Conference Paper

Application of Encapsulated Anthocyanin Pigments from Purple Sweet Potato (*Ipomoea Batatas* L.) in Jelly Drink

Tensiska, Herlina Marta, Yana Cahyana, and Nur Syadza Amirah

Department of Food Industrial Technology, Faculty of Agro-industrial Technology, Padjadjaran University

Corresponding Author:
Tensiska
tensiska_agam@yahoo.com

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Abstract

Since the beginning of the 21^{th} century, demand for natural food additives especially food colorant has increased sharply. The natural one were considered safer, while the synthetic ones are less safe. One source of natural food colorant is anthocyanin pigments from purple sweet potato (*Ipomoea batatas* L.). Anthocyanins have good stability at acidic pH especially pH 2-4 so that it can be applied for food products such as beverages with low pH like jelly drink. Nevertheless, anthocyanins are very sensitive to the temperature and light. It is thus important to store food containing anthocyanins in proper condition. The purpose of this study was to determine the proper storage conditions and to predict shelf life of jelly drinks containing encapsulated anthocyanin pigments from purple sweet potato. This research used experimental methods by regression analysis of four treatments and four replications. The treatments were storage of jelly drink in (1) Room temperature with light exposure (25°C ± 2°C), (2) Room temperature without light exposure (25°C ± 2°C), (3) Refrigerator temperature with light exposure (5°C ± 2°C) and (4) Refrigerator temperature without light exposure (5°C ± 2°C). Parameters analysed were: (a) intensity of red color; (b) total anthocyanins; (c) pH value. Observations were made on days 0, 5, 10, 15, 20, 25 and 30. The results showed that the temperature and light affected the decrease in colour intensity, and total anthocyanins of jelly drink. The best treatment was stored at refrigerator temperatures without light exposure, has the smallest decreasing of total anthocyanins (46,03%) and decreasing of red intensity (3,19%) during 30 days storage in which the shelf life spanned to around 200 days based on the color intensity.

Keywords: anthocyanins pigments; purple sweet potato; regression analysis.

1. Introduction

Since the beginning of the 21st century, the demand on natural food additives has increased dramatically because it is believed to be safer for consumers compared to the synthetic ones. One source of natural food colorant is anthocyanin pigments from purple sweet potato containing around 923 mg per 100 g of material [1].

According to Jackman and Smith in Hendry and Houghton [2] anthocyanins are unstable to pH, temperature, oxygen, and light. Anthocyanin degradation may occur not only during the extraction process of plant tissue but also during processing and storage of food [3]. Temperature is an important factor in the degradation of anthocyanins. Heating can alter the structure of anthocyanins in the equilibrium reaction of cation form in which flavium is converted into colorless chalcon [3]. Anthocyanin color changes are also influenced by the degree of acidity (pH). Anthocyanins will be more stable at acidic pH and display the red color, but in base conditions, anthocyanins are purple to blue [4].

Light, like heat, can degrade anthocyanin pigment and form colorless chalcon. The energy released from light triggers photochemical reactions or photooxidation, leading to the opening of the ring of anthocyanin. Longer exposure causes further degradation and the formation of other derivatives such as 2,4,6 trihidroksibenzaldehid and acid substituted benzoate [5]. One method that can be done to increase the stability of anthocyanin is coating the pigment with polymers such as carbohydrates and proteins called microencapsulation. Microencapsulation can protect pigment due to chemical and physical influences on the product application [6]. According to Canovas, et al [7], encapsulation is the process by which a thin wrapper that is continuous envelops solid particles, liquid droplets and gas cells that forms the thin walls.

Anthocyanin pigment is stable at low pH, ie about pH 2-4 Markakis [8], making it suitable to be applied in a jelly drink. Jelly drink is a drink gel that can be made from pectin, agar, carrageenan, gelatin or other hydrocolloid compounds with addition of sugars, acids and without other additives [9]. Based on the mentioned findings above, it is necessary to study the stability of anthocyanin pigments from purple sweet potato applied in jelly drink during storage in a wide variety of conditions, namely the room temperature in the lightexposure, the room temperature of with no light exposure, the refrigerator temperature in the light exposure, and refrigerator temperature with no light exposure.

2. Materials and Method

The main raw material used was purple sweet popato tuber varieties Ayamurasaki with 3 months of age was obtained from Cileles Village in Jatinangor, Sumedang, West Java (Indonesia). Supporting materials were distilled water, 1% tartaric acid, and dextrin (DE 15) 6%.

The tools used were analytical balance, magnetic stirrer, a set of centrifuges, filter cloth, beaker glass 1000 mL, spatula, rotary vacuum evaporator, a vacuum oven, grinder and glass tools. Raw materials for the manufacture of jelly drink was jelly powder (plain), water, sugar, potassium citrate, citric acid, lychee flavoring, and Na-benzoate.

Material analysis used was potassium chloride buffer solution (0.025 M) pH 1, a solution of sodium acetate buffer (0.4 M) pH 4.5, Whatman paper no. 42, salt saturated aluminum chloride and concentrated HCl. The equipment used was a CIE-LAB box, pH meter, oven, desiccator, spektrofotometer UV 9200, refrigerator, refrigerator showcase, vacuum filter, a Buchner funnel, a cardboard box, a laptop using Adobe Photoshop software and SPSS statistical software.

This research using experimental method consisting of four treatments with four replications. Results were analysed using a statistical regression. The treatments were designed to test the application of anthocyanin pigment encapsulated in jelly drink which was stored in various storage conditions, namely:

A = Storage exposed to light at room temperature (25 °C ± 2 °C)

B = Storage in the dark at room temperature (25 °C ± 2 °C)

C = Storageexposed to light at refrigeration temperature (5 °C ± 2 °C)

D = Storage in the dark at refrigeration temperatures (5 °C ± 2 °C)

Observations were made every five days (0,5,10,15,20,25, and 30) during the 30 days of storage, including the intensity of the red color with CIE-lab method [10] and total anthocyanins by differential pH method - Lambert Beer [11], as well as the pH value. Observations were analyzed statistically using regression method in which the independent and dependent variables are as follows: the independent variable (X) was storage time, while the dependent variables (Y) were red intensity of jelly drink product, the degree of acidity (pH), and total anthocyanins. Stages of the research were as follows:

1. Extraction of anthocyanin pigment from purple sweet potato according to the method Tensiska *et al.* [12].

2. Microencapsulation of anthocyanin pigments made with addition of 6% maltodextrin.

3. Jelly drink preparation. Aquadest 100 mL was added into jelly powder containing 0.3% and 12% sucrose and heated until all ingredients were dissolved (75°C, 5 min). Further citric acid, K-citrate and Na-benzoate, lychees flavour were added. Then, product was cooled to 40°C and added 15,000 ppm anthocyanin pigment powder and packaged in plastic cup, sealed and stored according to the treatment.

4. Determination of the critical point with the hedonic test which presented three additional concentrations of anthocyanin pigment powder as 3000 ppm, 5000

ppm and 10000 ppm. Based on the results of the hedonic test, the addition of 3000 ppm concentration was not accepted by the panellists anymore with a* value was 54.90.

5. Storage jelly drinks at various conditions as follows:

    - Storage at room temperature exposed to light by the lamp 60 Watt (25°C). The distance between the lamp and product about 2 meters. The intensity of light used was 30 lux.
    - Storage at refrigerator temperature with light exposure. Jelly drinks stored at refrigerator showcase illuminated by lamp 30 Watt (5 °C). The distance between the lamp and product about 70 cm with the intensity of the light used for the irradiation was 86 lux.
    - Storage at room temperature without light exposure. Jelly drinks are stored in cardboard boxes at room temperature (25°C).
    - Storage at refrigerator temperature without light exposure. Jelly drinks was stored in a refrigerator at temperature of 5 °C.

6. Observation of anthocyanin pigments stability in jelly drinks and determining the shelf life by measuring of red colour intensity with CIE-Lab method [10] and measuring the concentration of anthocyanin with pH-Differential-Lambert Beer method [11], the pH value for 30 days on days 0, 5, 10, 15, 20, 25 and 30. Furthermore, the observation of color intensity is used to determine the shelf life. During storage, measuring the intensity of the color (L *, a *, b *). Data were used to calculate the AE where AE stated value overall color change. AE value calculated by equation [13]:

$$AE = [(\Delta L^*)2 + (\Delta a^*)2 + (\Delta b^*)2]^{\frac{1}{2}}$$

Furthermore, the shelf life can be calculated by linear regression of the intensity of the red color (a * ln) of jelly drink that are no longer accepted by the panelists (critical point).

Description:
a*= red intensity
L*= brightness
b*=yellow or blue intensity
ΔE = discoloration sample

3. Results and Discussion

Table 1: Equation Regression and determination coefficient value (R^2) of Intensity Red Color (a *) of Jelly Drink on Order 0 and Order 1.

Reaction Orde	Storage Condition	Regression Equation	R^2
0	A (room temperature, with light exposure)	y = 67.186 – 0.250x	0.963
	B (room temperature, without light exposure)	y = 67.596 – 0.206x	0.958
	C (refrigerator temperature, with light exposure)	y = 66.431 – 0.085x	0.891
	D (refrigerator temperature, without light exposure)	y = 66.746 – 0.071x	0.905
1	A (room temperature, with light exposure)	y = 4.212 – 0.004x	0.962
	B (room temperature, without light exposure)	y = 4.214 – 0.003x	0.981
	C (refrigerator temperature, with light exposure)	y = 4.198 – 0.002x	0.976
	D (refrigerator temperature, without light exposure)	y = 4.206 – 0.001x	0.909
Note: the order of 1 is the data that has been in Ln.			

3.1. Color intensity

Kinetics of decrease in the intensity of red color (a *) during storage could be explained by the reaction order. Zero-order reaction showed linear relationship between the concentration of reactants/products and time, while first-order reaction showed logarithmic changes in the concentration of the reactants/product and reaction time. Almost all foods undergo treatment process shows first-order reaction [14]. Data reduction in the intensity of the red color during storage were plotted into zero-order and first order. The data was plotted on a curve of the reaction zero order is the value of intensity of the red color (a *) (y) in several storage conditions as a function of time (x), while for curves first order reaction, the data was plotted is the value of the exponential of the intensity of the red color (ln a *) (y) as a function of time (x).Curve can be determined based on the regression equation and the coefficient of determination (R^2) of zero order and first order. The regression equation and the coefficient of determination (R^2) the intensity of red color (a *) zero-order and first-order can be seen in Table 1.

Overall most of the value of R^2 on first order greater than the zero order except on treatment storage in room temperature exposure to light. Therefore, the first order of the reaction kinetics was chosen as the decrease in the red intensity of jelly drinks were stored in various storage conditions. This is supported by Hendry and Houghton [2] which states that the kinetics of degradation of anthocyanins is first order. In addition Villota and Hawkes in Heldman and Lund [15] also states that the kinetics of degradation of red color (a *) anthocyanin described using first order reaction and will produce a linear relationship between the duration of storage and the value of the exponential intensity of red (Ln a*).

Based on Table 1, it can be seen from the slope value jelly drinks which stored at room temperature exposed to light was the greatest rate of decline among the three treatment that is equal to 0.004. This is because the anthocyanins are easily damaged

Table 2: Decrease of a* value Jelly Drink During Storage.

Storage Condition	Days storage							Decrease of a* value (%)
	0	5	10	15	20	25	30	
Room temperature, with light exposure	66.77	65.70	65.00	63.76	63.10	60.64	59.13	11.44
Room temperature, with light exposure	67.10	67.01	66.03	64.15	63.43	62.42	61.82	7.87
Refrigerator temperature, with light exposure	66.65	66.10	65.10	65.00	65.00	64.35	63.75	4.35
Refrigerator temperature, without light exposure	67.04	66.40	65.72	65.69	65.00	64.97	64.90	3.19

by the light and high temperatures both during processing and storage. Product which was stored in refrigerator temperature and was not exposed to light has smallest slope on these storage conditions. Accumulation chalcon compounds as a result of the degradation of anthocyanins are formed more slowly.

The rate of decrease in the intensity of red color of the jelly drink can be explained with the percentage decline in the value of a * of jelly drinks on a variety of storage conditions, as presented in Table 2. According to Table 2, percentage of the smallest decline of red intensity was jelly drink which stored in refrigerator temperature without light exposure. It shows that anthocyanins are more stable at low temperature storage and not exposed to light (5 °C ± 2 °C).

3.2. Colour Change in Overall (ΔE)

ΔE is an attribute value of the parameter changes of overall color. ΔE average value of the jelly drink can be seen in Table 3. The higher value of ΔE indicates that damage level of sample color was greater [16]. Based on Table 3, the difference in ΔE value at 30-th day between sample in room temperature with exposed to light and refrigerator temperature with exposed to light was 7.59. Meanwhile product which stored at room temperature was not exposed to light and refrigerator temperature was not exposed to light is 4.84. Difference in value of ΔE because of the temperature difference is quite large because the temperature is a major factor in the degradation of anthocyanin where higher temperatures can alter the structure of anthocyanin in the equilibrium reaction of flavium cation form become chalconcolorless. Damage due to temperature can occur in two stages. First, hydrolysis occurs in anthocyanin glycosidic bond resulting unstable aglycones. Second, opening the aglycone ring forming chalconcarbinol [3].The study proves that temperature is more influential than light in the degradation of anthocyanins

Table 3: Overall Color Change (ΔE) During Storage 30 Days.

Days of Storage	Storage at			
	Room temperature, with light exposure	Room temperature, with light exposure	Refrigerator temperature, with light exposure	Refrigerator temperature, without light exposure
0	0.00	0.00	0.00	0.00
5	1.47	1.36	1.28	0.66
10	2.95	3.53	1.57	1.41
15	5.58	3.99	1.65	2.30
20	7.56	5.25	2.33	2.80
25	9.77	6.76	3.15	2.92
30	11.47	7.96	3.88	3.12

3.3. Total Anthocyanin

Data of reduction in total anthocyanin during storage were plotted into zero order and first order (Table 4.) Based on Table 4, the R^2 value on one order was greater than the zero order except R^2 of product which stored at refrigerator temperature with exposed to light. Overall most value of R^2 of one order were larger, so that the reaction one order was chosen as kinetics of decrease of total anthocyanin in jelly drinks which stored in various storage conditions. Based on the Table 4, jelly drink which stored at room temperature with exposed to light hasthe highest slope (0.034). It means that each additional day, the value of ln total anthocyanins decreased by 0.034, while the product which stored at refrigerator temperature without light exposure has the lowest slope (0.020). Room temperature gives a greater influence in lowering levels of anthocyanin in jelly drinks. The higher of storage temperature can cause changing of flavilium cation become chalcon was running quickly, while at refrigerator temperature storage such changes tend to be slower, due to the hydration reaction also progresses slowly so that the structure of flavilium cations of anthocyanin more stable in jelly drinks.

This is consistent with Walford [17], that cold storage conditions in acidic medium, changes of alkaline kuinoidal and alkaline carbinol to form chalcon is running slowly. In addition, the storage with light exposure also contributed to lowering the total anthocyanin. This is due to anthocyanin is generally not resistant to UV rays and visible light because the light is able to trigger reactions that can open the phytochemical anthocyanin rings then formed chalcon colorless compound [9]. The decline percentage of total anthocyanins of jelly drinks on variety of storage conditions are presented in Table 5.

Table 4: Equation Regression and determination coefficient value (R^2) of Total Anthocyanin of Jelly Drink on Zero Order and First Order.

Reaction Order	Storage Condition	Regression Equation	Coef. Of Det. (R^2)
0	Room temperature, with light exposure	y = 43.654 – 0.949x	0.975
	Room temperature, without light exposure	y = 45.439 – 0.938x	0.990
	Refrigerator temperature, with light exposure	y = 45.681 – 0.783x	0.994
	Refrigerator temperature, without light exposure	y = 46.635 – 0.760x	0.972
1	Room temperature, with light exposure	y = 3.838 – 0.034x	0.983
	Room temperature, without light exposure	y = 3.823 – 0.026x	0.996
	Refrigerator temperature, with light exposure	y = 3.836 – 0.021x	0.990
	Refrigerator temperature, without light exposure	y = 3.854 – 0.020x	0.975

Table 5: The decrease percentage of total anthocyanins of jelly drinks on variety of storage conditions.

Storage Condition	Total Anthocyanins at Days		The decrease percentage of total anthocyanins (%)
	0	30	
Room temperature, with light exposure	46.3	15.52	66.50
Room temperature, without light exposure	46.4	16.72	63.96
Refrigerator temperature, with light exposure	46.0	21.41	53.40
Refrigerator temperature, without light exposure	45.6	24.61	46.03

Based on Table 5, the decrease percentage of total anthocyanin was higher than the decrease percentage of red color intensity (a*). This is due to the influence of water holding capacity of gel (carrageenan hydrocolloid. Hydrocolloid has water binding properties and form a three-dimensional structure (double helix). The double helix structure trap water so that the water in the gel is not easily separated. Anthocyanin pigments are water-soluble compounds that participate trapped in the gel and subtle when calculating the total anthocyanin.

3.4. pH

Anthocyanin generally stable in acidic conditions, namely in pH range 2-3 [8]. During the 30 days of storage, the pH value of beverages jelly decreased but did not change significantly (still range below pH 3) so that the determination of the zero-order reaction and the reaction order is not used. Jelly drink pH during storage of 30 days at various storage conditions can be seen in Table 6. The Regression Equation are presented in Tabel7.

Table 6: pH of Jelly Drink During Storage.

Treatment	Days Storage						
	0	5	10	15	20	25	30
A	2.66	2.64	2.64	2.64	2.62	2.62	2.57
B	2.68	2.64	2.63	2.63	2.61	2.62	2.59
C	2.67	2.65	2.63	2.61	2.60	2.59	2.59
D	2.68	2.67	2.64	2.61	2.61	2.61	2.59

Table 7: Regression Equation of Jelly Drink pH During Storage.

Storage Condition	Regression Equation
Room temperature, with light exposure	y = 0.980 – 0.001x
Room temperature, without light exposure	y = 0.983 – 0.001x
Refrigerator temperature, with light exposure	y = 0.978 – 0.001x
Refrigerator temperature, without light exposure	y = 0.985 – 0.001x

Based on Table 7, the value of slope for all treatment are equal to 0.001. It mean that temperature and light on four storage treatment did not significantly affect pH of the jelly drink. Decrease in pH that occur are still within the range of pH suitable for anthocyanin. This condition are able to maintain stability anthocyanin in the form of flavilium cations. According Brouillard [18], at pH 1-3, anthocyanin is dominated by flavilium cation form which is stable form of anthocyanins. Stability of jelly drinks pH in all types of treatment supposedly because of role of citric acid and potassium citrate were added to jelly drink. Citric acid is the acid that is good in maintaining the stability of pH, while potassium citrate is buffer system.

3.5. Estimation of Shelf Life

One factor that can guarantee the quality of the product is to know the shelf life of product. According to the Institute of Food Science Technology [19], the shelf life of food products is the interval between time of production until the moment of consumption where the product is in accordance with the conditions of consumers expectations. Estimation of shelf life may be determined by value of critical parameter (the critical point) color intensity jelly drinks that are no longer acceptable by panelists. The concentration of anthocyanin pigment powder which was added to the jelly drink at the beginning of storage was 15000 ppm. Furthermore, the determination of the critical point at which the hedonic test presented three additional powder concentration of anthocyanin pigments as 3000 ppm, 5000 ppm and 10000 ppm. Based on the results of the hedonic test, the addition of 3000ppm is the concentration that is not accepted by the panelists with red color intensity (a* = 54.90), or the value ln a * = 4. Furthermore, the ln a *value was critical point of the anthocyanin pigments substituted into the

Table 8: The Shelf Life of Jelly Drink Base on Red Color Intensity.

Storage Condition	Regression Equation	Critical Point Ln a* (y)	The Self Life (Days)
room temperature, with light exposure	y = 4.212 – 0.004x	4.00	52
room temperature, without light exposure	y = 4.214 – 0.003x		69
refrigerator temperature, with light exposure	y = 4.198 – 0.002x		96
refrigerator temperature, without light exposure	y = 4.206 – 0.001x		200

regression equation y value in order to obtain shelf life (x). Based on the value of the critical point, the shelf life of jelly drink on variety of storage conditions can be seen in Table 8.

It can be seen in Table 8 that jelly drinks were stored at refrigerator temperature with or without light exposure, has the shelf life that is relatively longer than the jelly drinks were stored at room temperature without exposure or exposure to light. Light is also a factor that can shorten shelf life. According to Rein [20], light can accelerate degradation reactions of anthocyanin. This is evidenced by decreasing in color intensity of light exposure treatment that higher than treatment with light exposure. The best storage conditions to maintain shelf life of jelly drink which added anthocyanin pigments is at refrigerator temperature without exposed to light. In addition, the relatively long shelf life which is supported with an acidic food systems of jelly drink.

4. Conclusion

1. Storage of jelly drinks were added encapsulated anthocyanin pigment from purple sweet potato on refrigerator temperature without light exposure is the most appropriate conditions as it can maintain the stability of total anthocyanins, color intensity, and degree of acidity (pH).
2. Jelly drink which was stored at refrigerator temperatures that was not exposed to light has the smallest decreasing of total anthocyanins (46.03%) and decreasing of red intensity (3.19%) during 30 days storage in which the shelf life spanned to around 200 days based on the color intensity.

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