UDC 547.97:665
DOI: 10.15587/2312-8372.2017.108369

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INVESTIGATION OF ANTHOICIANS AVAILABILITY IN SUNFLOWER SEED HUSKS

Розглянуто комплексний підхід, який передбачає теоретичне і експериментальне обґрунтування нової сировинної бази для одержання антоціанових пігментів та обґрунтування методів їх вилучення із запропонованої сировини. Проведено дослідження з якісного та кількісного визначення наявності поліфенольних сполук (суми антоціанів) у зразках лушпиння насіння соняшнику. Встановлено, що середній вміст антоціанів в перерахунку на ціанідин-3,5-діглікозид складає 0,42 %.

Ключові слова: *антоціанові пігменти, екстракція, вторинні продукти, лушпиння насіння соняшнику.*

1. Introduction

Sunflower is the main oilseed crop in Ukraine. Due to processing of its seeds more than 2 million tons of husks are annually received as secondary products (wastes).

It is known that the husks of sunflower seed are used as raw materials in hydrolysis industry. In agriculture, the husk of sunflower seed is used as mulch and fertilizer, as well as for loosening the soil. In cattle breeding farms, the flour obtained from husk and enriched with a hydration fuse is widely used. The main part of sunflower seed husks are used as an alternative fuel, since it has a number of advantages over the traditional energy resources. Combustion heat of the husks and gas can be compared as follows: 2 tons of husks are equivalent to 1 000 m^3 of natural gas.

Sunflower seed husks can also be used as a sorbent. For example, it is capable to remove radionuclides from aqueous solutions. Removing of radionuclides includes several stages. At the first stage a contact between the phytosorbent and the cleaning solvent occurs (pH = 3.0...9.0). The second stage involves separation of solvent from the solution with a phytosorbent – crushed husk of sunflower seed, preliminarily subjected to acid hydrolysis [1].

An anthocyanin pigments (AC) include a wide group of water-soluble vegetable pigments that cause a red, blue and violet color of fruits, flowers, leaves and other parts of plants. Anthocyanins are derivatives of the flavilium cation containing three to six hydroxyl groups that can be methylated. Fig. 1 shows the structural formula of anthocyanidins. The structural features of some anthocyanidins are given in Table 1.

Anthocyanidins (aglycones) are poorly soluble in water because of their structure, so they exist in an aqueous-soluble form as glycosidated derivatives in plants. The total content of monomeric anthocyanins in plants may reach up to 2 % [2–5].

At present, there is a tendency to extract and use mainly dyes with natural origin for food purposes. Thus, extracts of AC from plant raw material are used in the food industry as natural dyes for coloring a food products. AC have a high antioxidant activity [5], neutralize the effect of free radicals, suppress the growth of tumors, have beneficial effects on the human body. Due to the natural properties of anthocyanins, they help reduce the fragility of capillaries, improve the state of connective tissues, help prevent and treat cataracts [4].

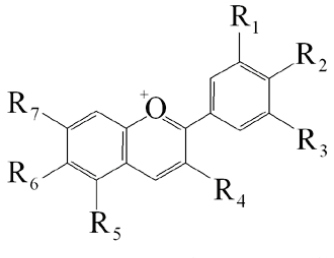

Fig. 1. Anthocyanins (flavilium cation)

Table 1

Structural features of some anthocyanidins

Anthocyanin	R_1	R_2	R_3	R_4	R_5	R_6	R_7
Cyanidine	-OH	-OH	-H	-OH	-OH	-H	-OH
Delphinidine	-OH	-OH	-OH	-OH	-OH	-H	-OH
Petunidine	-OH	-OH	$-OCH_3$	-OH	-OH	-H	-OH
Malvydin	$-OCH_3$	-OH	$-OCH_3$	-OH	-OH	-H	-OH
Pheonidine	$-OCH_3$	-OH	-H	-OH	-OH	-H	-OH
Rosinidine	$-OCH_3$	-OH	-H	-OH	-OH	-H	$-OCH_3$

For industries that produce sweets, confectionery and non-alcoholic beverages, a variety of harmless dyes is needed. A plant extracts, juices and concentrates that contain anthocyanins are of high importance in this spheres.

Anthocyanins aimed for coloring of food can be obtained sometimes from the wastes of edible berries processing [6]. However, the potential of local plant raw materials as a source of dyes has not been fully appreciated yet. So

ISSN 2226-3780

the task of finding the new sources, from where the natural dyes can be extracted in the future (in a necessary quantities and a low price), has still been actual.

2. The object of research and its technological analysis

The objects of research are the anthocyanin pigments from secondary fat and oil industry. Usually, for the production of such class of dyes are used as a raw materials fruits, flowers, leaves and other parts of plants, juice treatment wastes or, for example, refuse from dark grape varieties. In this study, the solution of the problem of anthocyanin pigments production is based on the search of a new raw material base for the extraction. A comprehensive approach based on the theoretical and experimental justification of a new raw material base for the producing of anthocyanin pigments is based on the research. This approach may promote to use an affordable and cheap secondary products (wastes) obtained from the processing of vegetable raw materials in fat and oil industry.

3. The aim and objectives of research

The aim of research is a justification of the source and methods choosing for anthocyanins production based on such branch as using of secondary products (wastes) of the fat and oil industry. It is necessary to resolve the next issues to achieve this goal:

1. A qualitative examination of polyphenolic compounds in the industrial samples of sunflower seed husks.

2. A quantitative examination of overall content of anthocyanins in the industrial samples of sunflower seed husks.

4. Research of the existing solutions of the problem

Natural dyes are more often obtained by extraction from vegetable raw materials with a suitable solvent. The most effective solvents are alcohols [7]. Their efficiency decreases according the next order: methanol, ethanol, i-propanol, n-propanol, n-butanol, i-butanol. Ethylene glycol, propylene glycol and glycerol are effective extractants among polyhydric alcohols. The most suitable mineral acids are hydrochloric, sulfuric, and phosphoric acids, while formic, acetic, lactic, tartaric and citric acids are the most suitable among the organic acids. Acids that are commonly used in food production, for example citric acid, are mainly used as a solvent.

Extraction of anthocyanin pigments is usually carried out with acidified aqueous and aqueous-alcoholic solutions. In order to optimize the extraction conditions of anthocyanins from plant raw materials, an influence of a raw material, solvent, pH, temperature and extraction time on the extraction ratio and stability of pigments was studied [8–11]. Water, ethanol, glycerin and water-glycerin mixtures were studied as an extractant and chokeberry, black currant, grapes and red rose petals were selected as a plant raw material.

In papers [5, 12], an aqueous glycerin solution was used in order to intensify process of colorants extracting. This allowed to increase the concentration of anthocyanin pigments and to reduce water content in the extract. The choice of glycerin as an extractant in this study was made due to its structure, non-toxicity, high boiling temperature, nonvolatility and more explosion safety as compared with ethanol.

In order to increase an accuracy of determining the amount of anthocyanins in the different parts of plants, food products, food colors, in the paper [13] is offered the method of extracting of anthocyanins. Extraction is carried out into a two-phase system of solvents: pentanol-1 – concentrated hydrochloric acid – water with a ratio: 10 – 12 : 10 – 12 : 2 – 4 at a temperature of 100–110 °C for 10–25 minutes. The total amount of anthocyanins is calculated using the difference of optical density at 530 and 655 nm before and after treatment of the extractant organic phase by the mixture of 30 % hydrogen peroxide and methanol.

Together with a mixture of chlorophylls, carotenoids, flavonols and flavones, anthocyanins in the paper [14] were also isolated anthocyanins by using a methanol solution and an herbal raw material of the Monarda tubular.

In paper [15], the anthocyanins of fruits were extracted by n-butanol and separated by chromatography on a column filled by a powdered cellulose using a mixture of n-butanol, acetic acid and water (4 : 1 : 2.5) as a solvent.

When extracting AC from dark plant material [5], the most efficient solvent as a mixture is water – glycerin extractant with a mass fraction of glycerin 50 %. The color of dark fruits is caused by pigments. Their structure predominantly consists of an active centers, than contains hydroxides in the neighboring carbon atoms. The part of their extraction increases with an increase in the content of the polyhydric alcohol with an α-glycol structure in the extractant as a result of the formation of chelate complexes by means of a hydrogen bond between the molecules of the solvent and the AC.

Concerning the extraction of natural dyes from other alternative natural raw materials, in [5] as a raw material was used dark grape varieties berries. Water, ethanol, glycerin and water-glycerin mixtures were examined as an extractant. The choice of solvents is defined by the possibility of their using in the food industry.

At present, the promising way of natural dyes obtaining is using of local plant raw materials or even the waste of its processing. Other perspective way of obtaining of natural dyes is the method of an extraction by an appropriate solvent or their mixture.

The authors of the work [7] found that the black color of some sunflower seeds can be converted to a solution in the form of a ruby red dye during extraction with a suitable solvent system. Such red color appears due to substance *helianthocyanine*. Moreover this substance was determined as belonging to the class of anthocyanins.

Based on the analysis, it can be concluded that such secondary product of the fat and oil industry as husk of sunflower seed can be used as a source of natural dyes.

5. Methods of research and experimental part

Samples of sunflower seed husks were used as raw material for the purposes of research. The presence of polyphenolic compounds in sunflower seed husks was determined according to work [16, 17] using qualitative reactions.

The moisture content in the samples was determined by the accelerated method according to the work [18].

A study of the total content of anthocyanins (polyphenolic compounds) in sunflower seed husks was held according to work [17] by measuring the optical density of the powder extracts solutions using spectrophotometer Specol 1300 (Germany) at a wavelength $\lambda = 490$ nm in a cuvette with a layer thickness of 10 mm.

6. Research results

The most appropriate raw material for qualitative and quantitative detection of anthocyanins is husk of sunflower seeds in the ground and previously defatting state.

At the first stage of the study, the presence of polyphenol compounds in the sunflower seed husks samples was qualitatively analyzed. Since there is no specific qualitative reaction to all polyphenol compounds, they can be determined by various reactions according to works [16, 17]. One of such reactions is based on the interaction of polyphenol filtrate with a 5 % of aluminum chloride solution. As a result, chelate complexes are formed due to hydrogen bonds between carbonyl and hydroxyl groups, as well as aluminum ion. Positive result occurs if a yellow-green color is appeared. Depending on the polyphenolic substances content in the test samples and a structure of the polyphenols, the color of filtrate is different in intensity and hue from slightly yellow to a distinct yellow-green color.

A reaction between metal magnesium or zinc and concentrated hydrochloric acid is often used for the detection of polyphenolic compounds. The appearance of pink or red staining in a test tube without magnesium indicates about presence of anthocyanin pigments. If to add just a single hydrochloric acid they will become a red coloured due to formation of oxonium salts.

In the second stage total content of anthocyanins in the samples of sunflower husks № 1–4 (in terms of cyanidin-3.5-diglucoside in absolutely dry raw material) was determined. An extraction of anthocyanins was carrying out by 1 % (m/m) HCl. Crushed raw material was treated by 100 ml of extractant (solvent) per 0.5 g of raw material at a temperature of 40–45 °C for 15 minutes. Moisture content in the industrial samples of sunflower seed husks was preliminarily determined by the accelerated method. Total anthocyanins concentration was calculated by using spectrophotometric method. The results of the experiments are presented in Table 2.

Table 2

The content of anthocyanins in samples of sunflower seed husks

Sample number	Moisture contents, %	Optical density d_{490}	Anthocyanin content, % m/m
1	3.3	0.683	0.78
2	3.3	0.368	0.42
3	8.9	0.036	0.044
4	8.8	0.025	0.03

According to the results showed in Table 2, it was determined that amount of anthocyanins in the samples of sunflower seed husks varies in the range 0.03–0.78 %. Its average content is about 0.42 %.

7. SWOT analysis of research results

Strengths. Among the strengths of this research, it is necessary to note the reasonable choice of raw materials for the production of natural dyes: complex processing of plant raw materials, as well as the using of the recovering raw materials base. According to the analysis of modern scientific literature, such information today is not available. The offered approach to the selection of raw materials will expand a raw material base for producing dyes by using secondary products (wastes). In addition, today there is in a tendency of obtaining and using mainly dyes from natural sources for food purposes. This will help to improve a quality of food that contains vegetable dyes (biologically active compounds of plant origin) and to use them for synthesis of a number of valuable pharmacological preparations.

Weaknesses. The weak side of this research is the fact that most of such dyes have instable color (during storage, heating, exposure to sunlight and oxygen of air). To prevent a discoloring it is necessary to obtain the dye immediately before use without concentration stage (i. e., concentration is sufficient for practical use); stabilization stage and storage. This will exclude, for example, a presence of acid in the extract that is necessary for oppression of microorganisms developing in the extract. Recycling of secondary products that increases energy costs is also the weak side of this research.

Opportunities. In the long term prospect, it is reasonable to conduct detailed studies on the search for renewable sources of raw materials for the natural dyes production. Such sources can be, for example, food wastes. It is also advisable to provide detailed studies on the production of other biologically active compounds of plant origin from food wastes. These biologically active compounds can have a wide range of pharmacological actions (antioxidants, enzyme inhibitors, etc.) and be further promoted for creation of valuable therapeutic, prophylactic, pharmacological preparations. Research in this sphere may promote more rational processing of the secondary products in food production.

Threats. Implementation of the results is usually very complicated task because the most preferable industrial dyes are synthetic due to their brightness and stability. Their color is stable and doesn't change under external factors and storage unlike most natural dyes. In addition, manufacturers have to cover additional expenses for medical expertise to prove its benefits for human health.

Thus, a SWOT analysis of obtained results allows to solve the next tasks:

– providing of complex examination to determine an influence of single factors on the extraction of natural dyes;

– developing the method of obtaining of natural dyes from secondary products of fat and oil industry, as well as other renewable natural sources;

– investigating of the individual characteristics of the obtained dyes in order to recommend their using in medical, chemical, pharmaceutical, perfume, cosmetic, food and other industries.

8. Conclusions

1. The reaction between polyphenols from filtrate obtained from the husk of sunflower seed with aluminum

ISSN 2226-3780

chloride solution was held. An appearance of yellow-green coloration can be used in qualitative analysis as an evidence of the presence of polyphenolic compounds.

2. An extraction of anthocyanins was provided from the samples of sunflower husk seed with an aqueous solution of HCl (1 % m/m). Total anthocyanins content was determined using a spectrophotometer (λ=490 nm, cuvette 10 mm). The average content of anthocyanins in terms of cyanidine-3.5-diglucoside is about 0.42 %.

References

1. Method for removal of radionuclides from aqueous solutions [Electronic resource]: Patent RU 2067328 C1 / Dontsov A. E., Lapina V. A., Ostrovskii M. A., Rubanov A. S., Rudak E. A.; assignee: Institute of Chemical Physics, RAS. – Appl. No. 93026484/25; Filed 21.05.1993; Publ. 27.09.1996. – Available at: \www/URL: https://patents.google.com/patent/RU2067328C1/en
2. Andersen, O. M. Flavonoids: Chemistry, Biochemistry and Applications [Text] / O. M. Andersen, K. R. Markham. – CRC Press, 2005. – 1256 p. doi:10.1201/9781420039443
3. Motohashi, N. Anthocyanins: Structure, Biosynthesis and Health Benefits [Text] / ed. by N. Motohashi. – Nova Science Publishers, 2013. – 325 p.
4. Ananieva, V. Research of dry plant concentrates – ingredient of a food health improvement [Text] / V. Ananieva, L. Krichkovska, A. Belinska, V. Dubonosov, S. Petrov // EUREKA: Physics and Engineering. – 2016. – Vol. 4. – P. 17–24. doi:10.21303/2461-4262.2016.000124
5. Perevertkina, I. V. Vliyanie golicerina na ehkstragirovanie antocianovyh pigmentov iz rastitel'nogo syr'ya [Text] / I. V. Perevertkina, A. D. Volkov, V. M. Bolotov // Himiya rastitel'nogo sir'ya. – 2011. – No. 2. – P. 187–188.
6. Vetchinkin, A. R. Estestvennye organicheskie krasiashchie veshchestva v pishchevoi promyshlennosti [Text] / A. R. Vetchinkin // Uchenye zapiski Saratovskogo gosudarstvennogo pedagogicheskogo instituta. – 1940. – Vol. 7. – P. 79–85.
7. Process for obtaining a dye substance of vegetal origin [Electronic resource]: Patent US 4156077 A / Pifferi P. G. – Appl. No. 05/737,768; Filed 01.11.1976, Publ. 22.05.1979. – Available at: \www/URL: https://www.google.com/patents/US4156077
8. Savvin, P. N. Issledovanie antioksidantnyh svoistv zheleinogo marmelada [Text] / P. N. Savvin, V. M. Bolotov // Himiya rastitel'nogo syr'ya. – 2008. – No. 4. – P. 177–179.
9. Harlamova, O. A. Natural'nye pishchevye krasiteli [Text] / O. A. Harlamova, B. V. Kafka. – Moscow, 1979. – 191 p.
10. Sposob polucheniia antotsianovogo krasitelia iz plodovogo syr'ia [Electronic resource]: Patent RU 222834 / Odin A. P., Hairutdinova A. D., Bolotov V. M. – Publ. 10.05.2004, BulI. No. 3. – Available at: \www/URL: http://www.findpatent.ru/patent/222/2228344.html
11. Perevertkina, I. V. Optimizatsiia uslovii ekstragirovaniia antotsianovyh krasitelei iz rastitel'nogo syr'ia [Text] / I. V. Perevertkina, A. D. Volkov, N. N. Titova, V. M. Bolotov // Himiya rastitel'nogo sir'ya. - 2014. – No. 2. – P. 137–141.
12. Method for production of anthocyan colouring agent from dark sorts berries refuses [Electronic resource]: Patent RU 2426755 C2 / Prervertkina I. V., Koltokova N. S., Titova N. N.; assignee: Voronezh State Technological Academy. – Appl. No. 2009124084; Filed 25.06.2009; Publ. 20.08.2011. – Available at: \www/URL: https://patents.google.com/patent/RU2426755C2/en
13. Sposob kolichestvennogo opredeleniia summy antotsianov [Electronic resource]: Patent SU 1744647 A1 / Roshal' A. D., Tsinovyi V. I, Orlov V. D. – Appl. No. 4640525/13; Filed 24.01.1989; Publ. 30.06.1992, Bull. No. 24. – Available at: \www/URL: http://rochal-home.univer.kharkov.ua/Papers/AC-1744647.pdf
14. Shanaida, M. I. Pihmentnyi sklad nadzemnoi chastyny Monarda fistulosa L. [Text]/ M. I. Shanaida, S. M. Marchyshyn // Aktualni pytannia farmatsevtychnoi i medychnoi nauky ta praktyky. – 2012. – No. 2 (9). – P. 31–32.
15. Lamort, C. Chromatographie et degradation d'anthocyanines de fruifs [Text] / C. Lamort // Rev. ferment. et inds aliment. – 1959. – Vol. 14, No. 2. – P. 70–72.
16. Korenskaia, I. M. Lekarstvennye rasteniia i lekarstvennoe rastitel'noe syr'e, soderzhashchie flavonoidy, kumariny, hromony [Text]: Handbook / I. M. Korenskaia, N. P. Ivanovskaia, I. E. Izmalkova. – Voronezh: IPTs VGU, 2007. – 80 p.
17. Gosudarstvennaia farmakopeia SSSR [Text]. Vypusk 2. Obshchie metody analiza lekarstvennoe rastitel'noe syr'e / Ministry of Health of the USSR. – Ed. 11. – Moscow: Meditsina, 1989. – 400 p.
18. Berezka, T. O. Kharchovi tekhnolohii. Metody kontroliu kharchovykh vyrobnytstv [Text] / T. O. Berezka, T. V. Matveeva, V. Yu. Papchenko. – Kharkiv: NTU «KhPI», 2011. – 168 p.

ИССЛЕДОВАНИЕ НАЛИЧИЯ АНТОЦИАНОВ В ШЕЛУХЕ СЕМЯН ПОДСОЛНЕЧНИКА

Рассмотрен комплексный подход, предусматривающий теоретическое и экспериментальное обоснование новой сырьевой базы для получения антоциановых пигментов и обоснование методов их извлечения из предложенного сырья. Проведены исследования качественного и количественного определения наличия полифенольных соединений (суммы антоцианов) в образцах шелухи семян подсолнечника. Установлено, что среднее содержание антоцианов в пересчете на цианидин-3,5-дигликозид составляет 0,42 %.

Ключевые слова: антоциановые пигменты, экстракция, вторичные продукты, шелуха семян подсолнечника.

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