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Research Article

Physicochemical Properties of Melanin from *A. auricula* Fruiting Bodies

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Abstract: The physicochemical properties of melanin from *Auricularia auricula* fruiting bodies were studied. The result showed that *A. auricula* fruiting bodies melanin powder was dark with a little red and yellow colored (L^* = 41.03, a^* = 2.26, b^* = 3.78). *A. auricula* fruiting bodies melanin was insoluble in both water and common organic solvents. It dissolved only in alkali aqueous solution and precipitated acidic aqueous solution (pH<3). *A. auricula* fruiting bodies melanin was gradually oxidative bleached by oxidant and was stable to reducer. It exhibited strong optical absorbance in a wide UV-VIS spectral range. Furthermore, these physicochemical properties were very similar to those of the melanin reported in the previous studies and to those of synthetic melanin. *A. auricula* fruiting bodies melanin could potentially be used in the food industry as a natural colorant.

Keywords: *Auricularia auricula*, fruiting body, melanin, physicochemical property

INTRODUCTION

Melanin is a dark-colored polyphenolic pigment produced from oxidative polymerization of phenolic or indolic compounds by tyrosinase (Chen *et al*., 2008). These natural pigments are synthesized by some fungi, plants, animals and several bacterial species (Dalfard *et al*., 2006). Melanins from different sources possess similar physicochemical properties, including strong light absorbance, unusual solubility and remarkable redox properties. In addition, melanin has a number of healthful functions, such as antioxidation (Tu *et al*., 2009), anti-HIV activity (Montefiori and Zhou, 1991) and immunomodulatory activity (Sava *et al*., 2001). These functions promise natural melanin with great development potential as a healthful food colorant.

Auricularia auricula is a precious macro-fungus distributed in the Northeast Provinces of China, which is a Tremellales fungus belonging to the Basidiomycotina and has been used as food and drug in China for a long time (Zhang *et al*., 1995). *A. auricula* fruiting bodies, a kind of edible black-brown mushroom, are rich in melanin and are increasingly popular as a "black food" in China. Melanin is considered to be one of the most important functional components in these "black food". However, there is little information available about physicochemical properties of melanin from *A. auricula* fruit-bodies in current reports. In this study, melanin was isolated and purified from *A. auricula* dried fruit-bodies and its physicochemical properties were investigated.

MATERIALS AND METHODS

Materials: Dried fruiting bodies of *A. auricula* were purchased from a local market in Dongning City (Heilongjiang Province, China), pulverized and sifted through a 40-mesh sieve. The powder (moisture content 12-15% on dry basis) stored in dark bags to prevent from moisture and light. All the chemicals and reagents used in the experiment were of analytical grade.

Isolation and purification of melanin: *A. auricula* fruiting bodies powder was washed with running water at a ratio of 30 mL/g (water/raw materials) for 5 min, followed by centrifugation at 4,000 g for 5 min. The precipitate was immersed into water at a liquid-solid ratio 50 mL/g and the initial pH was adjusted to 12.0 with 1 M NaOH. Then, the mixture was sonicated at 50°C for 30 min by an ultrasound cleaning bath (KQ250-DB, Jiangsu Province, China) working at an ultrasound input power of 250 W. Afterward, the sample was centrifuged at 4,000 g for 5 min and the supernatant containing melanin was obtained.

Purification of melanin was performed as described by Wu *et al*. (2008) 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 g for 20 min and the 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.

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Visual color of melanin: Visual color of melanin powder was measured using a Minolta colorimeter (CR-400, Minolta Camera Co. Ltd., Osaka, Japan) with the Hunter Lab color system. The color values were expressed as *L** (whiteness or brightness/darkness), *a** (redness/greenness) and *b** (yellowness/blueness).

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 UV-2802 diode array spectrophotometer (Unico Instrument Co. Ltd., Princeton, NJ, USA) to attain the solubility of melanin (Wang *et al*., 2006).

Redox properties of melanin: Redox properties of melanin were measured according to the basic procedure designed with minor modifications (Wang *et al*., 2006). 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.

UV-VIS absorption spectra of melanin: Melanin was separately dissolved in alkaline distilled water (pH 10.0) at final concentration of 20 mg/L. The UV-VIS absorption spectra of the melanin solution was scanned using a UV-2802 diode array spectrophotometer (Unico Instrument Co. Ltd., Princeton, NJ, USA) at wavelengths ranging from 200 to 800 nm.

RESULTS AND DISCUSSION

Visual color: Color values of *A. auricula* fruiting bodies melanin are shown in Table 1. Results from the colorimeter indicated that melanin presented lower *L** value (41.03), *a** value (2.26) and *b** value (3.78) in Hunter Lab color system. This might be caused by high conjugation degree of melanin which resulted weak spectral absorbance.

Solubility: As was shown in Table 2, the solubility experiments indicated that *A. auricula* fruiting bodies melanin 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 *A. auricula* fruiting bodies melanin was very similar to those of natural melanin previously reported and synthetic melanin (Babitskaya *et al*., 2000; Tu *et al*., 2009).

Redox properties: The experimental results showed that *A. auricula* fruiting bodies melanin exhibited marked redox properties (Table 2). They were gradually oxidative bleached by $KMnO_4$, $K_2Cr_2O_7$, H_2O_2 and NaOCl, illustrating that *A. auricula* fruiting bodies melanin could be decolorized by strong oxidant. However, the absorbance of the melanin remained almost unchanged in Na_2SO_3, indicating that melanin was stable to reducer. These results revealed that *A. auricula* fruiting bodies melanin presented the same redox properties of natural melanins previously reported (Babitskaya *et al*., 2000; Chen *et al*., 2008).

Table 1: Color values of *A. auricula* fruiting bodies melanin (mean±S.D., n = 3)

	L*	a*	b*
Color values	41.03±0.31	2.26±0.30	3.78±0.21

Table 2: Solubility and redox properties of *A. auricula* fruiting bodies melanin

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
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

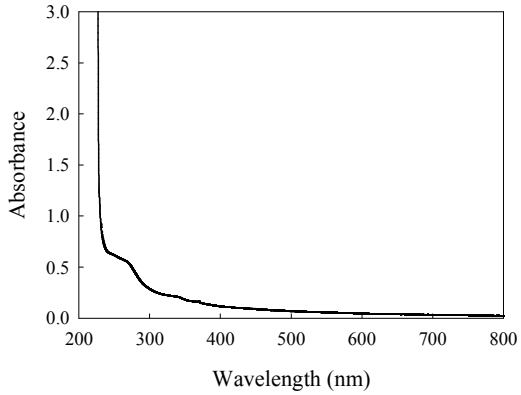

Fig. 1: UV-VIS spectra of *A. auricula* fruiting bodies melanin

UV-VIS absorption spectra: The UV-VIS spectra (200-800 nm) of *A. auricula* fruiting bodies melanin is shown in Fig. 1. Melanin absorbed strongly in the UV region and progressively less as the wavelength increased. According to a previous report (Riley, 1997), strong optical absorbance in a wide spectral range was one of the most conspicuous properties of melanin due to the high degree of conjugation in the molecule. However, *A. auricula* fruiting bodies melanin exhibited an additional shoulder at wavelength 260-280 nm. This property was similar to those of the melanins isolated from plant or animal, such as black tea (Sava *et al*., 2001), *Osmanthus fragrans*' seeds (Wang *et al*., 2006) and black-bone silky fowl (Tu *et al*., 2009). It was well known that normal proteins had an absorption maximum at about 280 nm. Therefore, *A. auricula* fruiting bodies melanin might contain a certain amount of protein.

CONCLUSION

In the present study, *A. auricula* fruiting bodies melanin was prepared by ultrasound-assisted extraction technology. Melanin powder presented lower values (L^*, a^* and b^*) in visual color. *A. auricula* fruiting bodies 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. It exhibited strong optical absorbance in a wide UV-VIS spectral range. *A. auricula* fruiting bodies melanin could be further used as a natural food colorant.

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