

# Technological Desition of Extraction of Melanin from the Waste of Production of Sunflower-Seed Oil

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**Abstract.** The research was realized in the field of the technology for re-use of waste of sunflower-seed oil production. A technological scheme of production of melanin from sunflower husk as a waste was developed. Re-cycling will give the opportunity to reduce the amount of waste and to obtain an additional source of income.

## 1. Introduction

Attention is paid to the problems of chemical and biotechnological utilization of waste of agricultural and food production, especially to the treatment of plant biomass. It is the necessity to utilize big volume of wastes and to get useful secondary materials with new properties. Sunflower-seed oil production is based on a technology with large volume of wastes of 5th degree of dangerous such as shells of sunflower seeds (husk). It consists of 100% of plant residue, so there are no dangerous properties. However, it is a problem of utilization of the waste by burial or combustion. There is a possibility of littering of enterprise territory or ignition in case of insufficient storage and transportation of big volumes of husk.

The following methods such as use of the husk as a part of the forage or mat in animal industry as well as an addition to compost for the growing of mushrooms, and for improvement of mechanical properties of soil are the most prevalent. Husk is known to be used for the production of decorative tiles, as a raw material for hydrolysis production, for alternative fuel, as a sorbent for the treatment of contaminated soils, for nutrient medium, humectants material, and lignocellulose raw material [1, 2, 3].

Despite the wide variety of directions of use of the husk of sunflower the real potential of the use of that substance did not answer the volumes of enterprises and the dumps which are accumulated. That wastes are difficult for access for microbial destruction. They contaminate soil, they need additional squares for storage, so it seems an ecological problem for the environment. Problems listed above is a background for the elaboration of new approaches for the re-cycling of the named waste.

Sunflower husk (seed shells) is a perspective feedstock raw material used in the manufacture of antioxidant products [4, 5]. Antioxidant properties depends on the content of phenolic polymers both of low-molecular and high-molecular nature. Those compounds are melanins and humic substances.

Low-molecular phenolic substances have following unique properties: they are able to protect cells from primary reactive oxygen species as well as from secondary reactive oxygen species such as lipid



peroxides. In addition, they are able to associate ferric and copper ions which activate peroxidation. However, their content in sunflower seed shells is low, that fact specifies the economic inexpediency of their separate extraction.

Melanins and humic substances are phylogenous or mycogenous high-molecular compounds. They have high stable paramagnetic centers as well as variety of functional groups. They are characterized by conjugate system of bonds in molecules. Their unique property is steady free radical state. Monomers of melanin are appeared as phenoxil or semi-quinone radicals. In oxidative reaction that natural polymers react not only in reduced hydroquinone form of phenols, but as a system of polyphenols and quinones which has semi-quinone radical as an obligatory intermediate component. Those compounds promote the high antioxidant activity of melanins. Also melanins are chelating agent against ferric and copper ions which initiate radical reactions.

Content of black melanin and humic melanin is up to 1.5% [1] and 12% [6] correspondingly.

Economical expedience of processing of this type of husk raw materials requires the development of methods for the simultaneous extraction of compounds belonging to different classes, such as melanins and humic substances, for their further use as antioxidants complex with high activity.

Now the most perspective method of the recycling of sunflower husk is extraction of melanin. Melanins which were got from the husk of sunflower are nitrogen-free compounds. They generate in the process of oxidation of pyrocatechin by enzymes. That melanins have high antioxidant and antiradical activity. That property gives the possibility of production of antioxidants, inhibitors of radical reactions, sorbents, biostimulants, and antiaging agent using melanins from the husk of sunflower [7].

## 2. Materials and methods

Chemical and element composition of husk was researched. Cross-sections of sunflower seed shells were prepared by standard anatomical method. Sections were impregnated by different colorants. So sudan was used to identify lipids, floroglucin was used to identify lignificated elements, and lugol solution was used to identify starch. Photographs were made by light microscope MX 300 with digital camera DCM 900 and computer program Scope Photo 3.0. Element compound was assessed by scanning electron microscope FEI Versa 3D LoVac with energy-dispersion detector EDAX Apollo X for micro-roentgen-spectroscopic analysis.

Biochemical investigations of final product were also carried out for assessing the rate of the lipid peroxidation. The substrate of the lipid peroxidation was suspension of chicken egg yolk. It contains two types of lipid-protein complexes which correspond to low and very low density lipoproteins of blood plasma. Out of all types of lipids, those lipoproteins are known to primarily affected by lipid peroxidation.

Described model system has the following advantages: commercially availableness, easy of lipoprotein extraction, stability during the storage and biochemical oxygen demand.

Chicken egg yolk was dried on filter paper and then it was solved in equal volume of phosphate buffer (40 mM  $\text{KH}_2\text{PO}_4$  + 105 mmol KCl, pH 7.5). The obtained phospholipid suspension was diluted 25-fold with the same buffer before use. Suspension may be stored for a week at 4°C.

The tests were realized by the following way. To the sample (volume 0.5 ml), 1 ml of lipoprotein suspension and 3 ml of 1% solution of orthophosphoric acid (pH 2.0) were added. Lipid peroxidation in the samples was initiated by addition of 0,1 ml solution of ferric sulfate (30 mg of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  in 10 ml of distilled water). Control sample did not contain antioxidants. Intimate mixtures were incubated in water thermostat for 20 min at 37°C.

Rate of lipid peroxidation was assessed by the amount of accumulated TBA-products (malonic dialdehyde) which reacts with thiobarbituric acid – TBA. After the incubation in each sample 1 ml of 0.6% solution of TBA was added. Intimate mixtures were heated in boiling bain-marie for 20 min, periodically stirring till it became of pink color. Then 4 ml of n-butanol was added to the mixture, samples were centrifuged for 10 min at 3000 rpm. Optical density of the butanol phase was assessed at 540 nm by photoelectrocolorimeter. The path length of cell was 0.5 cm. Butanol was used as a control.

Concentration of accumulated TBA-products was maid taking into account the coefficient of molar extinction of malonic dialdehyde which was  $1.56 \cdot 10^5 \text{ МОЛЬ} \cdot \text{СМ}^{-1}$ :

$$A = E_{\text{op}} \cdot 106 \cdot 4 \text{ ml} / 1.56 \cdot 10^5 \cdot 0.5 \text{ ml} = E_{\text{op}} \cdot 51.3, \quad (1)$$

if A is content of malonic dialdehyde ( $\mu\text{mol/l}$  or  $\text{nmol/ml}$ ); 4 ml is volume of butanol phase; 0.5 ml is volume of sample;  $E_{\text{op}}$  is optical density in samples and in control.

Antioxidant activity (AOA, %) was calculated using formula:

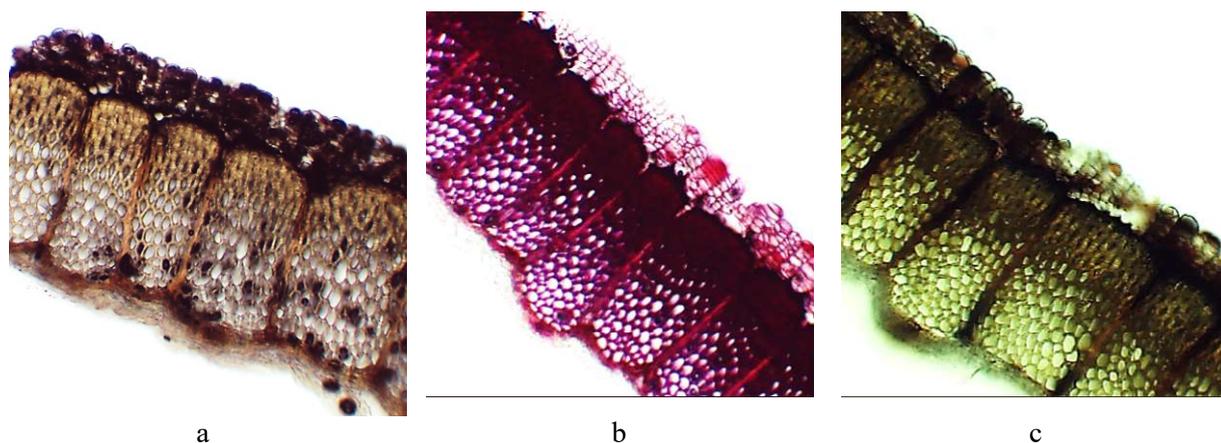
$$\text{AOA} (\%) = (E_{\text{control}} - E_{\text{sample}} / E_{\text{control}}) \times 100, \quad (2)$$

if  $E_{\text{control}}$  and  $E_{\text{sample}}$  are optical densities in samples and in control.

### 3. Results and discussion

The phytomelan layer is situated in the husk of the sunflower; it protects the seed from parasites. Husk of cultivars of sunflower differs by their color. They can be white, grey or black.

Husk was colored by sudan and floroglucinol due to the presence of lipids (fig. 1a) and lignin (fig. 1b) correspondingly. There was no starch in husk (fig 1c) because they were not colored by lugol. The content of lignin is known to be 25.1% ... 29.6%. Content of lignin depends on the oil content of the sunflower seeds; the more of oil the more of lignin.



**Figure 1** – The sections of husk impregnated by sudan, floroglucinol and lugol solutions

**Table 1** – Element content of the husk of sunflower

Color of husk	Element					
	C	O	Na	Mg	K	Ca
Black	64.36±3.7	32.8±3.1	0.27±0.09	0.57±0.17	1.03±0.31	1.02±0.25
White	62.01±2.0	32.1±3.7	–	1.37±0.16	1.43±0.51	2.83±0.92

The table 1 demonstrates that organic substances consists of only carbon and oxygen (hydrogen was not assessed by this method). So there was no nitrogen in those substances. There were no sufficient differences in contain of carbon and oxygen, but in white husk the content of mineral elements was more than in black ones.

The research of components of husk evidences that the useful components can be extracted from the material.

Searches of the optimal way of extraction of melanin from the husk of sunflower were realized at the laboratory. There is a patent for invention [8].

Due to melanins and humic substances as natural high-molecular compounds are slow-diffusible substances via cell walls, their extraction is a time-consuming process that entails significant intensification. The extraction of melanins from the raw materials is carried out preferentially at an elevated temperature and under the external power influences [9-12]. Despite the effectiveness of those ways of intensification their application to industrial production is not practicable due to high power consumption. As concerns economic expediency a perspective way of intensification of the process of extraction of melanins is vibrating influence [6, 8, 13]. It is possible to extract melanins from husk under standard conditions.

Duration of the process of extraction in vibrating extractor allows increasing of hydrodynamic conditions in large voids of raw material and decreasing of the retention time. Initiated convection promotes the increase of the rate of mass carry at the stage of diffusion and convection extraction. Vibration reduces dead diffusion space in extractor and enhances the coefficients of mass exchange. Experimental and calculated data of extraction of antioxidant are shown in table 2.

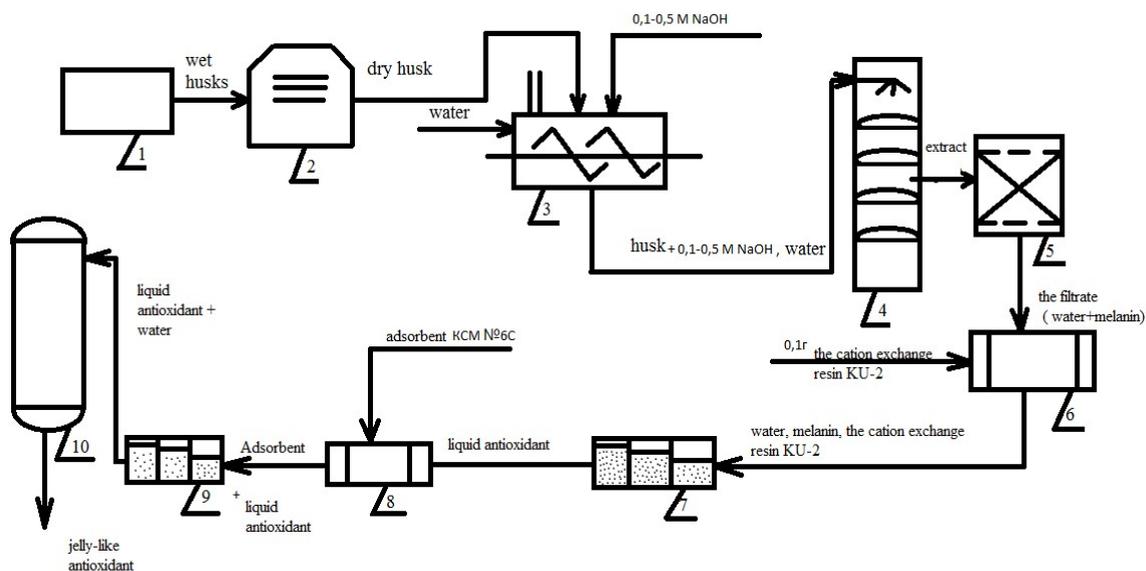
Elaborated method provides the increase of output of melanoid antioxidant on 40-170% and 27-98-time decrease of power consumptions.

Use of alkali solution as an extractant increases the solubility of melanins and humic substances, so this effect would improve the quality of the antioxidant and extend the output of the final product. Also the disintegration of insoluble complex of biopolymers such as cellulose-lignin-hemicellulose arises from the treatment of raw material by alkali solution. Use of sodium hydroxide solution as an extractant allows realizing the process at room temperature 18-22 °C without pre-heating.

**Table 2** – Dependence of output of the final product on the parameters of extraction

Variant	Extractant	Extraction time, min	Mass ratio husk: extractant	Power consumption for extracton of 1 kg of raw material, kW·h	Output of the final product, % of the mass of raw material
in accordance to <b>Ошибка! Источник ссылки не найден.</b>	water	55	1:10	12.76	3.55
in accordance to [14]	water	30	1:10	6.96	2.62
1	0.5M NaOH	30	1:7	0.13	7.04
2	0.1M NaOH	45	1:7	0.20	5.05
3	0.5M NaOH	45	1:7	0.20	8.67
4	0.1M NaOH	60	1:7	0.26	5.98
5	0.5M NaOH	60	1:7	0.26	9.67

Appropriate technology of production of melanin was elaborated and confirmed experimentally (fig. 2).



**Figure 2** – Technological scheme of the process of extraction of melanin from the husk of sunflower: 1 – bunker; 2 – lattice drying chamber; 3 – mixer; 4 – vibrating extractor; 5 – filter with settled bed; 6, 8 – tanks; 7, 9 – settlers; 10 – evaporation installation

Because of the necessity to extract some estimable components from solid phase of sunflower husk we used leaching as a main step of the technology. This process allows getting melanin which is useful for different branches of industry.

The process is realized in conditions of high temperature, heat supply and intensive mixing. We propose to use 0.1-0.5 M solution of sodium hydroxide. Taking in account all the peculiarities of the process we change an extractor. It is an apparatus equipped by oscillating drive component. The vibration process was used to intensify the process of extraction. The extractor has nickel coating to protect the apparatus in alkaliified medium. Nickel coating has high hardness and adhesion to the base part especially after the thermal treatment [15]. Nickel coatings are used in different branches of industry both as a back layer and all by itself for protective, decorative and special purposes. That coatings are characterized by hardness, corrosion stability, good reflecting power, and resistivity  $8.3 \cdot 10^{-2} \text{ Ohm} \cdot \text{m}$  [16]. Those coatings are applied for protective and decorative finish work of machine elements, instruments, apparatuses, and devises in industry. Also nickel coatings protect the equipment in severe corrosive conditions such as high temperature or special medium – alkalis and acids. It can be used as a back layer to coating of steel by other metals, it provides rigid adherence of coatings with the base. Nickel coating enhances the hardwearing of rubbing surfaces.

The process of leach needs some additional operations. Grinding husk is washed by water, then it follows to the bunker (1). Than it is dehumidified to free-flowing condition by hot air which has temperature 90-110°C in lattice drying chamber (2). The mass is mixed with 0.1-0.5 M solution of sodium hydroxide in mixer (3). The mass ratio of husk and extragent is 1 : (6÷7). The leach process is realized in vibrating extractor (oscillating drive component) (4).

After extraction the finished product is prepared by filtration of extract on the filter with scuttled bed (5). Filtrate and 100 mg of cation exchange resin KU-2 are mixed in a tank (6) under control of pH.

When pH runs to 7.0 the filtrate is separate from cation exchange resin in a settler (6). Then supernatant is baffled to tank (7), and adsorbent is added to the mixture. Adsorbent is precipitated in a settler (8). Dissolvent (water) is evaporated by boiling treatment in evaporation installation (10) [13].

Bag filter is recommended to provide high efficiency of filtration. Bladed rotary pump is preferable to realize continuous and uninterrupted flow of solutions.

Final product is dark-brown gel-like substance, it has sweet woody smell. It has antioxidant properties which connected with the presence of melanin. It enters into reactions with hydroperoxide, and the solution is discolored. The product reacts with potassium permanganate, the color changes into green, later the residue precipitates and the solution is discolored. The reaction of the final product with ferric chloride (III) is accompanied by precipitation of flaky residue and its following dissolution in the overbalance of reagent.

Final product contains polysaccharides as well as small amounts of hemicelluloses, soluble sugars and carbon acids besides the phenolic compounds which would increase its adsorbing activity.

Antioxidant activity of melanin was high (table 3, [6]).

**Table 3** – Antioxidant activity of product and residual quantity of TBA products

Material	Concentration of antioxidants, µg/ml	Antioxidant activity, %	TBA products, µmol/l
Sunflower husk	544.3	87.51±0.12	4.11±0.07
	216.5	71.09±0.19	9.40±0.10
	108.3	10.93±0.17	29.75±0.14
Control	0.0	-	32.44±0.12

### Conclusion.

A technological scheme of production of melanin from sunflower husk as a waste was developed. Final product has antioxidant properties which connected with the presence of melanin. Final product melanin can be applied as a prophylactic mean and medicinal agent for treatment of human diseases. It is a substance for food and perfumery industry. The simplest variant is addition of melanin to sun-protection cream. Presence of melanin in food products provides their long storage. In the process of digestion melanin partially dissimilates by assistance of microflora of intestinal tract. It controls the peristalsis and affects the microflora of intestine. Melanin plays a role of enterosorbent, so it is an active antidote and it helps in the cases of acute poisoning. Melanin brings out toxicants on the incipient stage of contamination before their absorption to blood. Melanin is applied for treatment and prophylaxis of disease of liver, neurological diseases such as stress and chronic fatigue syndrome, and oncological diseases. Treatment of melanin as an oxidant has the rejuvenating effect.

### References

- [1] Kartushina Yu.N. Perspektivy ispolzovaniya otkhodov masloekstraktsionnogo proizvodstva (luzgi podsolnechnika) s tselyu polucheniya melaninov /Yu.N. Kartushina. N.V. Gracheva. M.A. Danilova //Ekologiya i bezopasnost v tekhnosfere: sovremennyye problemy i puti resheniya. Tomsk: NI TPU. 2014. p. 90-93.
- [2] Gromyko N.V. Primeneniye podsolnechnoy luzgi v kachestve sorbenta dlya ochistki prirodnykh vod ot ionov tyazhelykh metallov /N.V. Gromyko //Innovatsionnaya nauka. № 1-3 (13). 2016. p. 41-42.
- [3] Kalashnikova L. I. Issledovaniye tekhnologicheskikh svoystv rastitelnykh otkhodov kak alternativnogo ekologicheskogo topliva /L.I. Kalashnikova. A.A. Ovchinnikova. A.V. Aleksandrova. A.A. Kalashnikova //Vektor nauki Toliattinskogo gosudarstvennogo universiteta. 4. 2011. p. 32-34.
- [4] De Leonardis A. A first pilot study to produce a food antioxidant from sunflower seed shells (*Helianthus annuus*) /A. De Leonardis, V. Macciola, N. Di Domenico //Eur J Lipid Sci Technol. 2005. 107:220-227.

- [5] Szydłowska-Czerniak A. Optimization of extraction conditions of antioxidants from sunflower shells (*Helianthus annuus* L.) before and after enzymatic treatment /A. Szydłowska-Czerniak, K. Trokowski, E. Sztyk //Ind Crop Prod. 2011. 33: 123-131.
- [6] Gracheva N.V. Sposob polucheniya melanina iz luzgi podsolnechnika i issledovaniye ego antioksi-dantnoy aktivnosti /N.V. Gracheva. V.F. Zheltobryukhov //Vestnik Kazanskogo tekhnologicheskogo universiteta. 2016. T. 19. 15. p. 154-157
- [7] Gabruk N.G. IK - spektroskopiya v izuchenii sostava kompozitov. poluchennykh iz rastitelnogo i zhi-votnogo syria /N.G. Gabruk. I.I. Oleynikova. A.V. Metelev [dr.] //Nauchnyye vedomosti BelGU. Seriya Este-stvennyye nauki. 2011. 15. 16 (110). p. 95-98.
- [8] Pat. 2578037 RF. MPK A61K36/28. B01D11/02. Sposob polucheniya melanoidnogo antioksidanta iz luzgi podsolnechnika /N.V. Gracheva. Yu.N. Kartushina. M.A. Danilova. A.B. Golovanchikov. V.F. Zheltobryukhov; VolgGTU. 2016.
- [9] Gracheva, N.V. Studies of the intensification of the extraction of biologically active substances from chaga using direct current electric fields /N.V. Gracheva, A.B. Golovanchikov //Pharmaceutical Chemistry Journal. 2010. T. 44. 11. p. 608-610.
- [10] Issledovaniye ekstraktov i melaninov griba inonotus obliquus (pers.) Pil.. poluchennykh posle obra-botki syria VChE-plazmoy / O.Yu. Kuznetsova, I.Sh. Abdullin, M.F. Shayekhov, G.K. Ziyatdinova, G.K. Bud-nikov //Uchenyye zapiski Kazanskogo universiteta. Seriya: Estestvennyye nauki. 2016. T. 158. 1. p. 23-33.
- [11] Pat. 2406514 Rossiyskaya Federatsiya. MPK A61K36/06. B01D11/02. A61P39/06. Sposob polucheniya vodnykh ekstraktov chagi /M.A. Sysoyeva, E.V. Sysoyeva, A.V. Sysoyeva, V.S. Gamayurova; zayavitel i patentoobladatel Kazanskiy gos. tekhnologicheskiiy un-t. № 2009113400/15; zayavl. 09.04.09; opubl. 20.12.10. Byul. 35. 5 p.
- [12] Pat. 1805968 Rossiyskaya Federatsiya. MPK A61K35/78. Sposob polucheniya sredstva, obladayushchego protivoyazvennoy i adaptagennoy aktivnostyu /G.L. Ryzhova, S.S. Kravtsova, I.V. Bogdanova, A.Ya. Korbut, V.I. Grebnev, M.P. Garbusenko, V.T. Pashinskiy, N.V. Grisel; Tomskiy gos. un-t. Tomsk. opubl. 30.03.93. Byul. 12. 5 p.
- [13] Kartushina Yu.N. Polucheniye melanina na osnove otkhodov masloekstraktsionnogo proizvodstva /Yu.N. Kartushina. M.A. Kirichenko. G.A. Sevryukova //Vestnik Kazanskogo tekhnologicheskogo uni-versiteta. 2016. T. 19. 16. p. 124-126
- [14] Pat. 2281779 Rossiyskaya federatsiya. mpk7 A61K 36/28. Sposob polucheniya prirodnogo melanoidnogo antioksidanta/L.A. Zhorina, R.N. Kashevatskaya, A.L. Ivanov, V.L. Ivanov; zayavitel i patentoobladatel L.A. Zhorina, R.N. Kashevatskaya, A.L. Ivanov, V.L. Ivanov. №2004134636/15. zayavl. 26.11.2004; opubl. 10.06.2006. Byul. 23. 6 p.
- [15] Dasoyan. M.A. Tekhnologiya elektrokhimicheskikh pokrytiy /M.A. Dasoyan. I.Ya. Palmskaya. E.V Sakharova. Lipetsk: Mashinostroyeniye. 1972. 464 p.
- [16] Voldman. G.M. Osnovy ekstraktsionnykh i ionoobmennykh protsessov v gidrometallurgii /G.M. Voldman. M.: Metallurgiya. 1982. 376 p.
- [17] Alekseyeva. T. N. Antimutagennyye svoystva rastitelnogo melaninovogo pigmenta /T.N. Alekseyeva. A.V. Oreshchenko. A.V. Kulakova [i dr.] //Khraneniye i pererabotka selkhozsyria. 2001. 5. p. 37-38.