

# Study of the Physico-chemical Properties and Antioxidant Activity of Extracted Melanins

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## Abstract

Ultraviolet (UV) light tends to cause skin damage; melanin can scavenge reactive oxygen species (ROS) produced from UV to protect skin from damage caused by free radicals. The purpose of this study was to investigate the physico-chemical properties and antioxidant activity of extracted melanins. Melanins were extracted from black tea (BT-melanin), black soybean (BS-melanin) and black-bone silky fowl (SF-melanin); they were then compared with synthetic melanin (SY-melanin). Three kinds of extracted melanins have absorbance ability in broad-spectrum (190-450 nm) wavelength. The experiment's results indicated that the solubility in 25°C water, organic solvents, 1 M NH<sub>4</sub>OH, and 1 M HCl of the three extracted melanins was similar to the solubility of synthetic melanin. The melanins also showed good stability in various light sources. The extracted melanins could chelate with Fe<sup>2+</sup> and Cu<sup>2+</sup>. An *in vitro* study showed that the extracted melanin enhanced the survivability of fibroblast cells with 25 µg mL<sup>-1</sup> concentration after UV irradiation (254 nm, 0.09 mW cm<sup>2</sup><sup>-1</sup>) (p<0.05). All of the extracted melanins increased glutathione peroxidase (GSH-Px) and catalase activity when exposed to UV light (p<0.001). They inhibited the peroxidation of lipid (TBARS) and scavenged superoxide anion (p<0.001) induced by UV irradiation. According to the above results, the melanins extracted from black tea, black soybean, and black-bone silky fowl are similar in their physico-chemical properties, and they have the capacity for anti-oxidation and photoprotection from UV irradiation.

**Keywords:** Physico-chemical properties, antioxidant activities, photoprotection, melanins

## 1. Introduction

Exposure to too much sunlight may cause erythema or ageing. Ultraviolet (UV) light especially tends to significantly affect skin and cause cell damage; even worse, it may result in skin cancer, for example, squamous cell carcinoma, basal cell carcinoma and melanoma (Reichrath, 2007). UV light also promotes gene mutation and DNA damage (Ravanat et al., 2001), produces reactive oxygen species (ROS) and increases oxidative damage (Herrling et al., 2006; Liu et al., 2009).

Some research indicates that melanin has a wide absorption spectrum because of its special structure and can protect skin from UV damage (Brenner & Hearing, 2008). Melanin contains some function groups, such as COOH, OH, SH and NH<sub>2</sub> groups; thus, melanin could supply and receive electrons and scavenge ROS produced from organisms or UV to protect from free radicals and lipid peroxidation (Bilgihan et al., 1995; Bochenek & Gudowska-Nowak, 2003; Geng et al., 2008a).

Melanins are a ubiquitous class of biological pigments; they play an important role in photoprotection and antioxidation. Recent advances in the chemistry of melanins have demonstrated their diversity. The various types of melanin show different physico-chemical properties; their photobiological properties may not be unique (Ortonne, 2002).

Black tea has a known antioxidant property resulting from its polyphenols and catechins components. In addition, it contains melanin. The black-bone silky fowl is a special kind of chicken. This chicken has a high level of melanin in its skin, bone membrane and organs. There is also a high level of melanin on the peel of black soybeans

Few reports were concerned about the physico-chemical properties and the capacity of antioxidation of these three kinds of melanin. In this study, we investigated the feasibility of extracting melanin from black tea, black

soybean and the skin of black-bone silky fowl. We also studied the physico-chemical properties of the extracted melanin and the capacity of the extracted melanin for photoprotection and antioxidation of fibroblast cells compared with the same capacity of synthetic melanin.

## 2. Materials and Methods

### 2.1 Melanin Extraction

Fully fermented black tea, black soybeans and silky fowl were bought at the market. The skin and the foot of the silky fowl were taken to extract the melanin.

#### 2.1.1 Crude Melanin Extraction

Black tea was boiled with water at a ratio of 1:10 (w/v) for 10 min, followed by filtration. The black soybeans were washed with water and soaked overnight to separate the skin. 10%  $\text{NH}_4\text{OH}$  was added to the black tea and black soybean skin, and the pH was adjusted to 10.5 for 12 h to extract the melanin. After extraction, the mixture was filtered and centrifuged at 5,000 g for 20 min to obtain the melanin crude extract. The extracted crude melanin was acidified with 2 M HCl to pH 2.5 at room temperature for 3 h and centrifuged at 5,000 g for 20 min.

The skin of the silky fowl was separated and washed. 10%  $\text{NH}_4\text{OH}$  was added to the chicken skin and blended. The mixture was boiled for 1 h and filtered. This crude extract was acidified with 2 M HCl to pH 2.5 and centrifuged at 5,000 g for 20 min.

Acid hydrolysis was applied to remove carbohydrates and proteins. The melanin was boiled in 7 M HCl for 2 h, followed by centrifugation at 10,000 g for 10 min and rinsing with water. Solvents (chloroform, ethyl acetate and ethanol) were used to remove the lipids. In practice, 100 mg of melanin were mixed with 50 mL of solvent, and left for 30 min. The melanin was collected after filtration.

#### 2.1.2 Purify Crude Melanin

The collected product was dissolved in 0.2%  $\text{NH}_4\text{OH}$ , and the solution was subjected to repeated precipitations. This was followed a previously reported procedure (Sava et al., 2001) and centrifugation at 6,000 g for 10 min. Melanin products were collected and analyzed.

The melanin solution was prepared using the following procedure. The filtered residue was dissolved in sterile distilled water in a slightly alkaline condition with  $0.5\text{ mol L}^{-1} \text{NH}_3 \cdot \text{H}_2\text{O}$  to pH 9, incubated at  $50^\circ\text{C}$  for 1 h and filtered. The ammonia was removed by a rotary evaporator under reduced pressure to a final pH of 7.5.

### 2.2 Physico-chemical Properties of the Melanin Determination

To determine the ultraviolet-visible absorption spectrum of the melanin, the absorption spectrum of the melanin solution was measured on a spectrophotometer (Hitachi U-1900, Japan). All of the absorption values were also determined with a spectrophotometer in the following experiments.

Firstly, to determine the dissolve capacity of different melanins in different solvents, 0.1 g of the melanin was added to 10 mL of water, aqueous acid, alkali (including  $\text{Na}_2\text{CO}_3$ , NaOH solution) and common organic solvents (including benzene, chloroform, ethanol, methanol, acetone, etc), and stirred at  $25^\circ\text{C}$  for 1 h. The solution was filtered and the  $\lambda_{\text{max}}$  value was determined to ascertain the dissolving capacity of the melanin.

Secondly, to determine the effect of different light sources on different melanins, 0.004 g  $100 \text{ g}^{-1}$  melanin solutions were put under natural light, in a dark place or under ultraviolet light (10 volt) at a distance of 30 cm for a specific time (10 min) and the  $\lambda_{\text{max}}$  value was determined.

Thirdly, to determine the pH effect on the stability of different melanins, the 0.0063 g  $100 \text{ g}^{-1}$  melanin solutions were adjusted to a pH of 3, 5, 7, 9 and 11 by NaOH or HCl. The samples were kept at  $25^\circ\text{C}$  for 30 min, and scanned with an absorption spectrum of 190–220 nm (determined to be a good absorption spectrum of melanin). The influence of pH on melanin was observed.

Finally, to determine different melanin chelating ability with different ions, the 15  $\text{mg L}^{-1}$  solutions of  $\text{CuSO}_4$ ,  $\text{MgSO}_4$ ,  $\text{CaCl}_2$  and  $\text{FeCl}_3$  were prepared and 1 mL of metal ions and 10 mL of 0.0063 g  $100 \text{ g}^{-1}$  melanin solutions were mixed. The  $\lambda_{\text{max}}$  value of homogenate was recorded.

### 2.3 In Vitro Cell Culture Experiment

#### 2.3.1 Cell Cultures

Human fetal skin fibroblasts (CCRC NO. 60300) were cultured in minimum essential medium (Sigma) (pH 7.2-7.4) supplemented with 10% bovine serum, penicillin-streptomycin solution  $100 \text{ unit mL}^{-1}$  and amphotericin B solution  $250 \text{ } \mu\text{g mL}^{-1}$ .

The cell culture was divided into the following groups: 1) negative control (no added melanin), 2) positive control (no exposed UV light), 3) SY-melanin (synthesis melanin); 4) BT-melanin (black tea melanin); 5) BS-melanin (black soybean melanin); 6) SF-melanin (silk fowl melanin). The final melanin concentration was  $25 \mu\text{g mL}^{-1}$ . Every group contained 15 flasks, and every flask 1 mL of  $5 \times 10^5$  cells  $\text{mL}^{-1}$  cell suspension was added for culturing. The experiment groups were exposed to UV light for 30 min  $\text{day}^{-1}$  for 5 days, except for the positive control. The successive 5-day UV dose was  $810 \text{ mJ cm}^{-2}$ .

### 2.3.2 Cell Survival Ability Determined

At the end of the cell culture, cell suspension samples were stained with 0.4% trypan blue to count the surviving cells with a hemocytometer.

### 2.3.3 Cell Homogenate Preparation

The cell suspensions (3 flasks were pooled) were centrifugated at 1,000 g for 5 min and the supernatant was removed. The cells were combined with 0.05 M Tris-HCl, put into liquid nitrogen for 15 min and then sonicated for 10 min by ultrasonic sieving. The above process was repeated 3 times and centrifugated at 2,500 g for 10 min. The supernatant was obtained and stored at  $-80^\circ\text{C}$  for antioxidant activity analysis.

### 2.3.4 Cell Protein Concentration Determined

The protein concentration was determined by the Lowry method (Lowry et al., 1959).

### 2.3.5 Antioxidant Activities Assay

Catalase activity was determined according to the method suggested by Ellerby and Bredesen (2000), with catalase (C-1345, Sigma Co, USA) used as the standard. Glutathione peroxidase (GSH-Px) activity was conducted following the method reported by Bhat et al. (1992).

Scavenging superoxide activity was determined following the method described by Shi and Dalal (1991). The reaction began after  $0.025 \text{ U mL}^{-1}$  xanthine oxidase was added to a cuvette that contained 0.90 mL of reaction buffer (including 50 mM potassium phosphate, pH 7.8; 1 mM EDTA; 100  $\mu\text{M}$  nitroblue tetrazolium (NDT); 0.25 g  $\text{kg}^{-1}$  triton x-100; 100  $\mu\text{M}$  hypoxanthine) and 50  $\mu\text{L}$  sample. The measurement was taken using a spectrophotometer at 560 nm for 5 min.

Thiobarbituric acid-reactive substance (TBARS) was determined according to the procedure reported by Fraga et al. (1988). The peroxidative damage to lipids resulted in the production of malondialdehyde (MDA), which reacted with thiobarbituric acid under conditions of high temperature ( $80^\circ\text{C}$ , 90 min) and acidity (trichloroacetic acid) generation of chromogen that can be measured spectrophotometrically at 535 nm. The unit was expressed as  $\text{nM MDA mL}^{-1}$ .

## 2.4 Statistical Analysis

The experiment data were subjected to statistical analysis by GLM using the SAS (statistical analysis system) for variance analysis while the significant differences among the groups were determined using Tukey's test (SAS, 1998) according to the following model,

$$Y = \mu + T_i + e_{ij}$$

Where Y denotes the dependent variable,  $\mu$  represents for the mean, T is the treatment effect and e denotes the random residual error term. All values were presented as means and SD; the level of significant difference was set at  $P < 0.05$ .

## 3. Results and Discussion

### 3.1 Physico-chemical Properties

#### 3.1.1 Appearance of Extracted Melanin

There is a slight color difference among the extracted melanins from different sources. There are two kinds of melanin: eumelanin and pheomelanin. The color of eumelanin is between deep brown and black; pheomelanin's color is between red and tawny. The different ratio of these two kinds of melanin causes the different colors. Among them, SY-melanin is the most black and SF-melanin is a color similar to SY-melanin. BT-melanin and BS-melanin are a deep brown color (Figure 1). The degree of black color can be listed as SY-melanin > SF-melanin > BS-melanin > BT-melanin. All of the melanins show granule particles.

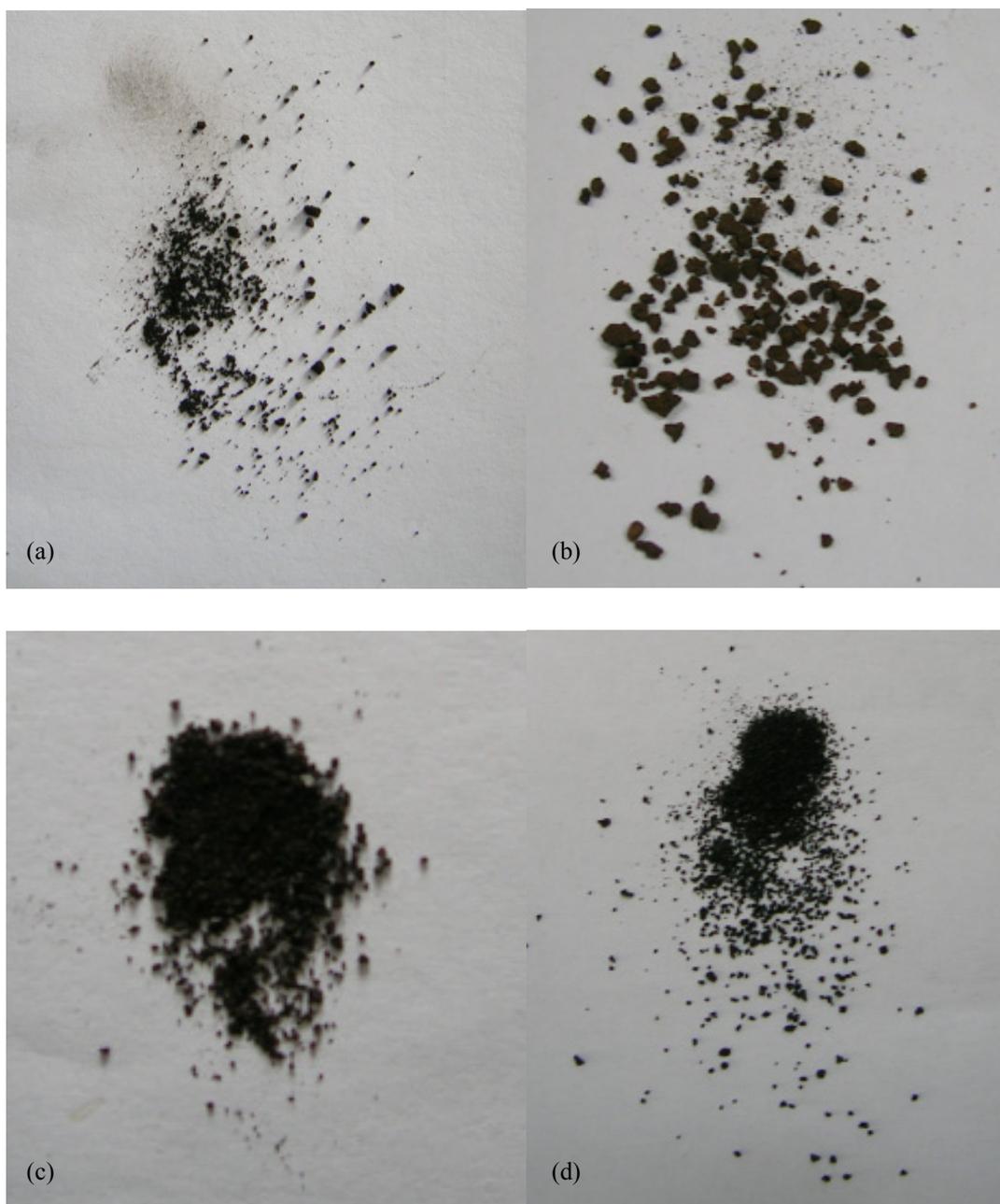


Figure 1. The appearance of melanin extracted from various sources  
(a) SY-melanin; (b) BT-melanin; (c) BS- melanin; (d) SF-melanin

### 3.1.2 The Extracted Ratio

The extracted ratio (melanin weight/stuffs weight) and relative concentration of melanin (melanin concentration/crude extracted melanin weight) are shown in Table 1. Table 1 reveals that the extracted ratio of BT-melanin is about 2% and the relative concentration is about 45%. The extracted ratio and relative concentration of BS-melanin are 0.16% and 26%; SF-melanin is 0.095% and 17%. BT-melanin has the highest extracted ratio and relative concentration; SF-melanin is the lowest.

Table 1. The extracted ratio and relative concentration of melanin extracted from various sources

Sources	Extracted ratio (%)	Relative concentration (%)
Black tea	2.005±0.010	45.33±0.58
Black soybean	0.166±0.003	26.67±1.53
Black-bone silky fowl	0.095±0.002	17.33±1.52

Values are mean±SD (n=3).

The extracted ratio = (the extracted melanin weight/ stuffs weight) × 100%.

The relative concentration = (melanin concentration/ crude extracted melanin weight) × 100%.

### 3.1.3 UV Spectrum of Melanin Solution

Melanin can be absorbed in wide spectrum visible light (400–700 nm) or UV light (340–200 nm), especially in the wavelength of UV. In the experiment, 20 µg mL<sup>-1</sup> of melanin solution was prepared and the spectrum was scanned using a spectrophotometer. Figure 2 shows the results. The spectrum of BT-melanin is similar to that of SY-melanin, and there is a classical shoulder wavelength at 275 nm (Sava et al., 2001). The spectrum of BS-melanin is similar to that of SF-melanin. This result shows that these three kinds of extracted melanin have similar absorption in UV light. The BT-melanin has the highest absorption solution. Novikov et al. (2001) indicated that melanin extracted from black tea exhibits photoprotection activity and showed that this melanin intensively absorbs radiation in UV and visible light.

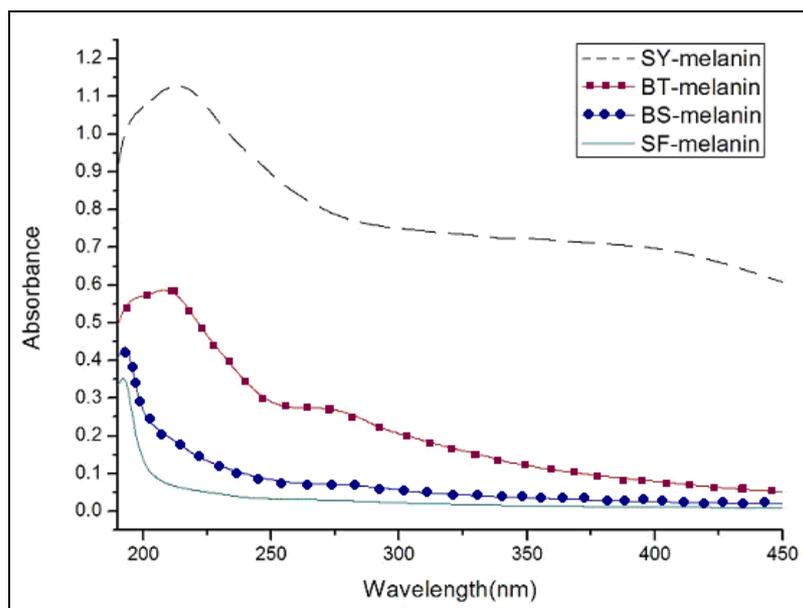


Figure 2. The UV spectrum pattern of melanin extracted from various sources. SY-melanin: Synthetic-melanin; BT-melanin: Black tea-melanin; BS-melanin: Black soybean-melanin; SF-melanin: Black-bone silky fowl-melanin

### 3.1.4 Physical Properties of Extracted Melanin

Table 2 shows the result of comparing the dissolving capacity of extracted melanins with synthesized melanin. The three extracted melanins could not dissolve in 25°C of water and organic solvents (methanol, ethanol, chloroform and acetone). They could dissolve in alkaline solution, which is a light yellow to brown color. The dissolving capacity of these three kinds of extracted melanins is similar to that of synthesized melanin. Melanin will precipitate in acid solution of a pH value of 3. This is also similar to synthesized melanin.

Table 2. Dissolved capacity of melanin extracted from various sources in different solvents

Tests	BT-melanin	BS-melanin	SF-melanin	SY-melanin
H <sub>2</sub> O at 25°C	N	N	N	N
Methanol	N	N	N	N
Ethanol	N	N	N	N
Chloroform	N	N	N	N
Acetone	N	N	N	N
1 N NH <sub>4</sub> OH (5 mg/10ml)	P	P	P	P
Precipitation by 1 N HCl (pH 2)	P	P	P	P

N: negative response; P: positive response.

### 3.1.5 The Effect of Light Sources on the Stability of the Melanin

UV in sunshine will injure organisms, but melanin is able to absorb UV light well. Organisms also produce melanin to protect themselves from UV damage. In this experiment, the three kinds of melanin solutions were placed in natural light, UV light and in dark conditions. Table 3 shows the results of the observations of the influence of different light sources on the melanin solutions. After 5 days of testing, the light absorbance of these three melanin solutions showed no difference to each other with respect to having been in natural light, UV light or in dark conditions. Thus, it is shown that these three kinds of melanin solutions will not be damaged by the visible spectrum and UV light. They have good stability when exposed to light.

Table 3. Effect of light sources on stability of the melanin

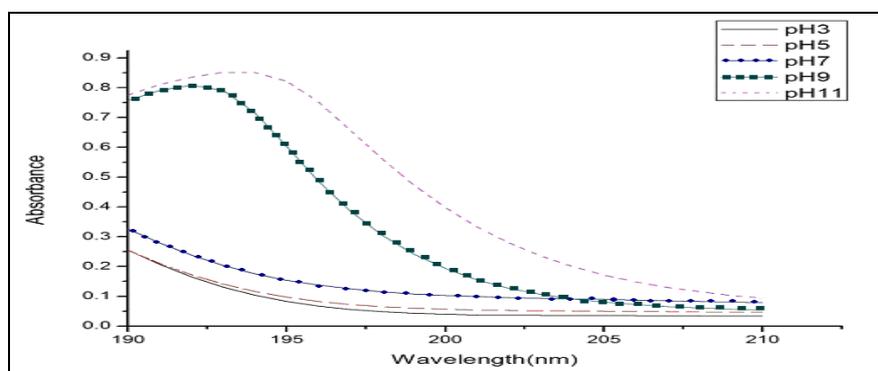
Groups	Light source	0 d	1 d	3 d	5 d
BT-melanin	UV light	0.454 ± 0.008	0.457 ± 0.013	0.457 ± 0.012	0.465 ± 0.014
	Nature light	0.455 ± 0.015	0.461 ± 0.018	0.462 ± 0.011	0.459 ± 0.015
	Dark	0.449 ± 0.026	0.455 ± 0.029	0.452 ± 0.031	0.447 ± 0.026
BS-melanin	UV light	0.457 ± 0.009	0.473 ± 0.013	0.476 ± 0.005	0.466 ± 0.011
	Nature light	0.466 ± 0.014	0.475 ± 0.004	0.479 ± 0.006	0.473 ± 0.020
	Dark	0.468 ± 0.013	0.459 ± 0.028	0.466 ± 0.016	0.457 ± 0.024
SF-melanin	UV light	0.248 ± 0.002	0.250 ± 0.007	0.249 ± 0.011	0.251 ± 0.010
	Nature light	0.249 ± 0.004	0.251 ± 0.005	0.257 ± 0.004	0.255 ± 0.003
	Dark	0.247 ± 0.003	0.249 ± 0.005	0.250 ± 0.004	0.257 ± 0.002

Values are mean ± SD (n=3).

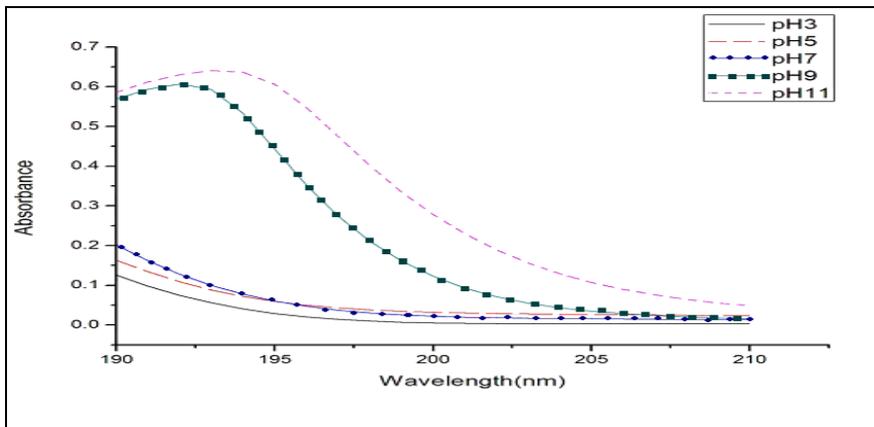
UV light is 10 volt.

### 3.1.6 The Effect of Different pH

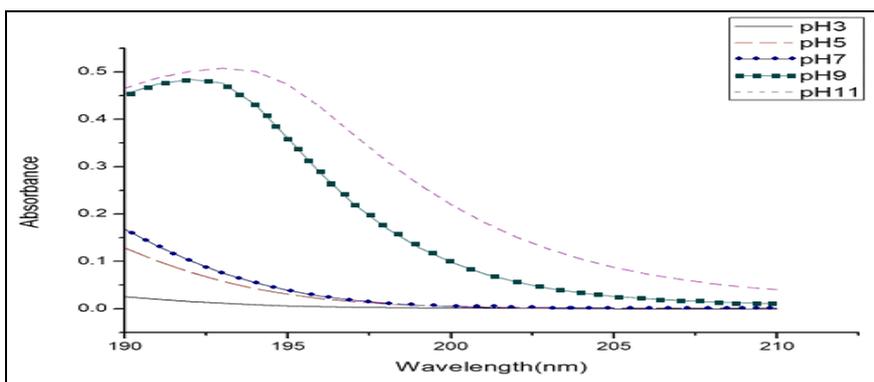
Figure 3 shows the absorbance of melanin solutions under different pH. Melanin can dissolve in alkaline solution and precipitate in pH 2-3. The result shows that the absorbance of melanin in alkaline is higher than in acid. When the pH decreases, the absorbance also decreases. The results of melanin extracted from different sources are the same. Thus, the solubility of these three kinds of melanin is obviously influenced by the pH value.



(a)



(b)

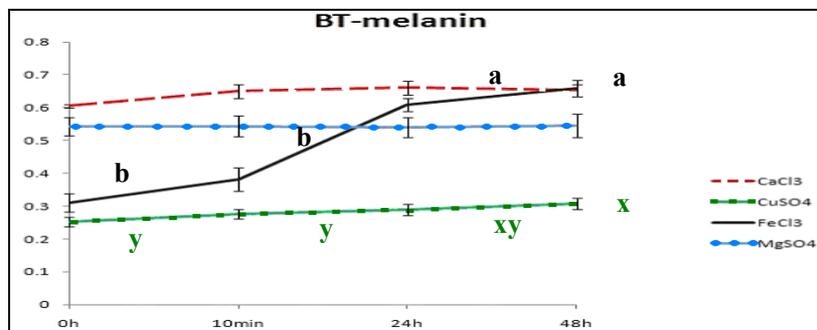


(c)

Figure 3. Effect of pH on absorption spectrum of melanin  
(a) BT-melanin; (b) BS-melanin; (c) SF-melanin

3.1.7 Effect of Metal Ions on the Stability of the Melanin

Figure 4 shows the result of different kinds of metal ions influenced by different melanins. The figure indicates that the three kinds of melanin showed great chelation with  $Fe^{2+}$ ; BS-melanin and SF-melanin showed good chelation with  $Cu^{2+}$ . These three kinds of melanin do not have apparent chelation with other ions. Wang et al. (2006) pointed out that  $Mg^{2+}$  will reduce the color of melanin, but we did not observe that phenomenon in this experiment. Szpoganicz et al. (2002) indicated that synthesized melanin chelated with  $Cu^{2+}$  and  $Zn^{2+}$  in different pH. Melanin can also be chelated with  $Ca^{2+}$  and prevent the DNA damage by  $H_2O_2$  (Hoogduijn et al., 2004). Bush and Simon (2007) also indicated that melanin has been implicated in maintaining calcium homeostasis in the cell. Hong and Simon (2005) revealed that cow choroid melanosomes had a higher binding capacity for the carboxylate-binding metal ions (e.g.,  $Mg^{2+}$ ,  $Ca^{2+}$ ). However, this phenomenon in these three melanins was not observed in this study.



(a)

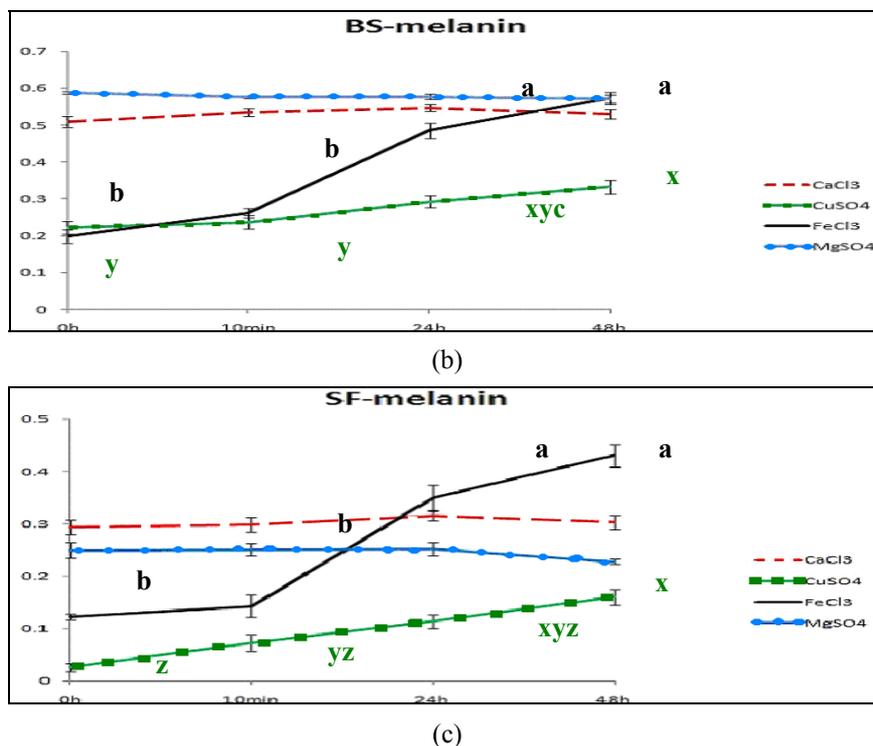


Figure 4. Effect of metal-ion on the stability of the melanin

Error bars represent standard deviations of means (n=3). (a) BT-melanin; (b) BS-melanin; (c) SF-melanin. Values are mean ± SD (n=3). <sup>a-z</sup> Means within the same color with different superscripts differ significantly (p<0.05).

### 3.2 The Antioxidation of Melanin

Yen et al. (2000) indicated that UV light could induce DNA mutation. It will cause damage to the DNA structure as well as play a role in gene mutation, diseases and cancers. Furthermore, UV will induce ROS and free radicals; this will generate oxidized damage for the organism and induce lipid peroxidation (Herrling et al., 2006; Jee et al., 2009).

#### 3.2.1 The Survival of Cultured Cells

The results are shown in Table 4: the survival rate of the negative control was obviously lower than that of the positive control. The survival rate of each test group was not obviously different from that of the positive control. This proved that UV will cause death to the fibroblast, and that SY-melanin, BT-melanin, BS-melanin and SF-melanin can protect the fibroblast from UV damage.

Table 4. The survival rate of cultured cells after UV irradiation

Experimental conditions	Cell density ( $\times 10^6$ cell/ml)	Survival (%)
MEM + SY-melanin	3.53 ± 0.29 <sup>a</sup>	96.98
MEM + BT- melanin	3.56 ± 0.25 <sup>a</sup>	97.80
MEM + BS- melanin	3.58 ± 0.18 <sup>a</sup>	98.35
MEM + SF- melanin	3.19 ± 0.12 <sup>a</sup>	87.64
Positive control	3.64 ± 0.14 <sup>a</sup>	100
Negative control	2.36 ± 0.30 <sup>b</sup>	64.84

Values are mean ± SD (n=5). <sup>a,b</sup> Means within the column with different superscripts differ significantly (p<0.05). Survival (%) = (treatment or negative control cells / positive control cells) x 100%. Positive control: cells no exposed UV irradiation. Negative control: cells exposed UV irradiation.

After UV radiation, the fibroblast will cause shape changing, damage and even death. This consistent with the results of Archambault et al. (1995) and Jee et al. (2009). They also reported that melanin could protect the skin from UV damage and scavenge ROS to avoid lipid peroxidation. Geng et al. (2008b) reported that the cell viability of fibroblast treated with varied doses of bacterial-derived melanin increased dramatically and became more resistant to UVA-induced apoptosis in comparison with the untreated control. Photoprotection against UV damage appears primarily to involve optical absorption/scattering by the melanin (Menter & Willis, 1997).

### 3.2.2 Influence on the Glutathione Peroxidase (GSH-Px) Activity of Fibroblast with Melanin Solutions Exposed to UV Irradiation

In Figure 5, the GSH-Px activity of BT-melanin, BS-melanin and SF-melanin groups show apparent promotion. The results compared to the negative/positive control were conspicuous ( $p < 0.001$ ). BT-melanin, BS-melanin and SF-melanin could increase the GSH-Px activity. GSH-Px activity of the SY-melanin group was not different from that of the positive control, but the catalase activity was apparently raised; this indicated that GSH has greater affinity to  $H_2O_2$ , and that it preserves catalase activity.

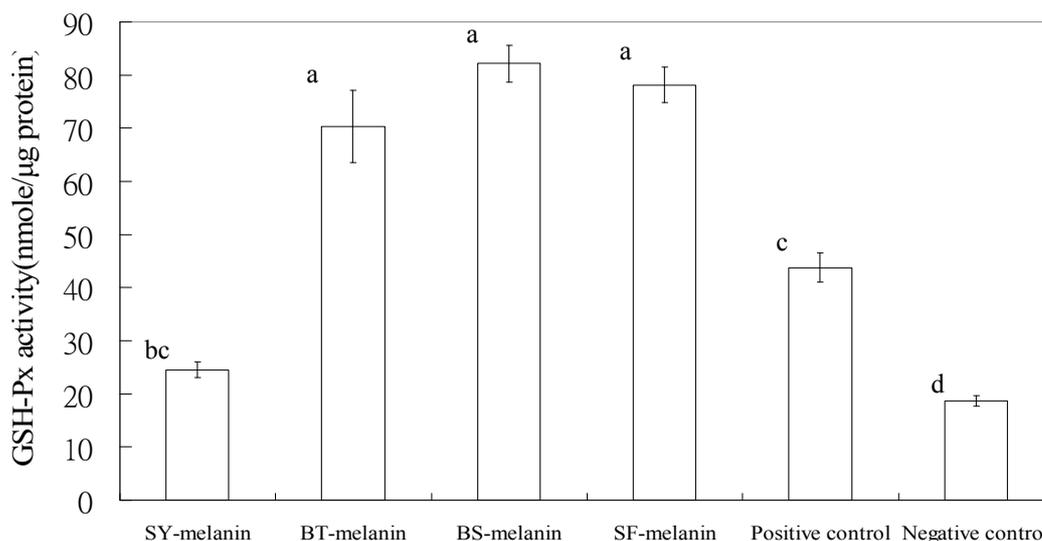


Figure 5. Influence on the glutathione peroxidase activity of fibroblast culture with melanin solutions exposed to UV irradiation. Each bar presented as the means  $\pm$  SD ( $n = 5$ ).

<sup>a,b,c,d</sup>Means with different superscripts differ significantly ( $p < 0.001$ ).

Hung et al. (2003) showed that melanin extracted from black tea could protect the liver cells of a mouse, avoid damage from free radicals induced by hydrazine (Hz) and maintain the GSH level. Geng et al. (2008b) added the melanin extracted from bacteria showed that they could protect cells from UV and  $H_2O_2$  damage.

### 3.2.3 Influence on the Catalase Activity of Fibroblast with Melanin Solutions Exposed to UV Irradiation

Figure 6 shows that the SY-melanin and BT-melanin groups could cause an obvious increase in the catalase activity. BS-melanin and SF-melanin show no difference compared to the positive control but are obviously higher than the negative control. The GSH-Px activity of these two groups was about doubled compared to the positive control. We conjectured that a high level of GSH was sufficient to remove  $H_2O_2$  induced by UV. Therefore, the catalase level was not influenced.

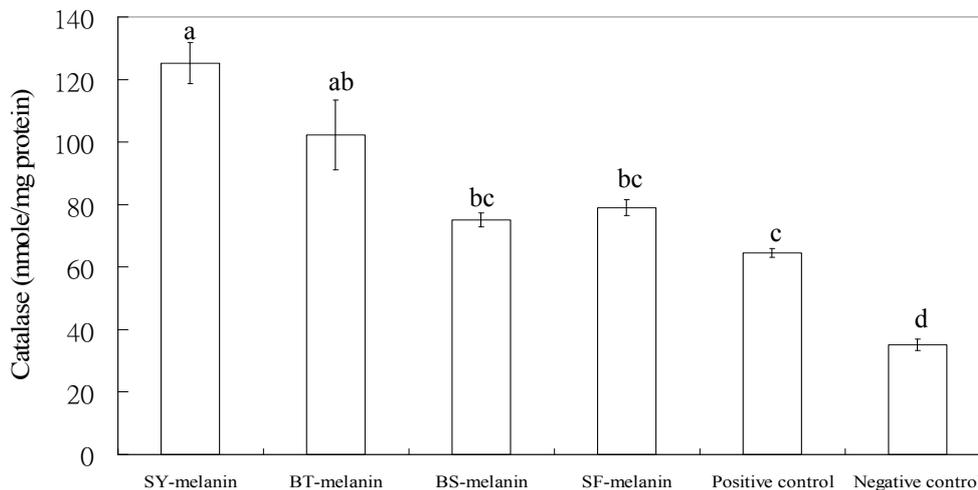


Figure 6. Influence on the catalase activity of fibroblast with melanin solutions exposed to UV irradiation.

Each bar presented as the means  $\pm$  SD (n = 5).

<sup>a,b,c,d</sup>Means with different superscripts differ significantly ( $p < 0.001$ ).

### 3.2.4 Inhibition of Lipid Peroxidation (TBARS) of Fibroblast Culture with Melanin Solution Exposed to UV Irradiation

Figure 7 demonstrates that all of the melanin groups show apparent inhibition of lipid peroxidation compared to the negative control ( $p < 0.001$ ). This indicated that melanin could inhibit the lipid peroxidation induced by UV irradiation and recover to a normal level. Bilgihan et al. (1995) reported that melanin extracted from the eye could effectively inhibit lipid peroxidation to prevent uveitis. Sava et al. (2003) indicated that melanin extracted from black tea could inhibit the MDA production and lipid peroxidation of liver cells when exposed to free radicals. Hung et al. (2007) added a different concentration of melanin extracted from black tea in mouse drinking water, which decreased the TBARS production of the kidney. Tu et al. (2009) also reported that the different doses of melanin extracted from silky fowl and synthesized melanin could both decrease lipid peroxidation of the egg. This experiment has the same result as in the above description and shows that melanin can inhibit lipid peroxidation.

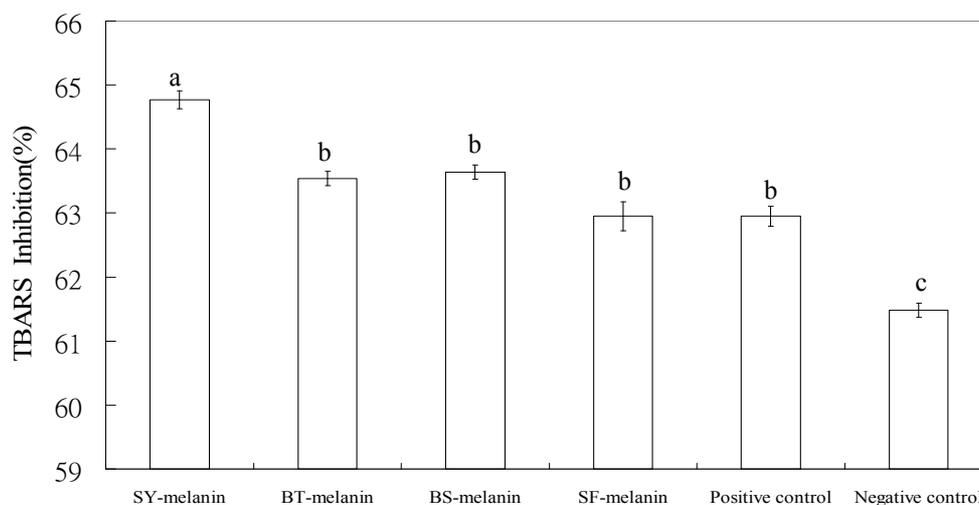


Figure 7. Inhibition of lipid peroxidation (TBARS) by fibroblast culture with melanin solution exposed to UV irradiation. Each bar presented as the means  $\pm$  SD (n = 5).

<sup>a,b,c</sup>Means with different superscripts differ significantly ( $p < 0.001$ ).

### 3.2.5 The Scavenging Capacity of Superoxide Anion Induced by UV Irradiation

The results are displayed in Figure 8, indicating that melanin has the scavenging capacity of superoxide anion. All the test groups could scavenge superoxide anion ( $p < 0.001$ ). At the same concentration ( $25 \mu\text{g mL}^{-1}$ ), the scavenging capacity of SY-melanin was 80%, and BT-melanin, BS-melanin and SF-melanin were 60%.

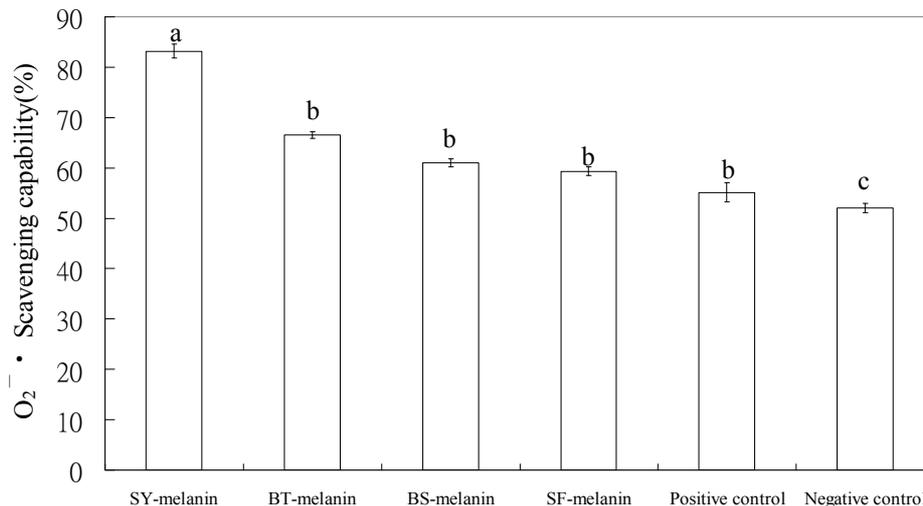


Figure 8. The scavenging capacity of superoxide anion induced by UV irradiation.

Each bar presented as the means  $\pm$  SD ( $n = 5$ ).

<sup>a,b,c</sup> Means with different superscripts differ significantly ( $p < 0.001$ ).

Blarzino et al. (1998) indicated that DHICA-melanin, DHI-melanin and Dopa-melanin had the scavenging capacity of superoxide anion and the scavenging capacity was higher than 90% with  $100 \mu\text{g mL}^{-1}$ . Tu et al. (2009) reported that melanin extracted from the muscle of silky fowl had the same ability of scavenging superoxide anion and the same effectiveness dependent on the concentration. Wang et al. (2008) reported that a significant decrease in singlet oxygen production was observed in the presence of eumelanin extracted from pig retinal pigment epithelium cells. Melanin interacted with free radicals via the simple one-electron transfer processes (Rózanowska et al., 1999). Many reports had shown that melanin had a great ability to scavenge superoxide anion, and our experiment also had the same result as the abovementioned literatures.

#### 4. Conclusion

The experiment results show that the melanins extracted from black tea, black soybean and black-bone silky fowl are similar in their physico-chemical properties, and have the capacity for antioxidation and photoprotection from UV irradiation.

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