

A METHOD FOR EXTRACTING EUMELANIN FROM INVERTEBRATE BIOMASS AND THE PRODUCT OBTAINED THEREFROM

TECHNICAL FIELD

5 The current disclosure relates to the extraction of eumelanin from invertebrate biomass and in particular the invention relates to a method for preparation of eumelanin by means of demineralisation and deproteination of invertebrate biomass and the product obtained therefrom.

BACKGROUND

10 Melanin is a broad term for a group of pigments found in most organisms. Most often it serves the function of photoprotection and is capable of converting UV radiation into heat. In microorganisms, melanin has been shown to protect against reactive oxygen species, high temperature and chemical stress. As melanin helps bacteria in its survival against the hosts
15 immune system, it is evaluated as a virulence and pathogenicity factor. In radiotropic fungi, melanin is suspected to be used for harvesting gamma rays in a similar way chlorophyll is used in plants for harvesting sunlight to produce energy. Melanin is found in the immune system of invertebrates and in cephalopod ink used for defense. In insect cuticles and plants, it acts as an important structural element.

20 Melanin is very heterogeneous and when isolated from natural sources, it is not easily separated from proteins and lipids which are always present. Also, melanin is often found as a mixture of multiple melanin types. Due to these difficulties, the exact structure of melanins remain unclear. Three main types of melanin are known, namely, eumelanin (brown/black), phaeomelanin (yellow/red) and allomelanin (catechol melanin) with eumelanin being the most
25 common variant. Melanin is produced in a process called melanogenesis. Besides isolation from natural sources, melanin can also be synthesized. However, synthetic eumelanin is expensive to synthesize as the reagents used are costly. Thus, it is preferable to extract eumelanin from natural sources instead of synthesising eumelanin.

30 A natural eumelanin reservoir are the ink sacs from cephalopods, which contain around 1 g eumelanin each, with around 15% of the ink (wet weight) consisting of eumelanin.

However, the available cephalopod ink sacs for eumelanin supply are severely limited, and is therefore not a reliable commercial source of melanin.

International Patent Application Publication No. WO 2010/064262 describes a process for extracting melanin from the fungus *Gliocephalotrichum*. After growing the fungus in liquid culture, the fungal biomass is removed from the culture liquid containing the melanin. The pH of the media is acidified by hydrochloric acid and the precipitate recovered by centrifugation or filtration. The precipitate is dried and re-dissolved at pH 11 or higher, dialyzed and precipitated.

Russian Patent No. RU2565178 describes the extraction of melanin from fungal spores. Mushroom fruiting bodies are colonized with mushroom eating beetles for 20 to 30 days. The resulting mass is treated with ethyl alcohol for 5 hours, separated by centrifugation, and boiled with water for 2 hours. After centrifugation, the precipitate is extracted with 0.5 M sodium hydroxide solution for 24 hours at 25 °C to 28 °C. After precipitation with hydrochloric acid, the precipitate is dissolved with 0.1 M sodium hydroxide and reprecipitated three times and subsequently dialyzed.

Chinese Patent No. CN103497535 describes a process for the extraction of melanin from animals with a focus on insects, where the raw material is crushed, extracted via an alkali solution at 70 °C to 100 °C, followed by an acid treatment and an extraction of melanin via an organic solvent.

Chinese Patent Application Publication No. CN104382953 describes the extraction of melanin from the black fungus *Auricularia*. Biomass is pulverized and treated with 1.5 mol/L sodium hydroxide under sonication to obtain a melanin solution. In the subsequent precipitation step, the pH is adjusted to 1.5 and stored at 80 °C for 6 hours to 12 hours. After centrifugation, the precipitate is dried or lyophilized and the melanin powder is refined by hydrolysis with 1 ml/L hydrochloric acid for 2 hours at 100 °C, filtered, extracted with an organic solvent and precipitated.

Ushakova et al., *Ukrainian Journal of Ecology*, 7(4), 424-431 (2017) used a process for extraction of eumelanin from *Hermetia illucens*. Insect biomass was ground, 10% sodium hydroxide solution added and stirred at 80 °C without air for 1.5 hours. Subsequently, 50% sodium hydroxide solution was added and the mixture stirred for 2 hours at 95 °C. Eumelanin

was recovered by precipitation through adjusting the pH of the liquid to 2 with concentrated hydrochloric acid. The precipitate was recovered by centrifugation, then neutralized and dried.

Ushakova et al., *Doklady Biochemistry and Biophysics*, 473(1), 102–105 (2017) extracted eumelanin from *Hermetia illucens* by extraction with 3 M to 5 M potassium hydroxide solution, followed by precipitation with concentrated hydrochloric acid at a pH of 2. After centrifugation, the eumelanin pellet was treated with 1% hydrochloric acid for 3 to 4 hours, centrifugated, washed with distilled water and dried in a desiccator.

The processes described above fail to sufficiently separate proteins, minerals and chitin from melanin so as to obtain sufficiently pure eumelanin. Furthermore, some of these processes, especially those that use fungi, are also expensive to run and would make the extraction of eumelanin commercially not viable.

SUMMARY

According to embodiments of the present disclosure, there is provided a method for extracting eumelanin from invertebrate biomass. The method may comprise: providing invertebrate biomass; rupturing the invertebrate biomass to form raw material; demineralising the raw material to form a first solid fraction; performing a deproteinising step on the first solid fraction to form a second solid fraction; performing a eumelanin liberating step on the second solid fraction; extracting a supernatant from the eumelanin liberating step; and precipitating and harvesting eumelanin from the supernatant.

According to some embodiments, the invertebrate biomass may be a melanised Arthropoda phylum species, and more specifically *Hermetia illucens*. The invertebrate biomass may comprise cuticles from prepupal stages, empty pupae shells or spent adult stage. According to some embodiments, rupturing of the invertebrate biomass may comprise of squashing, grinding, and perforation of the invertebrate biomass.

According to some embodiments, demineralising the raw material may comprise: adding a first acidic solution having a concentration of between 2 M to 5 M to the raw material to obtain a first mixture; treating the first mixture for a first period of time at a temperature between 25 °C to 50 °C; and extracting the first solid fraction from the first mixture by filtration or centrifugation. According to some embodiments, the first acidic solution may be hydrochloric acid. Adding the first acidic solution may be performed to obtain a first solid-to-

liquid ratio of between 1:8 and 1:30. The first period of time may be between 1 hour to 24 hours. According to some embodiments, treating the first mixture further comprises stirring the first mixture at a speed of between 10 rpm to 500 rpm during the first period of time before the first solid fraction is extracted.

5 According to some embodiments, demineralising the raw material may further comprise washing the first solid fraction with water and receiving a first washing liquid, checking the first washing liquid for pH level and rewashing the first solid fraction with water until the first washing liquid pH level is within 5 to 7.

 According to some embodiments, the deproteinising step may comprise: adding a first
10 alkaline solution having a concentration between 0.5 M to 2 M to the first solid fraction to obtain a second mixture; treating the second mixture for a second period of time at a temperature between 25 °C to 40 °C; and extracting the second solid fraction from the second mixture by filtration or centrifugation. The first alkaline solution may be sodium hydroxide. Adding the first alkaline solution may be performed to obtain a second solid-to-liquid ratio of
15 between 1:5 and 1:30. The second period of time may be between 30 minutes to 5 hours.

 According to some embodiments, treating the second mixture may further comprise stirring the second mixture at a speed of between 10 rpm to 200 rpm during the second period of time before the second solid fraction is extracted.

 According to some embodiments, the deproteinising step may further comprise the step
20 of washing the second solid fraction with water and receiving washing liquid, checking the second washing liquid for pH level and rewashing the second solid fraction with water until the second washing liquid pH level is within 5 to 7.

 According to some embodiments, the eumelanin liberating step may comprise: adding a second alkaline solution having a concentration of 3 M to 6 M to the second solid fraction to
25 obtain a third mixture; and treating the third mixture for a third period of time at a temperature between 80 °C to 100 °C. The second alkaline solution may be sodium hydroxide. Adding the second alkaline solution may be performed to obtain a third solid-to-liquid ratio of between 1:10 and 1:24. The third period of time may be between 2 hours to 6 hours. Treating the third mixture may further comprise stirring the third mixture at a speed of between 10 rpm to 500
30 rpm during the third period of time.

According to some embodiments, extracting the supernatant may comprise extraction by filtration or centrifugation of the third mixture or may further comprise washing a residue obtained from the filtration or centrifugation of the third mixture with water to obtain a wash liquid. According to some embodiments, the water used to obtain a wash liquid is at a volume of 1 to 3 times the volume of the second alkaline solution. The wash liquid may be combined with the supernatant prior to extraction of eumelanin. Insoluble substances may be removed from the supernatant by filtration or centrifugation.

According to some embodiments, precipitating and harvesting eumelanin from the supernatant may comprise: precipitating the eumelanin by adding a second acidic solution to the supernatant to obtain a fourth mixture, checking the fourth mixture for pH level, and adding the second acidic solution until the pH of the fourth mixture is between 1 to 2; and harvesting the eumelanin by filtration or centrifugation.

According to some embodiments, the method may further comprise the steps of: washing the harvested eumelanin with water at a eumelanin-to-water ratio of 1:20 to 1:60; recovering the washed eumelanin through filtration or centrifugation; and drying the recovered eumelanin by oven-drying, spray-drying or freeze-drying.

According to some embodiments, there is provided a composition obtainable according to any of the embodiments of the method. According to some embodiments, there is provided a eumelanin, which is obtainable by a process comprising the steps of: providing invertebrate biomass; rupturing the invertebrate biomass; demineralising the invertebrate biomass to form a first solid fraction; performing a deproteinising step on the first solid fraction to form a second solid fraction; performing a eumelanin liberating step on the second solid fraction; extracting a supernatant from the eumelanin liberating step; and precipitating and harvesting eumelanin from the supernatant.

BRIEF DESCRIPTION OF THE DRAWINGS

Features, aspects and advantages of the present disclosure will become better understood with regard to the following description and accompanying drawings in which:

FIG. 1 depicts a process of the eumelanin extraction process, in accordance with embodiments of the present disclosure.

FIG. 2 is a picture showing invertebrate biomass, in accordance with embodiments of the present disclosure.

FIG. 3 is a picture showing raw material obtained from the rupturing of invertebrate biomass, in accordance with embodiments of the present disclosure.

5 FIG. 4 is a picture showing the treating of the first mixture, in accordance with embodiments of the present disclosure.

FIG. 5 is a picture showing the first washing liquid obtained after washing the first solid fraction with water, in accordance with embodiments of the present disclosure.

10 FIG. 6 is a picture showing the first solid fraction obtained after the demineralising step and washing, in accordance with embodiments of the present disclosure.

FIG. 7 is a picture showing the treating of the second mixture, in accordance with embodiments of the present disclosure.

FIG. 8 is a picture showing the second washing liquid obtained after washing the second solid fraction with water, in accordance with embodiments of the present disclosure.

15 FIG. 9 is a picture showing the second solid fraction obtained after the deproteinising step and washing, in accordance with embodiments of the present disclosure.

FIG. 10 is a picture showing the liberating of the eumelanin, in accordance with embodiments of the present disclosure.

20 FIG. 11 is a picture showing a pellet of eumelanin extracted by centrifugation, in accordance with embodiments of the present disclosure.

FIG. 12 is a picture showing the dried eumelanin obtained from the eumelanin extraction process, in accordance with embodiments of the present disclosure.

25 FIG. 13 is a graph showing spectra obtained using ultraviolet-visible spectroscopy (UV-Vis) to measure samples of synthetic melanin and extracted eumelanin, in accordance with embodiments of the present disclosure.

FIG. 14 is a graph showing spectra obtained using Fourier Transform Infrared Spectroscopy (FTIR) (from 400 cm^{-1} to $4,000\text{ cm}^{-1}$) to measure samples of synthetic melanin and extracted eumelanin, in accordance with embodiments of the present disclosure.

DETAILED DESCRIPTION

The present disclosure provides for opening mechanically macroscopic entrapments filled with contaminants, followed by demineralization with hydrochloric acid to loosen the structure, to maximize surface accessibility for the weak alkaline solution treatment, which
5 removes protein while retaining a maximum of eumelanin. A subsequent, harsher treatment with alkaline solution, is strong enough to liberate the majority of eumelanin with much less protein contamination than other methods. By avoiding extreme, highly concentrated alkaline solutions, the costs for subsequent melanin recovery and purification are minimized. Thus, eumelanin can be produced in larger amounts and in higher purity compared with prior
10 solutions.

The invention relates to the extraction of eumelanin by: (a) rupturing invertebrate biomass for sufficient exposure of contaminants to solvents and solutions for their removal; (b) demineralization of the invertebrate biomass with acid solution for removal of minerals; (c) deproteination via alkaline solutions under mild treatment conditions for removal of proteins
15 while preventing eumelanin from leaching out; (d) eumelanin liberation with alkaline solutions under harsher conditions; (e) precipitation of eumelanin, followed by harvesting of eumelanin and subsequent washing and drying.

FIG. 1 depicts a process 100 of the eumelanin extraction process, in accordance with embodiments of the present disclosure. Invertebrate biomass is ruptured, demineralized with
20 acid to remove inorganic substances, deproteinated under relatively mild alkaline conditions to remove the protein fraction before the eumelanin is liberated in a hot alkaline treatment. Subsequently, the eumelanin is recovered by precipitation.

In step 102, the invertebrate biomass and therefore the raw material of the process can be obtained from any melanised species belonging to the phylum Arthropoda but preferably
25 from *Hermetia illucens* (Insecta: Diptera: Stratiomyidae), whose common name is the Black Soldier Fly. Preferably, the invertebrate biomass consists of the cuticles of the prepupal stages, empty pupae shells or spent adult stage, but it can be derived from any life stage.

In order to sufficiently expose remnant proteins and lipids to later treatments such as solvents and solubilization reagents, in step 104 the invertebrate biomass is ruptured by
30 subjecting the invertebrate biomass to squashing, grinding, perforation, or any other mechanical means to rupture the exoskeletons. Preferably, the torn pieces of exoskeleton from

any source remain as large as possible (> 5 mm) to facilitate separation of supernatant and filtrate in later steps. The invertebrate biomass prepared in such manner for the extraction and purification steps is subsequently termed “raw material” 106.

In step 108, the demineralization of raw material 106 is carried out by adding a first
5 acidic solution, preferably hydrochloric acid, having a concentration of between 2 M to 5 M but preferably at 3 M, to the raw material 106 to obtain a first solid-to-liquid ratio of between 1:8 and 1:30 but preferably 1:10. The first mixture should be treated for a first time period of between 1 hour to 24 hours but preferably 2 hours at a temperature of between 25 °C and 50 °C but preferably at 50 °C. During the first period of time, the first mixture is not stirred or stirred
10 in the range of 10 rpm to 500 rpm but preferably at 200 rpm. The first solid fraction 110, comprising demineralised raw material containing the eumelanin, is extracted from the first mixture by filtration or centrifugation and subjected to washing with water to receive a first washing liquid. The pH level of the first washing liquid is checked, and the first solid fraction 110 is washed until the colour of the first washing liquid is nearly transparent and within a pH
15 in the range of 5 to 7 but preferably 7.

Next, in step 112, the deproteination of the first solid fraction 110 is carried out by adding a first alkaline solution, preferably sodium hydroxide, having a concentration of between 0.5 M to 2 M but preferably 1 M, to the first solid fraction 110 to obtain a second mixture with a second solid-to-liquid ratio of 1:5 to 1:30 but preferably 1:10. The second
20 mixture should be treated for a second time period of between 0.5 hours to 5 hours but preferably 1 hour at a temperature of between 25 °C to 40 °C but preferably at 40 °C. The temperature is not allowed to exceed this range in order to prevent excessive liberation of eumelanin from the first solid fraction 110 and therefore to allow maximum eumelanin recovery. During the second period of time, the second mixture is not stirred at all or stirred in
25 a range of 10 rpm to 200 rpm but preferably at 200 rpm. The second solid fraction 114, comprising demineralised and deproteinated raw material and containing the eumelanin, is extracted from the second mixture by filtration or centrifugation and subjected to multiple rounds of washing with water to receive a second washing liquid. The pH level of the second washing liquid is checked, and the second solid fraction 114 is washed until the colour of the
30 second washing liquid is nearly transparent and/or within a pH in the range of 5 to 7 but preferably 7.

Next, in step 116, the second solid fraction 114 which now comprises mainly eumelanin and chitin, a stronger treatment with a second alkaline solution, is applied. A second alkaline solution, preferably sodium hydroxide, having a concentration of between 3 M to 6 M but preferably at 3 M, is added to the second solid fraction 114 to obtain a third mixture with a
5 third solid-to-liquid ratio of 1:10 to 1:24 but preferably at 1:12. The third mixture should be treated for a third time period of between 2 hours to 6 hours but preferably 2 hours at a temperature of between 80 °C to 100 °C but preferably at 90 °C. During the third period of time, the third mixture is not stirred at all or stirred in a range of 10 rpm to 500 rpm but preferably at 300 rpm. The supernatant 118, which is solution containing eumelanin, is
10 extracted using filtration or centrifugation, leaving behind a residue. The residue, which consist mainly of chitin, is washed with water to recover any residual eumelanin. Water is added at a volume of between 1 to 3 times the original volume of the second alkaline solution added but preferably 1.5 times. A wash liquid is extracted and the wash liquid containing the residual eumelanin is pooled with the supernatant 118 which contains most of the eumelanin. For
15 increasing eumelanin purity further, insoluble substances such as chitin fibres are further removed by filtration or centrifugation.

In step 120, eumelanin 122 is recovered from the supernatant 118 by precipitation through adjusting its pH using a second acid solution, preferably hydrochloric acid to obtain a fourth mixture. The pH level of the fourth mixture is checked and the second acid solution is
20 added until the fourth mixture has a pH of 1 to 2 but preferably 2. The eumelanin 122 is harvested by filtration or centrifugation and washed with water in order to reduce excess salts. Water is added to the eumelanin 122 to obtain a eumelanin-to-water weight ratio of between 1:20 to 1:60, but preferably 1:30. The eumelanin 122 is recovered by filtration or centrifugation and dried through oven-drying, spray-drying or freeze-drying.

25 The following example is provided to illustrate the present invention and should not be construed as limiting thereof:

I. Example 1: Extraction of Eumelanin from 1,250 g of Raw Material

Production of Invertebrate Biomass.

The invertebrate biomass used was dried empty pupae shells from the Black Soldier
30 Fly (*Hermetia illucens*). The dried empty pupae shells were collected from an artificial rearing process and separated from macroscopic contaminants such as plastics, feed and frass (FIG. 2).

1,250 g of dried pupae shells were briefly ground up in a blender (Robot Coupe Blixer 4, France) to ensure rupturing of any un-enclosed pupae and dead adults and sufficient exposure of proteins, fats and minerals to subsequent process steps (FIG. 3).

5 The 1,250 g of raw material obtained after rupturing of the dried pupae shells was demineralized in batches of 100 g. 1 L of 3 M hydrochloric acid was added to each batch of 100 g ruptured shells. The mixture was kept on a temperature-controlled hot plate (Witeg MSH-20D, Germany) at 50 °C and stirred with an overhead stirrer (IKA RW20, Germany) at about 200 rpm for 2 hours (FIG. 4).

10 The demineralized first solid fraction was obtained by pouring the mixture repeatedly through a sieve and washed with water until the first washing liquid reached a pH of 7 (FIG. 5). The wet first solid fraction (FIG. 6) was dried and weighed. The dry weight of the first solid fraction obtained was determined to be 580 g, indicating a weight loss of approximately 54% from the original starting material. 1 L of 1 M sodium hydroxide was added to each batch of first solid fraction and the resulting mixtures were stirred at 200 rpm, for 1 hour, room
15 temperature (25 °C to 27 °C) (FIG. 7).

The second solid fraction was obtained by pouring the mixture repeatedly through a sieve and washed with water until the second washing liquid was clear, thus ensuring that most of the proteins had been removed (FIG. 8). The wet second solid fraction (FIG. 9) was dried and weighed. The dry weight of the second solid fraction obtained was determined to be 452 g
20 which indicates a weight loss of approximately 64% from the original starting material.

Eumelanin was then liberated from the second solid fraction by adding 6 L of 3 M sodium hydroxide to the second solid fraction, and the resulting mixture was stirred at 200 rpm, for 2 hours at 80 °C. The eumelanin-containing supernatant was extracted by draining the mixture through a sieve. 9 L deionized water was used to wash the residue and to capture more
25 eumelanin in the wash liquid.

The eumelanin-containing supernatant was placed in beakers and stirred at 200 rpm by a magnetic stirrer (Witeg MSH-20D, Germany). The pH was adjusted to 2 with 36.5% concentrated hydrochloric acid to precipitate eumelanin (FIG. 10). The mixture was centrifuged at 4,000 rpm for 5 min in a swinging bucket centrifuge (Eppendorf 5810R,
30 Germany) to pellet the eumelanin (FIG. 11).

After centrifugation, the liquid was discarded. The pelleted eumelanin was submerged in 1,500 ml deionized water and centrifuged at 4,000 rpm for 5 min. The supernatant was decanted, and the pelleted eumelanin was dried overnight in an oven at 60 °C. The pelleted eumelanin was dried (FIG. 12) and the dry weight of the eumelanin obtained was determined to be 52 g, which indicates a final eumelanin yield of 4.2% obtained from 1,250 g of invertebrate biomass.

The extracted eumelanin was characterised through measurement with ultraviolet-visible spectroscopy (UV-Vis) and Fourier Transform Infrared Spectroscopy (FTIR).

FIG. 13 is a graph showing spectra obtained using ultraviolet-visible spectroscopy (UV-Vis) to measure samples of synthetic melanin and extracted eumelanin. Sample 1 (Eumelanin from prepupae cuticles), Sample 2 (Eumelanin from pupae shells) and synthetic melanin (Merck/Sigma Melanin, United States) were obtained in dried powder form. A few milligrams of each sample were dissolved in 5 ml 2 M sodium hydroxide (NaOH) solution, vigorously mixed and spun down for 10 min to remove any residual, undissolved powder. The blackish liquid free of particles was transferred to a new container and diluted 1:1 with water. A quartz cuvette was thoroughly cleaned with ethanol, dried and filled with 950 to 980 µl ultrapure water. 20 to 50 µl of Sample 1, Sample 2 and synthetic melanin, were added to each cuvette to a final liquid volume of 100 µl and mixed thoroughly resulting in a very faint aqueous solution. Each solution was measured in a UV-Vis spectrophotometer (Agilent Cary 50, United States) at a maximum wavelength ranging from 190 nm to 1100 nm at a scan rate of 600 nm/min in Dual Beam Mode with baseline correction. For baseline correction, a solution was prepared consisting of 950 µl water and 50 µl 2 M sodium hydroxide solution. The absorption was plotted against wavelength in absolute and in semi-logarithmic representation.

Both samples of extracted eumelanin and the synthetic melanin exhibited a strong absorbance in the UV range decreasing with longer wavelengths. The absence of pronounced peaks at 260 nm and 280 nm indicate the absence of nucleic acids, lipids and protein (FIG. 13). The maximum absorbance was measured between 222 nm and 220 nm for Sample 1 and Sample 2 respectively, while the maximum absorbance for synthetic melanin was measured at 225 nm (see Table 1). The decrease in the range between 500 nm and 250 nm, yields a negative slope when plotted semi-logarithmically. The values of -0.0041 to -0.0043 obtained from measuring the samples are in line with reference values from literature for eumelanin from different sources (see Table 1).

Table 1 **Derived UV-Vis Values Obtained from Measurement of Samples**

	Maximum Absorption Wavelength [nm]	R²	Slope of log₁₀
Sample 1	222	0.9957 – 0.9959	- 0.0041
Sample 2	220	0.998 – 0.9981	- 0.0042 – -0.0043
Synthetic melanin	225	0.9842 – 0.9846	- 0.0042

FIG. 14 is a graph showing spectra obtained using Fourier Transform Infrared Spectroscopy (FTIR) (from 400 cm⁻¹ to 4,000 cm⁻¹) to measure samples of synthetic melanin and extracted eumelanin. Sample 1 (Eumelanin from prepupae cuticles), Sample 2 (Eumelanin from pupae shells) and synthetic melanin (Sigma-Aldrich, United States) were obtained in dried powder form. A few grains of each sample were mixed with FTIR-grade potassium bromide (KBr) (Sigma-Aldrich, United States) and ground with an Agate mortar. The powder obtained was pressed into a disc using a manual hydraulic 10 tons benchtop press (Perkin Elmer, United States). Each disc was analyzed using an FTIR spectrophotometer (Perkin Elmer Frontier, United States) with the settings 4,000 to 400 cm⁻¹ at a scanning rate of 4 cm⁻¹. 150 sample scans were used to obtain the spectrum for each sample. The spectra were plotted as transmittance (%) against wavenumber (cm⁻¹).

The spectra of Sample 1 and Sample 2 were found to have high similarities to the synthetic melanin sample and published reference values (FIG. 14) (see Table 2). Reference values were obtained from Mboniyirivuze et al., *Physics and Materials Chemistry*, 3(2): 25-29 (2015). Although the spectra obtained for Sample 1 and Sample 2 were found to have similarities to the synthetic melanin sample and reference values, there are clear differences, which indicates that the eumelanin extracted has chemical and structural differences from synthetic melanin.

Table 2 **FTIR Peak Values from Measurement of Samples in Comparison with Published Reference Peaks for Melanin**

	O-H or N-H stretching vibration modes	Stretching vibration of aliphatic C-H group		Bending vibrations modes of aromatic ring (C=C, C=N)	O-H bending of phenolic and carboxylic groups
Reference Wavelength	3422	2917	2839	1621	1374
Synthetic melanin	3422	2918	2850	1626	1384
Sample 1	3442	2919	2851	1646	1384
Sample 2	3433	2920	2851	1636	1384

The processes described above provides effective separation of proteins, mineral and chitin from eumelanin which results in sufficiently pure eumelanin. Furthermore, the processes described above are a cost-effective method of producing eumelanin. The processes described above do not incur the hefty costs associated with synthesizing eumelanin in the lab or growing fungi for the extraction of eumelanin as the invertebrate biomass used is derived from secondary by-products of the insect farming industry and thus can be cheaply obtained.

CLAIMS

1. A method of extracting eumelanin from invertebrate biomass, the method comprising:
 - providing invertebrate biomass;
 - rupturing the invertebrate biomass to form raw material;
 - demineralising the raw material to form a first solid fraction;
 - performing a deproteinising step on the first solid fraction to form a second solid fraction;
 - performing a eumelanin liberating step on the second solid fraction;
 - extracting a supernatant from the eumelanin liberating step; and
 - precipitating and harvesting eumelanin from the supernatant.
2. The method of claim 1 wherein the invertebrate biomass is a melanised Arthropoda phylum species.
3. The method of claim 2 wherein the invertebrate biomass is *Hermetia illucens*.
4. The method of claim 1 wherein the invertebrate biomass comprises cuticles from prepupal stages, empty pupae shells or spent adult stage.
5. The method of claim 1 wherein rupturing of the invertebrate biomass comprising of squashing, grinding, and perforation of the invertebrate biomass.
6. The method of claim 1 wherein demineralising the raw material comprises:
 - adding a first acidic solution having a concentration of between 2 M to 5 M to the raw material to obtain a first mixture;
 - treating the first mixture for a first period of time at a temperature between 25 °C to 50 °C; and

extracting the first solid fraction from the first mixture by filtration or centrifugation.

7. The method of claim 6 wherein the first acidic solution is hydrochloric acid.
8. The method of claim 6 wherein adding the first acidic solution is performed to obtain a first solid-to-liquid ratio of between 1:8 and 1:30.
9. The method of claim 6 wherein the first period of time is between 1 hour to 24 hours.
10. The method of claim 6 wherein treating the first mixture further comprises stirring the first mixture at a speed of between 10 rpm to 500 rpm during the first period of time before the first solid fraction is extracted.
11. The method of claim 6 further comprising washing the first solid fraction with water and receiving a first washing liquid, checking the first washing liquid for pH level and rewashing the first solid fraction with water until the first washing liquid pH level is within 5 to 7.
12. The method of claim 1 wherein the deproteinising step comprises:

adding a first alkaline solution having a concentration between 0.5 M to 2 M to the first solid fraction to obtain a second mixture;

treating the second mixture for a second period of time at a temperature between 25 °C to 40 °C; and

extracting the second solid fraction from the second mixture by filtration or centrifugation.

13. The method of claim 12 wherein the first alkaline solution is sodium hydroxide.
14. The method of claim 12 wherein adding the first alkaline solution is performed to obtain a solid-to-liquid ratio of between 1:5 and 1:30.
15. The method of claim 12 wherein the second period of time is between 30 minutes to 5 hours.

16. The method of claim 12 wherein treating the second mixture further comprises stirring the second mixture at a speed of between 10 rpm to 200 rpm during the second period of time before the second solid fraction is extracted.
17. The method of claim 12 further comprising the step of washing the second solid fraction with water and receiving a second washing liquid, checking the second washing liquid for pH level and rewashing the second solid fraction with water until the second washing liquid pH level is within 5 to 7.
18. The method of claim 1 wherein the eumelanin liberating step comprises:
 - adding a second alkaline solution having a concentration of 3 M to 6 M to the second solid fraction to obtain a third mixture; and
 - treating the third mixture for a third period of time at a temperature between 80 °C to 100 °C.
19. The method of claim 18 wherein the second alkaline solution is sodium hydroxide.
20. The method of claim 18 wherein adding the second alkaline solution performed to obtain a third solid-to-liquid ratio of between 1:10 and 1:24.
21. The method of claim 18 wherein the third period of time is between 2 hours to 6 hours.
22. The method of claim 18 wherein treating the third mixture further comprises stirring the third mixture at a speed of between 10 rpm to 500 rpm during the third period of time.
23. The method of claim 1 wherein extracting the supernatant comprises extraction by filtration or centrifugation of the third mixture.
24. The method of claim 23 further comprising washing a residue obtained from the filtration or centrifugation of the third mixture with water to obtain a wash liquid.
25. The method of claim 24 wherein the water used to obtain a wash liquid is at a volume of 1 to 3 times the volume of the second alkaline solution.

26. The method of claim 24 further comprising combining the wash liquid with the supernatant prior to extraction of eumelanin.
27. The method of claim 23 further comprising removing of insoluble substances from the supernatant by filtration or centrifugation.
28. The method of claim 1 wherein the precipitating and harvesting eumelanin from the supernatant comprises:

precipitating the eumelanin by adding a second acidic solution to the supernatant to obtain a fourth mixture, checking the fourth mixture for pH level, and adding the second acidic solution until the pH of the fourth mixture is between 1 to 2; and

harvesting the eumelanin by filtration or centrifugation.

29. The method of claim 1 further comprising the steps of:

washing the harvested eumelanin with water at a eumelanin-to-water ratio of 1:20 to 1:60;

recovering the washed eumelanin through filtration or centrifugation; and

drying the recovered eumelanin by oven-drying, spray-drying or freeze-drying.

30. A composition obtainable by a method according to any of claims 1-29.

31. A eumelanin, which is obtainable by a process comprising the steps of:

providing invertebrate biomass;

rupturing the invertebrate biomass to form raw material;

demineralising the raw material to form a first solid fraction;

performing a deproteinising step on the first solid fraction to form a second solid fraction;

performing a eumelanin liberating step on the second solid fraction;

extracting a supernatant from the eumelanin liberating step; and
precipitating and harvesting eumelanin from the supernatant.