

PREPARATION OF MELANIN FROM SILKWORM WASTES AND STUDYING ITS PHYSICAL AND CHEMICAL CHARACTERISTICS

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ABSTRACT: The paper presents a scheme for preparation of melanin from silkworm wastes and studies of its physicochemical characteristics and individual composition. The optimal conditions for the isolation of melanin from silkworm wastes, providing the maximum yield of the target product, have been established. It has been established that the main substance of melanin isolated from silkworm wastes is 5,6-dioxiindole homopolymers. The black dry substance, the content of the target product with an average molecular weight of 58300 ± 112 and a density of 1.0 g/cm³ is 96-98 %. The maximum molar absorption is observed at a wavelength of 555 nm, it is well soluble in water, oils and organic solvents.

KEYWORDS: waste, silkworm, melanin, molecular weight, composition, GC-MS.

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INTRODUCTION

It is known that melanins are unique natural high-molecular pigments that have an irregular structure and complex chemical composition [1-2] and are characterized by high UV, radio-, gastro-, hepatoprotective, antioxidant activity [1-5]. Due to the unique properties of melanin, they are widely used as therapeutic and biologically active additives [1-5]. Typically, melanins are prepared by biotechnological methods using fungi and bacteria as raw materials [2, 4, 6]. Despite this, the research is being conducted on the search for alternative sources of melanins [7-9]. In this regard, the most promising for the production of melanins is the silkworm wastes, which accumulates in large quantities in the season and is characterized by low. In this connection, the development of a technology for the isolation of melanins from silkworm wastes, the study of the composition of melanin extracts and their certain properties is relevant.

The purpose of the work is to develop a technology for the isolation of melanin from silkworm wastes, to study the composition of melanin extracts by GC-MS and some properties.

MATERIALS AND METHODS OF RESEARCH

To isolate melanin, silkworm wastes were used as a raw material, and water, methanol, ethanol, propanol, butanol, and acetone were used as an extract. The composition of the extracts was studied by IR spectroscopy, gas chromatography-mass spectrometry (GC-MS) [10], and gel chromatography was used to determine the molecular weight [11].

RESULTS AND THEIR DISCUSSION

The isolation of melanin from silkworm wastes was carried out according to the scheme [12]. According to which the raw materials for the isolation of melanin have been dried up to a moisture content of 4-6 %, crushed to a size of 0.1-0.3 mm. The deproteinization of the raw material was carried out with the solutions of various concentrations of NaOH (from 2.5 to 15 % with an interval of 2.5%) in a ratio of 1:10 at 90-95 ° C for 4.0-6.0 hours, as a result melanin complex is formed. From the resulting hydrolyzate, melanin was precipitated by the addition of concentrated HCl and by centrifugation at a speed of 5000 rpm for 15 minutes. The acid reprecipitation procedure was carried out three times, after which the melanin precipitates were purified by gel chromatography on a Sephadex G-75 sorbent and lyophilized. The findings on the yield of melanin from the concentration of NaOH are presented in the form of a graph (Fig. 1).

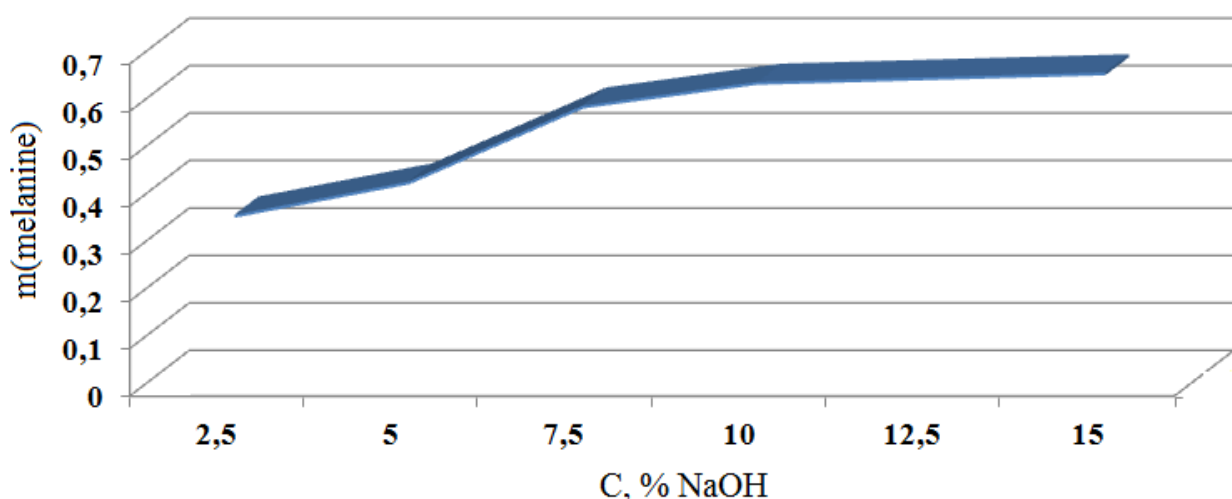


Fig. 1. The dependence of the yield of melanin on the concentration of NaOH

The graph shows that the optimal concentration is 10 % NaOH solution, at which the yield of melanin from the silkworm wastes makes 0.63 %.

The IR spectrum of the melanin sample in tablets was taken in the range from 4000 to 400 cm^{-1} (Fig. 2).

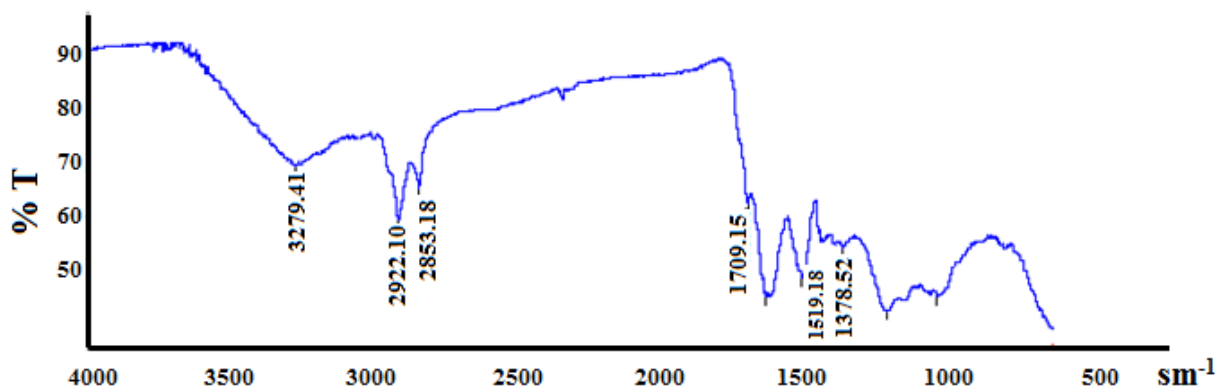


Fig. 2. IR spectra of melanin isolated from silkworm wastes

As a result of IR spectrum interpretation, it was found that the absorption band in a wide frequency range of 3250-3500 cm^{-1} is due to stretching vibrations of OH groups of alcohols and phenols, which are connected by inter- and intramolecular bonds. The presence of an absorption band in the spectra with wave numbers of 1140–1230, 1310–1420 cm^{-1} is characteristic for stretching and deformation vibrations of the C – O and OH groups of phenols, which indicate the presence of phenolic fragments in the melanin structure. Absorption bands at frequencies of 1633–1644 cm^{-1} are characteristic of C = O vibrations of quinones. The presence of an absorption bands with a wave number of 1519–1538 cm^{-1} , 1444–1448 cm^{-1} in the spectrum corresponds to the absorption band of stretching vibrations of aromatic C – C bonds, which indicates the presence of aromatic fragments in the structure of the studied melanin. An absorption band with a wave number of 1709 cm^{-1} corresponds to carbonyl fragments. The presence of absorption bands in the spectrum in the range of 2852–2853 cm^{-1} and 2921–2922 cm^{-1} confirms the presence of $-\text{CH}_2-$ fragments in the studied structure. The absorption bands in the spectra in the range of 1120–1150 cm^{-1} , 1000–1030 cm^{-1} indicate the presence of polysaccharide fragments. The absorption spectrum of the solutions of isolated melanin was taken. In this case, the maximum molar absorption is observed at a wavelength of 555 nm.

The molecular weight of melanin was determined using gel chromatography on Sephadex G-75. To do this, a calibration chart was built using as standards: bovine serum albumin ($M_r = 67000$), egg albumin ($M_r = 45000$), cytochrome C ($M_r = 13000$), trypsinogen ($M_r = 25000$). Standard proteins with known relative molecular weights and melanin were passed through a column packed with Sephadex G-75, and eluent volume was measured to displace each sample. The detection of samples at the exit was carried out by a photometric detector at $\lambda = 280$ nm. The protein concentration in the preparations is 1 - 2 mg / ml. From the calibration graph the equation for calculating the molecular weight was obtained:

$$\lg M_r = 5,286 - 0,775 \cdot \left(\frac{V_e}{V_o} \right),$$

where M_r is the molecular weight; V_e is the volume of the solution, in which the analyte leaves the column; V_o is the free volume of the column.

The calculated relative molecular weight of melanin is 58300 ± 112 .

Isolated melanin was used to study individual composition by chromatographic methods. For this, various organic solvents and water were used as an extractant. The extraction of the isolated melanin (2 g)

was carried out with organic solvents - 96% ethanol, acetone, 95% butanol, water at room temperature for 6 hours in a ratio of 1:10, then the extract was decanted. In the extracts, the content of insoluble substances was determined by the gravimetric method. Interesting data were obtained with butanol extract. Therefore, in the present work, the main attention will be paid to the study of the composition of the butanol extract of the isolated melanin.

Extraction was carried out with 95% butanol. To isolate lipids from the butanol extract, the solution was treated with hexane in a 2:1 ratio. The obtained extract was used to study the individual composition by gas chromatography and chromatography-mass spectrometry.

GC-MS analysis was performed with a Perkin Elmer mass detector under the following conditions: a capillary column with the size of 30 m x 0.32 mm packed with HP-5 with a layer thickness of 0.25 μm using a temperature programming mode: in the range from 100 to 150 °C heating 12.5 °C / min; in the range from 150 to 300 °C with heating of 3 °C / min. Isothermal mode at 300 °C - 15 min. Evaporator temperature - 300 °C. The flow rate of the carrier gas - helium - 2.5 ml / min. Mass spectrometric analysis conditions: quadrupole mass filter, electron ionization of 70 eV, ion source temperature of 200 °C. The identification of individual components from the composition of the samples was carried out using databases of mass spectra and retention characteristics [13–16].

As a result of identification from the composition of the butanol extract, in addition to melanin, were found sterols (monosterol and instodiol), triterpen (squalane), phenols (7-hydroxy-6-methoxy coumarin glycoside, methyl diphenyl acetate, 1,1-diphenyl-2-propanone, serine acid, serine aldehyde, 2-allyl-3-ethoxy-4-methoxyphenol), carbohydrates (glucose, galactose, arabinose, xylose), which correspond to the data of the authors [10]. The amount of the main substance is 96-98 %, it is well soluble in water, oils and organic solvents.

Thus, the main substance of melanin isolated from silkworm wastes is 5,6-dioxiindole homopolymers. The black dry substance, the content of the target product with an average molecular weight of 58300 ± 112 and a density of 1.0 g / cm^3 is 96-98%. The maximum molar absorption is observed at a wavelength of 555 nm, it is well soluble in water, oils and organic solvents.

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