Improvement of betalains stability extracted from red dragon fruit peel by ultrasound-assisted microencapsulation with maltodextrin

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ABSTRACT
Natural betalains can be potential food additives because of their antioxidant activities, but they have poor thermal stability. In this study, betalains were extracted from red dragon fruit peel, and then encapsulated with maltodextrin by ultrasound method to increase the physicochemical properties of betalains microcapsules. The encapsulation efficiency of the betalains was above 79%, and the particle size and Zeta potential values were 275.46 nm and −29.01 mV, respectively. Compared to the control sample, onset temperature and DPPH free radical scavenging of betalains microcapsules under the modest ultrasound treatment (200 W, 5 min) was increased by 1.6 °C and 12.24%, respectively. This increase could be due to the ability of ultrasonification to create interactions between maltodextrin and betalains (as evidenced by FT-IR). Therefore, modest ultrasound treatment can be used for microcapsulation to improve the stability of betalains, and then expand the application of betalains in heat processed food field.

1. Introduction

Dragon fruit also known as the pitaya or pitahaya (Hylocereus species), is a popular tropical fruit in the world due to its attractive appearance, sweet and juicy taste and high nutritional value [1]. According to the color of fruit pulp, it can be divided into red, yellow and white dragon fruit [2]. In recent years, red dragon fruit (Hylocereus monacanthus) with the red purple color has attracted more consumer interest because it is rich in natural pigments of betalains, water-soluble nitrogen containing pigments. These compounds have been reported to have antioxidant and anti-inflammatory activities, anticancer properties, antilipidemic effects and antimicrobial capacity [3]. Recent research has illustrated that pitaya fruit can have a beneficial effect on gut microbiota and gut immune boosting properties [4]. However, the weight of dragon fruit peel accounts for 30–35% of the whole fruit, and is discarded in fruit processing resulting in waste of resources and environmental pollution [5]. The peel of red dragon fruit are rich in the biological compounds, especially betalains [6]. Therefore, red dragon fruit peel could be used as a resource for betalains extraction.

Betalains have a drawback as a natural food colorant in that they have poor thermal stability, which limits their application in the food industry. It has been reported that betalains are unstable in the presence of light, oxygen, high water activity and temperature (>50 °C) [7]. Microencapsulation technology is one of the most effective methods to protect the bioactive compounds in the food field because these unstable compounds are wrapped in the interior of the more stable matrices, and shield the influence of external factors, as well as do not change the structure and biological activity of them [8]. In recent years, in order to improve the stability of betalains, some researchers have evaluated the effect of different encapsulating agents [9]. However, it has been reported that the wall material and preparation conditions could affect the encapsulation efficient and stability of betalains because of the interaction between betalains and wall material [10].

Ultrasound technology is widely applying in food industry as dispersing tool, which can enhance the mass transfer and the contact area between two phases by producing physical phenomena and caviation effect [11]. As the consequence of the cavitation, the uniformity of the two-phase mixing is improved, and the particle size of the...
microcapsule is reduced that could improve the encapsulation efficiency and stability [12]. Ultrasonification has been used in the extraction of bioactive ingredients from a number of fruits and vegetables [13]. Moreover, some previous studies have been reported that ultrasound can promote the interactions (hydrophobic forces, covalent bonding) between compounds in the solution dispersion system [14,15]. However, the application of ultrasound in the production of betalains microencapsulation by freeze drying has not been studied. Therefore, in this study, the betalains of red dragon fruit peels were extracted by the ultrasound techniques and subsequently betalains microcapsules were prepared using maltodextrin as wall material with the aids of ultrasound and freeze drying. The effects of ultrasound treatment on the physicochemical properties and antioxidant activity of betalains microcapsules were also investigated.

2. Materials and methods

2.1. Materials

Red dragon fruit was purchased from the local market (Zhenjiang, China). Absolute alcohol was purchased from Sinopharm Group Co., Ltd. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Maltodextrin (DE value of 15.3, pH 5.62) was supplied from Yuanye biological Co., Ltd (Shanghai, China). All other reagents were analytical grade and purchased from the local authorized supplier.

2.2. Extraction betalains from the red dragon fruit peel

The pulp and green parts of peel of fresh red dragon fruit was removed to obtain red dragon fruit peel. Five hundred grams of red dragon fruit (cut into 3 ~ 4 cm) was extracted with 300 mL of 60% ethanol solution (v/v) at room temperature (25 ± 1 °C) for 20 min with a high-speed blender (L12-P312, Joyoung Co., Ltd, Hangzhou, China) for 10 s on and 3 s off, and then separated by a centrifugation at 4000 g for 10 min (DL-6-B, Shanghai Anting Scientific Instrument Factory, Shanghai, China). For sufficient extraction betalains, the above extraction steps were repeated until the precipitant colorless. Collected supernatant was concentrated by a rotary vacuum evaporator (B-100HB, BUCHI, Switzerland) with 35 °C for 30 min. Finally, the concentrated extract was fixed to 1 L and stored at 4 °C for further uses.

2.3. The content of betalains and extraction yield

Betalains concentration in extraction was determined according to the method reported by Skalicky, et al. [16] with some modifications. One milliliter of extraction was diluted fourfold by ultrapure water to an optimal absorbance value of 0.4 to 0.5. Ultrapure water was used as the blank. Betalains absorption was determined at 480 nm and 540 by UV–Vis spectrophotometer (UV-1601, Beijing Beifen-Ruili analytical instrument Co., Ltd., China). The concentration of betalains and extraction yield were calculated using the following equation 1 to 3.

\[
C_a = \frac{A_a \times D_I \times M_{e1}}{c_I \times L} \quad (1)
\]

\[
C_b = \frac{A_b \times D_I \times M_{e2}}{c_I \times L} \quad (2)
\]

\[
Yield(\%) = \frac{(C_a + C_b) \times V}{m_0} \times 100 \quad (3)
\]

where \(C_a\) and \(C_b\) represent the concentration of betacyanin and betaxanthin (mg/L), respectively; \(D_I\) represents the dilution ratio (5); \(A_a\) and \(A_b\) represent the absorption at 540 and 480 nm; \(M_{e1}\) and \(M_{e2}\) are the molecular weight of betacyanin (550 g/mol) and betaxanthin (308 g/mol), respectively; \(c_I\) and \(c_2\) represent the mole extinction coefficient of betacyanin (60000 L/mol) and betaxanthin (48000 L/mol), respectively; \(L\) is the light through the distance (1 cm); \(V\) is the total extraction volume of betalains (L); \(m_0\) is the weight of red dragon fruit peel used to extract betalains (mg).

2.4. Determination thermal stability of betalains

The degradation rate constant and half-time period of betalains were determined using the reaction rate as reported by Fernandez-Lopez, et al. [17]. 5 mL of betalains solution was heated in the water bath at 60 °C for different treatment time (30, 45, 60, 75 and 90 min), and then heated solution was immediately cooled to stop thermal degradation. Finally, the degradation rate constant and half-life period of betalains solution were calculated as following equation 4 and 5.

\[
\ln \frac{c_0}{c_t} = kt \quad (4)
\]

\[
t_{1/2} = \frac{\ln 2}{k} \quad (5)
\]

where \(C_0\) represent the concentration of betalains at initial; \(C_t\) represent the concentration of betalains for heating t time (mg/L); \(k\) represents the degradation rate constant (h⁻¹); \(t\) represents the heat time (h); \(t_{1/2}\) represent the half-life period of betalains (h).

2.5. Preparation of betalains microencapsulates

10% (w/v) of maltodextrin was dissolved in water at 60 °C for 3 h by a magnetic stirrer (H05-1, Shanghai MeiyinPu Instrument Manufacturing Co., Ltd, China). The maltodextrin solution was cooled to room temperature and stored at 4 ± 1 °C for 24 h to ensure complete hydration. The betalains extraction was mixed with maltodextrin solution for 1:1 (v/v). Then, the mixed solution was sonicated at selected ultrasound power (100, 200, 300 and 400 W) for 1, 2, 5 and 10 min by ultrasonic homogenizer (SCIENTZ-IID, Ningbo Scientz Biotechnology Co., Ltd, China). The detailed sonication treatment was showed in Table 1. Finally, the sonicated solution was pre-frozen in −60 °C for 6 h, and then dried by a vacuum freeze dryer (SCIENTZ-10 N, Ningbo Scientz Biotechnology Co., Ltd, China) for 48 h. Finally, the betalains microcapsule was collected in desiccator and stored in darkness to avoid light degradation.

2.6. Determination of the encapsulation efficiency of betalains

The encapsulation efficiency of betalains was determined using the method described by Zhang, et al. [18] with some modifications. 0.2 g of betalains microcapsule was added into 10 mL of 60% ethanol solution with stirring by a magnetic stirrer at 90 r/min for 10 min, and then centrifuged at 4000 g for 10 min. Betalains content of supernatant was calculated by equations 1 to 3. Encapsulation efficiency of betalains (EE) was calculated as following equation 5.

\[
EE = \frac{C_a \times V}{m_0} \times 100 \quad (6)
\]
EE(%) = \frac{C_0 - C_1}{C_0} \times 100 \quad (5)

where \(C_0\) is the theoretical value of 0.2 g betalains microcapsules (mg/L); \(C_1\) is the supernatant of betalains content (mg/L).

2.7. Measurement of partial size and Zeta potential

The particle size and Zeta potential were determined by dynamic light scattering technique (Litesizer 500, Anton Paar GmbH, Austria). In order to avoid multiple scattering effects, small amount of betalains microcapsule was dispersed in ultrapure water, and the refractive index was set at 1.33. Each sample was determined tree times.

2.8. Morphology of betalains microcapsules

The morphology of betalains microcapsule was measured using scanning electron microscopy (Quanta FEG 250, FEI Co., Hillsboro, Oregon, USA). The sample was sputtered with a thin gold layer (SC7620, Quorum Technologies Ltd, UK) for 15 s. Finally, the sputtered coated sample was scanned and recorded SEM image with an accelerating beam voltage of 20 kV.

2.9. Fourier transform infrared spectroscopy (FT-IR)

The chemical structure of the betalains, maltodextrin, and betalains microcapsules were identified by FT-IR spectrophotometer (Nicolet iSS50, Thermo Fisher Scientific Inc., USA) according to the method of Zhang, et al. [19]. 2 mg of sample was blended with 100 mg of KBr and pressed into KBr pellet. The pellet was scanned in the spectral range of 400–4000 cm\(^{-1}\). The spectrum of sample was obtained by automatically removing the background spectrum under same operating conditions.

2.10. Differential scanning calorimetry (DSC)

Thermal analyses of betalains microcapsule was determined by a DSC (STA449C, NETZSCH Co., Germany) according to the method described by Mahdi, et al. [20] with some modifications. 5 mg of betalains microcapsule powder was heated on an aluminum pan from 20 to 300 °C at heating rate of 10 °C/min under the protection atmosphere of nitrogen (50 mL/min).

2.11. Assessment of antioxidant activity

The antioxidant activity of betalains microcapsule was examined by DPPH radical scavenging activity using the method described by Zhang, et al. [21] with some modifications. Briefly, 0.1 g of betalains
microcapsule was mixed with 5 mL ultrapure water, and centrifuged at 4000 g for 10 min. Then, 2 mL of supernatant solution was mixed with 2 mL of 0.1 mmol/L DPPH solution (dissolved in absolute ethanol), which was incubated in darkness at room temperature for 30 min. Finally, the absorbance of sample solution was monitored at 517 nm by UV–Vis spectrophotometer. The antioxidant activity of betalains microcapsule was calculated using the following equation 6.

\[
\text{AA}(\%) = \left(1 - \frac{A_1}{A_0}\right) \times 100
\]  

(6)

where AA represents the antioxidant activity; \(A_0\) is the absorbance of the control that the water replaces of the sample solution; \(A_1\) is the absorbance of the sample solution; \(A_2\) is the absorbance of the blank that ethanol replaces the DPPH solution.

2.12. Statistical analysis

All results are reported as the mean ± standard deviation (n = 3) using SPSS software (Version 17.0, IBM, York, USA). Variance analysis was analyzed by Origin software (Version 9.0, Origin Lab, Massachusetts, USA). Significance analysis was performed by Tukey’s multiple-comparison test and significance level was set at 0.05.

3. Results and discussion

3.1. Concentration of betalains and extraction yield

Betalains are a group of water-soluble pigments present in the peel and flesh of red dragon fruit, which mainly consist of betacyanin and betaxanthin [7]. The concentration of betacyanin and betaxanthin extracted from red dragon fruit peel was 29.43 mg/L and 20.60 mg/L, respectively, which was in agreement with concentration of betacyanin extracted from red dragon fruit peel in study of Rodriguez, et al. [22]. However, this value was higher than that of 13.8 ± 0.85 mg/100 g reported by L. C. Wu, et al. [23]. The extraction yield was 0.31% in this study, which was higher than those reported by previous studies [24,25]. The reason for these phenomena may be related to the discrepancy in the growing environment, varieties and extraction method of the red dragon fruit [26].

3.2. Thermal stability of betalains

The concentration of betacyanin and betaxanthin in the extraction solution after prolonged heating time at 60 °C is shown in Fig. 1. From the Fig. 1, it can be seen that the betacyanin and betaxanthin contents were significantly reduced as the heating time extended to 90 min (p < 0.05). This result indicated that the betalains pigment was unstable at high temperature, which was consistent with previous studies [7]. Moreover, according to the degradation data, the correlation coefficient (R²) of thermal degradation dynamics fitting equation reached 0.98, which indicates that the degradation rate of betalains fitted first-order kinetic models. This result was consistent with previous report that the degradation reaction in natural extracts most followed the first-order kinetic models [27]. The degradation rate constant (k) and half-life period (\(t_{1/2}\)) of betalains solution at 60 °C were 0.1312 and 2.724 h, respectively, which was in accordance with Fernandez-Lopez, et al. [17]. It was reported that the k value and \(t_{1/2}\) of Opuntia stricta fruit extraction in different heating temperatures were 0.1 h\(^{-1}\) and 2.98 h at 50 °C, 0.35 h\(^{-1}\) and 1.7 h at 70 °C, and 0.91 h\(^{-1}\) and 0.79 h at 90 °C, respectively. These results indicated that temperature could promote the thermal degradation of betalains. Therefore, increases in temperature should be avoided during the microencapsulation of betalains. So, the freezing drying technology is an ideal encapsulation method to obtain the better performance betalains microcapsules.

3.3. Encapsulation efficiency of betalains

The encapsulation efficiency (EE) of betalains under different ultrasound treatment is shown in Fig. 2. From Fig. 2a, it can be seen that the EE increased as the ultrasound power increased, and the maximum EE of 79.88% was obtained at 200 W, while the EE was decreased to 51.6% with ultrasound power continual increase to 400 W. In addition, the influence of ultrasound time also appeared to generate a similar trend, in that EE was increased as the ultrasound time increased, and the maximum EE of 79.88% was obtained at 5 min, while the EE was decreased to 50.04% with ultrasound time continues increase to 10 min (Fig. 2b). Compared to the control (without ultrasound treatment), the best EE of betalains was achieved with 200 W for 5 min. These results indicated that the modest ultrasound treatment could promote the formation of betalains microcapsules because the mechanical effect by
ultrasound could promote the uniformity of the solution [28]. However, excessive ultrasound effect could destroy the betalains microcapsules due to the cavitation effect produced by ultrasound. This result was in accordance with previous studies [29].

3.4. Particle size and Zeta potential

The particle size of microcapsules could play a key role in their physicochemical properties and food application [30]. The microcapsules size distribution and Zeta potential of betalains microcapsules at the different ultrasound treatment is shown in Table 2. According to the result, the particle size of betalains microcapsules without ultrasound treatment was 409.45 ± 19.39 nm. However, the particle size of betalains microcapsules treated by ultrasound treatment was decreased significantly (p < 0.05), and the smaller particle size of betalains microcapsules (275.46 ± 20.68) was obtained by 200 W for 5 min. The Zeta potential is an important parameter that indicate the stability of the particles in the solution [31]. The higher absolute value
of Zeta potential indicates higher electric charge to produce strong interaction force among particles to keep the particles stable in the solution [32]. In this study, all the betalains microcapsules showed a negative value, and samples treated by ultrasound (200 W for 5 min) processed the highest absolute Zeta potential (-29.01 ± 1.74), which indicated that modest ultrasound conditions could promote the stability of betalains microcapsules in solution. Moreover, a previous study has illustrated that higher Zeta potential of microcapsules could have a higher core material embedding efficiency [33], which was proved in this study.

3.5. Morphology of betalains microcapsules

The morphology of betalains microcapsules by different ultrasound treatment is shown in Fig. 3. From the Fig. 3, it can be seen that the betalains microcapsules were irregular rigid solids, which was similar with the morphology of microcapsules by freezing drying in a previous study [34]. Moreover, the morphology of betalains microcapsules appeared to be agglomerated microcapsules by ultrasound treatment at 200 W for 5 min. However, as the ultrasound power increased, the betalains microcapsules became irregular flakes and contain more fragments, compared to the control sample, which may be due to the mechanical, cavitation and micro-jets effects produced by ultrasound treatment. Furthermore, Jyothi, et al. [35] reported that the wall material, drying method and the grinding process directly affects the shape of the pigment microcapsules.

3.6. FT-IR of betalains microcapsules

FT-IR of betalains microcapsules by different ultrasound treatments is shown in Fig. 4. According to the characteristic absorption peak of maltodextrin, the absorption peak at 1415 cm⁻¹ was the stretching vibration mode of C–H bonds. The peak of 1370 cm⁻¹ was the bending vibration of –OH. The peak of 1153 cm⁻¹ was the stretching vibration mode of C–O bonds. The peaks of 1079 and 1024 cm⁻¹ were bending vibration mode of C–O–C. The peak of 853 cm⁻¹ was skeletal vibrations of pyranoid ring. These results were in agreement with a previous study.
As the ultrasound power increased, the intensity of peaks (1415, 1370, 1153, 1079, 1024 and 853 cm\(^{-1}\)) was decreased, and reached the lowest at 200 W. Moreover, as the ultrasonication treatment time increased, the intensity of characteristic absorption peak decreased initially, but increased after the ultrasonication treatment time exceeded 5 min. These results indicated that the modest sonication treatment (200 W, 5 min) could promote the interaction between maltodextrin and betalains, thereby limiting the vibration of the characteristic functional groups of maltodextrin, especially the groups of –OH and C-O-C that have the ability to form hydrogen bonds. Eslami, et al. [37] also reported that the optimal ultrasound treatment (200 W, 13.7 min) could promote the interactions between alginate-chitosan and the component (Vitamin D\(_3\)), such as hydrogen bonds or electrostatic forces, and increase the microencapsulation efficiency (92.86%) and loading capacity (30.1%) of vitamin D\(_3\).

3.7. DSC of betalains microcapsules

DSC analysis has been studied to examine the thermal characteristics of polymer and complexes [38]. The results of DSC of betalains microcapsules by different ultrasound treatment are shown in Fig. 5. As can be seen in Fig. 5, the onset temperature of maltodextrin was 101.2 °C, which was consistent with that suggested by Silalai and Roos [39]. All the onset temperatures of betalains microcapsules were lower than those of the maltodextrin, possibly because the presence of betalains reduced the onset temperature of complexes. Carolina Otalora, et al. [40] found that the interactions between the polysaccharides and betalains extraction, such as dipole-dipole and hydrogen bonding, could cause modifying the glass transition temperature of polymers. However, the onset temperature (99.8 °C) of betalains microcapsules by ultrasound treatment (200 W, 5 min) was slightly lower than maltodextrin, but higher than control (98.2 °C) and other sonicated samples (<96 °C), which showed that microcapsulation with maltodextrin could obviously increase the thermal stability of betalains, and optimal ultrasound treatment could promote the formation of more stable betalains microcapsules. This result was also similar with the findings of Li, et al. [41] who reported that the preparation of mulberry polyphenols (MP) microcapsules with β-cyclodextrin by optimal ultrasound treatment (450 W, 1.5 h) could promote the thermal stability detected by DSC, which the onset temperature of MP was increased from 94.12 °C to 131.97 °C. In addition, some studies indicated that ultrasound can be used as an efficient method to protect bioactivity compounds entrapped into microsphere [11].

3.8. Antioxidant activity of betalains microcapsules

DPPH free radical scavenging activity of betalains microcapsules by different ultrasound treatment is shown in Fig. 6. The antioxidant activity for ultrasound treatments ranged from 83.82% to 95.32%. The highest antioxidant activity of betalains microcapsules was obtained by 200 W for 5 min, which was increased of 12.24% than control sample. Moreover, the antioxidant activity of betalains microcapsules treated by ultrasound except BM4/5 was significantly higher than control sample (p < 0.05). This phenomenon was due to the ultrasound treatment promote more of betalains content embedding the microsphere, then increased the antioxidant activity of microcapsules, which was consistent with above the encapsulation efficiency of betalains. Moreover, previous studies have found that the low ultrasound treatment could increase the stability of polyphenols-microcapsules and in vitro antioxidant activities [42].

4. Conclusions

Betalains were extracted from the peels of red dragon fruits and subsequently encapsulated with the wall material of maltodextrin prepared by ultrasound assisted freeze drying method in order to promote thermal stability of betalains. The optimal ultrasound treatment for betalains microcapsules was power 200 W for 5 min. Under the optimal condition, the encapsulation efficiency of betalains was above 79%, and the particle size and Zeta potential were 275.46 nm and –29.01 mV, respectively. The betalains microcapsules showed thermal stability and bioactivity as determined by DSC and in vitro antioxidant activity, compared to the without ultrasound treated sample. Therefore, appropriate ultrasound treatment introduced into the microcapsule preparation can improve the stability of betalains, which is expected to make better use of plant natural pigments in the food processing.

CRediT authorship contribution statement

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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