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Microencapsulation of anthocyanins from red cabbage

[1*]Hongmei, Z. and [2]Meng, Z.

[1]*College of Food Science, Shanxi Normal University, Linfen, 041004, PR China*
[2]*College of Life Science, Shanxi Normal University, Linfen, 041004, PR China*

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Abstract

Natural anthocyanin from red cabbage is unstable in food industry. In this study, the response surface methodology (RSM) was used to optimize the microcapsule technology for anthocyanin and microencapsulated anthocyanins stability was also determined. Microcapsule technology was investigated with spray drying technology using anthocyanin freeze-dried in vacuum as core material and the maltodextrin (16-20DE) and gum acacia as wall materials. Optimized encapsulation rate conditions were as follows: relative content of maltodextrin in wall material was 54.48% and the ratios of core material to wall material (RC) was 7.07% and solid contents was 20.33%, respectively. Microencapsulated anthocyanin exhibited better stability when it was exposed to different temperatures compared with the control group.

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Introduction

Natural pigments have been received intensively studies, due to their safety, nontoxicity and biological activity (Timberlake and Henry, 1986; Delgado-Vargas *et al*., 2000). Anthocyanin is a kind of organic pigment; it is abundant and possess very good coloring ability (Gomes *et al*., 2006; Rimpapa, Toromanovic *et al.*, 2007), and has been widely used in food industry (Francis, 1989). Previous studies demonstrated the beneficial role of anthocyanin in human health, which might be attribute to its antioxidant abilities (Wang and Stoner, 2008; Galvano *et al*., 2009; Gonzalez-Barrio *et al*., 2010). Because of the presence of unsaturated bonds in the molecular structure, anthocyanin is easily oxidized under native conditions, including light, heat and pH, all of which may limit its application. Previous studies were mainly focused on the factors that could affect the stability and color of anthocyanin that from different origins, including red cabbage (Dorota Walkowiak-Tomczak, 2007) and Isabel grapes (Bordignon-Luiz *et al*., 2007).

Red cabbage belongs to the family of Crucifer. Red cabbage has been extensively studied, due to its attractive color and potential physiological functions, which might from the presence of anthocyanin (Dorota Walkowiak-Tomczak, 2007; McDougall *et al.*, 2007). Anthocyanin is the major pigment of red cabbage (Tanchev and Timberlake, 1969), and its component is constructed of cyanidin-3-diglucoside-5-glucoside 'cores', which are non-acetylated, mono-acetylated or di-acetylated with p-coumaric, caffeic, ferulic and sinapic acids. Previous studies extracted anthocyanin using high pressure CO_2 from red cabbage (Zhenzhen *et al*., 2010) and also explored its stability during storage (Dorota Walkowiak-Tomczak, 2007; McDougall *et al*., 2007); however no research focused on the anthocyanin microcapsulation.

Compared with artificial pigment, natural anthocyanin is instable, therefore, a novel and inexpensive technology that can improve its stability is necessary for the food industry. Embedding is a good technology to widen its application through avoiding degradation during transportation, storage and usage. Microencapsulation is one of effective embedding methods, and has been widely used in the food industry (Shahidi and Han, 1993); there is still no report regarding the anthocyanin encapsulation from red cabbage. On current study, we encapsulated and optimized freeze-dried anthocyanin using spray drying technology. We determined the optimized conditions for anthocyanin encapsulation and also measured the stability of products.

Materials and Methods

Plant materials

Red cabbages were from in Linfen County, Shanxi Province, China. After harvested, cabbages were washed with running tap water, and cut as 0.2×0.2 cm cubes and were frozen at -20°C for further use.

Anthocyanin extraction

Anthocyanins were extracted using ultrasonicator (model: KQ-250DB, Kunshan Ultrasonic Limited

*Corresponding author.
Email: *zhuhongmei1976@163.com*
Tel: 86-03572051247

Company, Jiangsu, China) according to established protocol in our lab. Briefly, red cabbages were broken down in 0.5 M acidified ethanol at 40 W, and the ratio of materials to solvent was 1:20 and extraction time was 60 min at 60°C. Extracted solution was centrifuged at 4000 rpm for 15 min and supernatant was concentrated using rotary evaporator.

Anthocynins purification and preparation

Extracted anthocyanins were purified according to previous report, using AB-8 macroporous resin absorption method (Hongmei *et al*., 2006). Concentrated anthocyanins were added to chromatograph column stuffed with AB-8 macroporous resin. Distilled water was used to remove impurities, including soluble sugar, mineral and protein, until distilled water become clean. Then anthocyanins was eluted with ethanol and ethanol was removed using low pressure (0.1 MPa) to get concentrated anthocyanin liquid. Finally, those liquid was further dried for 24 hours to get the anthocyanins powder.

Determination of anthocyanins contents

Total anthocyanins was quantified by a pH-differ-ential method according to previous report with small modifications (Fuleki and Francis, 1967). Two pigment samples (1 ml) were diluted 10 times respectively, using different diluted buffers (pH 1.0 and pH4.5). The absorption value of each sample was measured at different wavelength (546 nm and 700 nm, respectively), and the values were defined as $A_{546nmp}H1.0$, $A_{546nmp}H4.5$, $A_{700nmp}H4.5$ and $A_{700nmp}H4.5$. All absorbance values were obtained according to the Lambert-beer law against different-pH buffers as a blank. The absorption of anthocyanins was calculated according to the following equation: $A=(A_{546nmp}H1.0-A_{700nmp}H1.0)-(A_{546nmp}H4.5-A_{700nmp}H4.5)$. Anthocyanin contents were calculated as follows: anthocyanin contents (mg/g)=$A/\varepsilon l\times M_w\times$Dilution Factor$\times V/m$, where *A* was absorbance value, ε was molar absorbance, l was length of tube, M_w was molecular weight of cyanidin-3-glucoside, *V* (mL) was volume of extraction, and *m* (g) was weight of red cabbage. Calculation of anthocyanin contents were based on cyanidin-3-glucoside with the molecular weight of 445.2 and molar absorbance of 29600 (KIrca and Cemeroglu, 2003).

Microencapsulation preparation

RSM was used to determine the optimum microcapsule conditions. Content of malt dextrin (16-20DE) in wall material containing malt dextrin and gum acacia (CM), ratio of core material to wall material (RC) and solid content were obtained. For evaluating the values of these 3 factors, 3 levels were selected for each independent variable, coded as A, B and C, and Box-Behnken design was used for getting the best microcapsule design. Independent variables and levels were designed as table 1a. Optimal results were obtained through detecting embed rate as response values and dependent variable. The wall and core materials were mixed with 50°C distilled water firstly, and then fed into spray drier (Model: L-117, Laiheng Limited Liability Company, Peking, China) working at 160°C inlet and at 90±5°C outlet, with the speed of 10 ml/min.

Evaluate of embedding rate of microencapsulation

Microencapsulation efficiency was evaluated using embedding rate according to previous report (Xintian *et al.*, 2009). Total 1.0 g microencapsules powder was dissolved in 20 ml ethanol for the above layer pigment before pigment content of supernatant separating through centrifugation was measured using method of 2.4. The amount anthocyanin residues on the surface of microcapsule were measured as (S_0). Microencapsules (1.0 g) was ground and dissolved in 20 ml ethanol, and then centrifuged at 12000 g. The supernatant was removed and the precipitants were dissolved. After 3 times repeats, all the supernatants were combined for next use. The anthocyanin content of mixture was measured as G_0. The embedding rate (R_e) was calculated as follows: $R_e=(1-S_0/G_0)\times100\%$

Determine effect of temperature on microcapsule stability

Microcapsule pigment powder and freezing pigment powder were put in brown bottle at temperature of 20°C, 30°C, and 40°C respectively, to analyze the effect of temperatures on stability of anthocyanin. Total 1.0 g microcapsule at different temperature was weighted and repeatedly extracted in ethanol at pH2.0 until the supernatant became colorless. The anthocyanin contents of filtrate were determined according to 2.4 methods. The reaction rate constant of thermal degradation k was determined by the Arrhenius equation (KIrca and Cemeroglu, 2003; Reyes and Cisneros-Zevallos, 2007): $\ln(C_t/C_0)=-kt$, the thermal degradation half-life $t_{1/2}=0.693/k$, thermal degradation rate of $P=(1-C_t/C_0)\times100$, in which C_t was red cabbage pigment concentration at t time, C_0 was the initial concentration of the purple cabbage pigment and t was the reaction time.

Microcapsule structure analysis

The outside structure of microcapsules was observed by both light microscope (DM1000, Leica,

Table 1a. Results of Response Surface test for anthocyain microcapsule of red cabbage

No.	CM /% A	RC /% B	Solid content/% C	Embedding rate /%
1	40.00(-1)	6.00(-1)	20.00(0)	91.55±0.21
2	60.00(1)	6.00(-1)	20.00(0)	93.70±0.13
3	40.00(-1)	8.00(1)	20.00(0)	93.10±0.31
4	60.00(1)	8.00(1)	20.00(0)	94.3±0.29
5	40.00(-1)	7.00(0)	15.00(-1)	94.1±0.33
6	60.00(1)	7.00(0)	15.00(-1)	94.9±0.53
7	40.00(-1)	7.00(0)	25.00(1)	94.7±0.27
8	60.00(1)	7.00(0)	25.00(1)	95.6±0.14
9	50.00(0)	6.00(-1)	15.00(-1)	93.3±0.21
10	50.00(0)	8.00(1)	15.00(-1)	94±0.32
11	50.00(0)	6.00(-1)	25.00(1)	93±0.18
12	50.00(0)	8.00(1)	25.00(1)	93.4±0.24
13	50.00(0)	7.00(0)	20.00(0)	96.2±0.33
14	50.00(0)	7.00(0)	20.00(0)	96.4±0.12
15	50.00(0)	7.00(0)	20.00(0)	95.9±0.11
16	50.00(0)	7.00(0)	20.00(0)	95.7±0.15
17	50.00(0)	7.00(0)	20.00(0)	95.5±0.30

Japan) and scanning electron microscope (KYKY-2800B, Beijing, China) according to the manual. Microcapsule were coated with gold 20 mA/120 s under vacuum condition and observed surface using an accelerating voltage is 10 kv.

Statistic analysis

Response surface data of microcapsule were analyzed by Design Expert7.0. Else data were processed by SPSS12. $p \leq 0.05$ was considered as significant for all statistical analysis. All experiments were carried out in triplicate and data were shown as mean±SD (standard deviation).

Results and Discussion

Anthocyanins microencapsulation analysis

Table 1a shows the results of response surface based on 17 combinations and 5 replicates of center point. The embedding rate was analyzed as showed on Table 1b, and the regression model and correlation coefficient were calculated as follows: Embedding rate (%)=-46.5600+0.9081A+31.1363B+0.7720C-0.0238AB+0.0005AC-0.015BC-0.0069A^2-2.0888B^2-0.0171C^2 (R^2=0.9441). *R* square value indicated that the regression model works well, and the dependent variables in the model could reflect the change of variation. Furthermore, variance analysis in table1.b confirmed the model, with probability *(P)* value of regression model reached to significance but not the lack of fit test.

Table1.b shown the embedding rate affected by CM, and the linear ($P<0.01$) and quadratic ($P<0.01$) effects on microencapsulation rate reached to significance. Thus, the overall effects were curvilinear as shown in the contour map (Figure 1a). The results suggested that embedding rate increased quickly when the CM level was lowest; however, at the end, the embedding rate became lower. In such case, the influence of solid contents on embedding rate was slight, as its linear and quadratic effects were not significant. Data in table1.b also indicated that RC provided significant linear ($P<0.05$) and quadratic ($P<0.001$) effects on embedding rate. Figure 1b showed the overall curvilinear effects from RC, of which showed the contour map of influence of RC and solid content on embedding rate. At lower range of RC, the embedding rate increased rapidly before it reached to peak, whereas the embedding rate decreased rapidly when the RC was in the higher level. Figure 1c showed the contour graph of the effect of RC and CM on embedding rate, which further demonstrated the data in Figure 1a and Figure 1b.

Response surface not only illustrated the effect of independent variable but also calculated the best

Table 1b. Variance analysis for response surface quadratic model

Source	Sum of squares	df	Mean square	F value	P value
model	27.27	9	3.03	13.13	0.0013**
A	3.19	1	3.19	13.82	0.0075**
B	1.32	1	1.32	5.72	0.0480*
C	0.020	1	0.020	0.087	0.7770
AB	0.23	1	0.23	0.98	0.3557
AC	0.0025	1	0.0025	0.011	0.9200
BC	0.023	1	0.023	0.098	0.7639
A^2	2.00	1	2.00	8.66	0.0216*
B^2	18.37	1	18.37	79.62	< 0.0001**
C^2	0.77	1	0.77	3.32	0.1114
Residual	1.62	7	0.23		
Lack of fit	1.08	3	0.36	2.71	0.1795
Pure Error	0.53	4	0.13		
Cor total	28.88	16			

Table 2. Degradation velocity and half life of RCA and RCA-microcapsule

Factor		Sample	Degradation velocity constant ($\times 10^{-3}$/d)	Half life/ d
Temperature	20	RCA	2.7	256
		RCA-microcapsule	2.4	289
	30	RCA	3.5	198
		RCA-microcapsule	2.9	239
	40	RCA	6.0	115
		RCA-microcapsule	3.5	198

procedure condition. First-order partial derivatives function to each variable was used to obtain three equations of linear equations, through which the best process condition was calculated. The optimum conditions derived from response surface test were CM54.48%, RC7.07%, and solid contents 20.33%. Under such conditions, the anthocyanins could be well embedded.

The stability of anthocyanins microcapsules

Stability of red cabbage anthocyanins in microencapsulated powders was evaluated under different storage temperature conditions. Our results indicated that the degradation rate of anthocyanin gradually was increased when the temperature increased (Figure 2). At the same temperature, embedding anthocyanins had a smaller degradation rate than those of not embedded anthocyanins, which indicated that stability of RCA-microcapsule was improved. What is more, the degradation kinetics of anthocyanins in microcapsuls followed kinetics of the first order during the storage. These results were in accordance with the previous reports regarding the degradation of anthocyanins. Degradation kinetics and colour of anthocyanins in aqueous extracts of purple-and red-flesh potatoes (Reyes and Cisneros-Zevallos, 2007) and anthocyanins in blackberry juice (Weidong and Shiying, 2007) followed first-

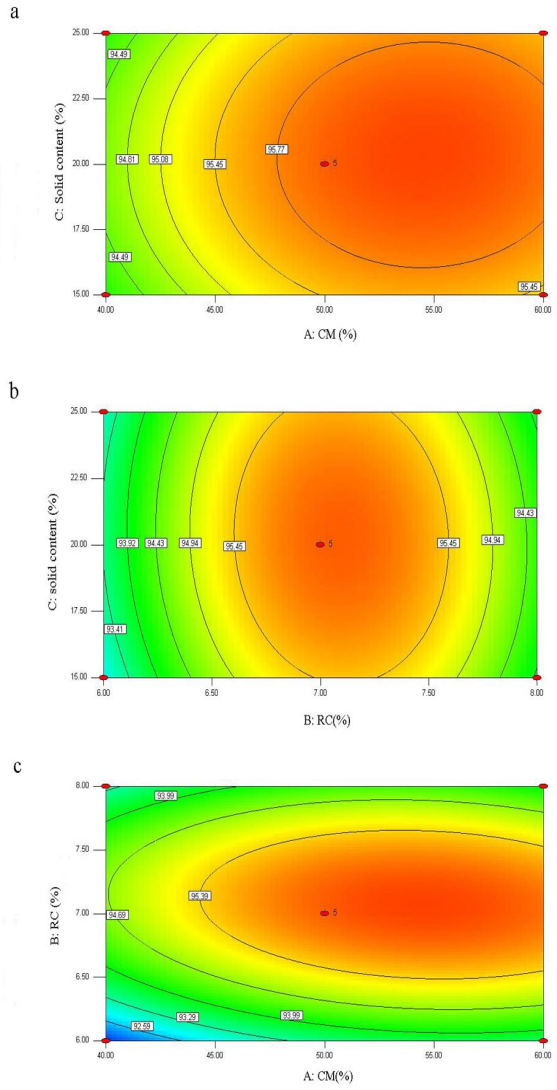

Figure 1. Contour plots for interact effect of CM, RC and solid content on embedding rate(%) of RCA. a is the interact effect of CM and solid content at a constant RC of 7% on the embedding rate of RCA; b is the interact effect of RC and solid content at a constant CM of 50% on the embedding rate of RCA; c is the interact effect of CM and RC at a constant solid content of 20% on the embedding rate of RCA.

order reaction kinetics. Research from another group suggested that the degradation kinetics of betacyanins microcapsules followed a pseudo–first order behavior (Saénz *et al*., 2009). The reason for this discrepancy seems from the different test temperature and different experimental material. Our data suggested that when the temperature exceeds more than 60°C, the degradation kinetics always followed a pseudo–first order behavior (Data was not shown). Degradation reaction rate constant k and half-life $t_{1/2}$ listed in Table 2 also confirmed that rate constant was increased and half-life was decreased with the increased temperature. The degradation reaction rate constant of RCA-microcapsule was smaller than

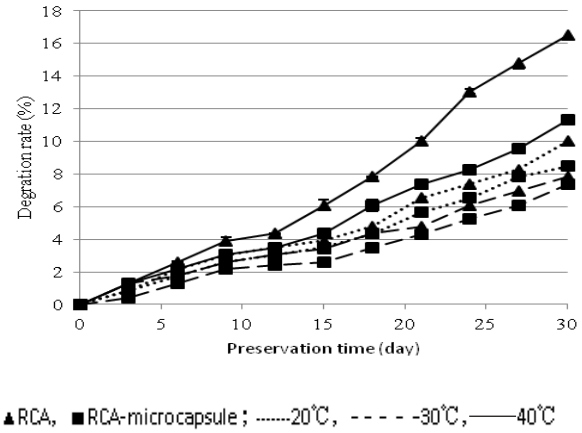

Figure 2. Effect of heat preservation time on the degradation rate RCA and RCA-microcapsule

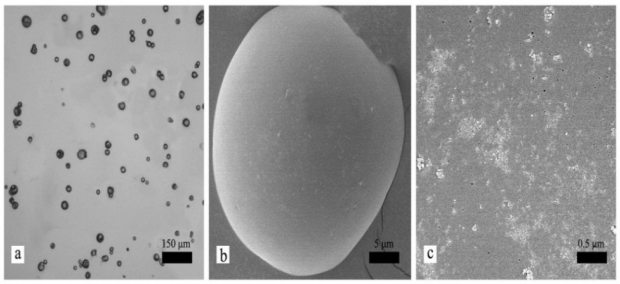

Figure 3. Microstructure of anthocyanins microcapsules under both Light microscope and SEM. a is light microscope images of microcapsules; b is SEM images of microcapsules; c is SEM images of microcapsules outer surface.

those of RCA, while half-life was prolonged.

Particle size and microstructure

The microstructure of anthocyanin microcapsules was observed under both light microscope and SEM. Figure 3a showed the light microscope structure of red cabbage anthocyanin microcapsules. The image clearly showed that appearance of microcapsule anthocyanin was spherical that with raised and smooth outer surface. Figure 3b and c were the anthocyanin microcapsules microstructure obtained under the SEM, and Figure 3c suggested that the particle size of powders was ranged from 30 μm to 50 μm. As shown in Figure 3b and c, the microcapule particle looked like spherical shape with smooth surface spheres, which further confirmed the images in Figure 3a.

Conclusion

In this study, we optimized the microcapsule process of the red cabbage anthocyanins. Using response surface methodology, a mathematical regression quadratic model was established. The response surface optimization was CM

54.48%, RC 7.07% and solids content 20.33%, and under such conditions, the embedding effects reached the theoretical maximum value of 96.10. Microencapsulated anthocyanins half-life was elongated when it was exposure to different temperature, which provided another way to improve the anthocyanint stability.

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References

Bordignon-Luiz, M.T., Gauche, C., Gris, E.F. and Falcão, L. 2007. Colour stability of anthocyanins from Isabel grapes (*Vitis labrusca* L.) in model systems. LWT-Food Science and Technology 40: 594-599.

Delgado-Vargas, F., Jimenez, A.R. and Paredes-Lopez, O. 2000. Natural pigments: carotenoids, anthocyanins, and betalains--characteristics, biosynthesis, processing, and stability. Critical Reviews in Food Science and Nutrition 40(3): 173-289.

Dorota Walkowiak-Tomczak, J.C. 2007. Colour changes of a preparation from red cabbage during storage in a model system. Food Chemistry 104: 709-714.

Francis, F.J. 1989. Food colorants: anthocyanins. Critical Reviews in Food Science and Nutrition 28(4): 273-314.

Fuleki, T. and Francis, F.J. 1967. A new developing solvent for paper chromatography of various phenolic compounds, sugars and amino acids. Journal of Chromatography 26(2): 404-411.

Galvano, F., Salamone, F., Nicolosi, A. and Vitaglione, P. 2009. Anthocyanins-based drugs for colon cancer treatment: the nutritionist's point of view. Cancer Chemotherapy and Pharmacology 64(2): 431-432.

Gomes, R., Parola, A.J., Lima, J.C. and Pina, F. 2006. Solvent effects on the thermal and photochemical reactions of 4'-iodo-8-methoxyflavylium and their consequences on the coloring phenomena caused by anthocyanins in plants. Chemistry 12(30): 7906-7912.

Gonzalez-Barrio, R., Borges, G., Mullen, W. and Crozier, A. 2010. Bioavailability of Anthocyanins and Ellagitannins Following Consumption of Raspberries by Healthy Humans and Subjects with an Ileostomy. Journal of Agricultural and Food Chemistry 58(7): 3933-3939.

Hongmei, Z., Yongbin, H., Zhenxin, G. and Gongjian, F. 2006. Adsorption and Desorption characteristics of Macroporous Resins to Purple Sweet Potato Pigments. Transactions of the Chinese Society of Agricultural Engineering 22(5): 153-156.

KIrca, A. and Cemeroglu, B. 2003. Degradation kinetics of anthocyanins in blood orange juice and concentrate. Food Chemistry 81(4): 583-587.

McDougall, G.J., Fyffe, S., Dobson, P., and Stewart, D. 2007. Anthocyanins from red cabbage – stability to simulated gastrointestinal digestion. Phytochemistry 68(9): 1285-1294.

Reyes, L.F. and Cisneros-Zevallos, L. 2007. Degradation kinetics and colour of anthocyanins in aqueous extracts of purple-and red-flesh potatoes (*Solanum tuberosum* L.). Food Chemistry 100(3): 885-894.

Rimpapa, Z., Toromanovic, J., Tahirovic, I., Sapcanin, A. and Sofic, E. 2007. Total content of phenols and anthocyanins in edible fruits from Bosnia. Bosnian Journal of Basic Medical Sciences 7(2): 117-120.

Saénz, C., Tapia, S., Chávez, J. and Robert, P. 2009. Microencapsulation by spray drying of bioactive compounds from cactus pear *(Opuntia ficus-indica)*. Food Chemistry 114(2): 616-622.

Shahidi, F. and Han, X.Q. 1993. Encapsulation of food ingredients. Critical Reviews in Food Science & Nutrition 33(6): 501-547.

Tanchev, S.S. and Timberlake, C.F. 1969. The anthocyanins of red cabbage *(Brassica oleracea)*. Phytochemistry 8(9): 1825-1827.

Timberlake, C.F. and Henry, B.S. 1986. Plant pigments as natural food colours. Endeavour 10(1): 31-36.

Wang, L.S. and Stoner, G.D. 2008. Anthocyanins and their role in cancer prevention. Cancer Letters 269(2): 281-290.

Weidong, W. and Shiying, X. 2007. Degradation kinetics of anthocyanins in blackberry juice and concentrate. Journal of Food Engineering 82(3): 271-275.

Xintian, G., Zhijian, W., Nize, S., Aiping, F. and Rongshu, W. 2009. Efficient methods for the extraction and microencapsulation of red pigments from a hybrid rose. Journal of Food Engineering 94: 122-128.

Zhenzhen, X., Jihong, W., Yan, Z., Xiaosong, H., Xiaojun, L. and Zhengfu, W. 2010. Extraction of anthocyanins from red cabbage using high pressure CO_2. Bioresource Technology 101: 7151-7157.