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# Determination of the true density of chaga by gas picnometry in atmospheric air

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Abstract. The considerable interest in the study of chaga (Inonotus obliquus) as an extraction material is due to the unique chemical composition and a wide range of biologically active substances used in various industries. An important parameter reflecting the content of chaga substances in the volume is the true density or the cell wall density not given in the literature. The porous structure of the fungus and the irregular geometric shape of chaga determined the choice of gas pycnometry as a method to measure the true volume and the subsequent determination of chaga cell walls density. After critical analysis, an alternative, theoretically substantiated method for determining the true volume of a porous body in atmospheric air was proposed. The experimental data results were processed to determine the true density of chaga cell walls, which ranges from 1.411g/cm<sup>3</sup> to 1.588g/cm<sup>3</sup>. The maximum density was in the central part of the fungus. To the periphery of the fungus, there is a decrease in the cell wall density and the lowest density is at the point where the birch fungus adjoins the tree trunk.

#### 1. Introduction

Currently, a great number of scientific papers are devoted to the study and research of the birch fungus or chaga (Inonotus obliquus) due to the wide range of substances it contains: biologically active compounds, polysaccharides, phenols, pigments, tannins, etc. [1, 2]. Some researchers deal with the medical applications of this fungus [3-5], some study the food component [6, 7], the attention of others is focused on the physicochemical processing [8,9] of the fungus. With regard to physicochemical processing, a significant amount of research is devoted to the extraction of various substances used in the above industries. The study of the kinetics of heat and mass transfer, mathematical description, and simulation of the extraction process require the knowledge of the characteristics of chaga as a material [10]. One of the important characteristics that determine the quantitative measure of a material is density [11]. In view of its plant origin, chaga has a porous structure. Therefore, in this fungus, as in other porous materials, we can distinguish between the average density and density of cell walls, or true density [12, 13]. From literary sources, it is known that chaga body consists of three layers: the outer sclerotium, the second fungal fruit, and the third inner layer immediately adjacent to the tree trunk. From the point of view of industry and science, the sclerotium and fungal fruit are of the greatest importance, since they contain the bulk of the valuable components of chaga [1, 14, 15]. However, the

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same sources do not provide any chaga density information which would allow the necessary process calculations. It is known that material density at different humidity has different values [16, 17]. This fact suggests that density is a more embracing parameter for various calculations in comparison with mass or volume. It should be noted, as in the case of wood and other porous materials, it is the fungus cell wall density (the true density) that can reflect the content of the chaga substance in the volume.

There are many methods to determine the density of solids. Normally, the density is determined by dividing the mass of the test sample by its volume. It should be noted that body weight can be determined by weighing and body volume by measuring linear dimensions, if the sample is a regular-shaped body. In practice, the investigated samples are largely of irregular geometric shape, therefore, their volume is determined using other methods. Among them are ultrasonic, pycnometric (liquid or gas displacement method), mercury porosimetry and other methods [18-20]. To determine the true volume of a porous body, the most preferred method is gas pycnometry in helium [21]. Essentially, the body displaces a volume of gas equal to its own volume. The use of gases as a displacement medium is more practical since no sample wetting occurs, thereby its further usability is retained. This helium method is widely used in industry and is standardized all over the world. Helium pycnometry, with all the advantages, has its drawbacks. These include the need to use helium of a certain purity and a rather laborious measurement procedure in the course of experiments.

This work aims at developing an instrument to measure the volume of porous bodies by air pycnometric method, determining the true density of chaga and studying the nature of its change over the cross section of the material.

#### 2. Methods and materials

For experimental studies, chaga was collected in the autumn of 2020 in the forests of central Russia. The fungus segment, from which the samples were further cut, was taken by two cuts perpendicular to the tree trunk. Subsequently, this segment was divided into 31 samples, the ordinal values of which are shown in figure 1. Samples numbered 1-9 represent the inner zone of the fungus in contact with the tree trunk. Samples 10, 18, 19-22, 27, 28-31 represent the surface zone of the fungus (sclerotium). The remaining samples are the main fungal fruit.



Figure 1. Segment of dried and cut chaga.

Samples 1-9 represent the inner zone of the fungus in contact with the tree trunk. Samples 10, 18, 19-22, 27, 28-31 represent the surface zone of the fungus (sclerotium). The remaining samples are the main fungal fruit.

To facilitate experimental studies, an installation was developed (figure 2), based on a pneumatic system with atmospheric air instead of helium. The installation consists of two Cylinders 1 and 2 of the same capacity, Valves 3, 4, 5, 6, Vacuum Pump 7 with Pressure Gauge 8 and Relief Valve 9, Cylinder 10 with water connected to the installation with Transparent Pipeline 11 with Scale 12.



**Figure 2.** Pilot installation scheme: 1 - sample vessel, 2 - measuring vessel, 3, 4, 5, 6 - valves, 7 - vacuum pump, 8 - pressure gauge, 9 - relief valve, 10 - vessel with water, 11 - transparent pipeline, 12 - scale, 13 - test sample.

The installation operation is as follows. Test Sample 13 is placed in the Sample Cylinder 1 while Valves 3, 4, 5, 6 open and the atmospheric pressure is set in Vessels 1 and 2  $P_{atm}$ . After that, Valve 5 is closed and Vacuum Pump 7 is turned on creating vacuum  $P_1$  in Vessels 1 and 2, recorded by Pressure Gauge 8. After providing the required draft, Valve 6 is closed, then Valves 3 and 4 are closed; the pressure in Pump 7 is leveled with the atmospheric pressure using Relief Valve 9. After sequential opening of Valves 5 and 3, the height of the water column  $\Delta h_1$  in Transparent Pipeline 11 changes due to the draft, which is recorded by Scale of 12. Then Valve 6 is opened and Valve 3 is closed, thereby setting the atmospheric pressure in the measuring system again, and the water column returns to its original level. After that, Valve 6 is closed and Valve 4 is opened again, the water from Cylinder 10 moves through Transparent Pipeline 11 due to the draft, where the change in the height of the water column  $\Delta h_2$  is recorded using Scale 12. By opening Valves 3, 4, 5, 6 and the Relief Valve, the measuring system is returned to its original position.

The above operations have the following theoretical interpretation. Taking into account the constancy of the system temperature, its state in Vessels 1 and 2 at atmospheric pressure  $P_{atm}$  and after removing part of the air can be reflected by equations (1) and (2) that obey the Boyle-Mariotte law:

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$$P_{atm}(V_{\nu} - V_{h}) = P_{1}(V_{\nu} - V_{h} - \Delta V_{1}), \tag{1}$$

$$P_{atm}V_{\nu} = P_1(V_{\nu} - \Delta V_2), \tag{2}$$

where  $P_1$  is the draft in the vessels after air removal,  $V_v$  is the volume of each vessel,  $V_b$  is the body volume sought for,  $\Delta V_1$  is the volume of air removed from the sample vessel,  $\Delta V_2$  is the volume of air removed from the measuring vessel.

Expressing  $P_{atm}$  from equations (1) and (2), we arrive at:

$$P_{atm} = \frac{P_2(V_v - V_b - \Delta V_1)}{(V_v - V_b)},$$
(3)

$$P_{atm} = \frac{P_2(V_v - \Delta V_2)}{V_v}.\tag{4}$$

Equating the right-hand sides of equations (3), (4), and, solving with respect to the sought body volume  $V_b$ , we obtain the equation for calculating the volume of the studied body:

$$V_b = V_{\nu} \left( 1 - \frac{\Delta V_1}{\Delta V_2} \right). \tag{5}$$

For practical application of expression (5), certain explanations should be provided.

In course of the experimental studies, we replace relation  $\frac{\Delta V_1}{\Delta V_2}$  with relation  $\frac{\Delta h_1}{\Delta h_2}$ , this is explained by

the following expression

$$\Delta V = \frac{\pi \cdot d^2}{4} \Delta h,\tag{6}$$

where d is the diameter of the Transparent Pipeline,  $\Delta h$  is change in the height of the water column.

It should be noted that at the same draft, the volume of air removed from the cylinders will depend on the volume of the sample under study, that is, an increase in the volume of the sample under study with  $P_1 = cons't$  will result in a decrease in the volume of the air actually removed from the cylinders. This indicates the need for calibration of the installation. In addition, in order to facilitate further calculations, we should get rid of  $V_v$ . For this, taking into account equation (6), expression (5) can be represented in the following form

$$\frac{\Delta h_1}{\Delta h_2} = 1 - \frac{V_b}{V_v}.\tag{7}$$

For calibration, we used end gauges of various volumes as reference body  $V_b$ . The vacuum pressure during the calibration and experiments was -70 kPa. As a result, a calibration diagram graph (figure 3) was obtained, where ratio  $\frac{\Delta h_1}{\Delta h_2}$  is represented on the abscissa axis and body volume  $V_b$  corresponding to this ratio is represented on the ordinate axis.

For simplicity, the graph is characterized by the following equation

$$y = -59.073 \cdot x + 59.32,\tag{8}$$

where x is relation  $\frac{\Delta h_1}{\Delta h_2}$ .

In the experiments on the developed installation, the volume of each dried chaga sample was determined by 10-fold measurement. Each sample was weighed using a Vibra electronic balance with an accuracy of 0.001g.

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Figure 3. Calibration diagram graph.

#### 3. Results

The results of the experimental data on the determination of chaga cell wall true volume and density by gas pycnometry in atmospheric air are presented in table 1.

No.	Sample	$\Delta h_1$	Sample	True	No.	Sample	$\Delta h_1$	Sample	True
of	weight,	Ah	volume,	density,	of	weight g	Ah	volume,	density,
sample	g	$\Delta \Pi_2$	cm <sup>3</sup>	g/cm <sup>3</sup>	sample	weight, g	$\Delta \Pi_2$	cm <sup>3</sup>	g/cm <sup>3</sup>
1+2+3	8.553	0.90384	5.927	1.443	18	6.68	0.93021	4.370	1.529
4	5.241	0.94287	3.622	1.447	19	2.794	0.97292	1.847	1.513
5	5.107	0.94461	3.519	1.451	20	4.255	0.95799	2.729	1.559
6	5.285	0.94304	3.612	1.463	21	4.53	0.95197	3.084	1.469
7	5.544	0.9409	3.738	1.483	22	3.596	0.96442	2.349	1.531
8+9	6.617	0.929	4.441	1.490	23	3.438	0.96754	2.165	1.588
10	4.878	0.9506	3.165	1.541	24	3.878	0.96183	2.502	1.550
11	5.714	0.94179	3.686	1.550	25	3.12	0.97007	2.015	1.548
12	4.711	0.95315	3.015	1.563	26	2.783	0.97311	1.835	1.516
13	4.297	0.95628	2.830	1.519	27	3.199	0.96598	2.257	1.418
14	4.049	0.9604	2.586	1.566	28	3.291	0.96498	2.316	1.421
15	3.071	0.97124	1.946	1.578	29	2.883	0.97036	1.998	1.443
16	3.415	0.96657	2.222	1.537	30	2.479	0.97474	1.739	1.425
17	4.133	0.95826	2.713	1.524	31	1.982	0.98041	1.404	1.411

Table 1. Calculation of the true density of chaga.

Samples 1, 2 and 3 were small, therefore, to improve the measurement accuracy, their parameters were determined simultaneously. A similar situation is for samples 8 and 9.

For a clear reflection of the nature of the true density distribution over the cross section of the fungus, a contour plane is presented (figure 4).

According to table 1 and figure 4, the maximum value of chaga true density was 1.588g/cm<sup>3</sup>, the minimum 1.411g/cm<sup>3</sup>. It should also be noted that the highest value of the true density corresponds to the central part (the fungal fruit) of the fungus. The density of chaga cell walls decreases to the periphery. Finally, at the point where the fungus adjoins the tree trunk, the true density of the fungus is the lowest.

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Figure 4. Distribution of chaga segment true density (g/cm<sup>3</sup>) on the contour plane.

#### 4. Conclusions

The high anti-inflammatory, antioxidant, antiviral, and stimulating properties of chaga (Inonotus obliquus) account for the wide interest of researchers to the study of its properties and structure, processing methods, and applications. An important parameter that determines the physicochemical parameters of the extracted components and biologically active substances is the density of the extracted material. The plant origin, and therefore the porous structure, import different types of chaga density. The parameter that is closest to the actual material density is the true density or the cell walls density which is the most accurate measure of the chaga substance content in the volume. However, literature sources do not provide any reliable information about the density of the birch fungus. In finding the chaga true density parameter, the main challenge is determining the volume of the body due to the irregular geometric shape of the fungus. Critical analysis of the gas pycnometry method made it possible to develop and theoretically substantiate a method for determining the true volume of a porous body in atmospheric air. The data obtained using the pilot installation allowed us to determine the true density or the cell wall density Inonotus obliquus. It was found that the true density of chaga is 1.411-1.588g/cm<sup>3</sup> and the nature of its distribution tends to increase from the periphery to the center. For the subsequent study of chaga extraction process kinetics and the process control, additional studies will be carried out to determine the density of chaga, which will allow us to assess its dependence on the content of various components, humidity, growing conditions, season, orientation of location on the tree, etc.

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