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Effect of conventional and ultrasonic-assisted extracts on betacyanin content of red dragon fruit (*Hylocereus polyrhizus*)

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Abstract

The red dragon fruit (*Hylocereus polyrhizus*), an available and popular agricultural source in Vietnam, is potential material for food colourants because of the presence of betacyanin. For the purpose of using dragon fruit extract to add colour to processed foods, the study on the effects of extraction methods (conventional and ultrasound-assisted) on the flesh and peel of the fruit was carried out using water as an extraction solvent. The obtained results showed that using ultrasonic waves in extraction enhanced the total betacyanin content in red dragon flesh and peel extract. Multiple regression analysis for betacyanin and the independent variable was obtained with a high coefficient determination (R^2>0.8). The highest total betacyanin content predicted by the models were 27.49 mg/100 mL and 22.28 mg/100 mL corresponding to extracted at a ratio of 83.74% red dragon flesh for 13.15 mins and the ratio of peel to the water of 90.63:9.37 for 12.91 mins. The obtained result could be applied to the food industry to use the achieved as a natural food colour for further processing.

1. Introduction

Food colour is one of the factors that strongly influence the sensory value, especially the attraction of consumers. In recent years, consumers are increasingly concerned about the safety of synthetic pigments. Industrial production of natural colours is gaining more interest (Azeredo, 2009). The extractions of colours from natural fruits, plants, and flowers as well as the application of the natural colours to the food industry are a new area of research in the world. These materials are rich sources of natural pigments worldwide, with thousands of different species able to produce attractive and applicable colours such as blue from butterfly pea flowers, and red from roselle calyx (Susanti *et al.*, 2020). Moreover, natural pigments were obtained from natural materials by solvent extraction (Sajilata and Singhal, 2006). Food and Drug Administration (FDA) assigned an "exempt colour additives" label to specify that colourings are free from the certification process (Meggos, 1995). One of the potential raw materials is the red dragon fruit (*Hylocereus polyrhizus*), an available and popular agricultural source in Vietnam. Red dragon fruit has high moisture content and is rich in fibre, organic acids, protein, phosphorus, vitamin C and other minerals such as potassium, magnesium, and calcium (Delgado-Vargas *et al.*, 2000). In particular, the study of Rebecca *et al.* (2010) published that at least seven betacyanins in this material were found, which contribute a deep purple-red colour to the flesh and peel. Betacyanins from red dragon fruit are potential colourants and antioxidants, which can be applied to many food products (Castellar *et al.*, 2003). Rodriguez *et al.* (2015) reported that the skin of the red dragon fruit contained sustainable amounts of the same health-promoting betacyanins as found in the edible part of the fruit, were 30.18±3.01 mg/100 g of fresh peel and 42.71±2.48 mg/100 g of fresh flesh. Betacyanins extracted from red dragon peel can be applied to beauty and health products as a natural red-purple colourant and antioxidant. The utilization of betacyanins in fruit peel contributes to the reduction of food wastage. Utilizing the flesh and skin of the fruit not only improves the economic value of the fruit but also reduces environmental pollution and a sustainable economy. The high molecular absorption index of betacyanins and their colouring ability is comparable to synthetic colourants (Strack *et al*., 2003).

Betalains are water-soluble compounds that are nitrogen-containing pigments (Khan and Giridhar, 2015). In terms of chemical properties and structure, betacyanin and anthocyanin are related (Naderi *et al*., 2012). Therefore, anthocyanin extraction methods can be applied to betacyanin (Sajilata and Singhal, 2006), however, betacyanins are more hydrophilic. Specifically,

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they are soluble in three common polar solvents (water, methanol, ethanol and their mixtures). In recent years, the safety and convenience, as well as the reasonable economy of the solvents used in the extraction process have received more and more attention, especially in green extraction. Therefore, the choice of solvent is a necessity. The disadvantage of using methanol and ethanol showed that after extraction, another system is needed to remove the solvent from the solution. Therefore, aqueous solvents are not only safe but also convenient in extracting betacyanin from red flesh dragon fruit. Furthermore, using traditional techniques in combination with modern technology such as ultrasonic-assisted extraction can improve extraction efficiency (Ramli *et al.,* 2014). Comparative studies between conventional and ultrasonic-assisted extraction were performed because the conventional method is convenient while ultrasonic-assisted extraction is effective in terms of commercially economy (Ramli *et al.*, 2014). Therefore, the objective of this study was to determine and compare total betacyanin contents in the peel and flesh of red dragon fruit using two technical extractions (conventional extraction and ultrasound-assisted extraction) with/without water addition.

2. Materials and methods

2.1 Sample preparation

Mature red dragon fruits were harvested 42 days from flowering, which was provided by Biochemistry-Plant Physiology Department, Can Tho University, Vietnam. Red dragon fruit was treated with an ozone generator (Z755, Vietnam) in a tank containing 5 litres of water with a concentration of approximately 3 ppm for 5 mins (fixed ratio of water: red dragon fruit is 3:1). The ozone method is applied to the washing process of raw materials to limit microorganisms, reduce residues of pesticides and prolong storage time (Ly and Thuy, 2017). After treatment, the fruit was drained and separated fruit into the flesh and peel, which were put in dark polyamide zip bags and stored at -9±2^{o}C in the freezer (Sanaky, Japan) until further use.

2.2 Extraction procedure

About 10 g (peel or flesh of red dragon fruit) preserved material was ground and used for each treatment. The percentage of material used were 25, 50, 75 and 100 (%w/v), the remaining percentage is water. The time of the process was fixed from 3 to 18 mins at 30^{o}C. Conventional solvent extraction (CSE) was carried out using a magnetic stirrer (ARE, VELPScientifica Srl, Italy) while ultrasonic-assisted extraction (UAE) was carried out in an ultrasonic bath (Branson, 490 W, 42 kHz, USA). Triplicates of extraction treatments were conducted. After extraction, the mixture was filtered, and the filtered extract was submitted to analyses of total betacyanin content.

2.3 Determination of total betacyanin content (TBC)

McIlvaine buffer (pH 6.5) was used for analysis (Jamilah *et al.*, 2011). Brief, 0.1 mL obtained extract was diluted with 3.9 mL prepared buffer in cuvette prior to spectrophotometric analysis. For blank, 4 mL McIlvaine buffer was used. All determinations were performed in triplicate using a UV-Vis spectrophotometer at 538 nm.

The calculation of additional percentage TBC at each extraction time followed Equation 1.

$$\%\text{additional} = \frac{TBC_{UAE} - TBC_{CSE}}{TBC_{CSE}} \times 100 \quad (1)$$

Where TBC_{UAE} and TBC_{CSE} are total betacyanin content in the extract by UAE and CSE at each measured time, respectively

2.4 Data analysis

Values were expressed as mean±SD. Multiple regression analysis was used to evaluate the parameters of the selected model and measure optimal value using the statistical software Statgraphics Centurion XV.I (USA). As a primary criterion to select the best equation, the coefficient of determination (R^2) and *p*-value of the model were determined and used as the primary criterion to select the best equation (Guan and Yao, 2008).

3. Results and discussion

3.1 Comparison of extraction techniques for betacyanin in red dragon flesh and peel

3.1.1 Conventional solvent extraction

The TBC obtained in the extract from the peel or flesh of red dragon fruit when extracted at different ratios tended to decline when the percentage of water added was raised (Table 1). The TBC content in the extract using 75% raw material and 25% water was slightly higher when compared with the sample without using water. Perhaps, this is because water could enhance the swelling of the cellular material, increasing the contact surface area between the plant substrate and the solvent, leading to an increase in the extraction yield (Hayat *et al.*, 2009). The pigment is water-soluble related to betacyanins' highly hydrophilic properties. Several hydroxyl groups (-OH) exist in the betacyanin structure leading to charge polarization and hydrogen bonding which is responsible for betacyanin's hydrophilic properties (Schmid, 2001). However, the increase in water content in the extraction leads to a decrease in the content per unit volume of the extract, leading to

Table 1. Effect of CSE on TBC in flesh and peel extracts from red dragon fruit

Material	Percentage of material used (%)	Extraction time (min)					
		3	6	9	12	15	18
Flesh	100	21.24±0.08	21.92±0.27	22.76±0.32	22.90±0.11	22.65±0.05	21.85±0.43
	75	25.01±0.02	25.84±0.13	27.18±0.03	27.34±0.07	27.24±0.04	26.49±0.59
	50	16.79±0.50	16.94±0.04	18.03±0.49	18.13±0.14	17.74±0.15	16.86±0.42
	25	12.83±0.10	13.71±0.14	13.80±0.25	13.94±0.18	13.89±0.02	13.70±0.49
Peel	100	17.60±0.18	18.54±0.13	18.98±0.02	18.99±0.03	18.97±0.10	17.93±0.09
	75	20.43±0.43	20.62±0.16	20.70±0.05	20.95±0.02	20.92±0.03	20.04±0.36
	50	12.84±0.21	13.84±0.01	14.35±0.07	14.39±0.20	14.33±0.03	13.59±0.37
	25	9.72±0.04	10.85±0.13	10.91±0.06	11.47±0.31	10.90±0.07	9.72±0.15

Values are presented as mean±SD.

analytical results on the total extract obtained also lower, because betacyanin is well soluble in water (Woo *et al.*, 2011). Betacyanins also are easily oxidized, they have contacted the presence of oxygen in solution and broken down into betamic (yellow) and amine (colourless) (Delgado-Vargas *et al.,* 2000). The small amount of water used leads to incomplete and inadequate extraction of betacyanin, but a high water percentage could cause waste at a later stage in the extraction process. Since the dissolution of substances into the solvent is a physical process, an increase in the amount of solvent would be facilitated the contact of biological substances with the solvent, and the higher permeability of cell walls had a positive effect on the extraction yield of bioactive compounds, but the efficient extraction will not continue to increase once equilibrium has been reached (Cacace and Mazza, 2003). Using water in extraction creates an optimum solvent for betacyanin extraction. It is also a simple, low-cost method that is as effective as organic solvents (Cai and Corke, 1999). The study of Castellar *et al.* (2003) studied betacyanin pigment in *Opuntia* fruit and reported that purified water was more effective than ethanol, moreover, it is the cheapest, abundant and environmentally friendly solvent. The results also showed that when increasing the extraction time from 3 to 12 mins, the TBC also tended to increase gradually in both the flesh and peel extracts. Specifically, the highest TBC was obtained when extracting using 75% of the raw materials for 12 mins, which was 27.34±0.07 mg/100 mL in flesh extract and 20.95±0.02mg/100 mL in peel extract. However, when using 75% raw material for extraction, the TBC in obtained solution was not significantly higher when extraction time was increased to 12 mins. The study of Vaillant *et al.* (2005) reported that TBC in flesh red dragon fruits ranged from 32 mg/100 mL to 47 mg/100 mL and 30.18 mg/100 mL in fruit peel. Different cultivation methods lead to differences in the physicochemical properties of fruits (Knapp *et al.,* 2018).

3.1.2 Ultrasonic-assisted extraction

A similar trend was found when increasing the percentage of water used in the extraction process, the amount of betacyanin obtained tended to decrease even with the assistance of the ultrasound. The time of ultrasonic-assisted extraction has a positive effect on the TBC obtained in the extract (Table 2), the obtained results are quite similar to the results of Raj and Dash (2020). After 15 mins of extraction, the TBC began to tend to decrease with increasing extraction time because the equilibrium point had begun to be reached. The efficiency of UAE extraction of natural pigments from annatto seeds was found in the study of Magazù *et al.* (2008), the amount of pigments collected gradually increased at the beginning of the process and began to decrease at the end of the extraction. In the early stages of extraction, some activities such as swelling and hydration of the plant material have occurred and membrane cell was collapsed by ultrasonic waves (Filgueiras *et al.,* 2000). The yield extraction was

Table 2. Effect of UAE on TBC in flesh and peel extracts from red dragon fruit

Material	Percentage of material used (%)	Extraction time (min)					
		3	6	9	12	15	18
Flesh	100	21.24±0.08	21.92±0.27	22.76±0.32	22.90±0.11	22.65±0.05	21.85±0.43
	75	25.01±0.02	25.84±0.13	27.18±0.03	27.34±0.07	27.24±0.04	26.49±0.59
	50	16.79±0.50	16.94±0.04	18.03±0.49	18.17±0.14	17.74±0.15	16.86±0.42
	25	12.83±0.09	13.71±0.14	13.80±0.25	13.94±0.18	13.89±0.02	13.70±0.49
Peel	100	17.60±0.18	18.54±0.13	18.98±0.02	18.99±0.03	18.97±0.10	17.93±0.09
	75	20.43±0.43	20.62±0.16	20.70±0.05	20.95±0.02	20.92±0.03	20.04±0.36
	50	12.84±0.21	13.84±0.01	14.35±0.07	14.39±0.20	14.33±0.03	13.59±0.37
	25	9.724±0.04	10.85±0.13	10.91±0.05	11.47±0.31	10.90±0.07	9.72±0.15

Values are presented as mean±SD.

increased when the solvent diffused into the plant cell, leading to increased contact area, and substrates began to easily dissolve into extract (Wang *et al.*, 2012; Xu *et al.,* 2014).

However, betalains are known to be very sensitive to heat. As a consequence of the heat, several betalain degradation reactions occur (Lim *et al.,* 2011). When treated at a high temperature for a long time, betacyanin compounds in red flesh dragon fruit would be lost, the study of Raj and Das (2020) showed that their decomposition rate increased when the extraction temperature was above 30^{o}C.

3.1.3 Comparison of efficiency extraction by techniques

The study clearly showed that the UAE improved the TBC in the extract, which was higher than that of the extract by CSE (Table 3). This effect is mainly due to the effects of ultrasound waves leading to fragmentation, erosion and detachment on the surface of the fruit matrix (Machado *et al.,* 2019). These effects combined with heating facilitate solvent access to the interior of the matrix which improves mass transfer during extraction (Koubaa *et al.*, 2016). Overall, since the pigment is closely bound to the cell membrane of the fruit, extraction can be improved through some improved methods such as ultrasound-assisted, and microwave-assisted (Kulkarni and Rathod, 2014; Sivakumar *et al.,* 2011). The results showed that UAE increased the TBC in the extract when increasing the extraction time (from 3 to 15 mins) with the ultrasonic-assisted method, the TBC tends to increase (from 8.76% to 15.82% in the fruit flesh extract and from 6.79% to 17.74% in the fruit skin extract). The comparison showed that the highest increase in TBC was 17.74 and 15.82% respectively in the flesh and skin after extraction for 15 mins, compared with the traditional extraction method. The short extraction time is not enough to dissolve the betacyanins in the cells into the solvent. Extraction time is very important, too short or too long time may reduce quality. Ultrasound creates holes, disrupting the cell walls, allowing solvents to transfer mass, thereby increasing extraction efficiency (Chemat *et al.,* 2017)

3.2 Multiple regression analysis for betacyanin in red dragon fruit extract by ultrasonic-assisted extraction

Multiple regression of the extraction ratio of material to water (flesh and peel of red dragon fruit) (A) and extraction time (B) was performed by the response surface method. Optimal values were evaluated through TBC in the extract. Using the optimization method to select the best percentage of material used and extraction time for betacyanin extraction in flesh and peel of red dragon fruit. The process of extracting betacyanin from the red flesh/peel of dragon fruit was carried out based on the dissolution of betacyanin in water with the assistance of ultrasonic waves. The R^2 statistic indicated that the model as fitted explains 80.03% and 85.73% of the variability in red dragon fruit flesh and peel extract, respectively (Table 4). In addition, since the P-value of both models were less than 0.05, there is an indication of possible serial correlation at the 95% confidence level. Moreover, the standardized Pareto chart showed that almost the coefficient parameter had P-value < 0.05, except for AB interaction (Figure 1). In determining whether the model can be simplified, notice that the highest P-value on the independent variables was over 0.05.

The model can be simplified as Equations 2 and 3 corresponding to the correlation between predicted betacyanin content and independent variables in red dragon fruit flesh and peel extract.

TBC in flesh extract (mg/100 mL) = -3.04 + 0.62A + (2)

TBC in peel extract (mg/100 mL)= -2.33 + 0.44A + (3)

Table 3. The percentage of TBC increase in red dragon fruit flesh and peel extract (%) by the UAE compared to CSE

Material	Extraction time (min)					
	3	6	9	12	15	18
Flesh	8.76±0.01	9.23±0.01	10.08±0.03	12.26±0.01	15.82±0.01	10.79±0.02
Peel	6.79±0.02	7.96±0.01	9.07±0.02	11.22±0.01	17.74±0.01	11.53±0.01

Values are presented as mean±SD.

Table 4. Analysis of Variance for betacyanin in red dragon flesh and peel extract

Source	Red dragon flesh					Red dragon peel				
	SS[a]	Df[b]	MS[c]	F-Ratio	P-Value	SS	Df	MS	F-Ratio	P-Value
Lack-of-fit	1804.05	5	360.81	52.9	0	1255.79	5	251.159	79.35	0
Pure error	450.119	66	6.81999			208.892	66	3.16504		
Total (corr.)	2254.17	71				1464.69	71			
Coefficient of determination	R^2= 80.03%; R^2 Adj. = 78.52%					R^2= 85.73%; R^2 Adj. = 84.66%				

[a]Sum of square, [b]Degree of freedom, [c]Mean of square

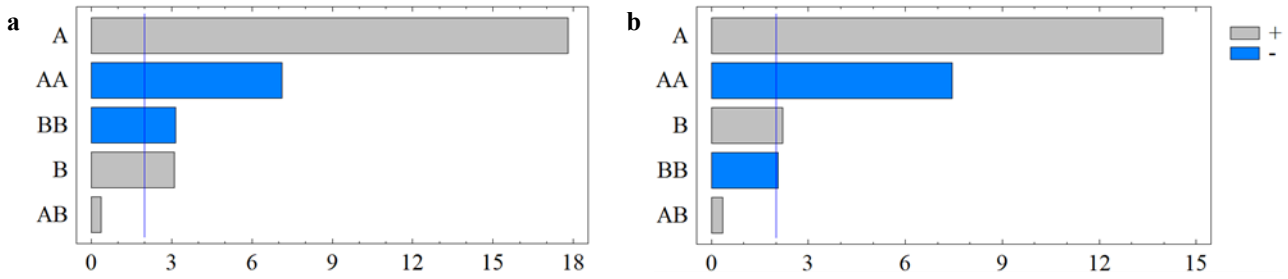

Figure 1. Standardized Pareto chart for betacyanin in red dragon (a) flesh and (b) peel extract

Where A is the percentage of material used (%), B is extraction time (min)

Adding water to the extraction process increases the mobility of compounds, creating an environment for soluble compounds, especially betacyanin which is a good water-soluble compound. Since the dissolution of substances into the solvent is a physical process, an increase in the amount of solvent facilitates the contact of biological substances with the solvent, resulting in a higher mass transfer but the efficiency of the extraction of biological compounds does not continue to increase. Optimal analysis results showed that the betacyanin content in fruit flesh reached the highest level of 27.49 mg/100 mL when extracted at a ratio of 83.74% red dragon flesh: 16.26% water for 13.15 mins (Figure 2a). The combination of factor levels maximizes total betacyanin content in peel extract over the indicated region (Figure 2b). The obtained results achieved that betacyanin content reached the highest level of 22.28 mg/100 mL when extracted at the ratio of 90.63% and the extraction time was 12.91 mins.

4. Conclusion

The ultrasonic-assisted extraction could be a promising extraction process to improve the extraction of betacyanin from red dragon fruit (flesh and peels). The multiple regression analysis models showed good fitting and adequacy to the experimental data and the high correlation of models indicated that it can be employed to optimise extraction conditions. With available extraction techniques, the obtained extract had nice colour, high content of bioactive compounds and antioxidant activity will be a promising technique to increase applicability in the food industry.

Conflict of interest

The authors declare no conflict of interest.

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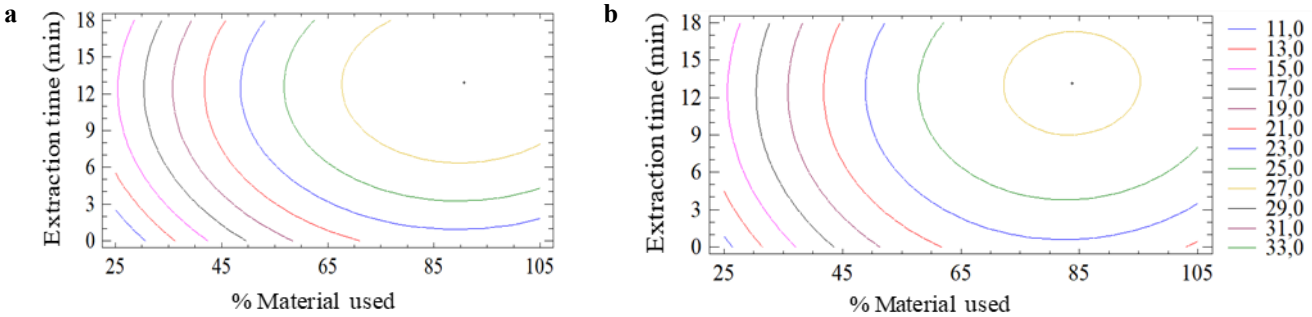

Figure 2. Estimated RSM contoured plot for TBC in red dragon (a) flesh and (b) peel extract

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