

**RESEARCH OF THE INFLUENCE OF THE MODE
PARAMETERS OF THE WATER-VACUUM EXTRACTION
PROCESS ON THE YIELD OF BIOLOGICALLY ACTIVE
SUBSTANCES INONOTUS OBLIQUUS**

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ABSTRACT

Inonotus obliquus or chaga birch mushroom has a fairly wide range of applications in the cosmetic, food and pharmaceutical industries due to the wide range of biologically active substances it contains. The analysis of foreign and domestic studies testifies to the ongoing research in the field of extraction of valuable components from chaga, however, issues of intensification of processes and the development of new methods of extraction remain topical. One of these methods is the water-vacuum extraction of chaga, which consists in alternating the stages of the process at atmospheric and reduced pressure. The aim of the study is to determine the optimal time parameters of the individual stages of the water-vacuum extraction of chaga to optimize the operating parameters of the process. The object of the study was chaga collected in forest plantations in central Russia in the spring season, and crushed to particles ranging in size from 0.1 to 1.2 mm. The experiments were carried out on a laboratory version of a vacuum extraction unit, which allows for the classical infusion and extraction of chaga at various variations in the pressure of the medium. Studies have shown that preliminary evacuation of dry raw materials and the introduction of a rarefied medium into the process of water extraction of chaga makes it possible to intensify the extraction process and has a positive effect on the yield and activity of extractive substances. It has been established that the preliminary evacuation of crushed chaga makes it possible to increase the efficiency of subsequent extraction and to increase the yield of valuable components by 15-18%. The optimal time for this stage was 5 minutes. The effective duration of the infusion step at atmospheric pressure was 30 minutes, and the subsequent evacuation is expediently carried out for 10 minutes. Based on the results of the presented work, the optimal scheme for carrying out the process of water-vacuum extraction of chaga was determined.

Keywords: *chaga birch mushroom, water extraction, vegetable raw materials, rarefied medium, extractive substances.*

INTRODUCTION

The significant content of biologically active substances and antiseptics in natural plant raw materials causes an increased interest in their use in medical preparations. This direction is especially important for modern medicine, when in matters of protection against infections, preference is given to environmentally friendly methods of strengthening immunity and herbal medicines.

One of the raw materials of this origin is the chaga birch mushroom, which has established itself as a storehouse of biologically active substances necessary for the human body. Chaga is successfully used in the pharmaceutical industry as an enhancer of the action of other drugs, as well as as an independent remedy for treating diseases of the gastrointestinal tract and cardiovascular system. Chaga is famous for its fairly high content of antioxidants, thanks to which it can be used as a prophylactic anticancer agent [1], [2]. The acids present in chaga have a therapeutic effect on the human body and normalize metabolism. The chromogenic complex contained in chaga slows down the development of cancer cells in the initial stages and relieves pain in the subsequent stages of the development of the disease. Chaga is also successfully used as food additives due to its high content of fiber, resins, polysaccharides and acids, which help to maintain the immune system and improve appetite [3]. The content of sterols in chaga helps to lower blood cholesterol and reduce the risk of cardiovascular disease. The tannins present in it can fold protein in the blood and contribute to the formation of a protective film on the mucous surfaces of organs. The use of decoctions based on chaga allows you to stimulate the work of brain tissues by improving the functioning of neurons. In general, we can say that chaga is a good carrier of a complex of therapeutically active substances, with the correct extraction of which it is possible to obtain sufficiently effective components for the production of medicines, as well as food additives that stimulate human immunity.

A fairly large number of works have been devoted to the study of chaga, the particular interest of which is caused by various compounds of chaga passing into its aqueous extraction. For example, in a study by A.A. Shivrina, E.V. Lovyagin and E.G. Platonov [4], a chemical analysis of chaga and a concentrate based on it was carried out, which showed that the investigated mushroom has a fairly good pharmaceutical potential.

In the work of V.F. Korsun and K.A. Treskunov [5] analyzed raw chaga, which found that chaga also contains lectins belonging to the class of complex glycoproteins, the content of which contains magnesium and calcium ions. The study found that the content of these substances allows drugs based on chaga to exhibit a hypoglycemic effect, to reduce the blood sugar content of diabetic patients, due to the ability to reversibly bind carbons.

Liuping Fan and others [6] in their work found that the chaga birch mushroom contains a water-soluble polysaccharide (ISP2a), which not only showed antitumor activity, but could also significantly increase the body's immunity. The studies also found that the ISP2a polysaccharide allowed an increase in lymphocyte counts and an increase in TNF- α content.

Despite all the above advantages of this raw material, there is a problem of extracting biologically active substances from raw chaga. Studies [7,8] have shown that it is the extraction methods that directly determine the future concentration, quantitative yield, and therapeutic activity of the extracted substances. Today, most of the pharmaceutical enterprises of the Russian Federation extract the necessary components from chaga by the method of water extraction, which is characterized by the simplicity and low cost of the hardware design of the process [9, 10]. It is also generally accepted that with water extraction there is a greater preservation and extraction of biologically active substances relative to other methods. Currently, there are various options for the extraction of plant raw materials with water, but they all have their obvious disadvantages, such as the duration of the process and the relatively low yield of extractives.

Studies have shown that the introduction of a rarefied medium into the process of water extraction of chaga allows for a deeper impregnation with the extractant by washing it out of the pores, and the alternation of the stages of the process at atmospheric and reduced pressure has a positive effect on the yield and activity of extractives [11], [12], [13].

The purpose of this work is to study the process of water-vacuum extraction of chaga and determine the optimal time parameters of individual stages of extraction in relation to the process of preliminary evacuation, the optimal time for infusion of the extract at atmospheric pressure and subsequent extraction with a decrease in the pressure of the medium.

METHODS AND MATERIALS

Chaga collected from forest plantations in central Russia (the Republic of Mari El) in the spring season was used as a test material. The choice of the place and time of collection was determined by studies [14], [15], in which the unambiguous dependence of the influence of these factors on the concentration of therapeutically active substances in chaga, and, consequently, on the value of the extracts obtained, was proved.

Raw chaga was crushed using a drum crusher and separated into fractions using a set of sieves. In experimental studies, chaga particles were used, the size of which varied in the range of 0.1-1.2 mm. Distilled water (hydromodule 1:10) was used as an extractant.

The studies were carried out on a specially designed laboratory setup that allows both classical aqueous extraction by infusion and extraction using a rarefied medium at various pressure parameters (Figure 1). The method of water-vacuum extraction consists in alternating the stages of extracting target components at atmospheric pressure and during the process of evacuation.

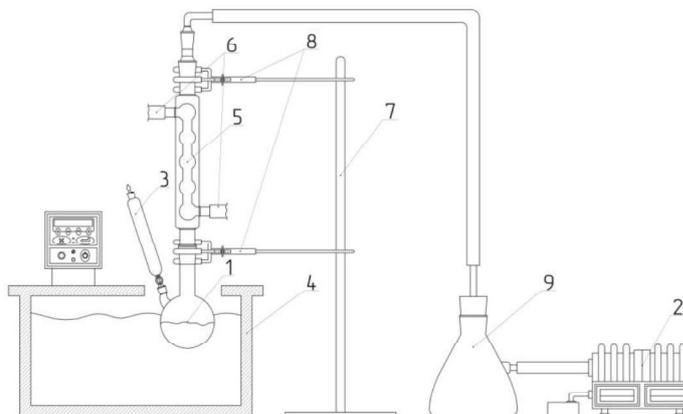


Fig. 1. Laboratory version of the vacuum extraction unit: 1) thick-walled flask with a spout; 2) vacuum pump; 3) a volumetric flask with a tap; 4) liquid thermostat; 5) refrigerator; 6) refrigerator taps; 7) tripod; 8) presser feet; 9) Bunsen flask.

The presented vacuum extraction plant operates as follows: crushed raw chaga is loaded into a flask with thick glass 1, which is subjected to a pre-evacuation process by turning on a vacuum pump 2 (pre-evacuation stage). Next, the chaga is poured with an extractant through a volumetric flask with a tap 3. The pressure is brought to atmospheric and infusion occurs (extraction stage at atmospheric pressure). The thermostat 4 is used to maintain a constant extraction temperature. The duration of the holding stage is set by the experiment plan. Next, the stage of evacuation begins. The refrigerator 5 installed at the top of the flask 1, in which the refrigerant circulates through the outlets 6, helps to minimize the loss of the extract during infusion and to exclude the ingress of liquid in the process of lowering the pressure of the medium. The presented structure is held by a tripod 7 and fastened with the presser feet 8. To completely exclude the ingress of liquid into the vacuum pump, a Bunsen flask 9 is used, through which the entire installation is directly connected to the vacuum pump 2.

According to the research plan of the process of water-vacuum extraction, as a result of experiments, the duration of such stages was determined as (Figure 2):

- stage of preliminary evacuation of raw chaga;
- stage of infusion at atmospheric pressure;
- the stage of extraction during evacuation.

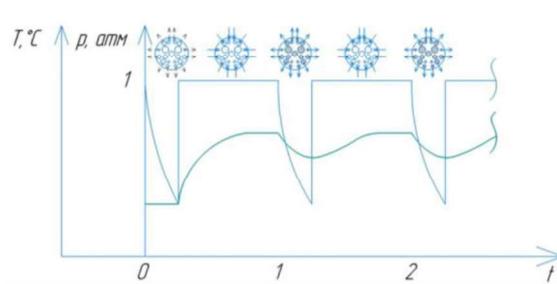


Fig. 2. Scheme of the process of water-vacuum extraction

RESULTS

The experiment to determine the pre-evacuation time was carried out while maintaining a constant extraction temperature and infusion time of the extract for 60 minutes. Crushed raw chaga was immersed in a flask and subjected to a preliminary evacuation process for 5, 10, 15, and 20 minutes at a medium pressure of 0.1 atm. Then the extractant preheated to 60 ° C was added to the raw material and the infusion process was carried out. The resulting extract was dried for 12 hours at a temperature of 70 ° C to obtain a dry residue. The dry residue was determined by the gravimetric method.

The results of the yield of extractive substances depending on different values of the time of preliminary evacuation of raw chaga are shown in Figure 3.

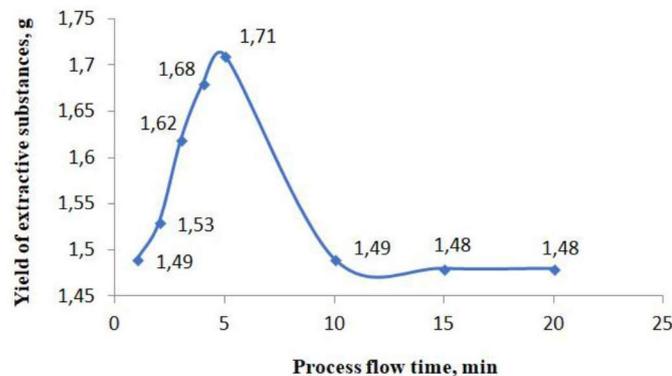


Fig.3. Graph of the influence of the pre-evacuation time on the yield of extractives

Based on the data obtained, it was found that the most optimal time for the pre-evacuation process is 5 minutes, since at this time parameter the highest concentration of biologically active substances was observed relative to other time values.

The experiment to determine the duration of the infusion stage at atmospheric pressure was carried out as follows. Raw chaga was subjected to a preliminary evacuation process for 5 minutes, then the extract was infused for 15, 30, 45 and 60

minutes at a constant temperature of 60 ° C. The weight of the obtained extract (dry residue) was determined as described above. The results of the experiment are shown in Figure 4.

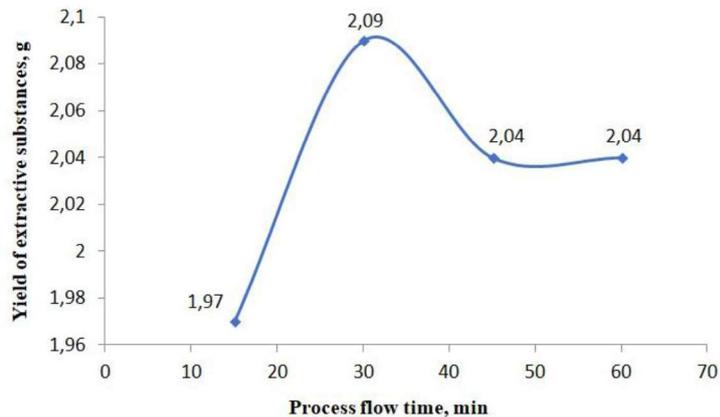
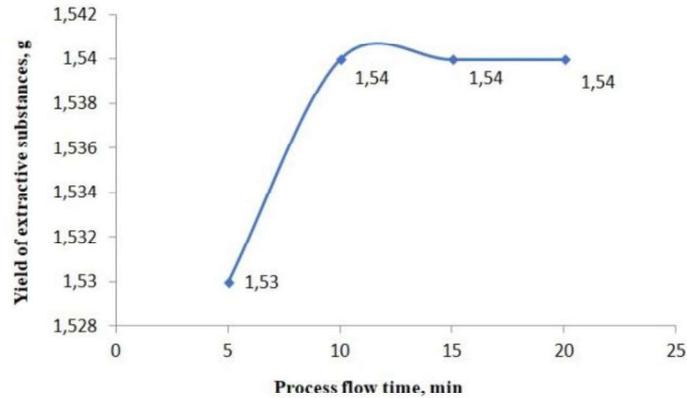


Fig. 4. Graph of the influence of the duration of the infusion stage at atmospheric pressure on the yield of extractives

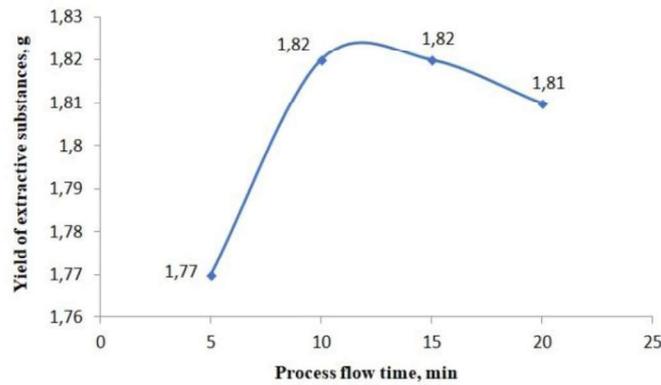
As can be seen from the graph, the highest yield of extractives is observed when infused for 30 minutes. A further increase in the extraction time leads to a decrease in the yield of the target components by 3.5%.

The experiment to determine the optimal extraction time with a decrease in the pressure of the medium (the stage of evacuation) was carried out in two ways: without preliminary evacuation of raw chaga and with the inclusion of this stage in the experiment. The experiments were carried out with the same aforementioned parameters.

The results of the experiments carried out to determine the duration of the vacuuming stage for various methods of preparing dry raw materials are shown in Figure 5.



a)



b)

Fig.5. Influence of the duration of the evacuation stage on the yield of extractive substances: a) without a preliminary evacuation stage; b) with preliminary evacuation

In the first case, without preliminary degassing, chaga was infused with a heated extractant for 30 minutes at atmospheric pressure, then the extraction process was carried out with a decrease in pressure (vacuum stage). The time of the evacuation stage was varied in the range of 5, 10, 15, and 20 minutes at a medium pressure of 0.1 atm.

In the second case, the raw chaga was subjected to a preliminary evacuation process for 5 minutes. Further, the process proceeded according to the scheme described above, while maintaining the parameters of the experiment. The yield of extractives was determined by the gravimetric method based on the dry residue.

From the presented graphs it can be seen that in both cases the maximum yield of the target components is achieved with a vacuum duration of 10 minutes. Further continuation of the depressurization stage does not affect the extraction efficiency. The inclusion of the stage of preliminary degassing of dry chaga intensifies the



subsequent extraction process, which is expressed in an increase in the yield of extractive substances by 15-18% with an equal duration of this stage.

CONCLUSION

Today, the topical direction of industry and scientific research is the use of natural plant materials in the production of medicines and cosmetics, as well as food additives. Chaga birch mushroom is in high demand among the listed fields of activity because it contains a huge amount of biological substances useful for the human body. The analysis of the applied methods and methods for the extraction of biologically active substances confirms the relevance of studies devoted to the intensification of extraction processes and the development of new methods for the extraction of valuable components from the chaga birch mushroom. Based on this, a method of water-vacuum extraction of chaga was developed, which consists in alternating the modes of the pressure of the medium in the apparatus, corresponding to different stages of heat and mass transfer processes.

Studies have shown that preliminary evacuation of dry raw materials and the introduction of a rarefied medium into the process of water extraction of chaga makes it possible to intensify the process and has a positive effect on the yield and activity of extractive substances. To work out the operating parameters and the practical implementation of this method, experimental studies were carried out to determine the duration of each stage of the water-vacuum extraction process relative to the maximum yield of extractive substances. Experimentally, the following optimal time parameters of individual stages have been determined with respect to the maximum yield of extractive substances. The duration of the stage of preliminary evacuation of dry raw materials is 5 minutes. Infusion at atmospheric pressure is optimal for 30 minutes, and subsequent evacuation - for 10 minutes. It is proved that the introduction of the stage of preliminary evacuation of dry material makes it possible to intensify the subsequent extraction process and to increase the yield of extractive substances by 16-18%. Based on the results of the presented work, the optimal scheme for carrying out the process of water-vacuum extraction of chaga was determined, which consists in the following. Crushed raw chaga with a particle size of 0.1 to 1.2 mm must be subjected to a preliminary evacuation process for 5 minutes at a pressure of 0.1 atm. Next, pour the extractant preheated to 60 ° C and infuse at atmospheric pressure while maintaining this temperature for 30 minutes. After that, lower the atmospheric pressure to 0.1 atm within 10 minutes. The resulting extract must be separated from the meal and dried.

These parameters ensure the efficiency of the extraction process and contribute to the production of extracts with high therapeutic properties.

REFERENCES

[1] Gubernatorov V. V., Khasanshin R. R., Safin R. R. The use of chaga in medicine and biomedical technologies / In the collection: Youth and the XXI century-2017. materials of the VII International Youth Scientific Conference: in 4 volumes. 2017. pp. 296-298.

[2] Shashkina M. Ja., P. N. Shashkin, A. V. Sergeev., Chemical and medico biological properties of chaga (*Inonotus obliquus*) / *Pharmaceutical Chemistry Journal*, Russia, vol. 40/ issue 10, pp. 37-44, 2006.

[3] Parfenov A, Vyshtakalyuk A, Sysoeva M, Sysoeva E, Latipova A, Gumarova L and Zobov V 2019 Hepatoprotective effect of inonotus obliquus melanins: in vitro and in vivo studies *BioNanoScience* 9:2 528-38.

[4] Shivrina A. N., Lovyagina E. V., Platonova E. G. On the chemical composition of chaga / *Chaga and its therapeutic use in stage IV cancer*. L., 1959. pp. 55-62.

[5] Korsun V. F., Krasnopolskaya L. M., Korsun E. V., Avkhukova M. A. Antitumor properties of mushrooms. - M.: Practical medicine, 2012. - 210 p.

[6] Liuping Fan, Shaodong Ding, Lianzhong Ai, Kequan Deng(2012) A water-soluble polysac-charide from chaga mushroom had potential as a natural antitumor agent with immunomodulatory activity. // *CarbohydrPolym*. 2012 Oct 1 ;90(2):870-4. Epub 2012 Jun 17. PMID: 22840014

[7] Safin R.R., Mukhametzyanov S.R., Gubernatorov V.V. Water vacuum-oscillating extraction of chaga / В сборнике: IOP Conference Series: Materials Science and Engineering. International Scientific-Practical Conference on Quality Management and Reliability of Technical Systems 2019. 2019. С. 012086.

[8] Liya Liang, Zesheng Zhang and Hao Wang 2009 Antioxidant activities of extracts and subfractions from *Inonotus Obliquus* *International Journal of Food Sciences and Nutrition* 60:2 175-84

[9] Safin R. G., Gubernatorov V. V., Safina A.V., Khuzeev M. V. Review of modern research in the field of extraction of biologically active substances from the birch fungus chaga for pharmaceutical and food industries / *Woodworking industry*. 2019. No. 3. pp. 93-103.

[10] Mazurkiewicz W 2006 Analysis of aqueous extract of *Inonotus obliquus* *Acta Poloniae Pharmaceutica* 63:6 497-501

[11] Safina A.V., Gubernatorov V. V., Garaev R. R., Razumov E. Yu. Intensification of the process of water extraction of chaga by periodically lowering the pressure of the medium / *Woodworking industry*. 2017. No. 2. pp. 50-53.

[12] Sung Hak Lee. Antitumor activity of water extract of a mushroom, *Inonotus obliquus*, against HT-29 human colon cancer cells / Sung Hak Lee, Hee Sun Hwang, Jong Won Yun // *Phytotherapy research*. P.-1784-1789.

[13] Razumov E.Y., Safin R.R., Sysoeva M.A., Khabibrakhmanova V.R., Gubernatorov V.V. Intensification of water extraction process of chaga by means of recurrent pressure reduction of the media / В сборнике: 18th International Multidisciplinary Scientific GeoConference SGEM 2018. Conference proceedings. 2018. С. 835-842.

[14] Safin R, Razumov E, Khasanshin R, Gubernatorov V and Saerova K 2018 Effect of growth conditions on qualitative characteristics of chaga mushroom *Int*.



Multidisciplinary Sci. GeoConf. Surveying Geology and Mining Ecology Management (SGEM 18th.) 781-88

[15] Razumov E. Yu., Safin R. R., Serova K. V., Gubernatorov V. V., Gubernatorova A. E. The influence of growing conditions on the quality indicators of chaga / Woodworking industry. 2018. No. 2. pp. 51-57.