Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(7):1560-1562

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Physicochemical properties and stability of melanin from black tea leaves

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ABSTRACT

Melanin is a natural pigment with great development potential as a healthful food colorant. In this paper, melanin from black tea leaves was extraction and purification and its physicochemical properties and stability were investigated. The experimental results showed that melanin was insoluble in both water and common organic solvents. It dissolved only in alkali aqueous solution and precipitated acidic aqueous solution (pH < 3). Melanin was gradually oxidative bleached by oxidant and was stable to reducer. These physicochemical properties were very similar to those of the melanin reported in the previous studies. Melanin had good resistance to light decomposition and heating treatment. Therefore, melanin from black tea leaves could be further used as a natural food colorant.

Keywords: black tea leaf, melanin, physicochemical property, stability

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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 [1]. Melanin, derived from such biological sources as Black-bone silky fowl [2], *Osmanthus fragrans*' seeds [3], *Auricularia auricula* fruit-bodies [4], and *Hypoxylon archeri* [5] of microorganisms, show similar physicochemical properties. These physicochemical 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 [2, 5], anti-HIV activity [6] 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 physicochemical properties of melanin, and melanin is considered to be one of the most important functional components in these "black food" [7]. However, there is little information available in literature about the investigation of physicochemical properties and stability of melanin from black tea leaves.

In this study, melanin was isolated and purified from black tea leaves and its physicochemical properties and stability were investigated.

EXPERIMENTAL SECTION

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.

Extraction and purification of melanin

The extraction process of melanin was carried out according to the method of Wang et al. [3] with proper

modification. 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 at 100 °C for 40 min. 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.

Purification of melanin was performed as described by Wu et al. [5] with proper modification. Melanin extract was first adjusted to pH 2.0 with 3 M HCl to precipitate melanin, followed by centrifugation at 10,000 rpm for 20 min and the pellet was collected. The pellet was washed with chloroform, ethyl acetate and ethanol for three times. Finally, the purified melanin was lyophilized and stored at -20 °C.

Solubility of melanin

The melanin (100 mg) was added to 10 mL of water, aqueous acid, alkali, or common organic solvents (such as ethanol, methanol, chloroform, acetone, aether, petroleum ether, benzene, ethyl acetate, butanol, etc.) under stirring for 1 h, and stood for 0.5 h, and then filtered. The absorbances of solutions were recorded at 400 nm in a a UV-2802 diode array spectrophotometer (Unico Instrument Co. Ltd., Princeton, NJ, USA) to attain the solubility of melanin [3].

Redox properties of melanin

Redox properties of melanin were measured according to the basic procedure designed with minor modifications [3]. Ten milliliter of 100 mg/L melanin solutions and 50 mL of different concentrations of $KMnO_4$, $K_2Cr_2O_7$, H_2O_2, NaOCl and Na_2SO_3 were mixed, and then the homogenate absorbance was determined at 400 nm.

Stability of melanin

Under different illumination time and different temperature, the preparation solution of melanin from black tea leaves was handled for a period of time, then observed the color change, and measured the OD values at 400 nm to determine melanin stability.

RESULTS AND DISCUSSION

Solubility

As was shown in Table 1, the solubility experiments indicated that melanin from black tea leaves was insoluble in both water and all common organic solvents (such as ethanol, methanol, chloroform, acetone, aether, petroleum ether, benzene, ethyl acetate, butanol, etc.). It dissolved only in alkali aqueous solution and precipitated in acidic aqueous solution (pH < 3). The solubility of melanin from black tea leaves was very similar to those of natural melanin previously reported [2, 8].

Table 1: Solubility of melanin from black tea leaves

Tests	Response results
Solubility in water	Negative response
Solubility in organic solvents	Negative response
Solubility in alkali aqueous solution	Positive response
Precipitation in acidic aqueous solution (pH < 3)	Positive response

Redox properties

The experimental results showed that melanin from black tea leaves exhibited marked redox properties (Table 2). It was gradually oxidative bleached by $KMnO_4$, $K_2Cr_2O_7$, H_2O_2 and NaOCl, illustrating that melanin from black tea leaves could be decolorized by strong oxidant. However, the absorbance of melanin remained almost unchanged in Na_2SO_3, indicating that melanin was stable to reducer. These results revealed that melanin from black tea leaves presented the same redox properties of natural melanin previously reported [7, 8].

Table 2: Redox properties of melanin from black tea leaves

Tests	Response results
Reaction with $KMnO_4$	Positive response
Reaction with $K_2Cr_2O_7$	Positive response
Reaction with H_2O_2	Positive response
Reaction with NaOCl	Positive response
Reaction with Na_2SO_3	Negative response

Effect of light on stability of melanin

The preparation solution of melanin from black tea leaves was placed under sunlight irradiation for 0, 3 and 6 h, respectively. Then, OD value at 400 nm was measured using a spectrophotometer and observed color changes. As was shown in Table 3, with the extension of the light duration, the OD value of melanin fell slightly, and its color had not changed significantly in visual. These indicated that light had less effect on melanin from black tea leaves.

Table 3: Effect of light on stability of melanin from black tea leaves

Illumination time (h)	0	3	6
OD_{400}	0.419	0.416	0.405
Color	Brown	Brown	Brown

Effect of temperature on stability of melanin

The effect of temperature on melanin stability was determined to ascertain the potential use of melanin as a natural colorant. Melanin was incubated at a constant temperature for 0.5 h. The results were shown in Table 4. When heating temperature of melanin rose, the OD values had little impact and the brown color of melanin was almost unchanged. The determination results revealed that melanin was stable to temperature.

Table 4: Effect of temperature on stability of melanin from black tea leaves

Heating temperature (°C)	0	20	50	80
OD_{400}	0.425	0.417	0.402	0.387
Color	Brown	Brown	Brown	Brown

CONCLUSION

In this study, melanin from black tea leaves was extraction and purification. Melanin was insoluble in both water and common organic solvents. It dissolved only in alkali aqueous solution and precipitated acidic aqueous solution (pH < 3). Melanin was gradually oxidative bleached by oxidant and was stable to reducer. Melanin from black tea leaves had good resistance to light decomposition and heating treatment. Light and temperature had little effect on melanin stability. Melanin from black tea leaves could be further used as a natural food colorant.

Acknowledgments

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

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