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DEPENDENCE OF PURIFIED RUTIN QUALITY ON ACTIVATED CARBON BRAND

E. V. Averyanova*, M. N. Shkolnikova, and A. V. Frolov

Biysk Institute of Technology (branch), Polzunov Altai State Technical University,
Trofimova Str. 27, Biysk, 659305, Russian Federation

* e-mail: lena@bti.secna.ru

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Abstract: The main problem of implementation of technology of rutin extraction from grass buckwheat *Fagopyrum sagittatum Gilib.* lies in purification of rutin raw. In this connection the following research object is determined: consideration of the possibility to use for rutin raw purification different commercial carbon brands as adsorbents by the method of preparative chromatography and assessment of their effectiveness to achieve the maximum degree of purification with minimum duration of the elution process. The article presents experimental data on purification of rutin raw sample, extracted from grass buckweat green material by the preparative chromatography method using wood- and coconut-based activated carbons of different brands as sorbents; besides, the following items are presented in the article: dependence of rutin sample melting temperature, qualitative and quantitative flavonoid content, authenticity on chlorophyll and red pigments content depending on sorbent layer height and elution duration in comparison with the GSO [State Standard Samples] control sample. To confirm the reliability of the obtained results, statistical processing of experimental data is conducted using the methods of correlation and regression analysis, as well as using the two-parameter normal distribution of values. It is demonstrated that the use of the following carbon brands, indicated in decreasing effectiveness order, can provide the best purity and stability of parameters values, characterizing the product: NWC-P, NWM-P, OU-A, OU-B; the conducted calculations indicate that the best correlation between the sorbent layer height in a column and the rutin samples quality parameter was achieved when the carbons of the brands NWM-P, OY-A and OY-B were used. Depending on the tasks, rutin purification degree may be regulated by sequential use of NWC-P and NWM-P carbons. When rutin is purified from proximate admixtures, chlorophyll and red pigments, NWC-P adsorbent allows to get a comparable result even when the layer height is from 50 to 70 mm respectively.

Keywords: Grass buckweat, rutin raw, activated carbon, purification, method of preparative chromatography

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INTRODUCTION

Nowadays, a special place in the pharmaceutical market is occupied by medicines and dietary supplements of plant origin, containing flavonoids, one of the most important qualities of which is the ability to increase strength of the capillary walls. P-vitamin activity of these medicines is associated with the antioxidant effect that is important in the treatment of chronic venous insufficiency, hypertension and other cardiovascular diseases, associated with the increased permeability of blood capillaries. The clearest representative of this group of substances is rutin, which not only has a pronounced capillary-strengthening, antioxidant and hepatoprotective action [1, 2], but also improves the treatment of various disorders, associated with physical fatigue [3].

Today the main industrial source of rutin is the buds of Japanese pagoda tree (*Styphnolobium japonicum L.*), however, there is no raw material base of this plant in our country. A promising domestic source of rutin and other flavonoids may be grass buckwheat (*Fagopyrum sagittatum* Gilib.), widely cultivated as a valuable food crop in the Russian Federation. It is known that the rutin content in the cultivated buckwheat sorts is from 2.0 to 8.7% on a dry basis. However, the sorts, the rutin content in which may be 12%, are selected [4, 5].

Despite the broad prospects of the use of grass buckwheat as a raw material for the rutin production, the main difficulty of the implementation of technology of rutin extraction lies in the stage of rutin raw purification and obtaining a product, suitable for use in pharmaceutical industry and dietary supplements production [6]. In view of this, studies have been conducted on the use of commercial carbons of different brands as adsorbents, used for rutin purification, by the method of preparative chromatography, as well as the assessment of their effectiveness to achieve the maximum degree of purification with minimum duration of the elution process was conducted.

OBJECTS AND METHODS OF STUDY

The study objects were rutin raw samples, extracted by the above-mentioned method [7, 8] and purified by the method of preparative chromatography on a layer of wood- and coconut-based activated carbon of six brands. Physical and chemical parameters of the carbons are indicated in Table 1.

Sample melting temperature was measured with the device PTP (M), qualitative and quantitative flavonoid content in the samples was measured by the method of HPLC on high performance liquid chromatograph Milikhrom A-02 with UV radiation and a chromatographic column from stainless steel O 2?75 mm, filled with a reversed-phase sorbent ProntoSIL 120-5C18 AQ, with a software package. The solution of 0.1% acetic acid and acetonitrile was used as an eluent. Flavonoid retention time is 25 minutes. Chlorophyll and red pigments content was measured by spectrometric method. The readings were taken from the devices Shimadzu UV-2401 PC UV-VIS RECORDIGPHOTOMETER (Japan) at wave length of 560 nm, 590 nm, 620 nm, 655 nm and 690 nm in a cuvette with the layer thickness of 1 cm. Isopropyl alcohol was used as a comparison solution. Microscopic examination of rutin samples was conducted with the electron microscope SK 14 28804.

Table 1. Physical and chemical parameters of activated carbons of different brands [9]

Carbon brands	Appearance	Adsorption activity by methylene blue, mg/g	Ash mass fraction, %	Moisture mass fraction, %	Bulk density, g/dm^3	Abrasion capacity, %
OU-A (GOST 4453-74)	Black fine-grained powder	225.0	4.6	4.7	270	60
OU-B (GOST 4453-74)	Black fine-grained powder	75.0	10.0	10.0	290	60
BAU-A (GOST 6217-74)	Black grains	60.0	6.0	10.0	240	60
BAU-MF (GOST 6217-74)	Black grains	70.0	10.0	10.0	Not regulated	60
NWC-P, FCC specification	Black powder	300.0	5.0	15.0	300	> 99
NWM-P, FCC specification	Black powder	280.0	10.0	15.0	300	> 96

RESULTS AND DISCUSSION

Rutin raw was extracted from grass buckweat green material by double extraction with ethanol solution of 70%. The output was 4.44 ± 0.05% (on absolute dry substance), contain of main substance in a sample was 75.74%, melting point was 162°C, crystals were gray-and-green. According to regulating documentation requirements, the rutin content shall be at least 95.0% (State Pharmacopoeia XI); in the GSO [State Standard Sample] sample it was at least 98.5%. For purification of rutin raw we offered the method of preparative chromatography on a layer of activated carbons of different brands – OU-A, OU-B, BAU-A, BAU-MF, NWC-P, NWM-P [10]. 99.5%-methanol was used as a solvent and eluent for chromatography.

In a column with a height of 360 mm and diameter of 15 mm activated carbon is placed (layer height is from 10 to 100 mm). Rutin raw solution is applied to the prepared sorbent layer, eluent feed to the column is produced at a speed of 1 drop in a second. Sorbent layer height in a column corresponds with the number of the sample of purified rutin.

After rutin raw purification with the use of activated carbons of different brands, the rutin samples were analyzed for compliance with the melting temperature, the content of flavonoids and authenticity on the presence of chlorophyll and red pigments in comparison with the GSO [State Standard Samples] control sample. All the used brands of carbons are activated and represented in 87–97 mas.% of composition of elements by carbon. The main structural element of the activated carbon sorption space at the organization molecular level is the graphite basal face, which is formed by carbon atoms in the state of sp^2-hybridization with delocalized fourth electron. Non-specific physical adsorption is realised on them by means of universal forces of dispersion interaction. Besides, non-specific electrostatic induction forces are expressed in carbon like in conductor by means of dipole direction in the sorbate molecule. Their intensity is determined by polarization capacity. Carbons express capacity to sorption by means of addition of these forces. At the same time, oxigenated functional groups and Bronsted acids are present on the surface initially and appear during activation process by overheated moisture vapour. They are represented by hydroxyl, phenol, carboxyl, carbonyl and lactone groupings, content and balance of which are different for different carbon brands [11]. It should be noted that acidic properties of the carbon surface change in the following sequence: NWM-P<NWC-P<OU-A, OU-B<BAU-MF<BAU-A. In other words, on the surface of NWM-P carbon there is minimum number of acidic groups from all the materials under consideration, and on the surface of BAU-A carbon this number is maximum. NWM-P and

NWC-P carbons express high reducing ability in comparison with BAU and OU carbons.

The research results of melting temperature determination are indicated in Fig. 1–3. According to the obtained dependences of Fig. 1, one can achieve the rutin highest melting temperature, which is one of the product characteristics, using NWC-P, NWM-P and OU-B carbons. Moreover, only when these brands of adsorbents are used, there is a dynamic in a positive index changing with carbon layer increase in a column. Rutin samples, purified on NWC-P carbon layer, are the closest to the control sample.

Processing of experimental data results on rutin raw purification with the use of the normal distribution is indicated in Fig. 2.

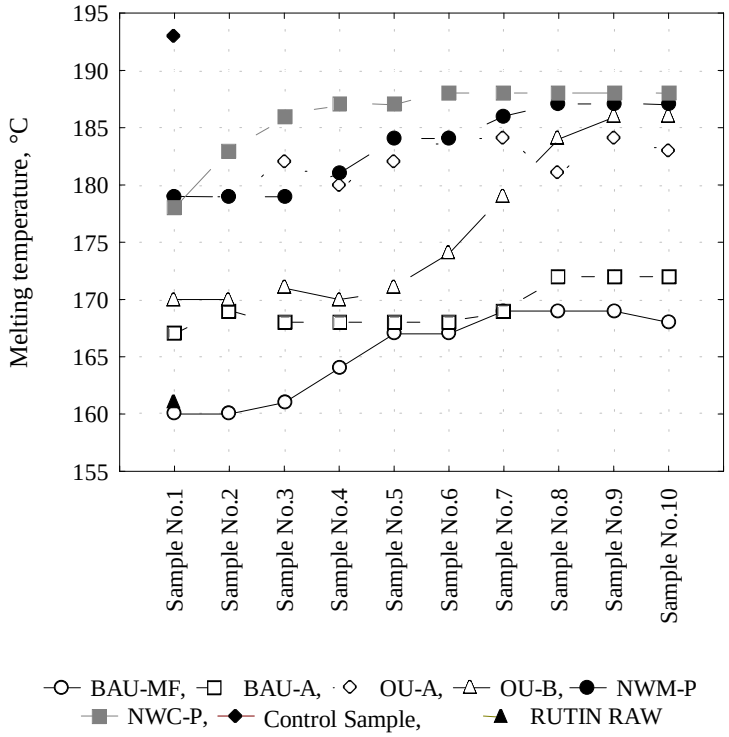


Fig. 1. Dependence of rutin samples melting temperature on carbon brand.

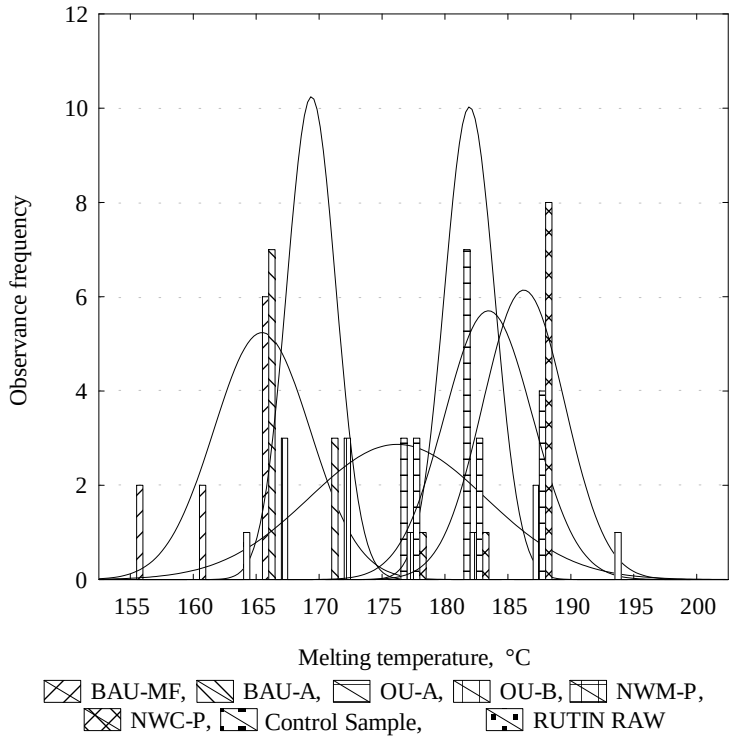


Fig. 2. Normal distribution of melting temperature values of rutin samples depending on carbon brand.

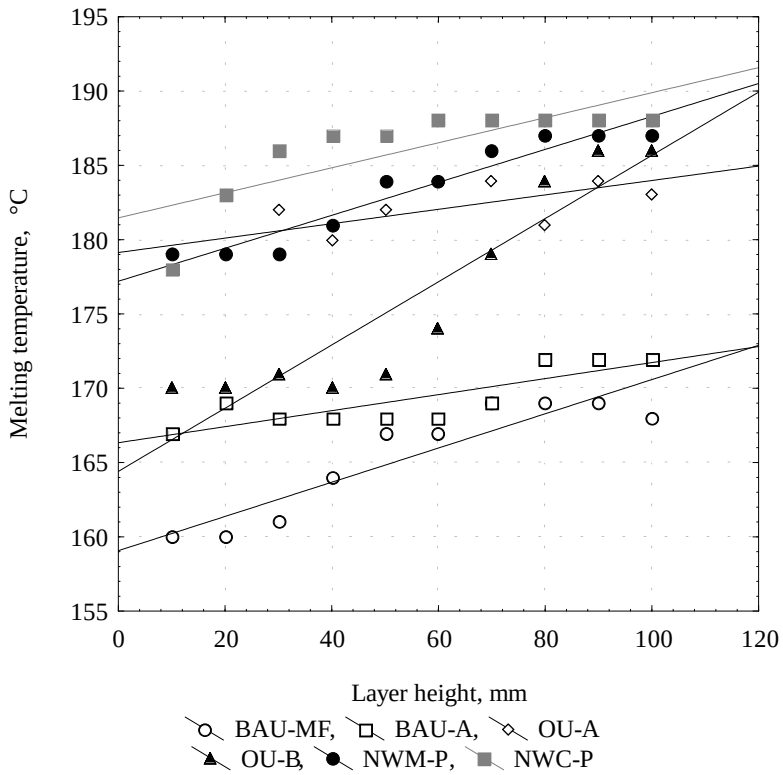
According to Fig. 2, the adsorbent efficiency of BAU-A and OU-A carbons nearly does not depend on the layer height in a column. Herewith, the use of OU-A carbon allows to get a stable result of melting temperature of 181.9°C with narrow distribution. BAU-MF adsorbent is absolutely ineffective, having wide distribution with an average value of 165.4°C. NWM-P and NWC-P adsorbents, having wide distribution, allow to get an average result of 183.3°C and 186.1°C respectively. Maximum purification degree for the carbons of these brands is determined by a much higher specific surface (1.5–2.0 times higher) in comparison with wood-based activated carbon. Coconut-based carbons are sorbents with pores of mixed type with nearly equal volume of micro- and mesopores, whereas wood-based activated carbons are characterized by a large proportion of macropores, acting as transporting channels which bring adsorbate molecules to adsorption space of the activated carbon particles. In this regard, wood-based adsorbents purification efficiency is directly connected with the layer height, it is expressed especially clearly in OU-V activated carbon which has the widest distribution.

Dependence of the use of the studied activated carbon brands on the layer height in a column concerning the melting temperature changing of rutin samples is indicated in Fig. 3.

The calculation results indicate that the highest correlation between the layer height and the achieved result is observed in NWM-P, OU-B and BAU-MF adsorbent brands with a direct correlation coefficient r = 0.9601, r = 0.9265 and r = 0.9159 respectively. Direct correlation coefficient value more that 0.75 indicates strong dependence between the layer height changing and the result obtained. Thereunder, when OU-A carbon is used for rutin raw purification, the results strongly depend on the layer height (r = 0.7381).

Qualitative and quantitative flavonoid contents in the rutin samples were defined with thin-layer chromatography (TLC) and high-efficiency liquid chromatography (HELC) methods. According to TLC, rutin raw contains a foreign admixture - quercetin, which is removed due to purification with the use of NWC-P and OU-B carbons regardless the sorbent layer height. The same effect can be achieved using OU-A and NWM-P carbons with the layer height in a column more than 40 mm. HELC analysis results are indicated in Fig. 4–6.

According to the data of Fig. 4, the greatest purification of the product can be achieved using NWC-P and NWM-P adsorbents. Moreover, only for these activated carbon brands there are stable positive dynamics of admixture reduction when the adsorbent layer in a column increases. However, rutin samples, purified with NWC-P carbon, are the closest to the control sample in their parameters.



Layer height, mm: BAU-MF: r = 0.9159; y = 159.066667 + 0.115151515•x
Layer height, mm: BAU-A: r = 0.8390; y = 166.333333 + 0.0539393939•x
Layer height, mm: OU-A: r = 0.7381; y = 179.133333 + 0.0484848485•x
Layer height, mm: OU-B: r = 0.9265; y = 164.4 + 0.212727273•x
Layer height, mm: NWM-P: r = 0.9601; y = 177.2 + 0.110909091•x
Layer height, mm: NWC-P: r = 0.7855; y = 181.466667 + 0.0842424242•x

Fig. 3. Dependence of rutin melting temperature on adsorbent layer height.

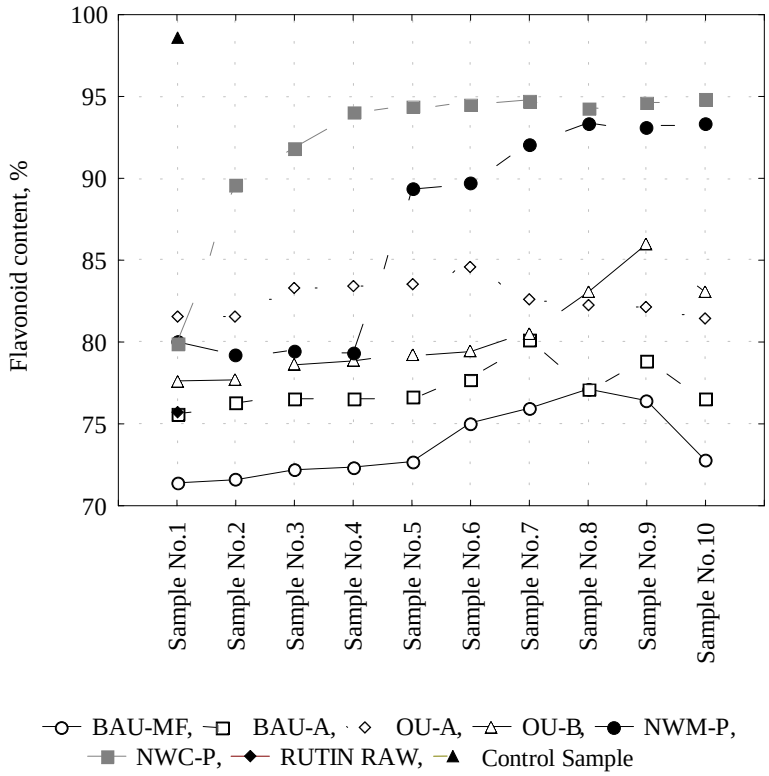


Fig. 4. Dependence of flavonoid amount content in rutin samples on carbon brand.

Processing of experimental data results on rutin raw purification with the method of normal distribution (Fig. 5) showed that the efficiency of BAU-A and OU-A adsorbents nearly does not depend on the layer height in a column. Herewith, use of OU-A carbon allows to get a stable result of 82.6% with narrow distribution. BAU-MF adsorbent is absolutely ineffective, having wide distribution with an average value of 73.8%, and NWM-P and NWC-P adsorbents, having the widest distribution, allow to get an average result of 86.9% and 92.3% respectively.

Dependence of the carbon brand efficiency on the layer height in a column is indicated in Fig. 6. The calculation results indicate that the highest correlation between the layer height and the achieved result is observed in NWM-P and OU-B adsorbent brands with correlation coefficients r = 0.9170 and r = 0.8940 respectively, for BAU-MF and NWC-P activated carbons correlation is less expressed with coefficients r = 0.7142 and r = 0.7235 respectively. Direct correlation coefficient value more that 0.75 indicates strong dependence between the layer height changing and the result obtained. If OU-A activated carbon is used as an adsorbent, there is no correlation among the studied parameters (r = -0.0579). Sorption rutin extraction is far less in comparison with its aglycone–quercetin, which may be connected with different adsorption nature, especially with the structure and the data size of polyphenol molecules. Functional groups on the activated carbon surface interact with phenol compounds due to Van der Waals forces in micropores and hygrogen bonds appearance in mesopores, herewith, the bigger and more polar glycoside molecule is adsorbed slower. Taking into account sub-acid nature of the split flavonoids, adsorption activity of the carbons, used in the present research, will be changed in a sequence, contrary to the surface acidic properties changing.

According to the results, obtained by mathematic treatment of experiments on determination of mass fraction of flavonoid amount, it can be stated that purification has a positive effect on the main substance content, extracted after the use of NWC-P and NWM-P carbons.

Rutin authenticity is confirmed by the results of determination of chlorophyll and red pigments content and is indicated in Fig. 7–9.

The obtained dependences of Fig. 7 show that the use of NWC-P, BAU-MF and BAU-A adsorbents allows to achieve the lowest chlorophyll and red pigments content, whereas using NWM-P carbon, a substantial increase in chlorophyll and red pigments content is observed.

Processing of experimental data results on rutin raw purification with the method of preparative chromatography with the use of normal distribution is indicated in Fig. 8.

According to Fig. 8, the efficiency of BAU-A and BAU-MF carbons nearly does not depend on the layer height in a column and allows to get a stable result of 0.00016% with narrow distribution. NWM-P carbon is absolutely ineffective in this case, having wide distribution with an average value of 0.00056%. It may be connected with the pore structure of the adsorbents under consideration: micropores, presence of which is typical for coconut-based carbons, are especially

suitable for adsorption of small molecules, whereas chlorophyll and red pigments are of quite big sizes. In this case, polymolecular adsorption is most effective which takes place in mesopores at sequential appearance of adsorption layers and which ends with pore filling by a mechanism of capillary condensation.

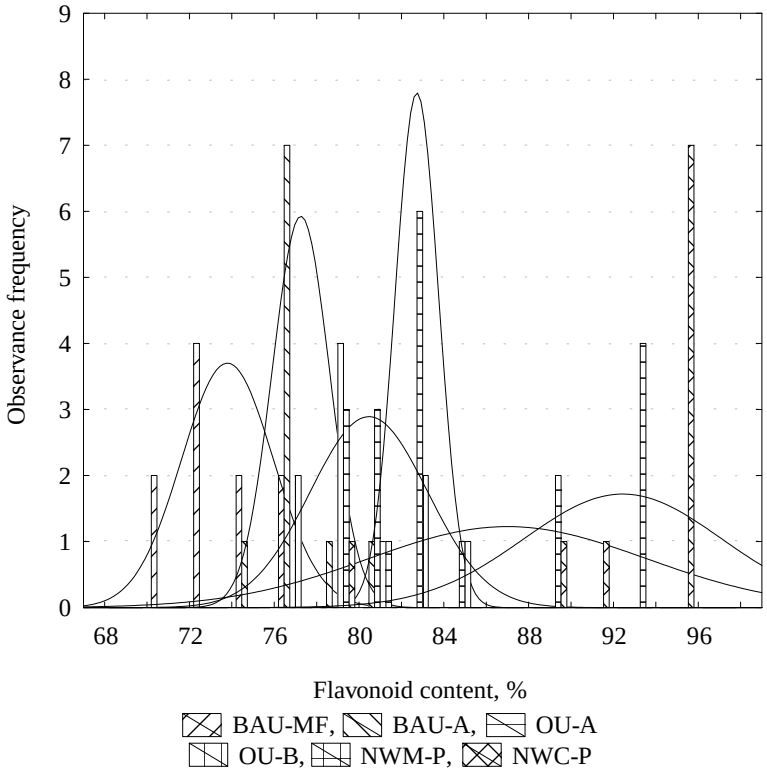


Fig. 5. Normal distribution of flavonoid amount content values in rutin samples depending on carbon brand.

Layer height, mm: NWC-P: r = 0.7235; y = 86.2166667 + 0.110842424•x

Fig. 6. Dependence of flavonoid amount content in rutin samples on adsorbent layer height.

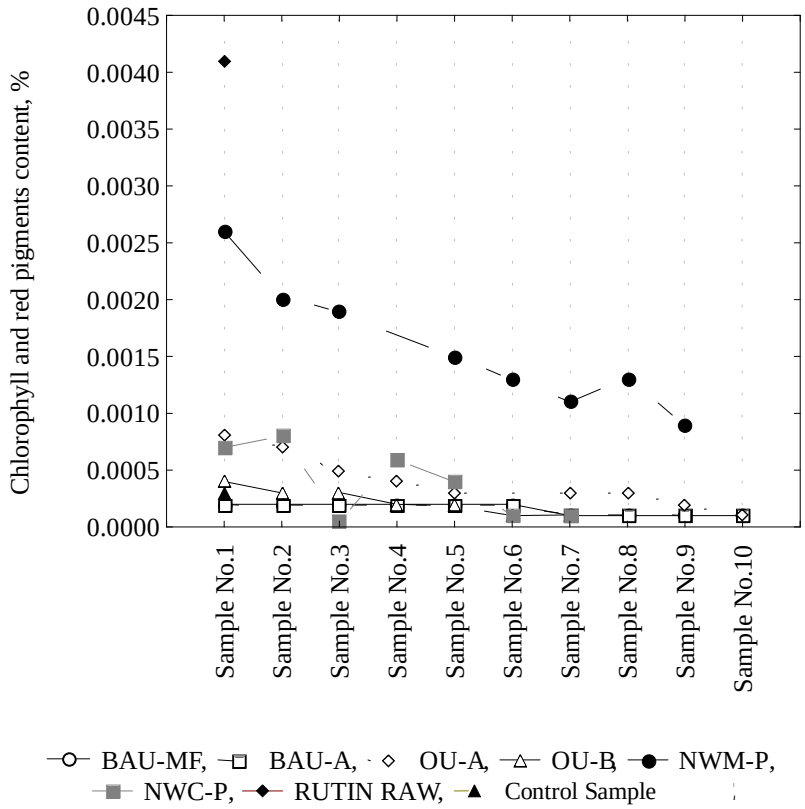


Fig. 7. Dependence of chlorophyll and red pigments content in rutin samples on carbon brand.

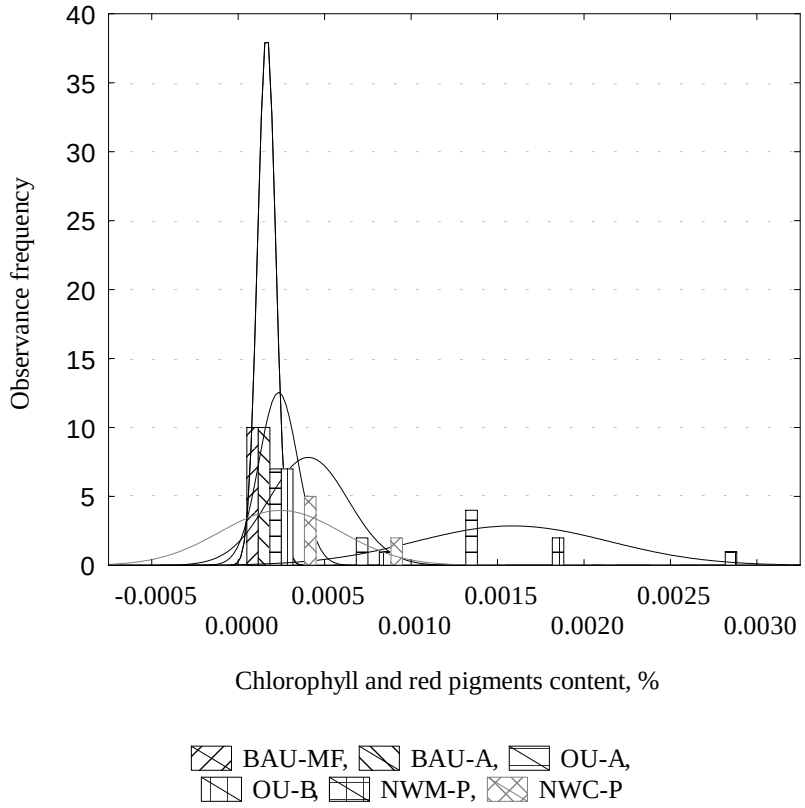


Fig. 8. Normal distribution of chlorophyll and red pigments content values in rutin samples depending on carbon brand.

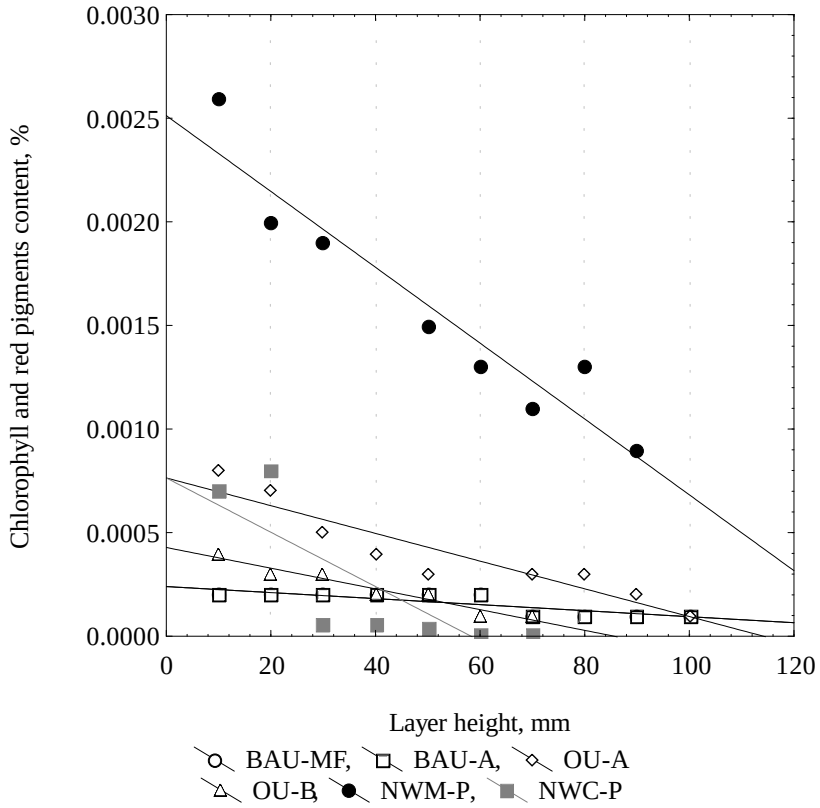
Dependence of the carbon brand efficiency on the layer height in a column is indicated in Fig. 9. The calculation results indicate that the highest correlation between the layer height and the achieved result is observed in OU-A, NWM-P and OU-B adsorbent brands with an invert correlation coefficient r = -0.9707, r = -0.9359 and r = -0.9359 respectively. OU-A and OU-B adsorbents are brightening carbons with mesopore volume of 0.7 cm^3/g and specific surface of 200–450 m^2/g, in contrast to other brands of activated carbons, for which these indexes are much lower and are 0.002–0.10 cm^3/g and 20–70 m^2/g respectively. According to the analysis of the obtained data, authenticity of all tested rutin samples is confirmed, which indicates strong correlation between the parameters under study and the adsorbent brand.

Thus, the use of the following carbon brands, indicated in decreasing effectiveness order, can provide the best purity and stability of parameters values, characterizing the product: NWC-P, NWM-P, OU-A and OU-B, excluding the authenticity index, where NWM-P carbon had the worst result.

The best correlation between the layer height in a column and product quality parameter was achieved, when NWM-P, OU-A and OU-B carbons were used, excluding the experiment on flavonoid amount content, where OU-A carbon showed no dependence.

Depending on the tasks, rutin purification degree may be regulated by sequential use of NWC-P and NWM-P carbons. Moreover, when rutin is purified from proximate admixtures, chlorophyll and red pigments, NWC-P adsorbent allows to get a comparable result even when the layer height is from 50 to 70 mm respectively. These data are proved by rutin samples micro-copying. After purification with NWC-P carbon, the crystal form is needle-like, identical to the GSO [State Standard Samples] control sample, which characterizes substance high purity. These data are indicated in Table 2.

Based on the results of Table 2, it should be noted that physical and chemical properties of the studied rutin samples No. 5 and No. 7 comply with State Pharmacopoeia XI after purification.



Layer height, mm: BAU-MF: r = -0.8528; y = 0.00024 - 0.00000145454545•x
Layer height, mm: BAU-A: r = -0.8528; y = 0.00024 - 0.00000145454545•x
Layer height, mm: OU-A: r = -0.9359; y = 0.000764189189 - 0.00000668918919•x
Layer height, mm: OU-B: r = -0.9707; y = 0.000428571429 - 0.000005•x
Layer height, mm: NWM-P: r = -0.9522; y = 0.00251295117 - 0.0000183014862•x
Layer height, mm: NWC-P: r = -0.7064; y = 0.000802857143 - 0.0000102142857•x

Fig. 9. Dependence of chlorophyll and red pigments content in rutin samples on adsorbent layer height.

Table 2. Rutin physical and chemical parameters

Rutin sample	Crystalls colour	Melting temperature, ◦C	Crystalls form	Crystalls size, μm	Rutin content, %	Flavonoids, different from rutin * (TLC data)	Ultraviolet spectroscopy	
							$D\ max^{I}$, nm	$D\ max^{II}$, nm
GSO	yellow-green	190	needle-like	0.33–153.30	98.56	1; 2	258.0	361.0
Rutin raw	greyish-green	162	irregular	1.66–203.13	75.74	1; 2; 3	258.0	361.0
Sample No. 5	yellow with the greenish cast	189	needle-like	0.42–159.00	96.25	1; 2	257.0	361.0
Sample No. 7	yellow with the greenish cast	189	needle-like	0.66–153.15	97.88	1; 2	258.0	361.0

*Notes.** 1 - kaempferol-3-O-rhamnoside; 2 - isoquercitrin; 3 – quercitrin.

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