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Physicochemical Properties and Stability of Microencapsulated Betacyanin Pigments from Red Dragon Fruit Peels and Flesh

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Abstract

Dragon fruit (*Hylocereus polyrhizus*) is known for its purple-coloured peels and pulp, which can be attributed to the presence of betalains. In this study, the potential of red dragon fruit as a source of natural colorant was investigated. Betacyanins were extracted from red dragon fruit peels and flesh in 1:3 ratio with water. Microencapsulation by spray-drying was done by adding 5% and 10% (w/v) maltodextrin (DE 11.8) to peels and flesh extracts, respectively. The spray-dried colorant powders all obtained <10% moisture content, 5.261-6.409 g/100g hygroscopic moisture content, and 5.317-7.349(mg/100L) betacyanin content. Morphological characterization revealed spherical, agglomerated particles with visible cracks on the surface. The stability study conducted showed that pigment retention was lowest at 70°C and highest at 4°C.

Keywords: *Hylocereus polyrhizus*; Red dragon fruit; Betacyanin; Microencapsulation; Physicochemical properties

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Introduction

Synthetic colorants, despite having lower production cost and greater stability, have raised several health and ecological concerns [1-3]. Concerns have also been raised about the deleterious effects associated with artificial food dyes [4-6]. Products containing natural ingredients are generally perceived by

the consumers to be of better quality, safer and healthier than those with synthetic compounds [7]. Recent trends show that consumers have been choosing products with natural colors due to their belief that these are healthier and safer, that is why there has been a growing interest in the development of natural colorants to replace synthetic colorants [8].

Anthocyanins are commonly used as natural colorants [9]. However, betalains are interesting alternative as they are more hydrophilic and have a higher tinctorial strength [10]. Thus, they are commonly used in the food industry as a source of natural red color. Betalains are water-soluble, nitrogen-containing natural pigments, which can be divided into two groups: red-violet betacyanins and yellow-orange betaxanthins [11]. The basic structure of betalains is betalamic acid linked to the molecule of cyclo-3,4-dihydroxyphenylalalanine (cyclo-DOPA) for betacyanins, and to the molecule of amino acid or amine for betaxanthins [10]. One of the common sources of betalain compounds is *Hylocereus polyrhizus* or red dragon fruit.

Dragon fruit or pitaya is one of the tropical fruits under the cactus family, *Cactaceae*. In general, there are two species of dragon fruits commonly found in the Philippine market, i.e. red dragon fruit (*Hylocereus polyrhizus*) and white dragon fruit (*Hylocereus undatus*). *H. polyrhizus*, widely known for its sweet taste and deep purple coloured peels and pulp, has been identified as a promising natural source of colorants [12].

In general, production and application of natural pigments encounter several problems, such as the use of large amount of raw materials, higher concentration to produce desired color, and lower stability [13-14]. Factors such as temperature, pH, water activity, light, and the presence of metal cations, greatly affect the stability of betalain pigments, subsequently leading to a loss in color and functional properties [10]. Microencapsulation is a process wherein a core particle is trapped in a carrier material to isolate it from an external environment which may affect it. Microencapsulation by spray-drying has been widely used due to its low-cost, flexibility, continuous production, and easy industrialization [15-18]. It involves dispersing of the core materials in a polymer solution and atomizing in a hot chamber to produce a powder with good quality and low water activity. Due

to its dried particulate form, the pigment can be transported and stored more easily [19-20].

Therefore, this study aims to microencapsulate red dragon fruit betacyanin pigments through spray-drying and establish the physicochemical properties of the colorant powder produced. It also aims to investigate the effects of different treatments on its stability. Knowing how factors such as pH, light, and temperature influence the colorant would be useful in its several applications in the industry.

Materials and Methods

Materials

The red dragon fruit used in this study was purchased from Silang, Cavite, Philippines. Maltodextrin (11.8 DE) and ascorbic acid (USP) were procured from Allyson's Chemical Enterprises (Quezon City, Philippines). Analytical grade hydrochloric acid (Sigma-Aldrich, USA) and sodium hydroxide pellets (Sigma-Aldrich, USA) were purchased from Belman Laboratories (Quezon City, Philippines).

Plant Processing and Storage

Freshly sourced dragon fruits were washed well to remove dirt. Prior to the experiment, the plant materials were stored at -50°C for preservation. The peels were separated from the flesh and then both were cut into smaller, uniform pieces.

Extraction of betalain from red dragon fruit peels and flesh

The pulps were subjected to liquid-solid extraction using deionized water (1:3) while the pulp were homogenized with water (1:3) using a laboratory waring blender. Both mixtures were extracted overnight at 4°C with periodic agitation, then separately filtered using cheesecloth to remove the other plant materials present in the extract.

Microencapsulation of betalain

Microencapsulation was done by completely dissolving maltodextrin (11.8 DE) at 5% and 10% (w/v) concentrations in the dragon fruit peels and pulp extracts, respectively. The microencapsulation process was carried out using a spray-dryer with the following parameters: 150 °C inlet temperature, 15 mL/min flow rate, and 2kg/cm³ air-flow pressure.

Physicochemical properties of spray-dried powder

Determination of moisture content

The moisture content was measured based on the Association of Official Analytical Chemists (AOAC) method. One gram of the microencapsulated powder was weighed in the pan and the temperature of Moisture Balance MOC-120 H was set to 105°C. The procedure was done in triplicate.

Color analysis

Quantification of color was done using Lovibond LC 100 Spectrocolorimeter calibrated with a white standard plate. The powder was contained in an optical cell and the L*, a*, and b* values were measured in triplicate.

Determination of betacyanin content

The betacyanin content of the microencapsulated powder was determined spectrophotometrically according to the method of Lim *et al* [21]. Approximately 0.2 g of the colorant powder was dissolved in deionized water and diluted in a 25 mL volumetric flask. The absorbance was read at 536 nm using L7 Double beam UV-Vis spectrophotometer and the betacyanin content was calculated using the formula:

$$\text{Betacyanin Concentration } \left(\frac{\text{mg}}{100\text{L}} \right) = \frac{A \times DF \times MW \times 1000}{\epsilon \times l}$$

wherein A = Absorbance (λ 538 nm), DF = Dilution factor, MW = Molecular weight of betacyanin (550 g/mol), ϵ = Molar extinction coefficients (60,000 L/mol cm), and l = Path length of cuvette (1.0 cm).

Hygroscopicity

The hygroscopicity of the microencapsulated powder was determined according to Cai and Corke [22]. One gram of the powder was weighed and stored at room temperature in a dessicator with saturated NaCl solution (67% RH). The weight of the sample was obtained after a week and the hygroscopicity was expressed as gram of adsorbed moisture (g/100 g) dry solids.

Particle Size Distribution

The particle size distribution analysis was conducted by UPLB Nanotech Lab. The samples were suspended in HPLC grade water and then subjected to Malvern Zetasizer NanoSeries Nano-ZS90. Particle size analysis was done using dynamic light scattering technique in automatic mode. Average particle size was determined through three measurements at 90°.

Morphological Characterization of the Microencapsulated Powder

The morphology of the microencapsulated powders was viewed using a scanning electron microscope (Phenom X, ThermoFisher Scientific). The powder samples were mounted on separate pin stubs and then applied with a thin layer of gold through sputter coating technique using a fine coater (JFC-1200 Fine Coater, Jeol). The average size of 100 particles in the image was obtained using ImageJ software.

Stability of the Pigment

The microencapsulated pigments from dragon fruit peels and pulps were subjected to different treatments. The powder was dissolved in different solutions and subsequently stored at various conditions. The absorbance was read at 536 nm at appropriate times. The pigment retention is expressed as

$$\text{Pigment Retention (\%)} = \frac{\text{Final betacyanin concentration}}{\text{Initial betacyanin concentration}}$$

Statistical Analysis

All measurements were done in triplicate ($n=3$). Data analysis was carried out using SPSS version 15 and Microsoft Excel 2013.

Table 1: Physicochemical properties of colorant powder from red dragon fruit extracts, spray-dried at 150 °C.

Powder		Moisture Content	Hygroscopicity (g/100g)	Betacyanin Content (mg/100L)
Peels (5% MD)	Batch 1	5.228	6.063	5.317
	Batch 2	4.847	5.261	5.281
Pulps (10% MD)	Batch 1	4.95	6.497	7.349
	Batch 2	4.7567	5.466	6.595

The hygroscopicity of the colorant powders ranged from 5.466 – 6.497 (g/100 g). Hygroscopicity refers to the ability of a material to take up water vapor from the atmosphere at constant temperature [24]. Addition of maltodextrin has been known to help decrease the hygroscopicity by absorbing water, thus forming a moisture-protective barrier on the surface of encapsulated material [25].

Betaxanthin is not present in red dragon fruit, making betacyanin responsible for its color [12]. Addition of higher maltodextrin concentration produced spray-dried powders with higher betacyanin content. Increase of solids content in the feed promotes bioactive retention, due to the ability of maltodextrin to reduce oxygen permeability of the wall matrix [26].

Results

Microencapsulation of Betacyanin Pigment

The physicochemical properties of the colorant powders can be seen in Table 1. All colorant powders obtained a moisture content of <10%, making it safe against microbial growth [23]. In comparison to feed with 10% (w/v) maltodextrin, the 5% (w/v) maltodextrin concentration produced colorant powders with higher moisture content compared to that of 10% maltodextrin.

Color was evaluated using Hunter 'L' (lightness), 'a' ('+a', redness and '-a', greenness), and 'b' ('+b', yellowness and '-b', blueness). Table 2 shows the L, a, b values of the colorant powders during week 0 and week 5. All powders had a '+a' value and '-b' value. Addition of 5% (w/v) maltodextrin to the extract produced colorant powders with higher 'L' and 'b' values, and lower 'a' values compared to the addition of 10% (w/v) maltodextrin. This agrees with the finding that L value is inversely proportional to the betacyanin content [27].

Powder		Week 0			Week 5			(ΔE)
		L	A	B	L	a	b	
Peels	Batch 1	66.8	21.9	-8.8	67.9	21.5	-7.4	0.8062
(5% MD)	Batch 2	66.1	26	-9.7	67.3	26.4	-9.1	0.6
Pulps	Batch 1	62.5	32.5	-18.9	63.5	34.3	-20.7	1.8
(10% MD)	Batch 2	64.4	30.3	-17.7	64	33.3	-19.9	2.2

Particle Size Distribution

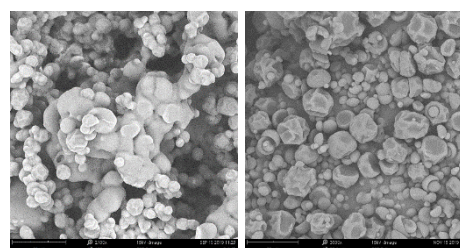
Particle size distribution affects the physical properties of the powders such as bulk density, angle of repose, flowability, rehydration capacity, solubility, and dispersibility [28]. The z-averages (d.nm) and PDI values can be seen in Table 3. The Z-average of pigments encapsulated with 10% w/v maltodextrin was higher with 921.1 ± 64.02 , while that of 5% (w/v) maltodextrin was 169.7 ± 21.33 . These results were consistent with previous findings that particle size increases with feed concentration. Other factors that affect the particle size include parameters in the formulation and spray-drying process such as droplet size and solubility of solute [29-30].

Table 3: Particle size distribution of red dragon fruit peels and flesh.				
	Z-average (d. nm)	RSD	PdI	RSD
Peels (5% w/v MD)	169.7 ± 21.33	12.60%	0.801 ± 0.092	11.40%
Flesh (10% w/v MD)	921.1 ± 64.02	6.95%	1.000 ± 0.000	0.00%

Morphology of microencapsulates

The microencapsulated betacyanin pigments are shown in Figure 1. The image reveals spherical, agglomerated particles with visible

cracks on the surface. Shrinking of the particles during drying and cooling caused the outer surface of the particles to dent [31]. Imperfections on the surface of the microparticles such as irregular shapes, cracks, and denting form due to the delay of the film formation process during the drying process [32].



a) Peels b) Pulps

Figure 1: Micrographs of microencapsulated betacyanin pigments of dragon fruit a.) peels and b.) Pulps

Stability of pigment

Table 4 shows the effect of different treatments and storage conditions in the betacyanin pigment retention of the spray-dried powders.

Effect of pH

In both samples, a huge difference in the color and betacyanin content in the acidic and basic medium has been observed. Both Treatment B solutions exhibited a yellow-brown color, in contrast to the pink solutions of Treatment A. Betacyanin is unstable in alkaline conditions because it undergoes dehydrogenation, producing a yellow betalamic acid and colorless

cyclo-Dopa 5-O- β -glucoside. This was evident in the hypochromic shift. On the other hand, acidic conditions allow for the regeneration of betacyanin through the recondensation of betalamic acid with cyclo-dopa 5-O- β -glucoside [33].

Table 4: Pigment retention of colorant powders in different treatments and storage conditions.

Treatment	PIGMENT RETENTION	
	PEELS	FLESH
Acidic (A)	52.38829	76.0274
Basic (B)	29.0545	29.01371
Oxidation	19.15655	23.29492
Light	61.96318	72.24462
Dark	76.22517	79.75032
4°C	93.42677	99.0339
50°C	59.8218	58.30059
70°C	17.88107	18.35831

Effect of light

Higher pigment retention was observed upon storage of the colorant powders in the dark. Betalains have been reported to have higher degradation rate under exposure to light. The mechanism proposed that the electrons of the betalain chromophore are excited to a more energetic state upon absorption of UV light. This results to an increase in the reactivity of the molecule and subsequently, a decrease in its activation energy [34].

Effect of temperature

Highest pigment retention was observed in 4°C, while the lowest was observed at 70°C. Temperature is said to be the most crucial factor on the stability of betalain pigments. It is said that betalain stability considerably declines within the temperature range of 50-80°C [35-36]. Thermal degradation of betacyanin in purple pitaya juices follows first-order reaction kinetics, and it is also a function of temperature and heating period. Upon thermal treatment, decarboxylation and dehydrogenation of

betanin occurs. This hypochromic shift from 538 nm to 505 nm results to an orange-red appearance and yellow tint [10,37,38].

Conclusion

Microencapsulation of betacyanin pigments from red dragon fruit peels and flesh produced powders with good color, moisture content, and hygroscopic values. The stability of microencapsulated betacyanin pigments from red dragon fruit peels and flesh is greatly affected by pH, light, and temperature. Results show that betacyanin content of colorant powders stored at 70°C decreased by 82-83%, while they were most stable at 4°C. Because of these results, red dragon fruit may further be developed as a source of natural colorant.

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