

Comparison of ultrasound assisted extraction and enzyme assisted extraction of betacyanin from red dragon fruit peel

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Abstract. This article was intended to extract betacyanin from the peel of red dragon fruit (*Hylocereus polyrhizus*) and used it as a natural colorant. In this study, enzyme and ultrasound techniques for the extraction of betacyanin from dried dragon fruit peel were compared. The ultrasonic power and sonication time levels were varied between 0.5, 1.5, 2.5, 3.5, and 4.5 W/g; and 2.5, 5.0, 7.5, 10.0, and 12.5 min. The enzyme concentrations were 0.25, 0.75, 1.25, 1.75, 2.25, and 2.75 %v/w. The results revealed that the maximum betacyanin content obtained by the optimal UAE condition (3.5 W/g and 7.5 min) was 0.3402 mg/g, and 9.47 % higher than that by the EAE method. The first- order kinetic extraction was used to describe the mechanism of extraction of betacyanin from red dragon fruit peels. The initial extraction rate (h) and extraction rate constant (k) of the UAE model were 30.80 and 27.81 % higher than those of the enzyme assisted extraction (EAE) model. The UAE treated only 5.0 min to obtain the highest level of betacyanin (0.323 mg/g), whereas the EAE took up to 20 min to achieve the maximal value (0.309 mg/g). The research clearly shows that the UAE method is a useful method for extracting betacyanin from dried red dragon fruit peel.

1 Introduction

The red dragon fruit or red pitaya (*Hylocereus polyrhizus*) belongs to the Cactaceae family from the genus *Hylocereus*. Dragon fruits are originated from Tropical Rain Forest area of Mexico. The variety is nowadays cultivated in many Asian countries such as Vietnam, Thailand, Malaysia, and Philippines [1]. The peel and flesh of the fruit are red in color [2-3]. The main coloring in red dragon fruit skin is betacyanin, and its color can be dissolved in water, ethanol, and methanol [3]. The color extracted from red dragon fruit peel remains stable despite pH changes, and shows a tinctorial strength up to three times higher than the anthocyanin [4]. Betacyanin can further be classified following its chemical structures including betanin-type, amaranthin-type, gomphrenin-type and bougenvillein-type [4]. For

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the peel of red dragon fruit, the major structure is betanin (*5-O-glucosides*) which exhibits high antiradical activities [5].

Nowadays, red beetroots (*Beta vulgaris*) are the most popular source of betacyanin which are available in the concentrated form [6]. Saponjac et al. [7] reported that the betacyanin content in red beetroot was 79.22 mg/100g dry weight. However, red beetroot extracts contain geosmin and pyrazines which relate to the formation of carcinogenic nitrosamines. In addition, these components cause for the unpleasant peatiness of this crop [7]. In contrast to red beetroots, the red dragon fruit peel extract does not have any negative impact on sensory properties [2-7]. Also, red dragon fruit is easily found in Viet Nam [1]. Thus, it can be said that the betacyanin extracted from the red dragon fruit peel has potential for coloring food products.

Extraction is a key process that aims to extract target components in plant tissues. Hot water extraction is one of the most traditional ways to extract bioactive compounds, but it necessarily takes high performance price in industry. Some previous researches are conducted that the enzyme-assisted [2-4] and ultrasound-assisted extraction [3] of phenolic compounds from plant sources can boost up the production yield in comparison with the ordinary extraction. However, there exists no studies on betacyanin extraction from dried red dragon fruit peel using the ultrasound assisted and the enzyme (Pectinex Ultra SP-L) assisted extraction.

Therefore, the purpose of this study was to compare the enzyme-assisted and the ultrasound assisted extraction of betacyanin from dried red dragon fruit peel using water as an extraction solvent. The authors also compared two kinetic extraction models with the goal to depict the kinetics of the ultrasound-assisted and the enzyme-assisted extraction.

2 Material and methods

2.1 Materials

The red fresh dragon fruits were purchased from Hoang Hau farm in Binh Thuan province, Vietnam. 100 kg of the peels were separated from the pulps using a stainless steel knife and then were cut into small cubes. After that, they were dried at 70°C for 14 hours to achieve the final moisture content of 6-8 %db (dry basis), and then stored in a laboratory at 4°C before the start of the experiments.

The Pectinex Ultra SP-L obtained from *Aspergillus aculeatus* were provided by Novozymes (Bagsvaerd, Denmark). The catalytic activity was 9500 PGU/mL. The optimum temperature and pH of preparation are 20–50°C and 3.0–4.5, respectively. Methanol (99.9 %) was from Sigma-Aldrich (Darmstadt, Germany), and NaOH anhydrous pellets (98 %) from Carlo Erba reagent (Burkina Faso, France).

2.2 Methods

2.2.1 Extraction procedure

The ultrasound-assisted extraction (UAE) was conducted using a horn-type ultrasonic probe with frequency of 40 kHz (VCX750 Vibracell; Sonic & Materials, Inc., Newtown, CT, USA). The effects of the ultrasound power and extraction time on the betacyanin content were studied. A 20 g of the dried dragon fruit peel were dispersed in 200 ml distilled water in Erlenmeyer flasks [2]. The ultrasound power and sonication time levels were varied between

0.5, 1.5, 2.5, 3.5, and 4.5 W/g dry weight material; and 2.5, 5.0, 7.5, 10.0, and 12.5 min. During the sonication, the samples were heated in a water bath (Memmert, WPE45, Germany) at 75–80°C [9]. The solid and mucilaginous materials were separated from the extract using vacuum filtration with a double layer of Whatman filter paper no.1.

In the enzyme assisted extraction (EAE), a 20 g of dried sample and 200 ml distilled water were put into 250 ml erlenmeyer flask for betacyanin extraction. Different amounts of Pectinex Ultra SP-L (0.25, 0.75, 1.25, 1.75, 2.25, and 2.75 %v/w dry weight of the dragon fruit peel) were added into the mixture and placed in an incubator with shaking at 35°C, 150 rpm. The enzyme extraction times varied from 5 to 30 min. After that, the enzymes were deactivated at 90°C for 1 min and filtrated to collect the clear juice.

2.2.2 Determining the betacyanin content

The concentration of betacyanin was determined using UV-spectrophotometry/NIR (Shimazu, UV-2600, Japan) where the extracts were diluted with water with the ratio of 1:10 [8]. The extract samples were then measured at 538 nm. The calculated betacyanin content (mg/g dry weight material) uses the following equation:

$$\frac{A_{538} \times MW \times V \times DF}{\delta \times W \times L} \times 100 \quad (1)$$

Where A_{538} : absorbance at λ_{max} (538 nm); L: path length of the cuvette (1.0 cm); DF: dilution factor; V: extract volume (mL); W: dried weight of extracting material (g). For betanin, δ : mean molar absorptivity (6.5×10^4 L/mol cm in water); and W: molecular weight of betacyanin (550 g/mol).

2.2.3 Color characterization

The color of liquid extracts was measured using a colorimeter Lovibond Tintometer PFXI-880L (Tintometer Ltd, USA). The color was assessed in accordance with the Commission Internationale de l'Eclairage (CIE) and expressed as L^* (lightness) a^* (redness) b^* (yellowness) system. The hue angle (h°) is calculated as follows:

$$h^\circ = \tan^{-1} \frac{b^*}{a^*} \quad (2)$$

2.2.4 First-order kinetic extraction

The first-order rate law can be used to determine the extraction rate constant of betacyanin from red dragon fruit peels. The general firs-order model was experessed as equation 3.

$$\frac{C_s - C_t}{C_s - C_0} = e^{-kt} \quad (3)$$

Where C_s is the betacyanin concentration in the extract at saturation (mg/g), C_t is the betacyanin concentration in the extract at a given extraction time t (mg/mg), C_0 is the initial betacyanin concentration in the extract (mg/g), k was the first-order extraction rate constant (mg/g.min). When $t=0$ and $C_0=0$, the first-order model can be written as equation 4:

$$\frac{C_s - C_t}{C_s} = e^{-kt} \quad (4)$$

The initial extraction rate (mg/g.min) can be calculated as follows [9]:

$$h = k \times C_s \quad (5)$$

The initial extraction rate h (mg/g.min), and the first-order extraction rate constant k (mg/g.min) were determined from the slope and intercept by plotting t/Ct against t .

2.3 Statistical analysis

All the experimental values obtained for UAE and EAE were statistically analyzed using Statgraphic Centrution XV (Statsoft Inc., Umeå, Sweden). The experimental results were expressed as mean±SD. The one-way analysis of variance (ANOVA) with a 95 % confidence level was carried out to determine differences among the group means.

3 Results and discussion

3.1 Ultrasound-assisted extraction (UAE) of betacyanin from dried dragon fruit peel

The effect of the ultrasonic power on the content of betacyanin is shown in Figure 1. As shown in Figure 1, the lowest content is observed for the ultrasound power of 0.5 W/g and sonication time of 2.5 min (0.2014 mg/g). When the ultrasound power increased from 0.5 W/g to 3.5 W/g, the betacyanin content in the extracts increased from 0.2014 mg/g to 0.3402 mg/g (68.92 % increases). Our results are similar with many authors who applied ultrasound to active component extraction from plants [10-11]. High ultrasonic power promoted extensive the disruption of cellular and allowed the intracellular components to be released into the solvent. In this study, the betacyanin content in the extracts slightly increased and leveled off with the increase of the ultrasonic time from 3.5 W/g to 4.5 W/g. However, high ultrasonic intensity induced the chemical reactions in the bulk solution and accelerated decomposition of components [11]. Therefore, the ultrasonic power of 3.5 W/g was chosen as the output power.

Figure 2 shows the effect of the experimental duration for extraction on the content of betacyanin. The increase in the sonication time from 2.5 to 7.5 min augmented the betacyanin content by 38.22%. However, the longer sonication time (from 7.5 to 12.5 min) did not significantly change the betacyanin content in the extracts. This process proves that in 7.5-min period, the effect of the ultrasound works more effectively. It may be that the increase in the sonication time directly related to the reduction of particle size, enhancing the mass transfer. In order words, the larger a contact area between a material and a solvent is created, the more betacyanin can be released into the solvent. Similar results were achieved by Ngoc et al. [9], Shan et al. [12]. In the research of Ngoc et al. [9], it confirmed that an extended sonication time had a greater influence on the reduction of particle size and enhanced the extraction yield [9].

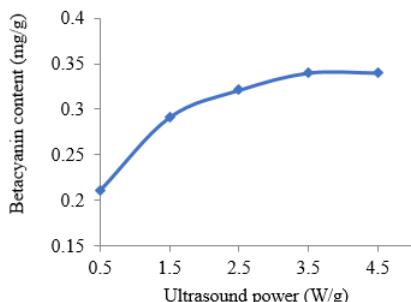


Fig. 1. The effect of ultrasonic output power on the content of betacyanin (extraction time: 2.5 min; solid to liquid ratio: 1:10; extraction temperature: 30°C).

In conclusion, the optimal extraction condition of UAE was the ultrasound power of 3.5 W/g and sonication time of 7.5 min. Then, this condition was applied in section 3.3.

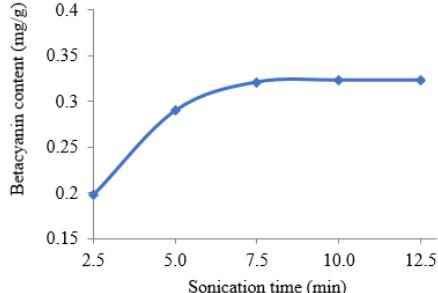


Fig. 2. The effect of sonication time on the content of betacyanin (ultrasound power: 3.5 W/g; solid to liquid ratio: 1:10; extraction temperature: 30°C).

3.2 Enzyme-assisted extraction (EAE) of betacyanin from dried dragon fruit peel

The degradation of the polysaccharide structure in middle lamella layer of the red dragon fruit tissue with the Pectinex Ultra SP-L was basically conducted to improve the betacyanin yield. As can be seen in Figures 3 and 4, the content of betacyanin increased with an increase in the enzyme concentration and treatment time. In Figure 3, the enzyme concentration changed from 0.25 to 1.75% v/w dry weight material, the betacyanin content significantly increased from 0.212 to 0.308 mg/g (31.59 % increases). This result could be attributed to the fact that the Pectinex Ultra SP-L could degrade pectin in the plant tissue and facilitate the release of betacyanin from the dried dragon fruit peel. Its finding is similar to Yazdi et al. [13], who reported that pectinase can disrupt the intracellular of plant materials, and enhance the inside pigments released into the solvent extraction [13]. However, the further increase in the enzyme concentration from 1.75 to 2.75 %v/w did not improve the betacyanin content in the extracts (from 0.308 to 0.309 mg/g). Because of economic issues, we selected 1.75 %v/w as an optimum enzyme concentration.

Figure. 4. showed the effect of the treatment time on the extracted betacyanin content from the dried red dragon fruit peel. According to the obtained results, when the enzymatic treatment time increased up to 20 min the concentration of betacyanin achieved a maximum of 0.309 mg/g and after that remained almost constant. A similar trend has been published by Lim et al. [14], who extracted betacyanin from the white dragon fruit peel. In this research, these authors documented that the highest betacyanin were observed after 5 min and after that the concentration remained constant [14]. Our study used a longer enzymatic treatment time than this study because of the difference in solubility and distribution of betacyanin in various plant materials used. Thus, the treatment time of 20 min was selected as an optimum extraction condition.

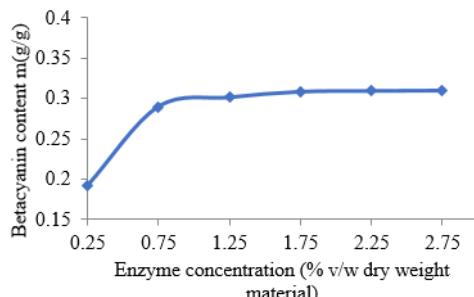


Fig. 3. The effect of enzyme concentration on the content of betacyanin (enzymatic treatment time: 10 min; solid to liquid ratio: 1:10; extraction temperature: 35°C).

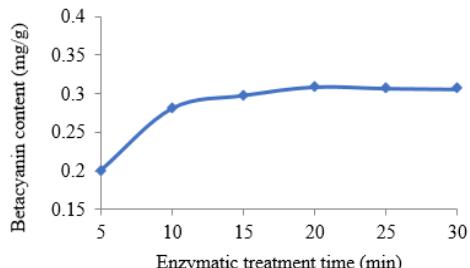


Fig. 4. The effect of enzymatic treatment time on the content of betacyanin (enzyme concentration: 1.75 %v/w; solid to liquid ratio: 1:10; extraction temperature: 35°C).

In summary, the conditions of the enzyme assisted extraction were the enzyme concentration of 1.75% v/w dry weight material and the extraction time of 20 min. Those conditions were then used in section 3.3.

3.3 Comparison of UAE and EAE of betacyanin from dried dragon fruit peel

The first-order kinetic models for betacyanin extraction using the UAE and the EAE can be studied and compared from the plot of $\log(C_s - C_t)$ against t . From the slope and the intercept of the plot, the maximum betacyanin concentration in the extract (C_s), initial extraction rate (h), extraction rate constant (k), and coefficient of determination (R^2) were calculated and shown in Table 1. As shown in Table 1, it can be seen that the first-order kinetic models for the extraction of betacyanin using the UAE and the EAE had a relatively high coefficient of determination ($R^2 = 0.993$ and 0.991, respectively). This result illustrated that the first-order kinetic model fitted well the experimental results.

Table 1. Comparison of the first-order kinetic parameters of betacyanin extraction in UAE and EAE.

Extraction methods	Maximum betacyanin content, C_s , (mg/g)	Extraction rate, k (mg/g.min)	Initial extraction rate, h (1/min)	R^2
EAE	0.306	0.501	0.155	0.993
UAE	0.323	0.694	0.224	0.991

As shown in Table 1, the initial extraction rate (h) and extraction rate constant (k) of the UAE model were 0.224 and 0.694, respectively and 30.80 % and 27.81 % higher than those of the EAE model. The maximum betacyanin concentration obtained in the UAE extract (C_s) was 0.323 mg/g, and 6 % higher than that of the EAE method (0.306 mg/g). In the UAE model (Figure 5A), the kinetic result (simulation and experimental) revealed that two-thirds of the betacyanin (> 0.288 mg/g) was recovered in the early sonication stage (0-5.0 min). The extended sonication time (from 5.0 min to 15 min) slightly increased in the yield (Figure 2). Meanwhile, in order to achieve two-thirds of the betacyanin content in the EAE model the extraction time rose up to 15 min. As our results suggest the ultrasound greatly shortened than the enzyme preparation. The results were contrary to Ngoc et al. [9] on protein extraction from defatted rambutan seeds. These authors argued that the ultrasonic treatment better accelerates the protein yield from defatted rambutan seeds than the enzymatic extraction.

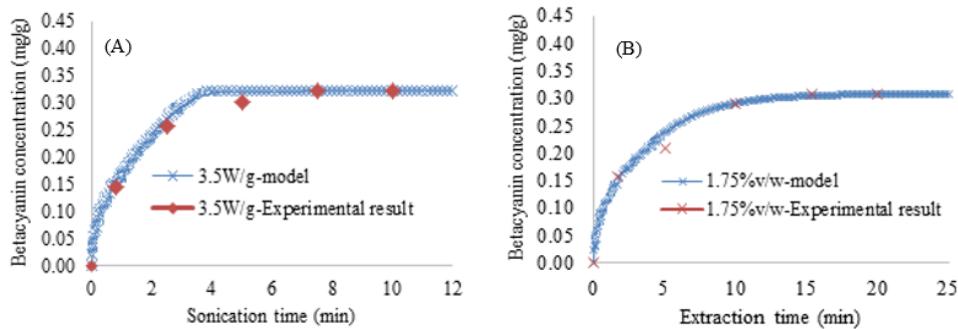


Fig. 5. Betacyanin concentration (C_t) in the extract during the time: (A) Ultrasound-assisted extraction (UAE), (B) Enzyme-assisted extraction (EAE).

3.4 Color characterization

The color characterization was performed to study the hue, saturation and intensity of color of the red dragon fruit peel extract using optimized extraction conditions (Table 2). As can be seen, the lightness (L^*) of the UAE extract was 22.85, which was lower than the EAE extract. The redness (a^*) and yellowness (b^*) of the UAE extract were higher than those of the EAE ($p < 0.05$). It means that the UAE method strongly affected to the red dragon fruit cell degradation, compared with the EAE method. These results also indicated that the extracts obtained from the UAE and EAE method contains a huge amount of pigments and betacyanins. The findings are similar with Bao and Pirak, Phuong & Tuan, who reported that dragon fruit peel is a good source of polyphenols and pigments [2-10].

Table 2. The color characterization (L^* , a^* , b^* , and h) of red dragon fruit peel extracts obtained from the EAE and UAE methods at optimum conditions.

Experiment	L^*	a^*	b^*	h
EAE extract (1.75 %v/w for 20 min)	23.98±0.79	6.98±0.72	2.48±0.54	19.56±0.68
UAE extract (3.5 W/g for 7.5 min)	22.85±0.79	8.25±0.60	3.35±0.71	22.10±0.64

4 Conclusions

The UAE method significantly improved the betacyanin yield from dried red dragon fruit peel compared with the EAE method. The ultrasonic treatment resulted in the shorten extraction time than the enzymatic treatment. A major diminution in the extraction time makes the extraction of thermally labile compounds fit. With this study, it can be indicated that UAE may become an ideal extraction method for dragon fruit betacyanin.

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