  is the concentration at time  $t$ ,  $C_0$  is the initial concentration, and  $K$  is the rate constant.

### Calculation of Half-Life Value

The half-life values of anthocyanin degradation and color changes were calculated using the following equations:

$$\text{Half-life for zero order: } t_{1/2} = \left[ \frac{A_0}{2k} \right] \quad (4)$$

$$\text{Half-life for first order: } t_{1/2} = - \frac{\ln 0,5}{k} \quad (5)$$

### Calculation of Activation Energy

Temperature dependence of anthocyanin degradation and color loss was determined by the Arrhenius equation as follow:

$$k = k_0 \exp^{-E_a/RT} \quad (6)$$

where  $E_a$  is the activation energy ( $\text{kcal mol}^{-1}$ ),  $k$  is the rate constant,  $k_0$  is the pre-exponential factor,  $R$  is the universal gas constant ( $1.987 \text{ kcal mol}^{-1}$ ), and  $T$  is the absolute temperature ( $^{\circ}\text{K}$ ).

### Simulated Digestion

The procedure of simulated digestion was adopted from the method outlined by McDougall et al. (2005)

consisting of two steps of digestion. The first step was pepsin/HCl digestion for 2 hours at 37 °C to simulate gastric conditions and the second step was bile salts/pancreatin digestion for 2 hours at 37 °C to simulate small intestinal conditions. Anthocyanin extract (2.5 mL) was made up to 20 mL with distilled water and the pH was adjusted to 1.7 with 5 M HCl. Furthermore, 315 units  $\text{mL}^{-1}$  pepsin was added and then incubated at 37 °C in a heated water bath shaker for 2 hours. After 2 h incubation, 2 mL aliquots of the post-gastric digestion were removed and frozen. The remainder was placed in a 250 mL glass beaker, then added with 4.5 mL of 4 mg  $\text{mL}^{-1}$  pancreatin and 25 mg  $\text{mL}^{-1}$  bile salts. The solution was fed into an Erlenmeyer flask containing 1 M  $\text{NaHCO}_3$  and incubated at 37 °C in a heated water bath shaker for 2 hours. The post-gastric IN and OUT samples were thawed when required and centrifuged, and the supernatants were assayed for anthocyanin contents.

## RESULTS AND DISCUSSION

### Anthocyanin Stability During Processing

Anthocyanin content was modified during the processing of PSP pasta. There was a significantly difference ( $P < 0.05$ ) in anthocyanin contents for each stage of processing of PSP into pasta (Table 1).

Anthocyanin content of fresh PSP was  $114.23 \pm 1.89 \text{ mg } 100^{-1} \text{ g}$  and slightly increased to  $156.38 \pm 3.55 \text{ mg } 100^{-1} \text{ g}$  after steaming. Similar research on PSP of Majesty variety also indicated that cooking affected anthocyanin content and steamed PSP had a higher amount of anthocyanin content than that of the fresh ones Lemos et al. (2015). Similar results were observed by Lachman et al. (2012) who explained that monomeric anthocyanin concentration in PSP increased 3.34 times after baking,

**Table 1. Changes in anthocyanin contents during processing of purple sweet potato pasta.**

Processing stage	Anthocyanin content ( $\text{mg } 100^{-1} \text{ g db}$ )
Initial condition (fresh purple sweet potato)	$114.23 \pm 1.89\text{e}$
Steaming purple sweet potato	$156.38 \pm 3.55\text{f}$
Steaming the dough	$82.08 \pm 2.74\text{d}$
Extrusion	$70.90 \pm 0.92\text{c}$
Drying	$59.16 \pm 3.37\text{b}$
Boiling	$21.21 \pm 0.82\text{a}$

Different letters within a column meant significant differences at  $p < 0.05$ .

4.2 times after boiling, and 4.5 times after steaming. In addition, Truong et al. (2012) found that steaming for 25 minutes in several varieties of PSP increased monomeric anthocyanin concentration. Steaming gave the best result in anthocyanin content of PSP (Tian et al. 2016). This condition can be explained by the presence of polyphenol oxidase (PPO) and peroxidase enzymes that cause degradation of anthocyanin. PPO activity is strongly influenced by the presence of oxygen, while the peroxidase is determined by the presence of H<sub>2</sub>O<sub>2</sub> compound. Destruction of PSP tissue such as cutting and pulping in the extraction process triggered PPO and peroxidase enzyme activity because it increased the availability of oxygen and peroxide compounds (Oren-Shamir 2009).

PSP pasta dough was formulated from the mixture of PSP, mungbean flour and tapioca starch. Therefore, anthocyanin content of the dough was smaller than that of steamed PSP. Anthocyanin content decreased slightly during extrusion and drying. Little losses of anthocyanin were found during extrusion (12%). An increase in temperature of 15 °C in the extruder may be responsible for the degradation of anthocyanin. This was also found in heat processes of rich flavonoid product at 50 °C for 90 seconds that caused flavonoid loss of 22% (Ioannou et al. 2012). In the drying process, evaporation may cause anthocyanin loss.

The largest anthocyanin loss occurred in the boiling process (Table 1). The water-soluble anthocyanin and also the high boiling temperature caused loss of anthocyanin. In red cabbage, boiling decreased total anthocyanin, while steaming and stir-frying increased anthocyanin content (Murador et al. 2015). Loss of anthocyanin in the boiling that involves high temperature was also encountered in the processing of PSP chips. The degradation occurred due to the Maillard's reaction during the cooking process with the presence of reducing sugars in the ingredients (Kita et al. 2013). The results showed that the loss of PSP pasta anthocyanin reached 74.16% during steaming of the dough.

Previous studies suggested that anthocyanin

degradation in heat-induced processes was associated with the increase in oxidation reactions. Chemical reaction causes a change in anthocyanin chemical structure. The opening of pyrylium ring and the formation of chalcone took place at the beginning of degradation process. The degradation continued with the occurrence of sugar hydrolysis and aglycone formation (Patras et al. 2010).

### Color Stability During Processing

Changes in anthocyanin contents at each stage of the process can also be seen from color changes of the product. Lightness (L\*), redness (a\*) and yellowness (b\*) changed during processing of PSP pasta. Lightness increased as the process proceeded, which was characterized by the increase in the L\* value. Before processing, the L\* value was the lowest.

Fresh PSP had the highest a\* value. This means that fresh PSP has a reddish degree greater than the processed PSP. It also had the largest b\* value, which means that fresh PSP has a yellowish degree higher than the processed PSP.

Color visualization was shown by plotting X and Y values in the approximate color region on CIE Chromaticity Diagram. According to X and Y values, the color of PSP pasta changed during processing. Fresh PSP showed purplish red and cooked PSP pasta (after boiling) was purplish pink. Change in color was correlated with anthocyanin loss during processing (Table 2). Loss of anthocyanin content was also reported in the processing of black carrot jams and marmalades (Kamiloglu et al. 2015). Alighourchi and Barzegar (2009) also reported correlation between total anthocyanin content and chromatic parameters of pomegranate juice.

### Anthocyanin Degradation and Color Stability During Storage

Anthocyanin degradation was also occurred during storage, at a rate strongly dependent on storage

**Table 2. Color stability of purple sweet potato pasta during processing.**

Processing stage	L*	a*	b *	X	Y
Fresh purple sweet potato	13.09 ± 4.12	46.07 ± 2.80	6.27 ± 0.69	0.61±0.042	0.24±0.017
Steaming purple sweet potato	24.50 ± 0.18	8.67 ± 0.57	-6.06 ± 0.52	0.34±0.004	0.29±0.002
Mashing to produce puree	27.08 ± 0.58	11.00 ± 0.45	-7.09 ± 0.28	0.34±0.001	0.28±0.001
Steaming the dough	36.93 ± 1.93	21.23 ± 2.60	-1.80 ± 0.72	0.39±0.006	0.30±0.007
Extrusion	31.65 ± 1.59	25.44 ± 0.81	1.08 ± 0.55	0.41±0.007	0.30±0.002
Drying	56.88 ± 1.68	21.52 ± 1.12	0.87 ± 0.18	0.38±0.003	0.31±0.002
Boiling	52.72 ± 1.64	13.63 ± 0.71	-11.66 ± 0.56	0.33±0.003	0.29±0.004

\*L = light, a = redness, b = yellowness.

temperature (Figure 1). The degradation occurred faster with the increase in storage temperature. Storage at 50 °C resulted in a fastest degradation. Arrhenius model was used to determine the effect of temperature on the kinetics of degradation process.

Increase in storage temperature enhanced the k value. PSP pasta storage at 50 °C showed the highest k value. Similar research also found that increase in storage temperature accelerated anthocyanin degradation (Alighourchi and Barzegar 2009; Kircha et al. 2007).

Anthocyanin degradation during storage followed the first-order reaction model as shown by the higher R<sup>2</sup> value of the model. Similar result was shown in anthocyanin degradation of PSP (Jie et al. 2013) and black rice (Hou et al. 2013). The half-life (t<sub>1/2</sub>) of anthocyanin of PSP pasta was 119.51, 60.80 and 32.85 days at 30 °C, 40 °C and 50 °C, respectively (Table 3). The half-life is required to determine anthocyanin stability associated with its lower bioavailability (less than 1% of the amount of anthocyanin consumed) (Milbury et al. 2010). The decrease in anthocyanin concentrations up to 50% reduced the functional properties of PSP pasta. Less concentration of anthocyanin consumed means less amount of its bioavailability. The presence of sugar and protein in the product affects anthocyanin degradation at high temperatures. Sugars and proteins cause Maillard reaction, which produces furfural compounds causing anthocyanin

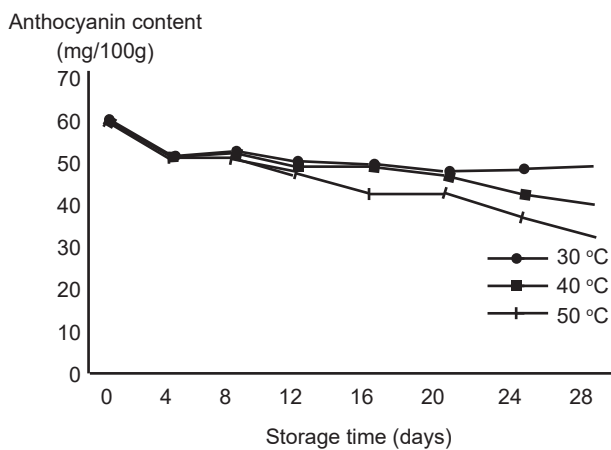


Figure 1. Degradation of anthocyanin of purple sweet potato pasta during storage at 30, 40 and 50 °C.

condensation (Tonon et al. 2010). The activation energy required for the degradation of anthocyanins at every rise in temperature of 10 °C was 52.60 Kj.

Anthocyanin degradation during storage was also shown by color change of the product. Storage temperature affected color changes and the changes were greater with increasing storage temperature. Results showed that H\* and L\* values increased during storage (Figure 2 and 3). Similar results were observed on storage of aqueous extracts of purple and red-flesh sweet potatoes (Reyes and Cisneros-Zevallos 2007) where the color of the products tends to be more reddish. The increase in L\* value resulted in paler appearance of the product. An increase in L\* value was reported to be related to the formation of translucent extract due to color fading in thermally treated anthocyanin aqueous solution over storage (Sui et al. 2016).

The activation energy for changes in H\* and L\* at every rise in temperature of 10 °C was 55.83 and 49.99 Kj, respectively (Table 4). The activation energy for change in L\* value was smaller than that for H\* value, which meant that in the storage of PSP pasta, change in lightness was faster than that in color visualization.

### Simulated Digestion

*In-vitro* simulated digestion models are widely used for determining anthocyanin stability in the gastrointestinal conditions. The results showed that the total monomeric anthocyanin after incubation for 2 hours at 37 °C in the simulated digestive system (PG) was  $19.78 \pm 3.71$  mg 100<sup>-1</sup> g and then decreased significantly after further digestion in

Table 4. Parameters of color changes of purple sweet potato pasta during storage at temperature of 30, 40, and 50°C.

Storage temperature (°C)	R <sup>2</sup>		k		Ea (Kj)	
	°H	L	°H	L	°H	L
30	0.873	0.702	0.978	0.563	55.83	49.99
40	0.888	0.659	1.479	0.253		
50	0.885	0.784	3.877	0.166		

Table 3. Degradation parameters of anthocyanin of purple sweet potato pasta during storage at temperature of 30, 40, and 50 °C.

Storage temperature (°C)	Zero order				First order			
	R <sup>2</sup>	k	T <sub>1/2</sub>	Ea (Kj)	R <sup>2</sup>	k	T <sub>1/2</sub>	Ea (Kj)
30	0.70	0.611	96.82	44.78	0.72	0.0058	119.51	52.60
40	0.91	1.113	53.14		0.92	0.0114	60.80	
50	0.96	1.827	32.38		0.96	0.0211	32.85	



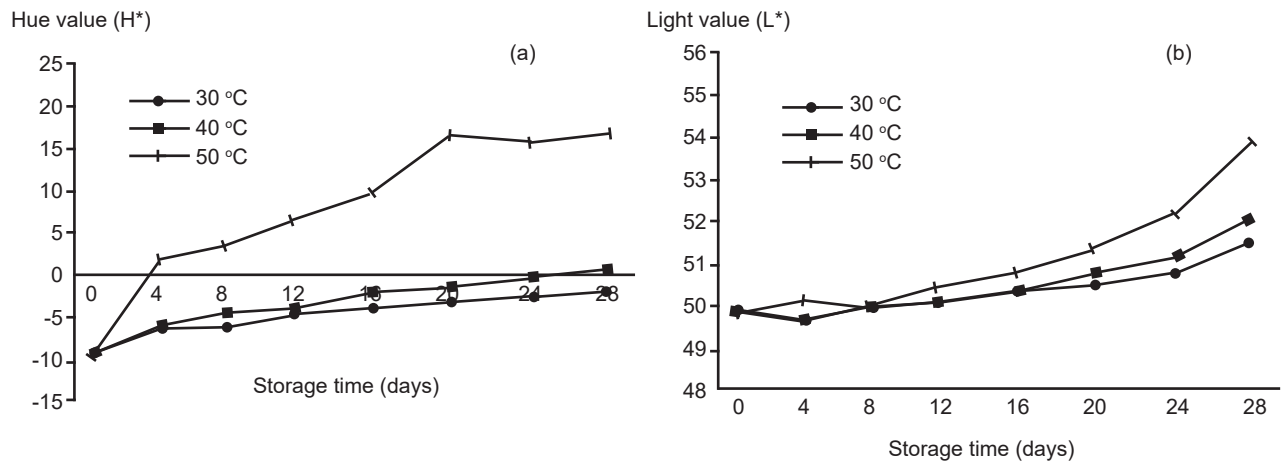


Figure 2. Changes in color of purple sweet potato pasta during storage at 30, 40 and 50 °C; a = Hue value (H\*), b = Light value (L\*).

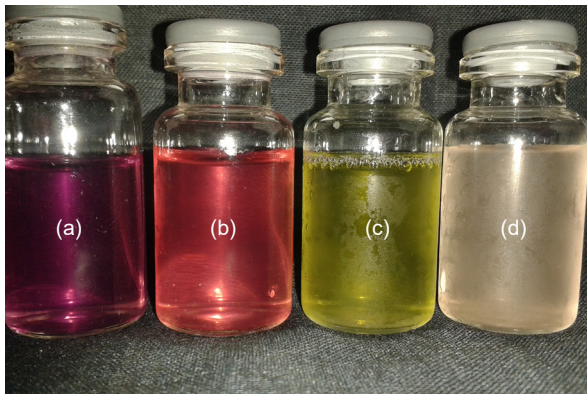


Figure 3. Changes in color of solution from gastric and intestinal system simulation: a = anthocyanin extract; b = postgastric; c = after adding simulated intestinal solution; d = after acidified.

the intestine containing bile salts and pancreatic enzymes. There was an increase in the total monomeric anthocyanin after incubation for 2 hours in the intestinal digestive system (OUT/colon) simulation. The amount of total monomeric anthocyanin was  $10.89 \pm 1.99 \text{ mg } 100^{-1} \text{ g}$ .

The total monomeric anthocyanin experienced significant losses during gastrointestinal simulation from the gastric fraction to the intestinal fraction. Liang et al. (2012) and Sengul et al. (2014) also reported the significant decrease in anthocyanin of mulberry extract after the fractional change from digestion in the stomach to the digestion in the gut. The loss of anthocyanins during digestion of digestive fraction in the intestine was probably due to an increase in pH, leading to the modification of anthocyanins into different phenolic components (Sengul, et al. 2014). Flavilium cations that influence the formation of anthocyanin color at low pH change gradually due to

the high pH of the fraction of intestinal digestion so that it becomes colorless. In this case, the flavilium cations turn into a colorless pseudo-base kalkan. The colored flavilium cations were stable at pH 2 or lower. However, at pH above 5, anhydro bases increased and became more stable at higher pH, forming ionized chambers (Liang et al. 2012). Identification of major anthocyanin content changes in pomegranate extract after co-digesting with several food components showed that cyn-3-glu was undetectable in the IN and OUT fractions (Sengul et al. 2014).

The increase in the total number of monomeric anthocyanins after passing the digestive system simulation in the intestine was associated with the decrease in the pH of the solution at the end of the incubation due to enzyme inactivity in the intestine. However, at this stage, precipitation process also occurred. The similar case was reported by McDougall et al. (2005). Precipitation of anthocyanins with insoluble complex compounds or components of pancreatin and bile salts during acidification led to the anthocyanin loss (LOSS) (McDougall et al. 2005).

The changes in anthocyanin structure due to pH changes in the digestive system simulation in the

**Table 5. Total monomeric anthocyanin of purple sweet potato pasta before and after *in vitro* gastric and intestinal digestion**

Fractional digestion	Anthocyanin content (mg 100 <sup>-1</sup> db)
Postgastric fraction	19,78 ± 3,71
IN (Intestinal fraction)	nd
OUT (Un-utilized fraction)	10,89 ± 1,99
Loss	8,89 ± 3,41

nd: undetectable.

gastric, intestines and after exit from the intestinal fraction (after acidification) can be seen from the color changes in the resulting solution (Figure 3). At the end of the gastric simulation (postgastric/PG), the color of the simulated gastric solution was pink (pH 2) and after the solution was added in the intestine (IN), the color was changed to green (pH 7). While after passing through the intestinal system and acidified (OUT), the color of the solution was very faded red (pH 2).

## CONCLUSION

Anthocyanin content of purple sweet potato pasta changed during processing, storage and simulated digestion. Anthocyanin content slightly increased in the steaming, but decreased during boiling, storage and simulated digestion with the highest loss at the boiling process. Anthocyanin losses during processing and storage was indicated by the color changes. Bio-accessibility of anthocyanin in the stomach digestive system was better than that in the intestine. Excessive boiling in the preparation of purple sweet potato pasta should be avoided because anthocyanin is soluble in water. Further study is required to minimize losses of anthocyanin of purple sweet potato pasta during processing and storage.

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