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Properties and Stability of Anthocyanin Pigments from Roselle in Jelly
สมบัติและความคงตัวของรงควัตถุแอนโทไซยานินจากดอกกระเจี๊ยบแดงในเยลลี่

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บทคัดย่อ

สกัดรงควัตถุแอนโทไซยานินจากดอกกระเจี๊ยบแดง โดยนำดอกกระเจี๊ยบสดต่อน้ำในอัตราส่วน 1:1 (โดยน้ำหนัก) ให้ความร้อนที่อุณหภูมิ 80 องศาเซลเซียส เป็นเวลา 5 นาที กรองแล้วนำไประเหยน้ำด้วยเครื่อง Rotary Evaporator จนมีปริมาณของแข็งที่ละลายน้ำได้เท่ากับ 20 °บริกซ์ นำมาใส่ในเยลลี่ โดยแปรปริมาณสารสกัดจากดอกกระเจี๊ยบเป็น 0.30, 0.40, 0.50 และ 0.60 เปอร์เซ็นต์ (โดยน้ำหนักทั้งหมด) เปรียบเทียบกับการใช้สีแดงสังเคราะห์ ที่ปริมาณ 0.02 และ 0.03 เปอร์เซ็นต์ (โดยน้ำหนักทั้งหมด) เมื่อนำมาวัดค่าสี พบว่า เยลลี่ที่ใช้รงควัตถุจากดอกกระเจี๊ยบแดงและเยลลี่ที่ใช้สีสังเคราะห์ในปริมาณ 0.60 และ 0.03 เปอร์เซ็นต์ ตามลำดับ มีค่าสีไม่แตกต่างกัน ($p>0.05$) และจากการเก็บรักษาเยลลี่ที่อุณหภูมิ 4 องศาเซลเซียส เป็นเวลา 4 สัปดาห์ พบว่า เยลลี่ที่ใช้รงควัตถุจากดอกกระเจี๊ยบแดงมีค่าความคงตัวของสีและความคงตัวของเจลสูงกว่าเยลลี่ที่ใช้สีสังเคราะห์ ทั้งยังมีค่าการยอมรับทางประสาทสัมผัสโดยรวมสูงกว่าเยลลี่ที่ใช้สีสังเคราะห์

คำสำคัญ: ดอกกระเจี๊ยบแดง แอนโทไซยานิน เยลลี่

Abstract

Anthocyanin pigment from fresh roselle (*Hibiscus sabdariffa* Linn.) was extracted with hot water (80 °C) at a ratio of 1:1 (by weight) for 5 minutes. Roselle pigment extract was passed through an hydraulic press, filter press and evaporated with a rotary evaporator until total soluble solid was 20 °Brix. Roselle pigment extract was used as a colorant in pectin jelly at concentrations of 0.30, 0.40, 0.50 and 0.60 percent (by total weight) compared with synthetic red colorants at concentrations of 0.02 and 0.03 percent (by total weight). The results showed that the color characteristics of jelly with 0.60 percent of roselle pigment or 0.03 percent of the synthetic colorants had no significant difference ($p>0.05$). During storing at 4 °C for 4 weeks, the jelly with roselle pigment showed more color retention and gel strength than jelly using the synthetic colorants. The acceptable sensory scores were also significantly higher for roselle than for those using the synthetic colorants.

Keywords: Roselle, Anthocyanins, Jelly

Introduction

Anthocyanins are water-soluble pigments responsible for red, blue, and purple colors in plants (deMan, 1990; Elbe, Schwartz, and Fennema, 1996). Commercial interest in anthocyanins has increased because these food compounds provide both color and health benefits, and for their potential use as natural pigments (Tsuda, 2008; Zhang, et al., 2008). Anthocyanins are unstable during processing and storage, and are generally relatively unstable, with greatest stability occurring under acidic conditions (Simpson, Richardson, and Finley, 1985). Anthocyanins degradation and brown pigment formation causes color loss in food products. Both the hue of the pigment and its stability are greatly impacted by substituents on the aglycone. Degradation of anthocyanins occurs not only during extraction from plant tissue but also during processing. The color and stability of an anthocyanin in solution is highly depended on the pH (Wong, 1989). They are most stable and most highly colored at low pH values and gradually loose color as the pH is increased. At around pH 4 to 5, the anthocyanin is almost colorless. This color loss is reversible, and the red hue will return upon acidification. This characteristic limits the application of anthocyanins as food colorant to products that have low pH.

Anthocyanins from many fruits and vegetables have been extensively studied

and are widely used (Chaovanalikit and Wrolstad, 2004; Jensen, et al., 2008; Lee, Durst, and Wrolstad, 2002; Skrede, Wrolstad, and Durst 2000). They are used as a food coloring, and are so widely distributed in human diets that large amounts of anthocyanins are ingested from plant-based foods, including berries (Espin, et al., 2000; Seeram, 2008). Blueberry juice concentrate has been used as a natural colorant in breakfast cereal (Carmire, et al., 2002). Rossele (*Hibiscus sabdarriffa* Linn.) typically has higher anthocyanin, so the dried Hibiscus flowers have been made a natural product. *Hibiscus sabdarriffa* Linn. has been known as a food, dye and beverage, and is used in many countries for the treatment of a variety of diseases.

The objectives of this study were to evaluate the performance of roselle pigments as a colorant in jelly, compared to a synthetic colorant, and to determine the color characteristics and stability, gel strength and sensory characteristics in jelly.

Materials and Methods

Materials

Synthetic red colorants used were 35% Ponceau 4R (E124), 4% Carmoisine (E122) and 1.8% Tartrazine (E102). Fresh roselle, pectin (grade 150), sugar (superfine sugar, Mitraphol Sugar Co., Thailand) and citric acid were purchased from a supermarket. Fresh roselle was harvested from Saraburi province, Thailand. Upon receipt at the Department of Food Science and Technology, University of the Thai Chamber of Commerce, the roselle was stored at 4 °C before subsequent sample preparation or processing.

Preparation of Roselle Pigments

Anthocyanin pigment was extracted from 1 kg fresh roselle with 1000 ml hot water for 5 min at 80 °C. The roselle extract was passed through an hydraulic press, filtered using a filter press and then evaporated by rotary evaporator until total soluble solid was 20 °Brix.

Preparation of Jelly

Jelly is fruit juice cooked with sugar to form a gel. Some fruits and fruit combinations require added pectin in addition to the natural pectin present in fruit itself. Jelly in this experiment was made with sugar (67.75% w/w), pectin (grade 150) (0.75% w/w) and citric acid (0.05% w/w) by dissolving ingredients in boiling water. Accurate amounts of pigment were incorporated by blending below 80 °C for 5 min, and then pouring into 70 ml plastic cups, sealed and stored at 4 °C until used.

Experimental Design and Storage

Four levels of roselle pigment (0.30, 0.40, 0.50 and 0.60% by total weight) and two levels of synthetic red colorants (0.02 and 0.03% by total weight) were used. Jelly color and gel strength were measured every week for 4 wks at 4 °C.

Instrumental Analysis

Jelly color was measured with a HunterLab digital color difference meter (HunterLab, Model DP-9000, USA). The L* (brightness or whiteness), a* (redness and greenness) and b* (yellowness and blueness) values were determined. Calculation of Chroma [$C = (a^{*2}+b^{*2})^{1/2}$] indicates color purity or saturation (High values are more vivid.). Hue angle ($H^0 = \tan^{-1} b^*/a^*$) indicates sample color. ($H^0 = O^0$ or 360^o = red; 90^o = yellow; 180^o = green; 270^o = blue). ΔE^*_{ab} indicates the total color difference between two samples, calculated as $[(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}$ (Francis, 1985). Color retention (%) for jelly was calculated by the formula: C-value at a storage time x 10^2 / C-value at zero storage time.

Gel strength was measured with Texture Lloyd Analyzer (Lloyd, Model LRX, UK). Texture analysis was performed using a 10-mm-dia cylindrical probe. Gel samples were compressed at 75% of initial height using a compression speed of 100 mm/min. Force from compression is shown by Newton force. Five samples were analyzed for each treatment.

Sensory Evaluation

Jelly products were evaluated using 9-point hedonic scale (1 = extremely dislike, 5 = neither like nor dislike, 9 = extremely like). Fifty-seven panelists (40 female and 17 male) recruited among undergraduate students of the University of the Thai Chamber of Commerce, tested the products, without any knowledge as to the formulation of the products. Jelly samples were evaluated after 24 hrs of storage at 4 °C. Samples were placed on white plates and evaluated for color, odor, appearance, texture, taste and overall acceptability. Each panelist received 2 samples (jelly containing roselle pigment and jelly containing synthetic colorant) coded with three digits. Testing was done in individual sensory booths under fluorescent light.

Statistical Analysis

Data from color characteristics and gel strength value were analyzed using a Completely Randomized Design (CRD), followed by Duncan's New Multiple Range Test to determine significant difference (Cochran and Cox, 1992). A T – Test procedure was used to analyze each sensory attribute comparing jelly containing

roselle pigment and jelly containing synthetic colorant. Significance of differences was defined at $p<0.05$.

Results and Discussion

The roselle pigment extract and synthetic colorants were added to pectin jelly products and the color characteristics, gel strength and acceptable sensory scores of jelly products were compared. Since L* and C values correlated with pigment content, the color change or color retention of pigment could be studied by determining L*, a*, b* and calculated C values for the jelly containing roselle and synthetic colorants (Table 1). The L*, a* and b* attributes increased with increased level of colorants. In jelly that used roselle pigment, the a* attribute increased significantly ($p<0.05$) from 5.43 in samples containing 0.30% roselle pigment, to 18.38 in samples containing 0.60% roselle pigment. The C values increased from 5.50 in samples containing 0.30% roselle pigment to 19.52 in samples containing 0.60% roselle pigment. In jelly containing synthetic colorants, the a* attribute increased from 11.86 in samples containing 0.02% synthetic colorants to 18.68 in samples containing 0.03% synthetic colorants, and the C values increased from 12.13 to 19.73. Hue angle (H^0) of jelly containing 0.60% roselle pigment had the same value ($H^0 = 19$) as hue angle of jelly containing 0.03% synthetic colorants, and showed a shade of red. The report by Cai and Corke (1999) on using red radish anthocyanin as a colorant in jelly, compared favorably with a similar hue angle using roselle pigment. Red radish containing anthocyanins had been reported as a natural pigment with favorable stability (Giusti and Wrolstad, 1996). High values of color attributes were obtained in samples containing 0.60% roselle pigment and 0.03% synthetic colorants; no significant differences between samples were found.

Table 1: Color Characteristics of Jelly with Roselle Pigment and Synthetic Colorant

Colorants	Pigment Level (%)	L*	a*	b*	C	H°
Roselle pigment	0.30	6.67^{c}	5.43^{c}	-0.88^{c}	5.50	350
	0.40	6.74^{bc}	6.60^{c}	-0.12^{c}	6.60	359
	0.50	7.45^{b}	13.19^{b}	3.02^{b}	13.53	13
	0.60	8.12^{a}	18.38^{a}	6.56^{a}	19.52	19
Synthetic colorant*	0.02	6.02^{c}	11.86^{b}	2.55^{b}	12.13	12
	0.03	7.82^{ab}	18.68^{a}	6.34^{a}	19.73	19

* Synthetic colorant (35% Ponceau 4R; 4% Carmoisine and 1.8% Tartrazine)

L* = lightness (0 = black, 100 = white); a* = redness/greenness (+ = red, - = green);

b* = yellowness/blueness (+ = yellow, - = blue)

^{a,b,c} Mean in a column (n = 5) with a different superscript letter is significantly different ($p<0.05$).

Table 2 presents L*, a* and b* values, chroma, hue angle, and total color difference between two samples (jelly containing 0.60% roselle pigment and jelly with 0.03% synthetic colorants) and color retention (%) of both jellies after 4 wks at 4 °C. The results showed that the total color difference (ΔE^*_{ab}) between jelly containing 0.60% roselle pigment and 0.03% synthetic colorants increased from 0.48 to 8.71 after 4 wks at 4 °C. The color retention of jelly containing 0.03% synthetic colorants decreased from 60.97% after 1 wk to 30.51% after 4 wks at 4 °C. Roselle pigment underwent pronounced degradation during storage at 4 °C from 99.74% color retention after 1 wk to 74.90% color retention after 4 wks. Skrede, Wrolstad, and Durst (2000) reported a 4% increase in anthocyanins following pasteurization, indicating that anthocyanins are well retained during pasteurization. The jelly containing 0.03% synthetic colorants showed lower color retention than the jelly containing 0.60% roselle pigment, indicating that roselle pigment extract can be used as colorant in pectin jelly products with accompanying high color stability.

Table 2: Color Stability of Jelly with 0.60% of Roselle Pigment and 0.03% of Synthetic Colorant at 4 °C

Colorants	L*	a*	b*	C	H°	ΔE^*_{ab}	Color Retention (%)
				At zero storage time			
Roselle	8.12	18.38	6.56	19.52	19	0.48	-
Synthetic	7.82	18.68	6.34	19.73	19		-
				At 1 wk storage time			
Roselle	4.93	18.52	6.02	19.47	18	7.59	99.74
Synthetic	3.50	11.51	3.49	12.03	17		60.97
				At 2 wk storage time			
Roselle	6.05	14.47	4.55	15.17	17	8.30	77.72
Synthetic	4.28	6.89	1.68	7.09	14		35.94
				At 3 wk storage time			
Roselle	3.91	14.20	3.75	14.69	15	8.57	75.26
Synthetic	2.33	6.05	1.64	6.27	15		31.78
				At 4 wk storage time			
Roselle	4.90	14.37	2.71	14.62	11	8.71	74.90
Synthetic	3.61	5.97	0.79	6.02	8		30.51

L* = lightness (0 = black, 100 = white); a* = redness/greenness (+ = red, - = green);

b* = yellowness/blueness (+ = yellow, - = blue).

ΔE^*_{ab} calculated relative to synthetic colorant (35% Ponceau 4R; 4% Carmoisine and 1.8% Tartrazine) at the same storage time.

Gel strength for jelly after storage at 4 °C is shown in Table 3. Jelly using roselle pigment lost strength from 0.97 N to 0.47 N after 4 wks at 4 °C, whereas gel strength of jelly containing the red synthetic colorants decreased from 0.80 N to 0.45 N after 4 wks of storage. Roselle pigment was extracted from fresh roselle, so jelly containing 0.60% roselle pigment extract showed higher gel strength than jelly containing 0.03% synthetic colorants.

Table 3: Texture Value of Jelly with 0.60% of Roselle Pigment and 0.03% of Synthetic Colorant at 4 °C

Storage Time (week)	Gel Strength of Jelly (N)	
	Roselle Pigment	Synthetic Colorant*
0	0.97[a]	0.80[a]
1	0.78[b]	0.65[ab]
2	0.68[bc]	0.53[b]
3	0.59[cd]	0.50[b]
4	0.47[d]	0.45[c]

* Synthetic colorant (35% Ponceau 4R; 4% Carmoisine and 1.8% Tartrazine).

[a,b,c,d] Mean in a column (n = 5) with a different superscript letter is significantly different ($p<0.05$).

Table 4 presents the average acceptability of the products tested. Mean color and appearance scores from the examining panel's evaluation were not significantly different for jelly containing 0.60% roselle pigment and 0.03% synthetic colorants. The acceptable sensory scores of jelly containing roselle pigment in odor, texture, taste and overall acceptability were significantly higher than jelly containing the synthetic colorants. The results indicated that roselle pigment extract can be used in jelly products with a higher overall acceptability score than synthetic colorants.

Table 4: Sensory Scores of Jelly with 0.60% of Roselle Pigment and 0.03% of Synthetic Colorant

Colorants	Sensory Scores					
	Color	Odor	Appearance	Texture	Taste	Overall Acceptability
Roselle Pigment	7.60[a]	7.73[a]	6.86[a]	7.06[a]	7.06[a]	7.73[a]
Synthetic Colorant*	7.53[a]	6.67[b]	6.73[a]	6.40[b]	6.46[b]	6.73[b]

* Synthetic colorant (35% Ponceau 4R; 4% Carmoisine and 1.8% Tartrazine).

Sensory scores based on 9-point hedonic scale (9 = extremely like, 1 = extremely dislike).

[a,b] Mean in a column with a different superscript letter is significantly different ($p<0.05$).

Conclusions

Results obtained indicate that 0.60% roselle pigment extract can be used in jelly products and has color retention and gel strength higher than synthetic colorants at level 0.03% (by weight of jelly). The jelly colored with roselle pigment showed a higher sensory score in odor, texture, taste and overall acceptability than jelly using the synthetic colorants. Fresh roselle (*Hibiscus sabdarriffa* Linn.), rich in anthocyanin pigment, can be used as a natural colorant in jelly products.

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