Homogenization and Microwave Irradiation Enhancements of Betacyanin Extraction from Pitahaya Peel and Its Stability

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***Abstract*—The aims of this research are to enhance extraction of betacyanin from pitahaya (dragon fruit - Hylocereus undatus) peel by using homogenization and microwave techniques and assessed pigment stability under the different pH and heat treatments. We assessed enhanced extraction with homogenization in distilled water at 2500 rpm at times of up to 20 minutes and 100W microwave power with times up to 30 seconds. After extraction, the stability test under the different pH of 3-7 and heat treatment levels (without heat treatment, pasteurization and sterilization) was also investigated. The homogenized extract after 20 minutes and microwave powered extract for 30 seconds led to the highest red color (measured by a* value) and betacyanin content when compared with each other processing time. The extracted pigment without heat treatment had higher stability than pasteurized and sterilized extract in the pH 4-6 range.**

***Index Terms*—extraction enhancement, pasteurization, pH, sterilization, colorant, betacyanin content**

I. Introduction

At present, consumers are increasingly concerned with the risk associated with synthetic colorants in foods. Thus replacement of the synthetic colorants with natural colorants has become important component of food ingredients, but they are less stable and more expensive. They are added to make products more appealing to consumers or to hide product defects. Natural colorants are usually extracted from several parts of plants, for example, flowers, leaves, fruits, roots and tubers. Natural compounds used as colorants, namely, carotenoids, anthocyanins, betanin and chlorophylls have attracted significant attention in recent years [1].

Betalains are found in beetroot, amaranth, basella alba, ulluco and pitahaya. They are composed of red-violet betacyanins and yellow-orange betaxanthins and are added to several low acidity foods [2].

Pitahaya (dragon fruit - Hylocereus undatus) is a fruit in the cactus family. In Northeastern Thailand, it is popular for the edible juicy white flesh and tiny black seeds which has many health-benefits, containing plentiful antioxidant pigments, high fiber and few calories. Waste pitahaya peel has potential as a natural colorant that may help prevent chronic disease [3]. Betalains are water–soluble pigments, thus pure water can extract more pigments than ethanol - water mixtures [4]. Microwave irradiation has been reported to increase betalain extraction efficiency from red beet and a short time cooling after the first irradiation delayed betalain degradation in the second irradiation [5]. The microwave assisted water extraction of pitahaya at 35^{o}C, for a 20g sample and 8 min treatment time, the betalain content was around 9 mg L-1 [6]. Betalains are stable in a wide pH range 3-7, but out of this range the visible absorption intensity decreases sharply [7]. In addition, the stability of betanin pigment found in 5-6 pH range [8]. Storage at 4^{o}C and pH 4 in the dark retained 80% of the anthocyanin pigment from pitahaya peel after 4 days [9]. Similar to the betacyanin, it retained the pigment when 0.25% ascorbic acid was added to red pitahaya juice and stored in the dark at 4^{o}C and pH 5-6 [10]-[12].

We studied enhancement of betacyanin extraction from pitahaya peel using two processes - homogenization and microwave - to assist water extraction. The extract color and betacyanin content was evaluated under different pH and thermal conditions.

II. Materitals and Methods

A. Chemicals

Analytical grade hydrochloric acid (HCl) 1 N, sodium hydroxide (NaOH) 1 N, citrate-phosphate buffer pH 5 and distilled water were used.

B. Sample Preparation

Good quality ripe pitahaya with white flesh and red skin, was purchased from a supermarket in Maha Sarakham (16.2026^{o}N 103.2771^{o}E) cultivated in Northeastern Thailand. Firstly, pitahaya was cut into twice cross sectional pieces and then manually separated the skin and edible portion. The peel was washed in clean water, chopped into small cubes, dried at 50oC for a day using hot air oven and ground. Sample powder which passed 1.02 mm apertures of a number 20 stainless steel mesh was sealed in a zip lock aluminium foil bag before use.

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C. Extraction

Sample powder was stirred with distilled water (5g in 100ml) in a 500 ml beaker and then left for 1 day (conventional extraction). Two methods of assisted extraction were evaluated - homogenization and microwave irradiation: - Again, 5g samples were stirred in 100 ml distilled water. The homogenizer was operated at 2500 rpm for 5-20 minutes. The microwave oven ran at 100 W for 5-30 seconds. The microwave irradiation used the short time to avoid betanin decomposition and consequent color change [13]. Then, the solution was filtered through a straining cloth and a number 100 fine screen mesh (0.198 mm aperture) leading to a clear red-violet solution. Triplicate samples were measured for color ($L^*a^*b^*$ color space) and betacyanin content and weighed the aqueous extract to calculate the yield.

$$Yield\ \% = (w_{ex}) / (w_{samp} + w_{solv})\ \text{x}100 \quad (1)$$

where w_{ex}, w_{samp} and w_{solv} are aqueous extract weight, sample weight and solvent weight, respectively.

D. Determination of Color and Betacyanin Content

A ColorFlex EZ spectrophotometer (Hunter Lab, VA) measured the color. L^* 0 (black) -100 (white), a^*(positive value or negative value mean reddish or greenish) and b^*(positive value or negative value mean yellowish or bluish) were recorded.

The betacyanin concentration was measured by absorbance using a UV-visible spectrophotometer (UV-1800 - SHIMADZU) at 538 nm. Betacyanin content (BC in mg/litre unit) calculated following [14]:

$$BC = (Abs \text{ x } D \text{ x } MW \text{ x } 1{,}000) / (\varepsilon \text{ x } L) \quad (2)$$

where *Abs* is the absorbance at λ = 538 nm, *D* is the dilution factor, *MW* and ε are the molecular weight and molar extinction coefficient (550 g/mol and 60,000 L/(mol cm) in H_2O) and *L* is cuvette thickness (1 cm).

E. Stability of Betacyanin in the Extract

The aqueous extract from homogenization and microwave enhancement was adjusted to pH values at steps of 1 in the range pH 3 to pH 7 with 1N HCl and NaOH using a 1.2:100 (v/v) ratio of aqueous extract to buffer solution for each pH. For each pH value, three levels of thermal treatment were used: without heat treatment, pasteurization (62^{o}C for 30 minutes) and sterilization (121^{o}C for 15 minutes). The color triples (L^*, a^*, b^*) were recorded. Hue angle (h^o) and chroma (C^*) for other color spaces were also recorded. Total color change (ΔE^*) was calculated from:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (3)$$

Betacyanin content was determined again from absorbance (equation 2) after pH adjustment and thermal treatment. Each experiment was duplicated and each sample was measured in triplicate.

F. Statistical Analysis

Data were analyzed by full factorial analysis of variance (ANOVA) and compared the differences of means by Duncan's new multiple range test at $p \leq 0.05$ [15].

III. Results and Discussion

A. Homogenization and Microwave Enhancements with Water Extraction

In this section, the optimum processing time for each method was assumed to be the highest betacyanin content prior to the stability test. Fig. 1 shows color coordinates (L^*, $a^*$and b^*) versus homogenization times. Increased processing time led to significant L^* increase in the first 15 minutes and then remained constant for the next 5 minutes. Conversely, b^* decreased significantly in the first 15 minutes and increased for the next 5 minutes whereas a^* increased significantly throughout the 20 minutes of homogenization. For microwave irradiation, L^* increased consistently with microwave irradiation time and b^* decreased significantly (see Fig. 2). However, both L^* and b^* showed only insignificant differences after then first 15 seconds of irradiation. a^* increased significantly in the first 15 seconds, then increased more slowly and seemed constant. The aqueous extract for both homogenization and microwave irradiation became more red-violet in color with processing time. Extracted colors for the three methods -conventional, homogenization and microwave - are compared in Fig. 3. Visually, the microwave extract look lighter and clearer than those of conventional and homogenized extracts. Because microwave extract uses shorter time and low irradiation power but the other methods have longer contact times. These corresponded to the reddish (a^*) in each method, the descending of reddish start from homogenized extract at 20 minutes, conventional extract left a day (not shown) and microwave extract at 30 seconds, respectively.

Microwave irradiation at all tested times resulted in the highest yields, whereas the conventional method led to the lowest yield (see Table I). The higher yield with microwave irradiation was due to the short processing time. With conventional and homogenization methods, most of the water was adsorbed into the sample cells and made them appear like jelly. Although, we tried to press the extracted solution from the sample, it was retained in the sample. Hence, the microwave yield was twice as high as that from other methods. We noted that the longer the contact time between sample and water, the more the sample cells became saturated and the redder the extract became.

Betacyanin content (BC) for both homogenizing and microwave methods increased with processing time. The highest and lowest BC could be obtained from 20 minutes of homogenization and 10 seconds of microwave. The BC of microwave for all levels of irradiation time were significantly lower than the conventional method but the BC for homogenization for over than 10 minutes was significantly higher. Therefore, the optimum processing times were 20 minutes for 2500 rpm homogenization and 30 seconds for 100 W microwave irradiation. These conditions were used to test the colorant stability in the next section. In addition, microwave at our mild

conditions led to a BC similar to that reported by Thirugnanasambandham and Sivakumar [6].

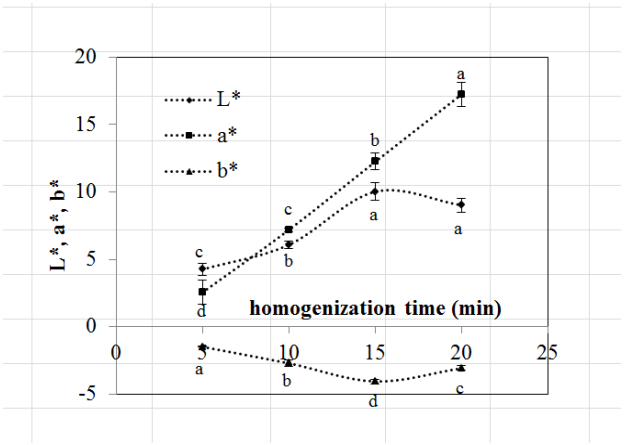


Figure 1. L*,a* and b* coordinates *vs* homogenization time.

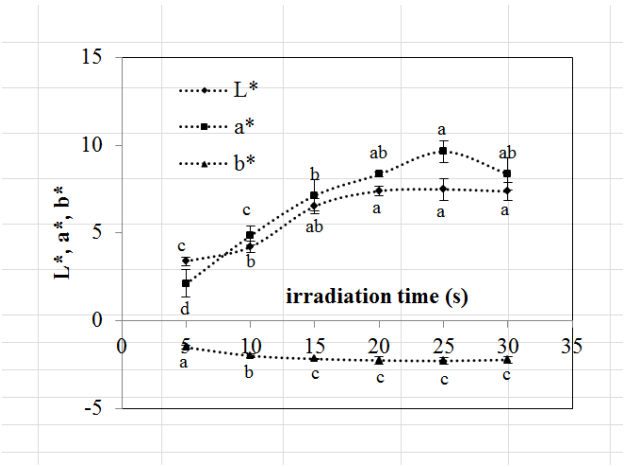


Figure 2. L*,a* and b* coordinates *vs* microwave irradiation time.

Figure 3. Extract colors after 20 min homogenization, left for a day (conventional method) and 30 sec microwave irradiation.

TABLE I. EFFECT OF EXTRACTION METHOD AND PROCESSING TIME ON YIELD AND BETACYANIN CONTENT

Extraction method	Processing time	Yield (%)	Betacyanin content (mg L^{-1})
Conventional	1 day	24 ± 2^{d}	30 ± 6^{b}
Homogenization	5 min	30 ± 3^{c}	11 ± 6^{cd}
	10 min	34 ± 1^{b}	30 ± 1^{b}
	20 min	28 ± 2^{c}	49 ± 2^{a}
Microwave	10 sec	53 ± 2^{a}	8 ± 1^{d}
	20 sec	53 ± 4^{a}	14 ± 4^{c}
	30 sec	50 ± 6^{a}	17 ± 2^{c}

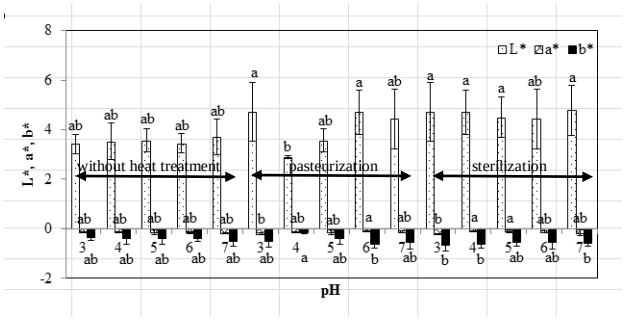
Note: Values are the mean ± standard deviation. Different superscripts (a, b and c) in the same column imply that the values are significant different ($p \leq 0.05$)

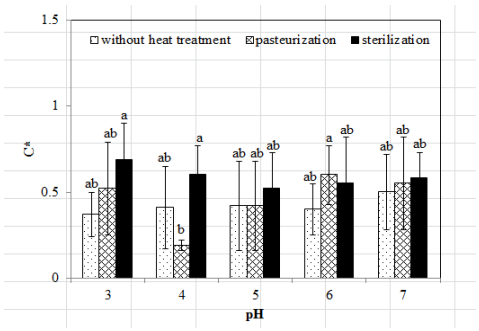
B. *Stability under Different pH and Heat Treatments*

Homogenized colorant extracts under different pH without heat treatment, were stable at all pH levels. This was consistent with the results of Jackman and Smith [7]. Over the pH 3 to 7 range, BC, L^*, a^*, b^*, C^*, ho and ΔE^* values were insignificantly affected - see Figs. 4a-e. With increasing levels of pasteurization, the BC was stable in the pH 4 to 6 range, but, it differed insignificantly at all pH levels. An increase of pH tended to a minor change of L^*, a^*, b^*, C^*, h^o and ΔE^* values. The later sterilization heat treatment, the BC was unstable in all levels of pH. Consequently, the BC, L^*, a^*, b^*, C^*, h^o and ΔE^* were insignificantly different for the whole pH range except a^* at pH 3.

Colorant after microwave extraction was tested for stability under different pH and heat treatments. A significant change in the a^*, b^* and ho resulted from pH change without heat treatment but the other parameters was not significantly changed - see Figs. 5a-e. With increasing levels of pasteurization, the BC was stable in the low acid to neutral range (pH 4 to7), but it was not stable in at high acidity (pH 3). An increase of pH led to a slight change of b* and ho values, but the remaining parameters was not affected. After increasing the heat treatment up to sterilization, the BC was unstable at pH 3 and 4. However, a^*, b^*, C^* and ho were significantly decreased at pH 6.

The homogenized extract was more stable than that the microwave extract because the thermal irradiation assisted degradation whereas mechanical agitation caused negligible degradation. Therefore, the microwave extract was also unstable during heat treatment stability test. For the stability of both homogenized and microwave extracts, the heat treatment level (temperature) was a key factor which mostly affected hue and BC. However, pH has a little effect on the color coordinates and BC. These results are consistent with measurements of color stability of ulluco tuber extract [16].





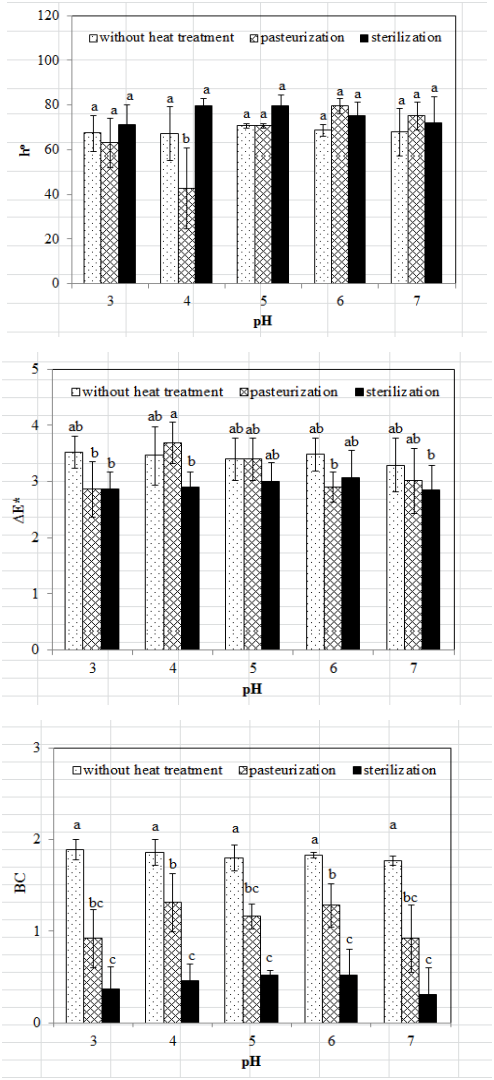
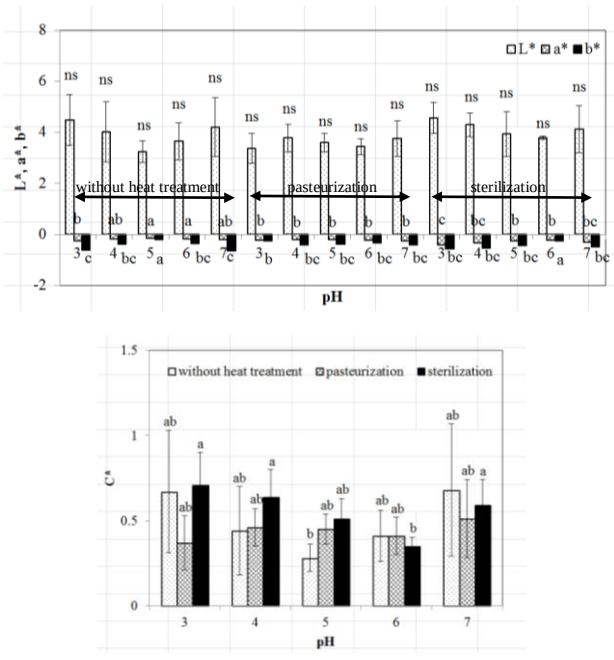


Figure 4. Stability of homogenized extract under different pH and levels of heat treatment (a) L* a* and b*, (b) C*, (c) h°, (d) ΔE* and (e) BC.



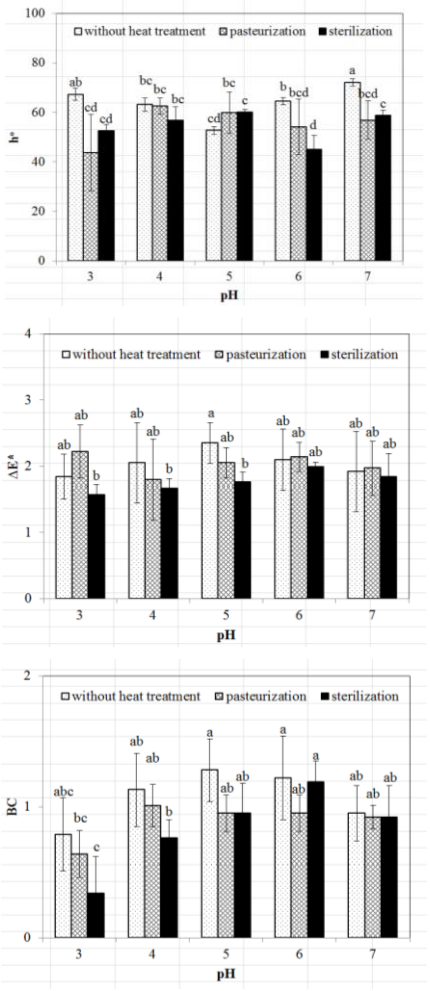


Figure 5. Stability of microwave extract under different pH and levels of heat treatment (a) L* a* and b*, (b) C*, (c) h°, (d) ΔE* and (e) BC.

IV. Conclusions

Pitahaya peel is a potential source of colorant, containing from 8 to 49 mgL^{-1} of betacyanin. The highest BC derived from 20 minutes homogenization assisted water extraction. Exposure to a 100 W microwave power for 30 seconds led to the lowest BC. The BC was increased with processing time but the yield tended in the opposite direction. The BC in pitahaya peel correlated with the a^* value increases with 'redder' samples. A major factor affecting BC and extract color was the level of heat treatment, while pH was a minor factor. All heat treatment levels changed the extract color from reddish to yellowish in the high acid (pH 3) region and reddish to pink in the low acid (pH 4-6). The BC stability increased in order from least with sterilization, then pasteurization and highest without heat treatment.

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