

EXTRACTION OF BETULIN FROM THE BIRCH BARK BALANCE AT PULP AND PAPER PRODUCTION

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(RECEIVED JANUARY 2020)

ABSTRACT

The technical bark was used to obtain betulin; the one which is a barking of birch balance at pulp and paper production. The bark was prepared by grinding it on a crusher of abrasive action and fractionating, thus, the main fractions were obtained: less than 1 mm (35.4%), from 3 to 4.5 mm (31.5%). The bark was separated during the grinding process, the bark prevails in the coarse fraction, while bast is found in the fine fraction. For comparison, model samples of various sizes of birch bark were obtained by hand cutting. Chemical processing of bark fractions and model birch bark samples were carried out by the use of super-high frequencies (SHF) extraction with ethyl alcohol. Raw betulin was settled from extracts. The yield of extractive substances and raw betulin was 12.1-21.9% and 5.1-16.7% from absolutely dry raw materials; the maximum yield was from a coarse fraction of the bark or from model samples with smaller particle sizes. The authors studied the kinetics of the yield of extractive substances depending on the duration of SHF-extraction for bark fractions and birch bark samples. The optimal duration of the extraction for bark fractions is 10 min, and for birch bark is 15 min. The quantitative content was determined by the high performance liquid chromatography (HPLC) method for the obtained samples of betulin raw material. The total content of triterpenoids in the samples is about 80%, the betulin share is between 70.3 - 72.1%, from the absolutely dry substances of the extract. Coarse fraction of the bark contains more of the main component as compared to other fractions. It is advisable to use the fine fraction of the bark as a source of phenolic substances.

KEYWORDS: Birch bark, extractive substances, betulin, SHF extraction, HPLC.

INTRODUCTION

The birch bark is of the greatest interest for the chemical processing of bark waste, as it has a rather unusual composition. The birch bark accounts for 16-20% of the total mass of the bark (Kislitsyn 1994). It contains up to 50% of extractive substances (ES) (Jaasketainen 1981), which have a huge potential for chemical processing in order to produce new products and biologically active substances (BAS). The main ES component of birch bark is betulin, as it is responsible for the white color of birch bark. The value of betulin as a biologically active substance is proven by scientific data. The works (Pokhilo et al. 1988, Hayek et al. 1989, Alakurtti et al. 2006, Kuznetsova et al. 2013) show that the mass fraction of betulin in the bark can vary a lot (10-35%) depending on the type of birch, the place and conditions of growth, the age of the tree and many other factors. Along with betulin birch bark extracts contain its oxidized derivatives: betulinic acid, betulinic aldehyde, methyl ester of betulinic acid, coffee-related betulin, etc. Some results of 20 types of betulin biological activity and more than 10 types for betulinic acid have been published (Krasutsky 2006).

The biological activity of betulin is in its hepatoprotective, antibacterial and antiviral properties, as well as antipyretic and wound healing effects of the substance (Tolstikov et al. 2005, Bębenek et al. 2019, Cole et al. 1991, Kazakova and Tolstikov 2008). Betulin derivatives that have antitumor and low toxic properties are crucially important, thus, they can be further used for making anti-cancer drugs (Zehra et al. 2019, Zhuo et al. 2018, He and Liu 2007, Fulda 2008).

Currently betulin is obtained from birch bark with the use of various organic solvents, most of which extract approximately the same amount of the main component (Kislitsyn et al. 2005). Betulin extraction can be intensified using the different methods: non-isobaric steam cracking (Kuznetsova et al. 2005), shock-acoustic treatment (Mikhailov et al. 1999), supercritical extraction with carbon dioxide (Edwardson et al. 2001, Armbruster et al. 2017), etc. Alcohol extraction in a SHF field is of special concern, since it allows reducing the process time by 10-15 times as compared with traditional extraction methods (Vivekananda et al. 2007, Chemat et al. 2013). Betulin, lupeol and betulinic acid may be isolated from outer birch bark in good yield and purity, using supercritical fluid extraction (Krasutsky et al. 2006).

MATERIALS AND METHODS

Technical birch bark from the Arkhangelsk pulp-and-paper mill was used as raw material. The bark was prepared by grinding it on the dr. Koerner, mode II machine; bark was not divided into birch bark and bast. The scheme of the grinding machine is given in Fig. 1. The grinding machine has two disks: fixed and non-fixed, and there is an adjustable slot between them. The bark is fed into the receiving hopper, it further goes into the slot, and is cut into pieces by the knives of the rotating disk. Fragments of the bark are collected in the container.

Bark's moisture and ash content was carried out according to generally accepted methods. The moisture content of the bark was 2.5%, while the ash content was 1.35%. The crushed mass is subjected to fractionation. The results show that the fractional content mainly consists of the less than 1 mm (35.4%) and the 3 to 4.5 mm fractions (31.5%). The middle fractions with 1-2 mm and 2-3 mm of bark are less representative. There is an insignificant amount of large particles of bark in the size from 4.5 to 5 mm (1.1% of the total mass of the bark).

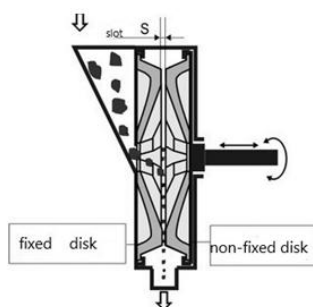


Fig. 1: Scheme of the grinding machine.

For comparison, model samples of birch bark, separated from bast, were prepared with the use of hand cutting into particles with fixed dimensions: 2.5 x 2.5 mm; 2.5 x 5.0 mm; 2.5 x 10.0 mm; 5.0 x 5.0 mm (tangential and longitudinal direction). Particle sizes are chosen in such a way that they differ from each other only in one direction. The geometrical characteristics of the samples of bark obtained during grinding, and samples of birch bark with hand cutting are given in Tab. 1.

Tab. 1: Geometric characterizes of bark and birch bark samples.

Type of grinding	Fraction	Fraction weight ratio (%)
Grinding machine	less 1 mm	35.4
	from 1 to 2 mm	19.0
	from 2 to 3 mm	13.0
	from 3 to 4.5 mm	31.5
Hand cutting	2.5 2.5 mm	-
	2.5 5.0 mm	-
	2.5 10.0 mm	-
	5.0 5.0 mm	-

The preparation of betulin was carried out according to the method developed by the authors and described in works (Tretjakov et al. 2015, Koptelova et al. 2013). Extraction was carried out on the device with a SHF-chamber, shown in Fig. 2.

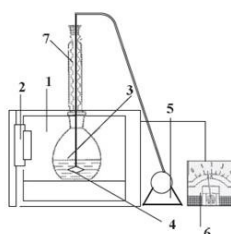


Fig. 2: Scheme of a device with a SHF-chamber (1 – the chamber with the SHF electromagnetic field, 2 – magnetron, 3 – extraction flask, 4 – mixer, 5 – mixer drive, 6 – Amperemeter, 7 – backflow condenser).

The fraction of crushed bark or birch bark sample weighing 6 ± 0.01 g was loaded into an extraction flask, then added 86% of ethyl alcohol, the liquid module (the ratio of solid and liquid phases) was 1:20. Then the flask was placed in the SHF chamber, connected to the backflow condenser, the mixers were turned on, so the bark was extruded at an energy consumption of $16 \text{ kWh}\cdot\text{kg}^{-1}$ of raw material. Sampling of extracts was carried out at regular intervals (every two min) to study the kinetics of ES extraction from bark and birch bark. The ES concentration increase and it was monitored by the use of the weight method (evaporation and drying of the sampled bulk solution). Later the resulting mass was filtered on a Buchner funnel with a water-jet vacuum pump. Raw betulin was obtained from the remaining ethanolic extract by the method of physical condensation.

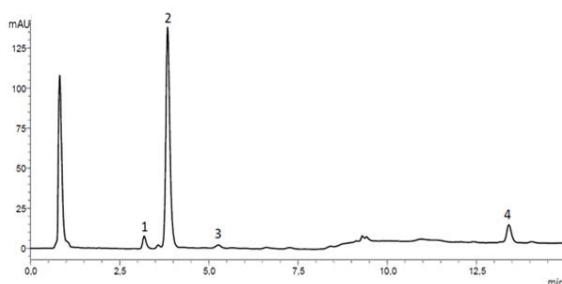


Fig. 3: Chromatogram of raw betulin sample (1- betulinic acid, 2- betulin, 3- erythrodiol, 4- lupeol).

The quantitative content of the main components was determined by high performance liquid chromatography (HPLC). The analysis was performed at the center 'Arctic', NarFU. To carry out an analysis 0.001 g sample was dissolved in 1 ml of methanol, the resulting solution with a concentration of $1 \text{ mg}\cdot\text{l}^{-1}$ was used for analysis. The analysis was performed in four replications. An example of the chromatogram of the analyzed sample is given in Fig. 3.

RESULTS AND DISCUSSION

According to the results of alcohol extraction, dependencies of the ES yield on the duration were obtained, and presented in Figs. 4, 5 and Tab. 2.

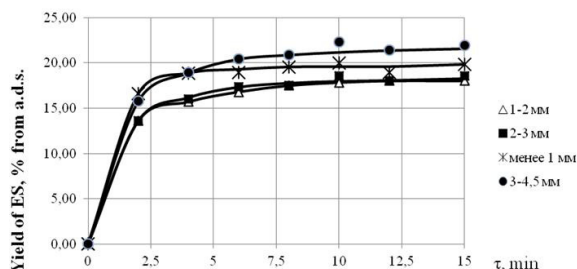


Fig. 4: Dependency of the ES yield on the duration of extraction for different bark fractions (abrasive crusher).

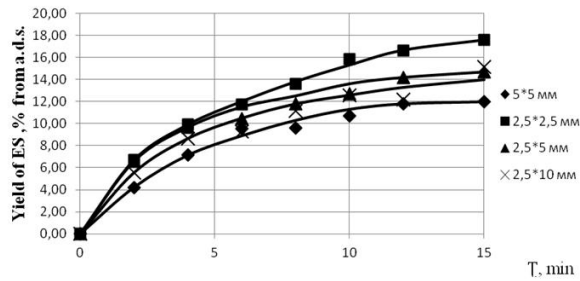


Fig. 5: Dependency of the ES yield on the duration of extraction for various birch bark fractions (model samples).

ES are extracted from the bark in the first 3-5 min of extraction, and then the ES yield is stabilized (Fig. 3). When the bark size is bigger the ES yield increases and reaches its maximum (22%) with the use of a fraction of 3-4.5 mm (Tab. 2). However, from a less than 1 mm fraction, the ES yield is significantly greater (19.8%) than when using intermediate fractions of 1-2 mm and 2-3 mm (18.3% and 18.6%, resp.). This may be because the fine fraction consists mainly of bast containing a large amount of tannins, which are also actively extracted during alcohol extraction. Consequently, during grinding, the bark is spontaneously divided into bark and bast, and bast is crushed faster.

Tab. 2: The yield of ES and raw betulin depending on the method and degree of bark grinding (birch bark).

Type of grinding	Sample	Yield of ES, (% from a.d.s.)	Yield of raw betulin, (% from a.d.s.)
Grinding machine	less 1 mm	19.8	5.1
	from 1 to 2 mm	18.3	9.8
	from 2 to 3 mm	18.6	12.4
	from 3 to 4.5 mm	21.9	16.7
Hand cutting	2.5 x 2.5 mm	17.6	13.4
	2.5 x 5.0 mm	14.4	11.1
	2.5 x 10.0 mm	14.8	9.2
	5.0 x 5.0 mm	12.1	9.9

On the contrary, in the case of model birch bark of hand cutting (Tab. 2), the maximum yield (17.6%) is observed when extracting the smallest sample (2.5 x 2.5 mm) and the minimum (12.1%) from sample (5.0 x 5.0 mm) with the largest tangential size and equivalent radius. An increase in the size of the bark, both in the tangential and in the longitudinal direction, decreases the ES yield because of diffusion phenomena. One can observe a significant increase in the ES yield in the range of 0-15 min for all samples when considering the dependency of the ES yield on the duration of birch bark extraction (hand cutting, Fig. 5), except for the sample with a large tangential size, for which the yield is stabilized starting only from 12th min of extraction.

If we compare the results of the extraction of bark - waste of birch wood from pulp and paper production and previously published (Koptelova et al. 2013), waste of birch round log of plywood production, the advantage of the second alternative in the yield of ES and raw betulin becomes

clear. The reason is the better quality of the bark in the birch round log than in the balance, as well as the removal of water-soluble substances during the process of bark soaking in warm water as part of the preparation of the round log for plywood production, which leads to an increase in the ES content.

The dependency of the yield of raw betulin on the bark particle size does not coincide with the dependence of the yield of extractive substances on the same parameter: the product yield is minimal (5.1%) from a less than 1 mm fraction, i.e. the content of betulin is reduced because of the high content of bast substances in it. The maximum (16.7%) was found during the extraction of a coarse fraction, which contains more bark than bast. When extracting model samples of birch bark, the yield of raw betulin corresponds to the average values obtained by extracting bark fractions. The maximum yield of raw betulin was 13.4% (from fine particles), the minimum - 9.2% (from the longest particles), which shows the difference in the penetration of the extractant and slowing the extraction in the longitudinal direction.

The structure of betulin raw material was studied by HPLC, thus, the study showed that of the dominant components were betulin (2), its derivative - betulinic acid (1) and in smaller quantities the its derivatives - erythrodiol (3) and lupeol (4). The numbers of the peaks correspond to the numbers of the formulas (Fig. 6) on the chromatogram (Fig. 3). All components belong to pentacyclic triterpenoids group.

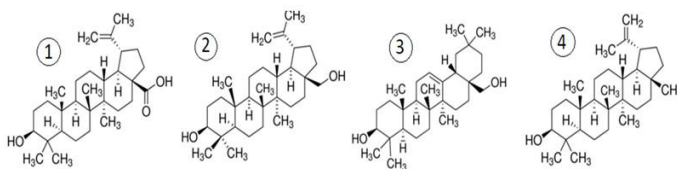


Fig. 6: Structural formulas of pentacyclic triterpenoids that make up the ES of birch bark (1- betulinic acid; 2- betulin; 3- erythrodiol; 4- lupeol).

The quantitative content of the main components in the samples of betulin raw extracts, determined by HPLC, are given in Tab. 3 (for absolutely dry substances).

As we can see in the Tab. 3, the content of the main component, betulin, is about 2.0% higher in samples obtained from the bark fraction of 3-4.5 mm. The content of the main derivative, lupeol, in a solution of betulin raw extract, obtained from a coarse fraction, is higher compared to the fine fraction.

Tab. 3: The extractive content of triterpenoids, (%) a.d.s.).

Component	Fraction from 1 o 3 mm					± Δ
	1	2	3	4	Average	
Betulinic acid	3.47	3.52	3.46	3.51	3.49	±0.05
Betulin	69.74	71.20	69.62	70.76	70.33	±1.23
Erythrodiol	1.15	1.16	1.13	1.15	1.15	±0.02
Lupeol	5.87	5.96	5.84	5.88	5.89	±0.08
Total	80.23	81.84	80.05	81.30	80.86	±1.36
Component	Fraction from 3 to 4.5 mm					± Δ
	1	2	3	4	Average	
Betulinic acid	3.40	3.27	3.28	3.25	3.30	±0.10

Betulin	73.91	71.46	71.74	71.49	72.15	±1.88
Erythrodiol	1.21	1.16	1.15	1.14	1.17	±0.05
Lupeol	6.77	6.57	6.76	6.77	6.71	±0.16
Total	85.29	82.46	82.93	82.65	83.33	±2.10

Note: ± Δ - confidence bounds of random error of the measurement result. The main component is in bold.

This fact can be explained by the partial presence of bast in the fraction of the bark of 1-3 mm, due to which other substances are extracted so the proportion of the main component decreases. The presence of very valuable derivatives of betulin (lupeol and betulinic acid) in betulin-raw extracts only makes the product more important as a source of biologically active substances.

CONCLUSIONS

Conclusions could be stated as follow: (1) During the grinding process of birch bark and its further fractionation, we observe separation of parts of the bark: the bark accumulates in coarse fractions, the bast in fine ones. (2) The yield of raw betulin in the extraction of bark from less than 1 mm fractions is 5% by raw material weight. This fraction can be further used to obtain polyphenolic compounds. (3) The yield of extractive substances was more than 20% with a 10 min process during the SHF extraction of grinded bark of birch balances in the pulp and paper industry. (4) The results of HPLC proved that the main component of betulin raw extract, obtained from the technical bark, which is a waste of pulp and paper production, is betulin (70.3 - 72.1%, from extractive a.d.s.). Barker refuse of birch balance should be regarded as a promising raw material for the extraction of biologically active substances.

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