



# BioTechnology

An Indian Journal

FULL PAPER

BTALJ, 11(7), 2015 [269-273]

## Optimization of extraction conditions of melanin from black tea leaves using response surface methodology

Yu Zou\*, Yuan Wu, Mixia Tian

College of Life Science, Dalian Nationalities University, Dalian, Liaoning, (CHINA)

E-mail: yuzou126@126.com

### ABSTRACT

Melanin is a natural pigment with great development potential as a healthful food colorant. In this paper, extraction conditions of natural melanin from black tea leaves were investigated. Temperature, duration and liquid-solid ratio were chosen to optimize extraction conditions using response surface methodology (RSM). The Box-Behnken experimental results showed the optimum extraction conditions as follows: a temperature of 97.92 °C, a duration of 44.93 min and a liquid-solid ratio of 106.76 mL/g. Under these conditions, the maximal melanin yield was 271.40 mg/100 g. The results of this study showed that black tea leaves could be used as a good source of the new-type natural melanin. © 2015 Trade Science Inc. - INDIA

### KEYWORDS

Black tea leaf;  
Melanin;  
Extraction condition;  
Optimization.

### INTRODUCTION

Frequent discovery of synthetic pigments' harmful effects on human health has led to public interest in natural pigments as alternatives in food industry, resulting in increasing study on natural colorants. Melanin is an irregular red, black or brown polymer produced from oxidative polymerization of tyrosine or indolic compounds<sup>[1]</sup>. Melanin, derived from such biological sources as Black-bone silky fowl<sup>[2]</sup>, *Osmanthus fragrans*' seeds<sup>[3]</sup>, *Auricularia auricula* fruit-bodies<sup>[4]</sup>, and *Hypoxylon archeri*<sup>[5]</sup> of microorganisms, show similar physical and chemical properties. These physico-chemical properties include strong light absorbance, unusual solubility and remarkable redox properties. As is shown in previous studies, melanin has a number of healthful functions, such as antioxidation<sup>[2,5]</sup>, anti-HIV activity<sup>[6]</sup>

and so on. These functions promise natural melanin with great development potential as a healthful food colorant.

Black tea is a Chinese traditional and famous food. Black tea leaves are rich in melanin and are increasingly popular as a "black food" in China. There have been reports about the research on extraction of melanin, and melanin is considered to be one of the most important functional components in these "black food"<sup>[7]</sup>. However, there is little information available in literature about the investigation of extraction of melanin from black tea leaves using an affective statistical technique. This paper was to develop an economical and efficient extraction of melanin from black tea leaves. Response surface methodology (RSM) was employed to optimize extraction conditions (temperature, duration and liquid-solid ratio) in order to obtain the maximal melanin yield.

## FULL PAPER

## EXPERIMENTAL

## Materials and reagents

Black tea leaves were purchased from a local market in Dalian City (Liaoning Province, China), pulverized and sifted through a 60-mesh sieve. The powder stored in dark bags to be kept from moisture and light. Synthetic melanin was purchased from Sigma Chemicals Co. (St. Louis, USA).

## Extraction of melanin

The extraction process of melanin was carried out according to the method of Wang et al.<sup>[3]</sup> with proper modification. About 2 g of black tea leaves powder was washed with running water at a ratio of 60 mL/g (water/raw materials) for 5 min, followed by centrifugation at 4000 rpm for 5 min. The precipitate was immersed into water and the initial pH was adjusted to 12 with 1 M NaOH. Then, the mixture was put into a conical flask for incubation extraction. After that, the sample was centrifuged at 4000 rpm for 5 min and the supernatant containing melanin was obtained and stored at 4 °C in the dark.

## Experimental design

The Box-Behnken experimental design with three factors and three levels was employed to optimize the extraction conditions in order to obtain the highest melanin yield. Temperature (*A*), duration (*B*) and liquid-solid ratio (*C*) were chosen as independent variables in this design. *A* (60, 80 and 100 °C), *B* (20, 40 and 60 mL/g) and *C* (80, 100 and 120 min) were determined as critical levels with significant effect on melanin extraction. The complete design consisted of seventeen combinations including five replicates of the center point (TABLE 1).

The experimental results were analysed by quadratic stepwise regression to fit the second-order equation (1):

$$Y = \beta_0 + \sum_{i=1}^3 B_i X_i + \sum_{i=1}^3 B_{ii} X_i^2 + \sum_{i=1}^3 B_{ij} X_i X_j \quad (1)$$

Where *Y* stands for melanin yield,  $X_1$ ,  $X_2$ ,  $X_3$  for independent variables,  $\beta_0$  for the model intercept and  $B_i$ ,  $B_{ii}$ ,  $B_{ij}$  for regression coefficients of variables for intercept, linear, quadratic and interaction terms, respec-

TABLE 1 : Independent variables and their coded and actual values used for optimization

Independent variable	Symbol	Code levels		
		-1	0	+1
Temperature (°C)	<i>A</i>	60	80	100
Duration (min)	<i>B</i>	20	40	60
Liquid-solid ratio (mL/g)	<i>C</i>	80	100	120

tively. The software Design-Expert 7.0.0 Trial (State-Ease Inc., Minneapolis, USA) was used to obtain the coefficients of the quadratic polynomial model.

## Determination of melanin

Melanin extract was first adjusted to pH 2.0 with 3 M HCl to precipitate melanin, followed by centrifugation at 10000 rpm for 20 min and the supernatant was discarded. The precipitated melanin was washed with chloroform, ethyl acetate and ethanol, and was then dissolved in 0.01 M NaOH. For the quantification of melanin, the optical densities of solution containing melanin at 400 nm were determined with a UV-2802 diode array spectrophotometer (UNIC, USA) and were compared to a synthetic melanin<sup>[8]</sup>.

## Statistical analysis

The experimental results obtained were expressed as means  $\pm$  SD of triplicates. Statistical analysis was performed using Fisher's *F*-test.  $P < 0.05$  was regarded as significant and  $P < 0.01$  as very significant.

## RESULTS &amp; DISCUSSION

## Analysis of Box-Behnken experiment

The extraction conditions including temperature, duration and liquid-solid ratio as independent variables were further optimized for the maximum melanin yield. The Box-Behnken design and the corresponding response values were shown in TABLE 2.

By using the Design Expert software, a second-order polynomial model describing the correlation between melanin yield and the three variables in this study was obtained in Eq. (2) below:

$$Y = -531.41 + 1.59A + 10.54B + 7.79C - 0.03A^2 - 0.13B^2 - 0.05C^2 + 0.03AB + 0.04AC - 0.01BC \quad (2)$$

The statistical significance of Eq. (2) was checked by *F*-test, and the results of analysis of variance

TABLE 2 : The Box–Behnken design and experiment data for melanin extraction

Run	A	B	C	Melanin yield (mg/100 g)	
	Temperature (°C)	Duration (min)	liquid-solid ratio (mL/g)	Observed value	Predicted value
1	0	-1	1	145.82±11.18	151.71
2	0	-1	-1	135.60±10.24	139.86
3	0	1	1	179.45±11.17	175.14
4	-1	1	0	143.57±10.28	138.10
5	0	0	0	218.55±14.70	234.90
6	-1	0	-1	165.22±12.47	176.51
7	0	0	0	266.54±18.32	234.90
8	1	1	0	226.56±10.19	242.08
9	0	0	0	200.52±12.21	234.90
10	1	0	1	272.41±19.98	261.09
11	0	0	0	253.65±17.19	234.907
12	1	0	-1	240.80±20.98	231.14
13	0	0	0	236.07±14.24	234.90
14	-1	-1	0	139.54±10.08	123.92
15	-1	0	1	138.66±9.87	148.26
16	0	1	-1	191.28±15.43	185.29
17	1	-1	0	182.50±12.91	187.40

TABLE 3 : Analysis of variance (ANOVA) of the response surface regression model

Source	Sum of squares	Degree of freedom	Mean squares	F-value	P-value
Model	32102.06	9	3566.90	6.54	0.0112
A	14019.75	1	14019.75	25.34	0.0015
B	2370.16	1	2370.16	4.28	0.0772
C	1.44	1	1.44	0.01	0.9607
A <sup>2</sup>	454.32	1	454.32	0.82	0.3949
B <sup>2</sup>	11227.08	1	11227.08	20.30	0.0028
C <sup>2</sup>	1728.71	1	1728.71	3.13	0.1204
AB	410.06	1	410.06	0.74	0.4178
AC	846.81	1	846.81	1.53	0.2559
BC	121.00	1	121.00	0.22	0.6542
Residual	3872.11	7	553.16		
Pure Error	2779.70	4	694.92		
Corrected Total	35974.17	16			

$R^2 = 0.8540$ ,  $adj R^2 = 0.9324$ .

(ANOVA) were shown in TABLE 3. The model  $F$ -value of 6.54 obtained by ANOVA indicated that the model was significant ( $P < 0.05$ ). For the model fitted, the coefficient of determination ( $R^2$ ), which could check the goodness of a model, was 0.9324 implying that the sample variation of 93.24% for the melanin yield was attributed to the independent variables, and only 6.76% of the total variation could not be explained by the model. These results suggested that the developed model

could adequately represent the real relationship among the parameters chosen.

#### Effects of temperature, duration and liquid-solid ratio on melanin extraction

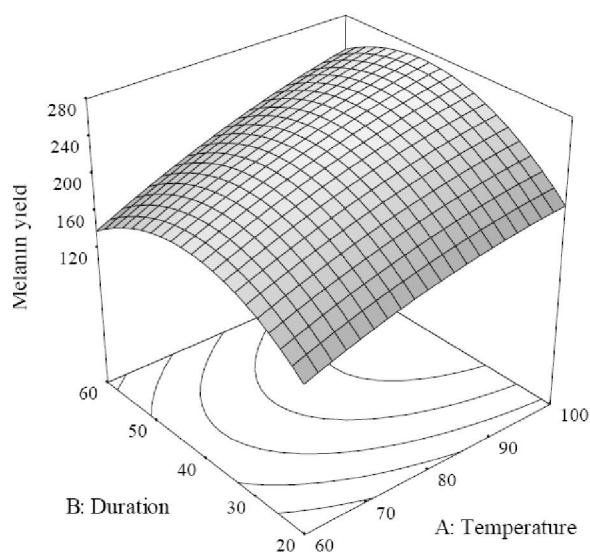
As was shown in TABLE 3, temperature had very significant linear effect ( $P < 0.01$ ) on melanin extraction; duration had very significant quadratic effect ( $P < 0.01$ ). However, none of the independent variables

## FULL PAPER

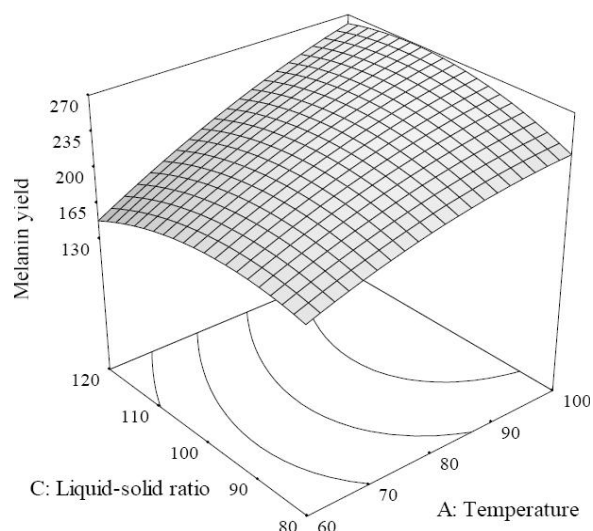
(temperature, duration and liquid-solid ratio) interacted significantly ( $P > 0.05$ ).

Figure 1 showed the effect of temperature and duration on melanin extraction from black tea leaves at a constant liquid-solid ratio of 100 mL/g. At a fixed duration, the melanin yield increased rapidly when temperature was raised. This indicated that extraction temperature was the principal effect on the melanin yield, which agreed with results reported by Wang et al.<sup>[9]</sup>. The melanin yield increased when duration was extended from 20 to 45 min but slowly decreased when duration continued to be extended. This phenomenon could be explained in terms of colorant degradation. The melanin might degrade due to long time treatment under high temperature condition<sup>[10]</sup>. Therefore, the optimum duration should be about 45 min in the present work.

Figure 2 showed the effect of temperature and liquid-solid ratio on melanin extraction from black tea leaves at a constant duration of 40 min. When liquid-solid ratio was set, the melanin yield increased rapidly when temperature was raised, which also implied that melanin yield was significantly influenced by temperature. At a fixed temperature, the variety of melanin yield was slight when the liquid-solid ratio increased, especially when the temperature exceeded 90 °C. However, increasing extraction temperature might result in more solvent volatilization, more energy cost and more



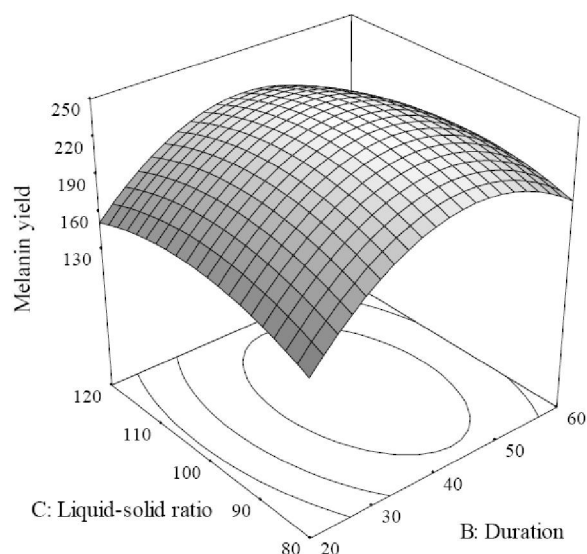
**Figure 1 :** Response surface plot showing the effect of temperature and duration on melanin extraction from black tea leaves. The liquid-solid ratio was constant at 100 mL/g.



**Figure 2 :** Response surface plot showing the effect of temperature and liquid-solid ratio on melanin extraction from black tea leaves. The duration was constant at 40 min.

impurities extraction<sup>[11]</sup>. Therefore, extraction temperature should not exceed 100 °C.

Figure 3 showed the effect of duration and liquid-solid ratio on melanin extraction from black tea leaves at a constant temperature of 80 °C. The increase of both liquid-solid ratio and duration accelerated extraction of melanin. However, above the optimal liquid-solid ratio (about 105 mL/g) and duration (about 45 min), the increase in liquid-solid ratio and duration would not further increase the melanin yield. This result was similar to those previously reported by Rodrigues et al.<sup>[12]</sup>. These findings make the whole process of melanin ex-



**Figure 3 :** Response surface plot showing the effect of duration and liquid-solid ratio on melanin extraction from black tea leaves. The temperature was constant at 80 °C.

traction economically more feasible and efficient in the potential application in food industry.

### Optimization of extraction conditions and verification of the model

According to the RSM test results, the optimal extraction conditions to obtain the highest melanin yield were determined as follows: a temperature of 97.92 °C, a duration of 44.93 min and a liquid-solid ratio of 106.76 mL/g. The melanin yield was 271.40±13.24 mg/100 g, and this value was not significantly different ( $P > 0.05$ ) from the predicted value of 272.26 mg/100 g. These data proved that the model designed in this study was valid.

### CONCLUSIONS

In this study, temperature, duration and liquid-solid ratio were chosen to further optimize melanin extractions by a three variable, three level Box-Behnken experiment design. The combination of temperature (97.92 °C), duration (44.93 min) and liquid-solid ratio (106.76 mL/g) was determined to obtain the highest melanin yield (271.40 mg/100 g). The experimental results showed that black tea leaves could be used as a good source of the new-type natural melanin.

### ACKNOWLEDGMENT

This work was supported by the Doctor Research Fund of Dalian Nationalities University (0701-110015).

### REFERENCES

- [1] P.A.Riley; *Int.J.Biochem.Cell Biol.*, **29**, 1235-1239 (1997).
- [2] Y.Tu, Y.Sun, Y.Tian, M.Xie, J.Chen; *Food Chem.*, **114**, 1345-1350 (2009).
- [3] H.Wang, Y.Pan, X.Tang, Z.Huang; *LWT*, **39**, 496-502 (2006).
- [4] Y.Zou, Y.Yang, B.Zeng, Z.Gu, Y.Han; *Int.J.Food Prop.*, **16**, 803-813 (2013).
- [5] Y.Wu, L.Shan, S.Yang, A.Ma; *J.Basic Microbiol.*, **48**, 217-221 (2008).
- [6] J.T.Manning, P.E.Bundred, P.Henzi; *J.Theor.Biol.*, **223**, 131-133 (2003).
- [7] S.R.Chen, B.Jiang, J.X.Zheng, G.Y.Xu, J.Y.i, N.Yang; *Food Chem.*, **111**, 745-749 (2008).
- [8] C.N.S.Santos, G.Stephanopoulos; *Appl.Environ. Microbiol.*, **74**, 1190-1197 (2008).
- [9] J.Wang, B.Sun, Y.Cao, Y.Tian, X.Li; *Food Chem.*, **106**, 804-810 (2008).
- [10] B.K.Tiwari, C.P.Donnell, P.J.Cullen; *J.Food Eng.*, **93**, 166-171 (2009).
- [11] L.Zhang, Z.Liu; *Ultrason.Sonochem.*, **15**, 731-737 (2008).
- [12] S.Rodrigues, G.A.S.Pinto, F.A.N.Fernandes; *Ultrason.Sonochem.*, **15**, 95-100 (2008).