Study on the Antioxidant Capability and Microencapsulation of Opuntia Ficus-indica Anthocyanins

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Abstract: Opuntia ficus-indica contains anthocyanins, flavonoids and other substances rich in biological activity. Among them, anthocyanins have antioxidant, antitumor, anticancer, blood sugar, and blood lipid-lowering effects. Through a DPPH free radical scavenging test, a hydroxyl free radical scavenging test, a superoxide anion scavenging test and a test of the total reducing power to determine the in vitro antioxidant capacity of opuntia ficus-indica anthocyanins, along with the use of complex agglomeration embedding technology, the Opuntia ficus-indica anthocyanins were microencapsulated to achieve protection and sustained release. The results showed that the DPPH scavenging ability, hydroxyl radical scavenging ability, and superoxide anion scavenging ability of Opuntia ficus-indica anthocyanins were significantly higher than those of ascorbic acid, with IC50 values of 0.59 mg/mL, 0.72 mg/mL and 0.80 mg/mL, respectively. Through single-factor combined with response surface test analysis, it was determined that the best conditions for embedding anthocyanins were a core-to-wall ratio of 1.2:1, a wall material concentration of 1.02 g/mL, and a pH of 3.36. Under these conditions, the predicted value of the prickly pear anthocyanin embedding rate was 64.50%. Under the conditions of microencapsulation, the stability of anthocyanins is significantly increased.

1 INTRODUCTION

The color of Opuntia ficus-indica is green or purple. There are thorns inside and outside the skin. The size depends on the variety. The flesh is purple and slightly sour. Opuntia ficus-indica is rich in essential amino acids, a variety of minerals and trace elements, vitamins, polysaccharides, flavonoids and other nutrients (Yahia,2011). Vegetables and fruits are rich in anthocyanins. Because of their special functions and effects, anthocyanins are used in many fields and are most widely used in medicine, food, cosmetics and other industries. There are a large number of studies and records of the functions of anthocyanins, which include antioxidation (Su, 2016), anticancer (Stoner, 2009), and antiaging (Leichtweis, 2019); moreover, they are a rich source of natural antioxidant sugar (Wang, 2019) and can lower blood lipids (Li, 2019), among other effects. Because anthocyanins contain different numbers and positions of hydroxyl groups and different types of binding sugar groups, they exhibit different antioxidant capabilities. The phenolic hydroxyl structure of anthocyanins is easier to oxidize into quinones, which allows anthocyanins to capture free radicals (Kim, 2016). Moreno et al. found that red wine contains mallow pigment and cyanidin, which causes red wine to have antioxidant capacity (Sanchez, 2003). Joseph et al. mainly studied the antioxidant components in the extracts of four Opuntia ficus-indica varieties. The combined flavonoids, ascorbic acid and carotenoids were separated from the extract. Opuntia ficus-indica with purple skin has stronger antioxidant activity than other varieties of fruit

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extracts. The data also show that opuntia ficus-indica is suitable as food (Kuti, 2004).

The principle of microencapsulation technology is to use embedding technology to embed unstable solid, liquid or gaseous substances in tiny closed capsules to achieve protection and controlled release effects. In this technology, the wall material is the material performing the embedding, the core material is the material to be embedded, and microencapsulation is the process of embedding (Zhang, 2015). Because anthocyanins are easily affected by factors such as pH, temperature, and light, their stability is reduced (Tarone, 2020); therefore, embedding anthocyanins through microcapsule technology will increase their stability. There have been studies on the extraction and purification of wild Opuntia ficus-indica anthocyanins, but there have been few studies on their antioxidation and microencapsulation preservation technology. This article hopes to investigate the antioxidant capacity and microencapsulation of Opuntia ficus-indica anthocyanins, to increase the development and utilization of wild Opuntia ficus-indica and to provide a theoretical basis for the follow-up in-depth study of anthocyanins in Opuntia ficus-indica.

2 MATERIALSANDMETHODS

2.1 Chemicals and Solvents

Opuntia ficus-indica (purchased in Hainan), anhydrous ethanol, sodium acetate, potassium chloride, concentrated hydrochloric acid, macroporous resin HPD-100, sodium acetate, potassium chloride, trichloroacetic acid, glacial acetic acid, ethyl acetate, 30% hydrogen peroxide, ferrous sulfate, salicylic acid, potassium ferricyanide, pyrogallol, hydroxymethyl aminomethane, formic acid, ascorbic acid (Tianjin Guangfu Technology Development Limited company), DPPH (West Asia Chemical Limited company), gelatin, an gum arabic (Shanghai Xiangrui Biological Technology Limited company)

2.2 Study on the Antioxidant Ability of Cactus Fruit Anthocyanins in Vitro

2.2.1 Extraction of Opuntia Ficus-indica Anthocyanin

The frozen Opuntia ficus-indica was thawed in a water bath, homogenized with a juicer, and frozen in an ultralow-temperature freezer at -80℃ until the sample became solid, after which it was placed in a vacuum freeze dryer. Two grams of powder was added to a 100-mL Erlenmeyer flask, and 50% ethanol at pH=2 (concentrated hydrochloric acid for pH adjustment) at a material-to-liquid ratio of 1:25 g/mL was added. A stir bar was added to the flask, after which the flask was sealed with a sealing film and placed in a magnetic stirrer at 60 ℃for 70 minutes of extraction. After that, it quickly entered the cooling state and was centrifuged for 15 min (the centrifuge speed was 4500 r/min). The supernatant was concentrated under reduced pressure at 50 ℃ and freeze-dried for 48 h to obtain the Opuntia ficus-indica anthocyanin extract.

To determine the Opuntia ficus-indica anthocyanin content, 1 ml of the extracted supernatant was placed in a 25-mL volumetric flask, 10 mL each of pH=1 and pH=4.5 buffer solutions were added to constant volume, and the solution was allowed to stand for 60 minutes. The absorbance was measured at 530 nm with an ultraviolet spectrophotometer. The content of Opuntia ficus-indica anthocyanin was calculated by the pH difference method using the following calculation formula (Ryu, 2018):

$$= \frac{A \times MW \times DF \times V}{\beta \times L \times m} \times 100 \text{（mg/100g）} \quad (1)$$

here: A=(A530-A700) pH1.0MW-The molecular weight of Bluebonnet-3-glucoside is 449.2 g/mol; -(A530-A700)pH4.5;

β-The molar extinction coefficient is 26900 L•mol-1•cm-1;

DF-The dilution factor; L-The optical path (cm);

V-Extract volume (mL); m-Raw material mass (g)

2.2.2 Determination of the Scavenging Capacity of DPPH Free Radicals

This article uses the Abdel (Abdel, 2018) method with slight modification. Two milliliters of different mass concentrations of Opuntia ficus-indica anthocyanin solution was added to a test tube with a stopper, and 0.2 mmol/L DPPH solution was added. The solution was shaken well and put in a dark place for 60 minutes. The blank group was treated without anthocyanin solution to remove the influence of sample color. The experimental blank control is the sample without DPPH. The scavenging rate of DPPH free radicals in the sample was calculated using ascorbic acid. After a controlled experiment was performed, the absorbance at a wavelength of 517 nm was measured:

$$\text{Clearance rate/\%}=\left(1-\frac{A_1-A_2}{A_0}\right)\times 100\ \% \quad (2)$$

where A_0 (517 nm)-is the absorbance of the blank tube solution without sample solution;

A_1 (517 nm)-is the absorbance of the reaction solution;

A_2 (517 nm)-is the absorbance of the control tube without DPPH solution

2.2.3 Determination of Hydroxyl Radical Scavenging Ability

This study was performed using a slightly modified protocol based on Cásedas(Sang, 2015, Cásedas, 2017): 1.0 mL of H_2O_2 (8.8 mmol/L), 1.0 mL of FeSO4 (10 mmol/L), and 1.0 mL of 10 mmol/L salicylic acid-ethanol solution were added to test tube, followed by 1.0 mL of sample solution and 1.0 mL of H2O2 to react in a 37℃ water bath for 0.5 h. Distilled water was used as a blank test and ascorbic acid was used as a control test. The absorbance was measured at 510 nm, and the clearance rate was calculated:

$$\text{Clearance rate/\%}=\left(1-\frac{A_1-A_2}{A_0}\right)\times 100\ \% \quad (3)$$

where A_0 (510 nm)-is the absorbance of the blank tube solution without sample solution;

A_1 (510 nm)-is the absorbance of the reaction solution;

A_2 (510 nm)-is the absorbance of the control group (containing 1.0 mL of H2O2 and 1.0 mL of sample solution)

2.2.4 Superoxide Anion Free Radical Scavenging Ability

In this paper, according to the method of Chen(Damar, 2012, Chen, 2020), 50 mmol/L of Tris-HCL buffer (4.5 mL, pH 8.2) and 4.5 mL of distilled water were added to a dry test tube, mixed well, and kept warm for 20 minutes (25 ℃), after which 1.0 mL of sample solution, 3.5 mL of distilled water, and 0.3 mL of the pyrogallol solution (concentration 3 mmol/L) preheated at 25 ℃ were added, and the solution was shaken quickly until it was uniform. The solution was then transferred to a cuvette, and the absorbance of the solution was measured at 320 nm every 0.5 min, stopping after 5 min. The increase in absorbance A0 (320 nm) within 1 min was calculated in the linear range. Ascorbic acid was used as a control experiment. The clearance rate can be calculated as follows:

$$\text{Clearance rate/\%}=\frac{A_0-A}{A_0}\times 100\ \% \quad (4)$$

where A_0 (320 nm)-is the autooxidation rate of pyrogallol.

A (320 nm)-represents the autooxidation rate of pyrogallol after adding the sample solution.

2.2.5 Determination of Total Reducing Power

Tsai (Tsai, 2002) and other methods were referenced to determine the reducing ability. Using the Prussian blue method, 1.0 mL aliquots of samples with different concentrations were measured and placed in 5 dry test tubes, and 3.0 mL of 0.2 mol/L phosphate buffer (pH=6.6) and 2.5 mL of hexacyanoferric acid were added in sequence. Potassium solution (concentration of 1%) was kept in a water bath (50 °C) for 20 minutes, removed and quickly cooled. Then, 2.5 mL of 10% trichloroacetic acid was added and centrifuged for 4500r,10 minutes, and the supernatant was extracted. Then, 3.0 mL of distilled water and 0.5 mL of 0.1% ferric chloride solution were added in sequence. After mixing them thoroughly, they were allowed to stand at room temperature for 10 min. Ascorbic acid was used as a control test, and then the absorbance was measured at 700 nm. The reduction ability of Opuntia ficus-indica anthocyanins was judged according to the absorbance value. The stronger the absorbance value, the greater the reduction ability(Cerezo, 2010).

2.3 Study on Microcapsules of Prickly Pear Anthocyanins

Weigh a certain amount of gum arabic and gelatin to dissolve them, then weigh a certain amount of anthocyanins and mix them with gelatin, homogenize and emulsify at a high speed for 3 min, while slowly adding the gum arabic solution, and stir with a cantilever agitator for 40 min (300 r~450 r), measure the absorbance value A, add 10% acetic acid solution dropwise, adjust the pH value to 4.0, stir at 50℃ for 1 h, cool to below 15℃, adjust pH 8 with 10% sodium hydroxide solution -9, add 4 mL 10% tannic acid, stir at low temperature for 3 h, stand still for 10 h, pour it into a centrifuge tube and centrifuge at 4500 r/min for 15 min, take the supernatant and measure its absorbance B.

Preparation of anthocyanin microcapsules and calculation of the embedding rate;

Certain amounts of gum arabic and gelatin were weighed to dissolve them, and then certain amounts of anthocyanins and gelatin were weighed and mixed homogeneously. Then, gum arabic was slowly added while stirring, the pH was adjusted with acetic acid, and the mixture was stirred at high speed at 50 ℃. After cooling, the pH value was adjusted again with sodium hydroxide and solidified with tannic acid. After standing for 10 hours, a solid powder was obtained by freeze drying.

The embedding rate is an important indicator used to evaluate the quality of microcapsules. The higher the embedding rate, the better the embedding effect, and the less exposed the core material, which increases the stability of the product, which can then be stored for a long time.

$$E=\frac{(A-B)}{A}\times 100\% \quad (5)$$

Where: A-The absorbance value of the solution before embedding

B-The absorbance value of the solution after embedding

E-The embedding rate (%)

The influence of the core-wall ratio on the process of anthocyanin microcapsules

Accurately weigh 5 portions of gelatin and gum arabic 0.5 g each, put them into a beaker containing 50 ml of distilled water and dissolve them in a water bath at 50°C, accurately weigh out 0.3 g, 0.5 g, 1 g, 2 g, and 3 g of anthocyanins, and wait for the gelatin. After dissolving the anthocyanin and gum arabic, put the anthocyanins into the gelatin solution for high-speed homogenization and emulsification, prepare microcapsules according to the above method, and study the core-to-wall ratio (3:1, 2:1, 1:1, 1:2, 1:3) Influence on the process of anthocyanin microcapsules.

2.3.1 The Influence of Wall Material Concentration on the Process of Anthocyanin Microcapsules

Accurately weigh 0.25 g, 0.475 g, 0.5 g, 0.75 g, and 1 g of gelatin and gum arabic, respectively, and dissolve them in a beaker containing 50 ml of distilled water in a water bath at 50°C. Weigh accurately 5 parts of anthocyanins, 1 g each, According to the above method to microcapsule, study the influence of different wall material concentration (0.5%, 0.75%, 1.0%, 1.5%, 2.0%) on the process of anthocyanin microcapsule.

2.3.2 The Influence of pH Value on the Process of Anthocyanin Microencapsulation

Accurately weigh 5 parts of gelatin and gum arabic 0.5 g each, put them into a beaker containing 50 ml of distilled water and dissolve them in a water bath at 50°C, accurately weigh 5 parts of anthocyanins 1 g each, prepare microcapsules according to the above method, and add them dropwise The pH value of 10% acetic acid solution was adjusted to 3.0, 3.5, 4.0, 4.5, 5.0, respectively, and the influence of pH value on the process of anthocyanin microcapsules was studied.

2.3.3 Box-Behnken Experimental Design

According to the principle of the Box-Behnken experimental design and the single-factor results, three factors that had a significant embedding rate of anthocyanin microencapsulation were selected: core-wall ratio X1, wall material concentration X2, pH X3 is the influencing factor, and the response surface test design with 3 factors and 3 levels is carried out. Table1shows the test factors and levels.

Table 1: Independent variables and levels for optimization

Level	Factor		
	X_1: Core wall ratio	X_2: Wall material concentration	X_3: pH
-1	2:1	0.75	3.5
0	1:1	1	4.0
1	1:2	1.5	4.5

2.3.4 Stability of Microcapsules

The effect of light on the stability of microcapsules

Two grams of Opuntia ficus-indica anthocyanin and Opuntia ficus-indica anthocyanin microcapsules were accurately weighed, dissolved in 50 mL of pH 3.0 citric acid-sodium citrate buffer solution, and placed under natural light at 0, 2, 4, and 6. Samples were taken at 8 and 10 days, and the absorbance was measured at 530 nm.

2.3.5 The Effect of Light on the Stability of Microcapsules

Fifty-two grams of Opuntia ficus-indica anthocyanin and Opuntia ficus-indica anthocyanin microcapsules were accurately weighed, dissolved in 50 mL of pH 3.0 citric acid-sodium citrate buffer solution, and placed in 20, 40, 60, 80, and 100 °C water baths. The absorbance values were measured after 3 h.

2.3.6 Stability of Microcapsules

The single-factor application SPSS 19.0 software was used to analyze the variance of the data, OriginPro8.5 was used for graphing, and the response surface was analyzed and graphed using Design-Expert 8.0.6 software.

3 RESULTS AND DISCUSSION

3.1 Antioxidant Ability Measurement Results

3.1.1 DPPH Free Radical Scavenging Ability Measurement Results

The lone pair electrons of DPPH free radicals have strong absorption at 517 nm. When free radical scavengers are present in the reaction system, the absorption will gradually disappear. This is because the free radical scavengers pair with DPPH single electrons. The relationship is proportional, so the ability of free radical scavengers can be measured by the reduced absorbance value(Corrales, 2019). Fig. 1 shows that the DPPH free radical scavenging ability of Opuntia ficus-indica anthocyanins is significantly higher than that of ascorbic acid at 0.2-0.6 mg/mL. The clearance rate reaches its maximum at 0.8 mg/mL, and the scavenging ability of ascorbic acid DPPH free radicals is higher than that of anthocyanins at 1.0 mg/mL.

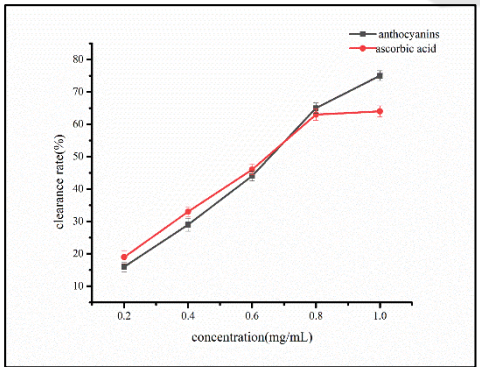


Figure 1: Determination of DPPH free radical scavenging rates.

3.1.2 Measurement Results of the Hydroxyl Radical Scavenging Ability

Fig. 2 shows that the scavenging ability of Opuntia ficus-indica anthocyanins was positively correlated with the scavenging ability at 0.2-0.4 mg/mL. The scavenging ability was significantly higher than that of ascorbic acid at the same concentration. Thereafter, the scavenging ability of anthocyanins was also stronger than that of ascorbic acid. This is because anthocyanins have an aromatic ring structure, so the provided hydrogen can react with hydroxyl radicals to generate inert substances. The hydrogen peroxide produced by ascorbic acid in its self-oxidation process can promote the generation of hydroxyl groups in the reaction, and the ability to scavenge hydroxyl radicals is low(Szymanowska, 2018). According to statistical analysis, the scavenging ability of anthocyanins on hydroxyl free radicals was significantly higher than that of ascorbic acid (P<0.05).

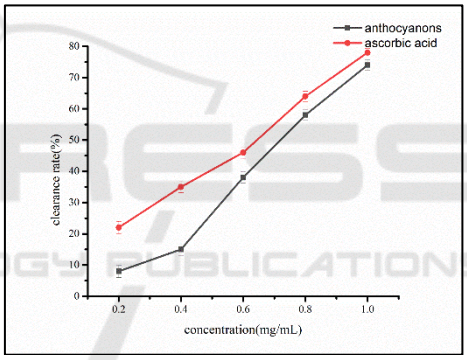


Figure 2: Determination of hydroxyl radical scavenging rates.

3.1.3 Superoxide Anion Free Radical Scavenging Capacity Results

Fig. 3 shows that Opuntia ficus-indica anthocyanins scavenge superoxide anions. Within a certain concentration range, the scavenging ability of Opuntia ficus-indica anthocyanins on superoxide anions increases with increasing concentration. The same concentration of anthocyanins has the effect of scavenging superoxide anions. The clearance of anthocyanins is significantly higher, and the clearance rate of anthocyanins is 1.23 times that of ascorbic acid at 0.8 mg/mL. The clearance rate of Opuntia ficus-indica anthocyanins reached 1.0 mg/mL.

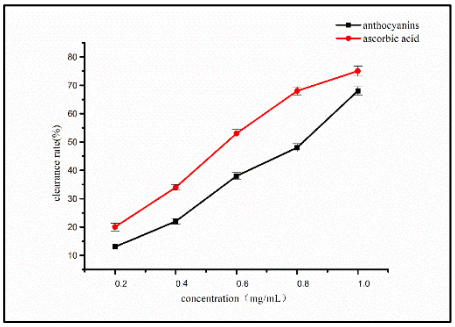


Figure 3: Determination of superoxide anion free radical scavenging rates.

3.1.4 Superoxide Anion Free Radical Scavenging Capacity Results

Fig. 4 shows that the total reducing power of Opuntia ficus-indica anthocyanins and ascorbic acid both show increasing trends, but the total reducing power of the same concentration of anthocyanins is lower than that of the same concentration of ascorbic acid, and the total reducing power of anthocyanins is lower than that of ascorbic acid. This may be due to the reaction of Opuntia ficus-indica anthocyanins with oxidants and their reducing power, but because the number of hydroxyl groups of anthocyanins was greater than ascorbic acid, the total reducing power is still slightly lower than that of ascorbic acid(Grobelna, 2019).

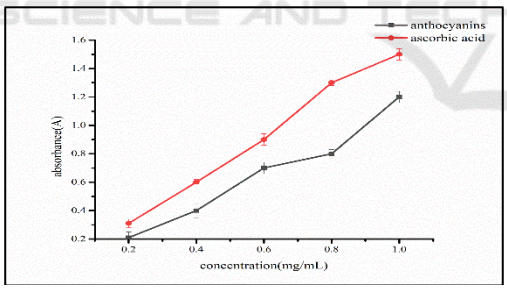


Figure 4: Determination of total reduction ability.

3.1.5 Comparison of the IC50 Values of Opuntia Ficus-indica Anthocyanin and Ascorbic

Table 2: The IC_{50} value of anthocyanin and ascorbic acid ascorbic acid antioxidant capacity

Table 2: The IC_{50} value of anthocyanin and ascorbic acid ascorbic acid antioxidant capacity.

name	DPPH clearance (mg/mL)	Hydroxyl radical scavenging ability	Superoxide anion scavenging capacity (mg/mL)
		(mg/mL)	
Anthocyanin	0.55	0.55	0.53
Ascorbic acid	0.59	0.72	0.80

3.2 Single-factor Test Results

3.2.1 Determination of the Optimal Core-wall Ratio for Anthocyanin Microencapsulation

While keeping other conditions unchanged, the different core-wall ratios that affect the embedding rate of the microcapsules were studied, and the results are shown in Fig. 5. The embedding rate first increased and then decreased with the wall-core ratio. When the wall-core ratio was 1:1, The embedding rate of microcapsules reached a maximum of 63.7%, and there was a significant difference between the sexes of each group (P<0.05). The results are the same as Gao Yan's optimal core-to-wall ratio in the preparation of capsaicin microcapsules by the complex coacervation method (Chen, 2018).

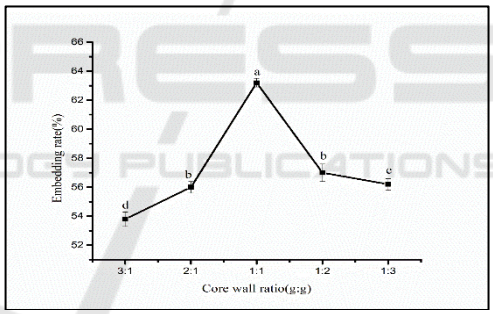


Figure 5: The relationship between the wall core ratio and the embedding rate.

3.2.2 Determination of the Optimal Wall Material Concentration for Anthocyanin Microencapsulation

Under other conditions unchanged, by changing the concentrations of the gelatin and gum arabic solutions, the influence of different wall material concentrations on the embedding rate of microcapsules was investigated. When the wall material concentration is large, a cohesion reaction will occur between the wall materials. As a result, empty sacs are generated, and the embedding rate is reduced; when the wall material concentration is small, the core material cannot be completely embedded (Meng, 2019), as shown in Fig. 6. As shown in the figure, the wall material concentration

was 1.0%, the embedding rate reached a maximum of 64.5%, and the difference between each concentration was significant (P<0.05).

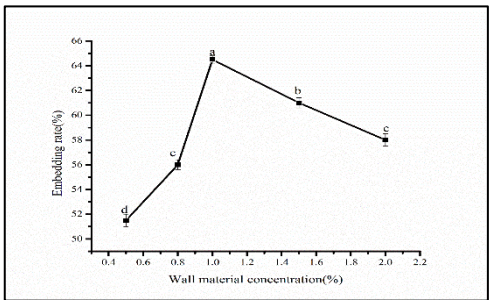


Figure 6: The relationship between wall material concentration and embedding rate

3.2.3 Determination of the Optimal pH Value for Anthocyanin Microencapsulation

Keeping other conditions unchanged, by changing the pH value, the effect of different pH values on the embedding rate of microcapsules was investigated, and the results are shown in Fig. 7. There were significant differences between the groups (P<0.05). When the pH was 4, the embedding rate reached its maximum of 63.2%. This may be because at pH 4, gelatin and gum arabic have a better charge–chemical balance. At other pH values, the polymer formed between the wall materials will change, which will reduce the affinity with anthocyanins, which will then reduce the embedding rate(Wang, 2016). This was the same as the optimal pH value in the preparation process of VE microcapsules described by Feng Yan et al.

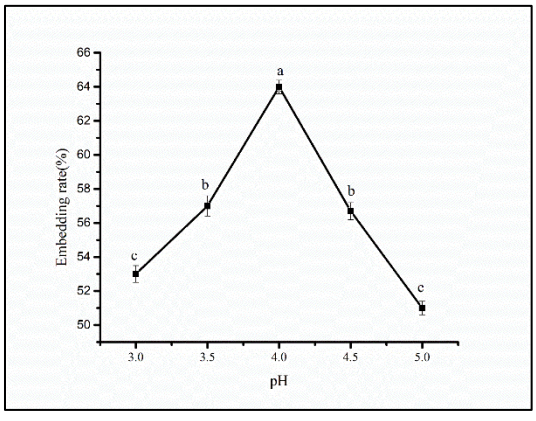


Figure 7: The relationship between pH and embedding rate.

3.3 Response Surface Test Design and Results

3.3.1 Response Surface Test Model Establishment and Results

According to the design principle of the Box-Behnken test, based on the above single-factor test, the wall-to-core ratio, the wall material concentration and the pH value were used as the three main influencing factors, and the anthocyanin microcapsules were optimized through response surface experiments (three factors and three levels) to determine the embedding rate (Y). According to statistical regulations, the regression fit various regression coefficients. Table 3 shows the specific experimental design and results.

Table 3: Box-Behnken design with the observed responses.

Numbering	X_1Core wall ratio /(g:g)	X_2Wall material concentration/(%)	X_3pH	Embedding rate/(%)
1	1:2	1	4.5	55.03
2	2:1	0.75	4.0	61.9
3	1:1	0.75	4.5	51.86
4	2:1	1.5	4.0	53.57
5	1:2	1.5	4.0	60.21
6	1:1	1.5	4.5	51.01
7	2:1	1	3.5	57.02
8	1:1	1	4.0	64.11
9	1:1	1	4.0	64.34
10	1:1	1	4.0	64.33
11	1:1	1.5	3.5	56.00
12	1:2	1	3.5	60.55
13	1:1	1	4.0	64.01
14	1:1	0.75	3.5	54.81
15	1:1	1	4.0	64.45
16	1:2	0.75	4.0	55.87
17	2:1	1	4.5	54.86

Design Expert 8.0.6 statistical software was used to perform regression fitting on the test data in Table 3 through stepwise regression and to obtain a quadratic polynomial regression model of 3 factors for the anthocyanin microcapsule embedding rate:

$$Y=64.25+0.53X_1-0.47X_2-1.95X_3+3.19X_1X_2-0.84X_1X_3-0.51X_2X_3-1.45X_{12}-4.89X_{22}+5.94X_{32}$$

Analysis of variance was performed on the model, and the results are shown in Table 4.

Table 4: ANOVA for the regression model

Source of variance	Sum of squares	Degree of freedom	Mean square	F value	p value	Significance
model	359.48	9	39.94	73.05	<0.0001	**
X_1	2.24	1	2.24	4.09	0.0828	
X_2	1.74	1	1.74	3.18	<0.1177	
X_3	30.50	1	30.50	55.78	0.0001	**
X_1X_2	40.64	1	40.64	74.33	<0.0001	**
X_1X_3	2.82	1	2.82	5.16	0.0573	
X_2X_3	1.04	1	1.04	1.90	0.2102	
X_1^2	8.83	1	8.83	16.14	<0.0051	**
X_2^2	100.80	1	100.80	184.35	<0.0001	**
X_3^2	148.33	1	148.33	271.28	<0.0001	**
Residual	3.83	7	0.55			
Lack of fit	3.70	3	1.23	37.42	0.0622	
Errors	0.13	4	0.033			
Total deviation	363.31	16				
R^2=0.9895						

By comparing the absolute value of the primary coefficient (from the multiple regression equation), the order of factors affecting the embedding of Opuntia ficus-indica anthocyanin microcapsules is pH value > core wall ratio > wall material concentration. From the results of the analysis of variance shown in Table 3, we can conclude that the significance level of the model was p=0.0022<0.05, meaning that the regression variance was significantly different; the coefficient of determination was R2=0.9895, meaning that the model was highly reliable; the proposed item was 0.0622, and the lack of fit item was not significant. This shows that the fitting model composed of the pH value, the wall-to-core ratio and the wall material concentration can be used as a prediction and analysis model for prickly pear anthocyanin embedding.

3.3.2 Response Surface Experiment Results

Fig. 8 shows the interaction of the core-wall ratio and pH on the embedding rate of Opuntia ficus-indica anthocyanin microcapsules. As shown in the contour map, the shape of the contour was close to an ellipse, indicating that the core-wall ratio and the pH value interacted strongly. Moreover, based on the slope of the response surface, the interaction between the core-wall ratio and the pH value was obvious. Because the ellipse or circle in the figure was in a closed state, it was the largest in this range.

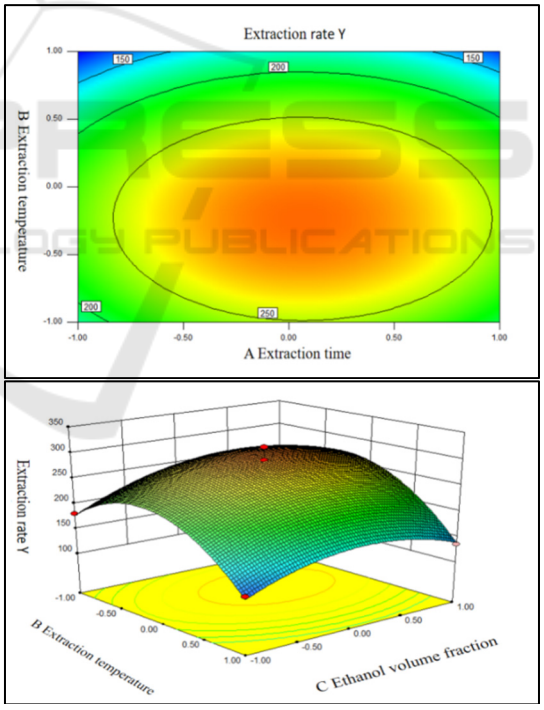


Figure 8: Response of contour plots and surface plots of the extraction yield under the interaction of the wall core ratio and the pH.

Fig. 9 shows the interaction between the core-wall ratio and the wall material concentration on the embedding rate of Opuntia ficus-indica anthocyanin microcapsules. As shown in the contour map, the

shape of the contour was elliptical, indicating that the core-wall ratio and the wall material concentration have a strong interaction. From the steep slope of the response surface, the interaction between the core wall ratio and the wall material concentration was obvious. Because the ellipse or circle in the figure was in a closed state, it was the largest in this range.

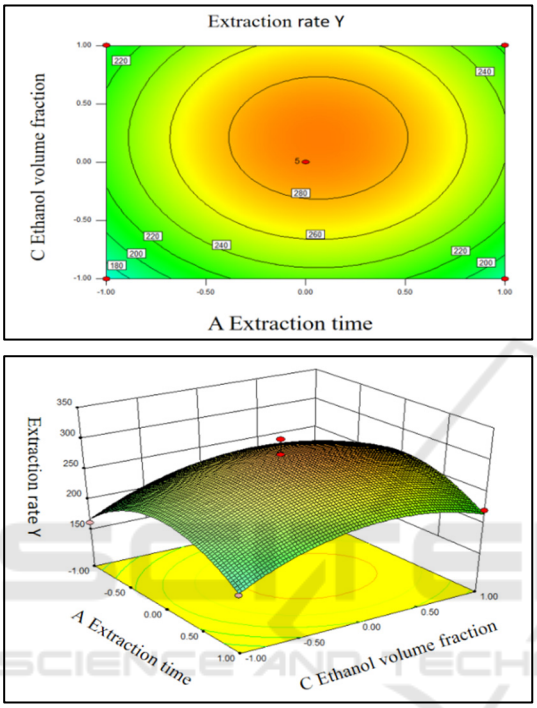
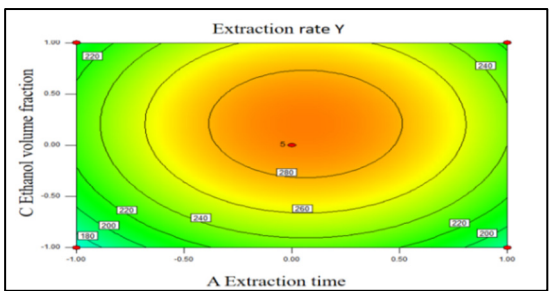


Figure 9: Response of contour plots and surface plots of the extraction yield under the interaction of the wall core ratio and the wall material concentration amount.

Fig. 10 shows the interaction of the wall material concentration and the pH on the embedding rate of Opuntia ficus-indica anthocyanin microcapsules. As shown in the contour map, the shape of the contour was circular, indicating that the two factors of wall material concentration and pH value did not have a strong interaction.



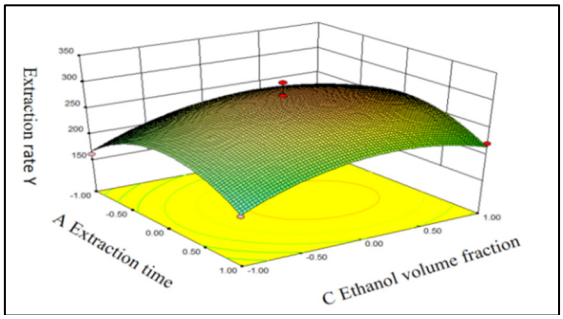


Figure 10: Responses of contour plots and surface plots of the extraction yield under the interaction of wall material concentration and pH.

From the analysis results of the Box-Behnken design model, the optimized process parameters of Opuntia ficus-indica anthocyanin microcapsules are a core-to-wall ratio of 1.2:1, a wall material concentration of 1.02%, and a pH of 3.36. Under these conditions, the predicted value of the prickly pear anthocyanin embedding rate was 64.50%. To verify the reliability of the Box-Behnken model, three experiments performed according to the optimal process of the model optimization revealed that the embedding rate of the Opuntia ficus-indica anthocyanin microencapsulation was 65.12%, demonstrating that the model optimized the Opuntia ficus-indica anthocyanin microcapsules. The process has certain application value.

3.4 Stability Analysis of Microcapsules

3.4.1 Analysis of the Stability of Microcapsules under Light

As shown in Fig. 11, as time increases, the absorbance values of the anthocyanins before and after the microcapsules show a downward trend, but the anthocyanins after microencapsulation decreased more slowly than the anthocyanins before microencapsulation. This finding indicates that after the microcapsule wall material embedded the Opuntia ficus-indica anthocyanin, the influence of light on the anthocyanin was reduced, thereby improving the stability of the anthocyanin.

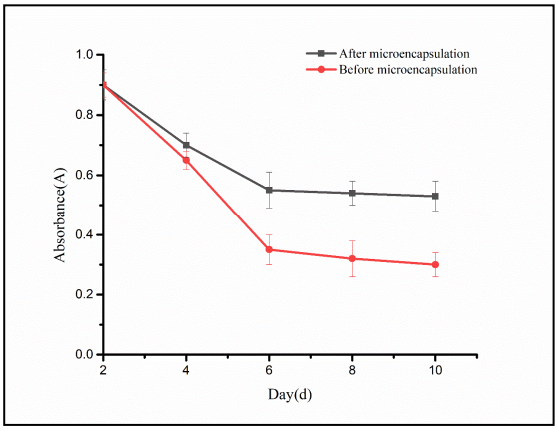


Figure 11: Relationship between light and microcapsule stability.

3.4.2 Analysis of the Stability of Microcapsules under Light

As shown in Fig. 12, within 3 h, as the temperature increased, the absorbance of anthocyanins gradually decreased, but it was clear that the decrease in absorbance of anthocyanins after microcapsules were formed was lower than that before microcapsules were formed. At 20 ºC, the absorbance values of the anthocyanins before and after microencapsulation were the same, indicating that the anthocyanins were stable at 20 °C, and then as the temperature increased, the absorbance values of the anthocyanins before and after microencapsulation had significantly different trends. These results show that after the Opuntia ficus-indica anthocyanins were embedded in the microcapsule wall material, the influence of temperature on the anthocyanins was reduced, thereby improving the stability of the anthocyanins.

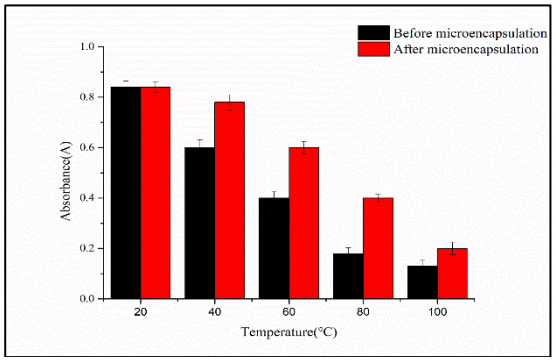


Figure 12: Relationship between temperature and microcapsule stability.

4 CONCLUSIONS

The antioxidant capacity in vitro of Opuntia ficus-indica anthocyanins was determined, and the results showed that the IC50 values of Opuntia ficus-indica anthocyanin and ascorbic acid were 0.55 mg/mL and 0.59 mg/mL for DPPH scavenging capacity, the IC50 values of the hydroxyl radical scavenging capacity were 0.55 mg/mL and 0.72 mg/mL, respectively, and the IC50 values of the superoxide anion scavenging capacity were 0.53 mg/mL and 0.80 mg/mL, respectively. Taken together, Opuntia ficus-indica anthocyanins were determined to have strong antioxidant capacity in vitro.

The process of optimizing the microencapsulation of Opuntia ficus-indica anthocyanins was determined by the compound coacervation method. The best process was a core-to-wall ratio of 1.2:1, a wall material concentration of 1.02%, and a pH of 3.36. Under these conditions, the predicted value of the prickly pear anthocyanin embedding rate is 64.50%. By comparing the effects of light and temperature on the stability of the anthocyanins before and after the microcapsules, the results show that the absorbance value of the anthocyanins after 6 days of light is 1.6 times that before and after embedding at 60℃. The anthocyanin stability is 1.4 times that before embedding. Taken together, the stability of anthocyanins is significantly increased after microencapsulation.

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