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**NOVEL EXTRACTION METHOD FOR OBTAINING
OF EXTRACTIVES WITH A HIGH CONTENT OF
BETULIN**

Summary of the Doctoral thesis

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Riga 2015

UDK 674.03:54(043.2)

Pā 990 n

Pāže A. Novel extraction method for obtaining of extractives with a high content of betulin. Summary of the Doctoral thesis.-R.:RTU, 2015.-32 p.

This work was supported by the European Regional Development Fund, contract No. 2010/0289/2DP/2.1.1.1.0/10/APIA/VIAA/058



EIROPAS SAVIENĪBA



EIROPAS REĢIONĀLĀS
ATTĪSTĪBAS FONDS

IEGULDĪJUMS TAVĀ NĀKOTNĒ

The Doctoral thesis is worked out at the Latvian State Institute of Wood Chemistry and Riga Technical University, Department of Polymer Materials Technology.



ISBN 978-9934-542-15-2

THE DOCTORAL THESIS IS WORKED OUT AT THE RIGA TECHNICAL UNIVERSITY FOR AQUISITION OF A DOCTORAL DEGREE IN ENGINEERING OF CHEMICAL TECHNOLOGY

The Doctoral thesis is openly defended on 14st October 2015 at 15:00 at the Riga Technical University, Faculty of Materials Science and Applied Chemistry, 3 Paula Valdena Street, room 272.

The Doctoral thesis is available at the library of the Riga Technical University, 10 Kipsalas Street, Riga, LV-1048 and the National Library of Latvia, 3 Mukusalas Street, Riga, LV-1423.

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CONFIRMATION

Hereby I confirm that I have worked out the present Doctoral thesis, which I submitted for consideration at the Riga Technical University for acquisition of a Doctoral degree in engineering sciences of chemical technology. The present Doctoral thesis is not submitted in other scientific institutions for acquisition of a scientific degree.

Aigars Pāže (signature)

Date: 16.06.2015.

The Doctoral thesis is written in Latvian; it contains Introduction, References review (10 chapters), Experimental (4 chapters), Results and discussion (8 chapters), Conclusions and the used References list. The Doctoral thesis contains 166 pages, 61 figures, 27 tables, 2 attachments and 232 references.

ACKNOWLEDGEMENTS

I am grateful to my Doctoral thesis supervisors Dr. sc. ing. Assoc. Prof. Mārcis Dzenis, Dr. sc. ing. Jānis Rižikovs and Dr. sc. ing. Jānis Zandersons for the guidance, timely assistance, patience and moral support during the development of the work.

I am grateful to Director of the Latvian State Institute of Wood Chemistry Uģis Cābulis for the provision of the infrastructure for working out the Doctoral thesis at the Institute's Department of Technological Research.

I would like to thank all my colleagues for the moral and practical support they have rendered me during the development of the thesis, for making the necessary analyses and helping to understand the results. Great thanks to the colleagues of the Latvian State Institute of Wood Chemistry: Ausma Tardenaka, Ance Pļavniece, Baiba Spince, Juris Grīniņš, Kristīne Meile, Māris Puķe, Prans Brazdausks, Vilhelmīne Jurkjāne as well as leading researcher of the Institute of Organic Sythesis Pēteris Trapencieris and colleagues from the Riga Technical University - Inese Mieriņa and Māra Jure for making analysis, helping and useful recommendations in the experimental work.

Sincere thanks to my wife, mother, sisters and friends for their faith and support.

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LIST OF ABBREVIATIONS

Oven dried	o.d.
Relative moisture content	w_{rel} .
Weight percent	wt%
Birch outer bark	BOB
Hours	h
Ethanol	EtOH
Petroleum ether with the range of boiling temperature 100-140°C	PE140

ESSENCE AND URGENCY OF THE PROBLEM

In industrial birch wood processing sectors, the heavy tonnage waste is bark, which is currently used only as a fuel [1]. It is not a rational solution, because the white outer layer of Latvian silver (*Betula pendula* Roth.) and downy (*Betula pubescens* Ehrh.) birch bark, in terms of its chemical composition, is a unique natural product, which contains a great amount of valuable extractives (~35 weight (wt) % from oven dry (o.d.) birch outer bark (BOB)). The extractives' basic mass (~75 wt% from o.d. crystals) is made up by an individual chemical compound - lup-20(29)en-3 β ,28-diol or betulin which has been known for more than 200 years in scientific publications, but in folk medicine, in healing bark powder compositions, it has been applied since ancient times [2]. However, just in the last decades, the first advancements have been made in using betulin and its derivatives against cancer and HIV-infection due to the progress in organic synthesis and the development of methods for research and analysis of the physico-chemical structure of individual substances; therefore, interest in the chemistry and synthesis of individual compounds from betulin and its derivatives (betulinic acid and lupeol), obtained from BOB extractives, has also increased substantially [3]. Thus, also betulin and its derivatives have several other valuable properties, which are very promising for use not only in pharmacy, but also in the food, cosmetics and polymer industries [2]. All this stimulates the search for a new, more efficient and less expensive isolation methods which could, with minor investments, be easily scaled up to large-scale industrial production.

Urgency of the issue

Industrial production of high value-added products is one of the cornerstones of the national development. Latvia is not rich in mineral resources; therefore, the pathway of the sustainable development of Latvian economy and welfare should be planned, constructing the plants, which use local renewable resources. However, to gain the maximum benefit, the manufacturing process should be planned out as rationally as possible, involving production residues in the manufacturing process, thus striving for the biorefinery concept or non-waste technologies. Currently, the veneer industry sector developed in Latvia would need changes in processing technologies, because for a long time, there has not been found a better way to use production residues than burning. Continuing to do so, the possibility to considerably increase the added value of the own products is not exploited, because production residues constitute a significant part of the processed veneer logs mass.

Human economic activities, which are associated with the greenhouse gases (carbon dioxide, methane, etc.) emissions, can increase the Earth's average temperature and affect the global climate change, which is accompanied by various natural disasters. With this in mind, the behaviour should prudent, so that, if

possible, to avoid the burning of residues and to search for an alternative use or recycling options. The searches for alternatives are stimulated also by the commitments undertaken by the Latvian State in 2002, when joining the Kyoto Protocol. The Kyoto Protocol is an international act, which was accepted in 1997 with the objective of limiting atmospheric pollution by greenhouse gases. The Protocol envisages several international level mechanisms to reduce global greenhouse gas emissions, and one of them is international emission trading. In this way, Latvia and other European Union countries, through trade with carbon dioxide quotas, for their part, maintain the emission balance. Hence, it can be concluded that, by choosing an alternative for burning, it is possible not only to participate in nature conservancy, but also to make an extra income on sales of the saved carbon dioxide quotas [4].

All over the world, the natural cosmetics market constantly grows, that generates a need for new cosmetic raw materials, which especially applies to functional additives such as emulsifiers, preservatives, antioxidants, etc. Functional additives for natural cosmetic products are often synthetically derived, because it is difficult to find additives of natural origin with the required properties [5]. In this respect, extractives of BOB are special, because, on definite processing, they simultaneously possess emulsifier, preservative and antioxidant properties, as well as pronounced skin healing properties [6, 7]. This, in turn, might be of interest for natural cosmetics producers, whose market is wide enough to be able to sell large amounts of Latvian extractives.

The aim of the work

To develop a novel method for isolating a chemical compound - betulin with a high yield and purity in an as simple and efficient as possible process, that could be scaled up to a large-scale industrial production in Latvia.

For the development of the thesis, the following goals were set out:

- ❖ Based on the literature, to investigate information about BOB preparation for triterpenoids extraction and its subsequent purification, using industrial birch bark residues as the raw material;
- ❖ To find and evaluate optimal conditions for extraction of triterpenoids in the Soxhlet apparatus;
- ❖ To choose cheap, selective solvents for extraction of triterpenoids as well as the most suitable intensification techniques of the extraction process and betulin purification options;
- ❖ Based on the obtained results, to combine and improve the known BOB processing technologies and develop a novel, effective method for isolation of high purity betulin in the extraction process;
- ❖ To choose rational options for use of betulin and birch bark processing residues (birch inner bark, extracted outer birch bark and mother liquor);

- ❖ To assess the feasibility of industrial manufacturing of betulin in Latvia.

For defending the thesis

- ❖ Industrial birch bark residues is suitable raw material for isolation of betulin.
- ❖ Preparation of BOB (separation from birch inner bark, pelletisation) improve the yield of the extractives with high content of betulin.
- ❖ Advanced extraction unit (intermediate crystallisation method, stirring, extracion at solvent boiling temperature) accelerates the obtaining process of high purity betulin by using non-polar solvents.
- ❖ Developed method of betulin production is economically beneficial and appropriate for Latvia's conditions.
- ❖ Betulin and the residues from betulin production are suitable raw material for several high value-added products.

Scientific novelty of the work

- ❖ The novel method for betulin isolation combines and improves the known methods for BOB flotation, granulation, extraction and betulin crystallisation.
- ❖ A novel method for BOB quality control is developed, that enables a prompt monitoring of the BOB purification process.
- ❖ For the first time, the use of an intermediate crystalliser for isolation of high purity betulin during the extraction process is investigated.
- ❖ Rational variants of the use of extractives and by-products are offered and practically tested, thereby pointing to the possibility of implementing the biorefinery concept for industrial birch bark.

Practical importance of the work:

- ❖ The developed BOB processing method significantly improves the efficiency of using birch wood and the total added value of veneer industry production; as a result, birch wood will become more competitive in the market.
- ❖ An original intensive mass exchange extraction pilot unit is designed and patented, and the optimal regime is developed for isolating crystalline betulin with high yield and purity in one stage.
- ❖ Using the obtained results, a technological proposal for industrial production of betulin and lupeol from processing residues of birch veneer logs is offered.

Approbation of the results of the work

The main scientific achievements and results of the thesis were presented at 7 scientific international conferences, with a positive assessment. There are 16 printed works on the themes of the thesis, including 4 articles (1, 4, 5 and 10) in scientific citation journals, 2 articles in conference proceedings (3 and 12), 6 items of abstract summaries (3, 7-9, 11 and 13), 1 patent (14) and 2 patent applications (15 and 16):

1. Rizhikovs, J., Zandersons, J., Dobeles, G., Paze, A. Isolation of triterpene-rich extracts from outer birch bark by hot water and alkaline pre-treatment or the appropriate choice of solvents // *Ind. Crop. Prod.*, - 2015, vol. 76, 209-214 p.
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5. Rižikovs, J., Zandersons, J., Pāže, A., Tardenaka, A., Spince, B. Isolation of suberinic acids from extracted outer birch bark depending on the application purposes // *Baltic Forestry*, 2014, vol. 20(1), 98-105 p.
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 14. Zandersons, J., Rižikovs, J., Pāže, A., Dobeles, G., Tardenaka, A., Spince, B., Jurkjāne, V. Tāss ķīmiskās pārstrādes paņēmieni un iekārta tā realizēšanai // Latvijas patents 14759 B (20.04.2014).
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SHORT SUMMARY OF THE THESIS

In the **Introduction** of the thesis, the topicality of the work is stated, the aims and objectives are formulated and the guidelines are set out.

The **first chapter** is a literature review, in which the prevalence of birch species, its differences, application and processing in Latvia are considered. The morphology of birch bark, basic components' content, and its chemical composition depending on the origin are given. Methods for BOB preparation are given, and characteristics of BOB extractives are considered. Appropriate methods for separation and purification of betulin, lupeol, as well as their parameters are analysed and discussed. The application potentialities of BOB extractives and birch bark processing residues are described.

The information compiled in the literature review allowed to find out an appropriate method for preparation of BOB, as well as the ways for intensifying betulin extraction and its purification.

The **second chapter** is the experimental part, in which the choice of raw material or industrial birch bark is justified, sample preparation is described, and the materials, methods and equipment used in the thesis are reflected. For the development of a novel betulin extraction method the following scheme was used, which can be seen in Fig. 1.

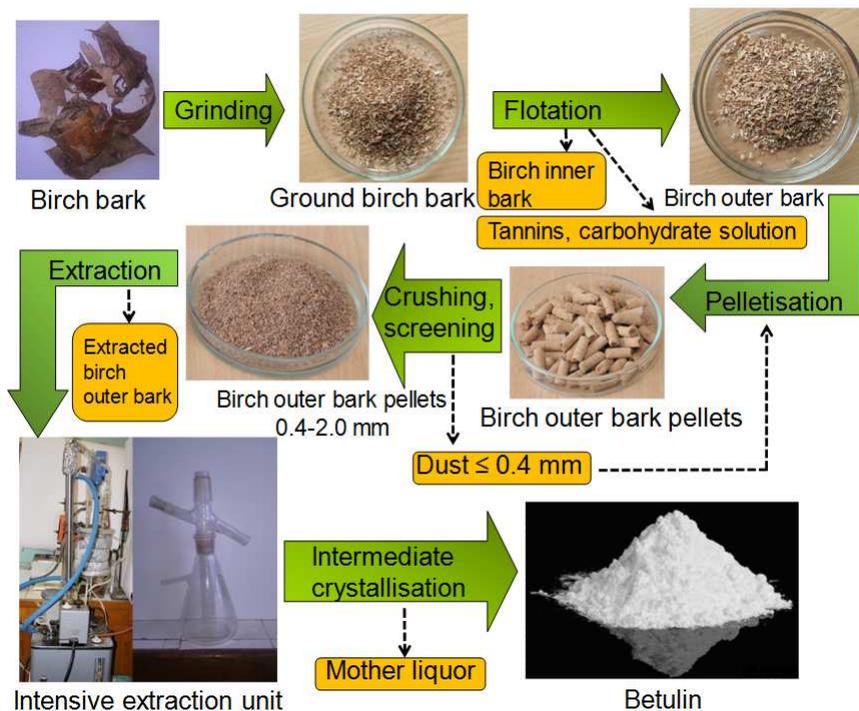


Fig. 1. Simplified experimental scheme for obtaining betulin from industrial birch bark

In the **third chapter**, the results obtained in chapters 1 and 2 are analysed, and an assessment is given.

Conclusions consist of the formulation of the obtained results, and the most important solutions are given.

In the **References list**, the sources used in the work are mentioned, based on which the avenues of research were defined.

RESULTS AND DISCUSSION

For the research as a raw material, the industrial residue of veneer manufacturing - birch bark was chosen, which is currently a relatively cheap and in great quantities available material in Latvia.

Characterisation of industrial birch bark

Experiments were started with the analysis of the characteristics of the raw material (industrial bark and its outer and inner bark), for which the chemical and elemental composition, and calorific value were determined (Tables 1-3).

Table 1
Chemical composition of industrial birch bark and its separate outer and inner bark layers

Components content, wt% from the o.d. raw material	Bark	Outer layer	Inner layer
Extractives	18.1	34.7	9.0
From which: betulin	8.4	25.7	0.6
Phenolic compounds (hydrolysable tannins etc.)	1.3	2.1	1.2
Monosaccharides	1.3	1.0	1.1
Other (triterpenoids, wax etc.)	7.1	5.9	6.1
Substances soluble in NaOH - EtOH solution *	32.5	53.6	25.2
From which: suberinic acids	12.0	38.7	1.1
Other (condensed tannins etc.)	20.5	14.9	24.1
Cellulose**	19.3	3.6	26.5
Lignin**	16.8	2.2	24.6
Other**	11.5	3.8	13.3
Ash	1.8	2.1	1.4

* a group of compounds, identified after extraction with ethanol (EtOH); ** components, identified after the isolation of extractives and separation of substances, soluble in 0.5 N NaOH EtOH solution

Table 2

Elemental analysis of industrial birch bark and its separate layers

Raw material	N, wt%	C, wt%	H, wt%	O, wt%
Outer layer	0.5	70.4	6.3	22.9
Inner layer	0.6	59.0	5.9	34.5
Bark	0.5	67.5	6.0	26.0

Table 3

Combustion heat of industrial birch wood, bark and its separate layers

Raw material	Combustion heat, MJ/kg
Birch wood	19.3-19.7
Bark	25.1-26.0
Outer layer	31.8-33.0
Inner layer	24.5-26.1

Combustion heat data show that the highest values are for BOB, which can be explained by a higher content of carbon and a lower content of oxygen elements

than in birch inner bark. However, from the chemical composition results, it can be concluded that industrial BOB contains a total of 34.7 wt% of extractives, from which 25.7 wt% is betulin, while birch inner bark contains a total 9 wt% of extractives which, if birch inner bark is not separated from BOB, would reduce the betulin content in extractives almost 3 times (the content of betulin in birch bark's extractives - 8.4 wt%).

Quality control of BOB

Since industrial birch inner bark contains a substantial amount of extractives with undesirable compounds, before extraction it was necessary to separate birch inner bark from the outer one as fully as possible to provide repeatable yields of BOB extractives with a high content of betulin. Therefore, to control the degree of separation of the birch inner bark or quality of BOB samples, it was necessary for a novel, effective method of control analysis.

During the experiments, correlations between the content of birch inner bark admixtures in BOB and the content of the easily and hardly hydrolysable polysaccharides or the heat of combustion were observed. By preparing a pure BOB and birch inner bark mixtures in different mass ratios and by making appropriate analysis calibration straight line was created, from which after the following results was possible to determine the content of birch inner bark in the sample (Fig. 2).

A method with the determination of the sample combustion heat turned out to be more accurate and faster. Outer birch bark can be regarded as qualitatively and sufficiently purified from birch inner bark if its combustion heat is above 32-33 MJ/kg (birch inner bark content would be below 4 wt%).

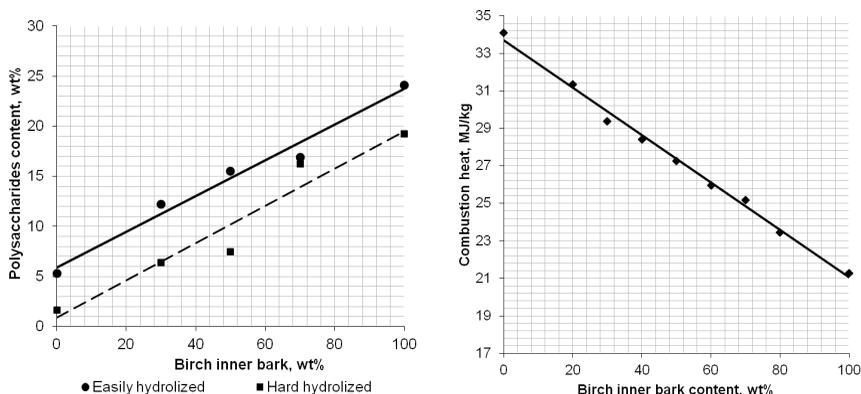


Fig. 2. Calibration straight lines for determination of the birch inner bark admixture from easily and hard hydrolysed polysaccharides and combustion heat values

The developed BOB quality control method would also be very useful for BOB extractives' production plants in order to promptly check the purity of the raw material and timely prevent the potential fluctuations of quality due to admixtures of birch inner bark in the raw material.

Preparation of BOB for extraction of triterpenoids

Taking into account the information available in the scientific literature, as the most suitable method for the separation of birch outer and inner bark in laboratory conditions, soaking or flotation in water of ground (through a 2 mm sieve) birch bark was chosen [8, 9]. During the flotation process, birch inner bark and wood particles gradually moisten and sink, but BOB particles remain floating. In order to determine the optimal duration of flotation, an experiment was carried out, in which several suspension of bark/water with the weight ratio 1/5 was formed. After a definite time, the material remaining on the surface was skimmed, dried and, by the developed BOB quality control method, the content of the birch inner bark admixture was determined. The experimental results are shown in Table 4.

Table 4

Yield of floated BOB depending on the duration

Parameters	Duration of flotation, h								
	0	1/4	1	3	5	12	24	48	120
Floating part – outer bark, wt% from bark	100	52.3	48.0	43.2	39.5	36.7	38.7	44.3	36.9
Sunken part – inner bark, wt% from bark	0	44.9	48.2	56.8	57.2	59.4	58.3	55.7	63.1
Combustion heat of the floating part, MJ/kg	26.2	29.4	30.8	31.3	32.2	32.4	32.3	33.5	32.5

Judging by the results of the experiment, in order to obtain a sufficiently pure BOB, flotation duration must be at least 5 h, in which the yield of BOB is 39.5 wt% from o.d. birch bark.

Since the ground, floated BOB has low bulk density (the mass of volume), pelletisation was carried out with the aim to increase the further extraction volume productivity per mass unit as well as the efficiency of the raw material transportation. The dried BOB (relative moisture content $w_{rel.}=3wt\%$) was pelletised on a laboratory-scale “Amandus KAHL” flat die type pelletiser with holes of diameter 6 mm. Experiments on the pelletiser showed that the BOB pelletised well,

with a yield of 98 wt% from the o.d. raw material. Visually better and mechanically more resistant pellets were obtained in the temperature range 70-90°C.

The first extraction experiment of outer birch bark pellets showed that their structure is too dense, because after 4 h mixing in heptane, on the average, 82% of pellets retained the form, which greatly hampered the process of diffusion of extractives from the volume of pellets. Therefore, a decision was made to reduce the size of pellets and to crush them through a 2 mm sieve. In the course of experiments it was also observed that the dust of BOB inhibits the diffusion of extractives from extraction area, so the particles below 0.4 mm was separated off and for further extraction experiments the fraction of particles with the size from 0.4 to 2.0 mm was used, which amounted 93.2 wt% from crushed pellets (Table 5). The remaining dust (≤ 0.4 mm) was used again for producing new pellets. Thereby, it was possible to increase the bulk density of the raw material from 263 to 500 kg/m³. Floated BOB, its pellets and the pellets fraction with a size of 0.4-2.0 mm are shown in Fig. 3. In its turn, the flow diagram of the BOB preparation process and the material balance of the process can be seen in Fig. 4.

Table 5

Distribution by fractions after crushing of BOB pellets through a 2 mm sieve

Size of fraction, mm	Distribution, wt% from o.d. raw material
> 1.00	42.1
0.80-1.00	24.1
0.63-0.80	13.8
0.40-0.63	13.2
0.20-0.40	5.9
≤ 0.20	0.9



Fig. 3. From left: floated BOB, its pellets and the fraction of pellets with the size from 0.4 to 2.0 mm

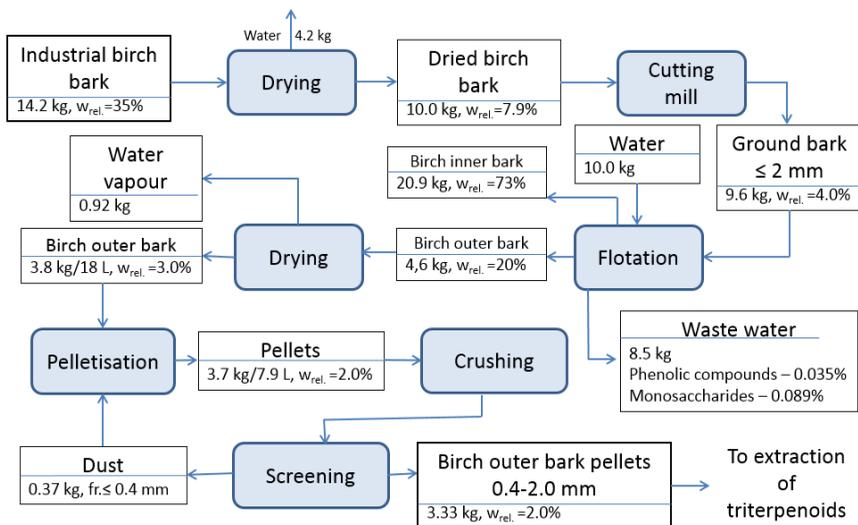


Fig. 4. Flow diagram for preparation of BOB with the material balance of process

Extraction of BOB in Soxhlet apparatus

When an appropriate raw material was prepared from industrial birch bark, extraction studies were started in a 200 ml Soxhlet apparatus using EtOH. For comparison purposes, fresh, untreated Latvian BOB was also taken, which differed by age (15 and 60 years), species (Latvian silver birch (*Betula Pendula* Roth.) and downy birch (*Betula pubescens* Ehrh.)), and sampling height (0-60, 60-130 and 130-210 cm from the roots). For the extractives isolated from the BOB samples, the yield and basic composition were determined; the results are summarised in Table 6.

It could be concluded that the compared species, age and sampling height factors for the BOB of both species affected relatively little the isolated amount of betulin. Only as the tree remained older, the content of betulin in the bark slightly decreased, which could be explained by a thicker bark and lesser need for protection from external exposure factors. However, it was revealed unexpectedly that the content of lupeol was 2-4 times higher in silver BOB. Similar content of lupeol in the extractives of industrial BOB indicated to their belonging to silver birch species as well as confirmed the wide use of silver birch in the veneer industry, described in the literature [10]. Belonging of industrial birch bark to the silver birch species was also identified using a specific indicator reagent - 2,4-dinitrophenylhydrazine, described in the literature [11]. The content of "other" compounds (monosaccharides, tannins, etc.), in the industrial BOB extractives was almost twice lower; hence, it can be concluded that, macerating birch veneer logs in hot water before debarking in the factory, a significant amount of water-soluble admixtures was removed, so veneer production residues are most suitable for the isolation of extractives with a high content of betulin than untreated birch bark [12]. During this

experiment, it was also emerged that the optimal time of extraction in a Soxhlet apparatus using polar EtOH was 11 h, with such an intensity of heating, in which the filling frequency of the siphon of the Soxhlet apparatus was 2 times/h.

Table 6

Yield and basic composition of EtOH extractives obtained from industrial and fresh BOB samples in a Soxhlet apparatus

BOB origin	Extractives	Betulin	Lupeol	Other
	wt% from o.d. BOB			
Industrial BOB	34.7 ± 1.0	25.7	2.2	6.8
Silver BOB, 60 years	36.2 ± 0.9	22.3	2.2	11.7
Silver BOB, 15 years, 0-60 cm	43.0 ± 1.1	25.6	1.9	15.5
Silver BOB, 15 years, 60-130 cm	44.7 ± 1.3	26.2	2.0	16.5
Silver BOB, 15 years, 130-210 cm	43.4 ± 0.8	27.1	2.1	14.2
Downy BOB, 60 years	36.0 ± 0.6	22.9	0.8	12.3
Downy BOB, 15 years, 0-60 cm	43.1 ± 1.2	27.7	0.6	14.8
Downy BOB, 15 years, 60-130 cm	37.1 ± 1.5	25.7	0.6	10.8
Downy BOB, 15 years, 130-210 cm	36.4 ± 0.2	23.3	0.5	12.6

Experimenting with solvents of different polarity, available in the laboratory, and as previously determining the yield of the extractives and their basic component content, an insight in the BOB components' solubility and solvents' selectivity with respect to triterpenoids was gained. The yield and basic component content of the pelletised industrial BOB extractives obtained in the Soxhlet apparatus with solvents of different polarity in the 11-h extraction are summarised in Table 7.

The results showed that, with decreasing solvent polarity, the solubility of triterpenoids and, along with it, also the total yield of extractives decreased; however, the selectivity of the solvent with respect to triterpenoids increased, as evidenced by up to 10-fold lower content of "other" admixtures in extractives of non-polar solvents, which by extraction more than 11-h maximum increased an average 2-fold (with cyclohexane the yield of extractives after 33-h extraction was 28.4% from o.d. BOB from which "other" admixtures was 1.5%).

In turn, a comparison of the yields of the industrial BOB extractives obtained in a Soxhlet apparatus with PE140, cyclohexane and EtOH, depending on the extraction time, shows that the extraction with non-polar solvents (PE140, cyclohexane) occurs 4-7 times slower (Figure 5).

Looking at the scanning electron microscope (magnification 100x and 1000x) industrial BT sample (size 1x1 cm) images of cross-section before and after extraction with EtOH in a Soxhlet apparatus can be seen with the extractive filled lumina, which judging by the information of literature is the "spring" cells (Figure 6). A characteristic feature of BOB is the formation of annual rings. The spring cells have thin walls, and in the radial direction, the cells lumina are thicker. White

betulin grains are located in these lumina. Further, in the stem periphery direction, 3-5 rows of strongly flattened cells with thick walls (autumn cells) are located, whose lumina are filled with tannins [14].

Table 7

Yield and basic component content of industrial BOB extractives obtained in a Soxhlet apparatus with solvents of different polarity in a 11-h extraction

Solvent	Extractives	Betulin	Lupeol	Other	Relative polarity [13]
	yield, wt% from o.d. BOB				
n-hexane	4.5 ± 0.4	1.5	1.8	1.2	0.009
PE140	7.8 ± 0.6	4.8	2.2	0.8	0.011*
n-heptane	4.1 ± 0.9	1.9	1.2	1.0	0.012
Cyclohexane	12.4 ± 0.5	10.2	1.5	0.7	0.006
Benzene	17.6 ± 0.8	11.1	2.0	4.5	0.111
Chloroform	33.3 ± 1.4	24.3	2.5	6.5	0.259
Dichloromethane	33.0 ± 1.0	23.3	2.2	7.5	0.309
Acetone	33.9 ± 0.9	25.0	2.0	6.9	0.355
EtOH	34.7 ± 1.0	25.7	2.2	6.8	0.654
Methanol	34.5 ± 1.2	24.3	2.4	7.8	0.762

* PE140 is a mixture of C7-C9 alkanes, for example, heptane (0.012), octane (0.012), nonane (0.009) solution; therefore, PE140 polarity is shown as an average value

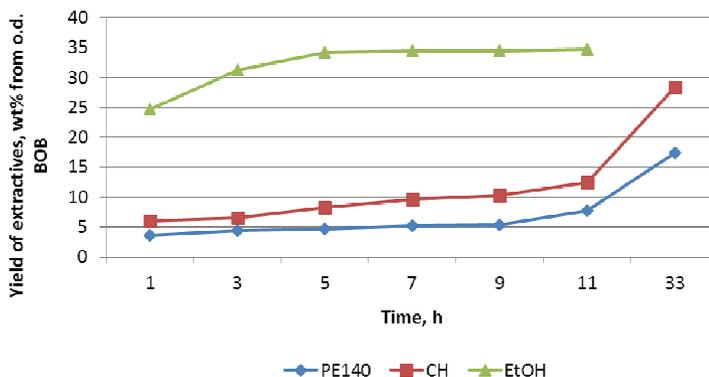


Fig. 5. Yields of BOB extractives, obtained with PE140, cyclohexane (CH) and EtOH in a Soxhlet apparatus depending on the extraction time

Experimenting with the extraction of industrial BOB, several disadvantages of the Soxhlet apparatus could be observed, which especially stand out using non-polar solvents. The observed problems and their simple, effective solutions, which were taken into account in the development and assembling of the novel extraction unit with higher productivity, are listed in Table 8.

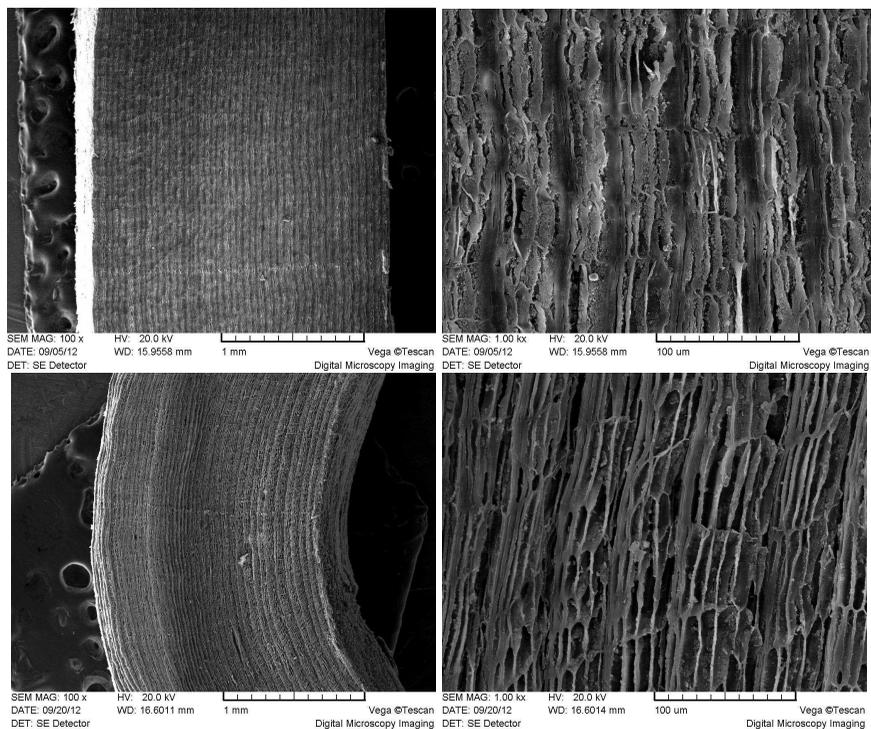


Fig. 6. Cross-section of industrial BOB samples before (top) and after (bottom) extraction with EtOH. Images are obtained with a scanning electron microscope at 100x and 1000x magnification at the Latvian State Institute of Wood Chemistry

Table 8

Technological solutions to Soxhlet apparatus' disadvantages for isolation of triterpenoids with non-polar solvents

Problem	Solution
Low temperature of the solvent in the siphon of the Soxhlet apparatus	Extraction is carried out at a temperature, which is close to the solvent's boiling
Slow diffusion of extractives from the BOB particles	Extraction is carried out in a turbulent regime or intensively stirring the BOB - solvent suspension
Slow mass exchange process – long waiting for the moment of siphon filling	The maximal addition of the fresh solvent and the rate of removal of the betulin concentrate must be ensured

Development and investigation of a novel extraction method and an original intensive mass exchange extraction unit

Solving the above mentioned problems with the possible technical improvements and ideas, under the guidance of Janis Zandersons, leading researcher of the Technological Research Department of the Latvian State Institute of Wood Chemistry, an experimental laboratory scale intensive extraction unit was developed, which, during experimenting, was scaled-up several times and whose front and side overall view is shown in Figure 7. However, the methods for extraction intensification as heating and intensive stirring, mentioned in Table 5, are not new; therefore, in an attempt to improve technically extraction process, attention was paid in the present work to the fact that, as a result of the difference in temperature, triterpenoids rapidly crystallised from saturated solution of non-polar cyclohexane and PE140 in large quantities. So, an idea emerged to place the cooled flask in the flowpath of concentrated extractives' solution, and so called intermediate crystalliser was invented. In this way, in my opinion, it was possible to speed up the triterpenoids isolation process, because it usually requires considerable time and labour, while the extractives are precipitated after the cooling or evaporation of the solution. The flow diagram of the original extraction pilot unit, and the technological solution of novel intermediate crystallisation method involved therein are shown in Figure 8.

The pilot unit consisted of three main stages - 1) a stainless steel extraction reactor with a geometric volume of 3.49 liters (usable volume 2.65 liters), manufactured from drawings; 2) a glass evaporator with a capacity of 3.5 liters; 3) an intermediate crystalliser, which consisted of a drain glass tube welded to a 0.5 liter Erlenmeyer conical flask, with a special glass pipes system placed therein, which were custom-made thanks to the glass master of the Latvian State Institute of Wood Chemistry (Figure 9). During extraction, the intermediate crystalliser was cooled in water with sodium chloride and ice.

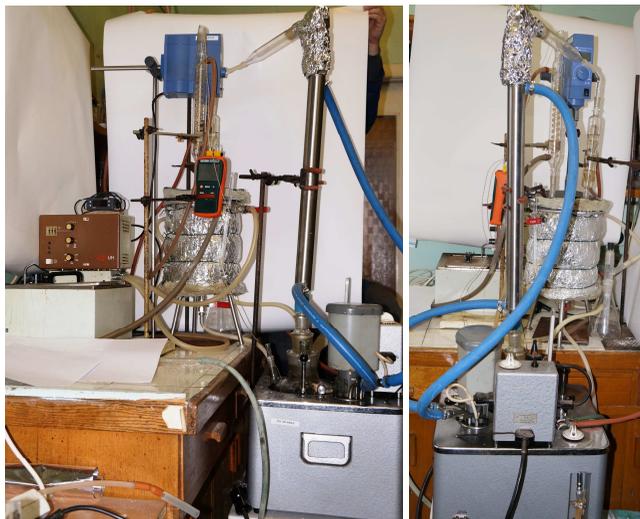


Fig. 7. Front and side overall view of the original intensive mass exchange extraction unit

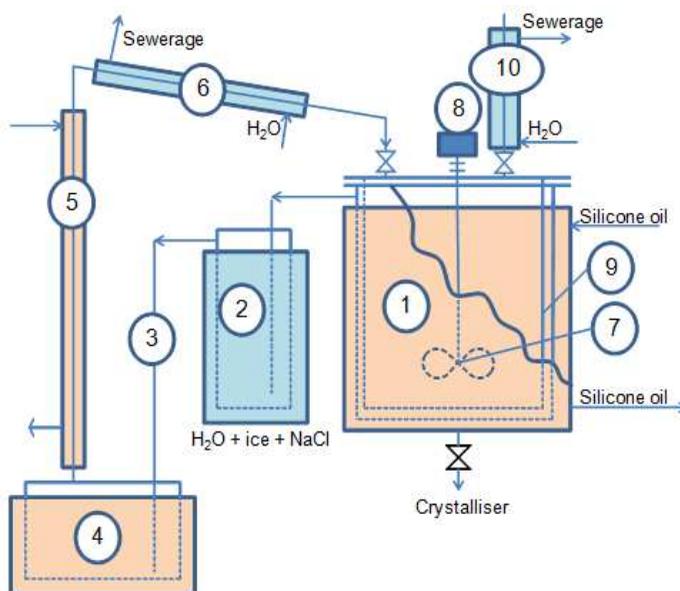


Fig. 8. Intensive mass exchange extraction unit: 1 – extractor with a heating jacket; 2 – intermediate crystalliser with a cooling jacket; 3 – tube for drain of mother liquor; 4 – evaporator; 5 – vapour exhaust pipe with a heating jacket; 6 – descent condenser; 7 – mixer; 8 – electromotor of the mixer; 9 – wire net basket; 10 – reflux cooler



Fig. 9. Intermediate crystalliser with a special glass pipes system and the second intermediate crystalliser flask for replacement during extraction

Often, due to a great amount of accumulated crystals in the intermediate crystallisation process, there were problems of the flow of the solution and the crystal losses from the intermediate crystalliser; therefore, it was decided to set up a second similar crystalliser flask for replacement. When the first crystalliser flask was full with crystals, it was quickly replaced with another one during the extraction process, and the precipitated crystals were filtered, but the separated mother liquor was returned in the cycle through the second crystalliser.

Experiments in the original extraction unit were started with the search for the most effective solvents for the intermediate crystallisation method (Table 9). The first tests of intensive extraction showed good results - the extraction intensity with non-polar solvents, compared to the case of a Soxhlet apparatus, improved an average for 4-5 times. As the best solvents with the highest average efficiency of intermediate crystalliser, the cyclohexane and petroleum ether fraction with a boiling range of 100-140°C (PE140) stood out, indicating the highest average crystallisation efficiency. In the extraction process with cyclohexane, up to 46 wt% was separated, but with PE140 – even up to 59% from the total yield of extractives. In this case, the betulin content in the crystalliser extractives from cyclohexane was 90.9 wt% and from PE140 - 91.6 wt%, which indicated its high purity. In the extraction process with polar solvents (EtOH and acetone) an intermediate crystalliser was ineffective, because the solubility of triterpenoids even at low temperatures remained high.

In general, non-polar solvents are more suitable for isolation of extractives with a high content of betulin due to the high selectivity, small losses of betulin in the mother liquor, undiluted with the moisture of the raw material, and the formation of betulin crystals with advantageous structure and properties for technological and chemical processes [9].

Table 9

Yields of industrial BOB* extractives from the intensive mass exchange extraction unit (IMEEU) and the intermediate crystalliser (IC) attached to it, compared with the Soxhlet apparatus, depending on the solvent and time of extraction

Solvent	Boiling temp., °C	Yield of extractives, wt% from BOB				Soxhlet app. 11 h	Average efficiency of IC, wt% from the total yield
		IMEEU					
		1.5 h		3 h			
		IC	Total	IC	Total		
Acetone	56.5	-	26.3	-	32.8	33.9	-
EtOH	78.4	-	27.3	-	33.3	34.7	-
n-hexane	68.7	0.3	3.1	0.9	6.9	4.5	10-13
Cyclohexane	80.7	1.6	6.7	6.1	13.2	8.3	24-46
n-heptane	98.4	1.0	2.8	1.6	6.5	4.1	25-36
Petroleum ether	80-110	0.6	2.1	1.8	5.2	3.8	29-35
Petroleum ether	100-140	2.2	4.6	6.2	10.6	7.8	48-59

* Fraction of floated, pelletised BOB with a size of 0.4-2.0 mm, $w_{\text{rel.}} = 3.0$ wt%

Exploring in the “Across” internet catalog the prices of the solvents used for extraction and others, it could be concluded that PE140 is the cheapest (Figure 10). Developing cost-effective extraction method was taken into account the important factor of solvent price, so it was decided to use only PE140 in the following intermediate crystallisation and other research in an intensive extraction unit.

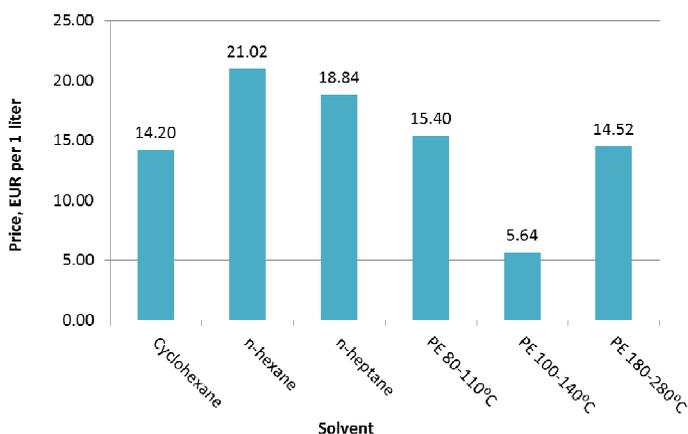


Fig. 10. Comparison of the solvent prices from the “Across” catalog per 1 L (the price converted from the price of 25 L of solvent (information obtained 02.04.2015))

Experiments of extraction process were started with the searches of optimal mass ratio of the solvent and BOB, where the sample weight was changed and loaded in the pilot unit extractor so that, during a 7 h extraction process, the maximum amount of extractives would be isolated. Experimental results are summarised in Figure 11.

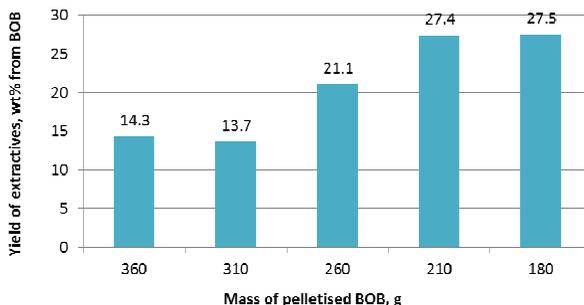


Fig. 11. Yields of extractives after the 7 h extraction process depending on the masses of the used BOB

Judging by the results, beginning with the 210 g BOB mass, in the 7 h extraction, it was possible to achieve almost the whole isolation of the triterpenoids present in BOB. 210 g of industrial BOB (fraction from 0.4 to 2.0 mm, $w_{rel.} \sim 3$ wt%) at the average bulk density 500 g/L takes ~ 0.41 liters, which is an average of 15.5% from the volume of the extractor wire net basket (2.65 liters). According to the calculations it can be concluded, that the optimal mass ratio of BOB pellets and PE140 (density - 740 kg/m³) in the extractor is 1:8.

Then, the solubility of extractives and saturation time of the solution in PE140 was studied. The concentration of extractives in PE140 at the boiling point already after 1 min was 6.3 g/L, which was related to the external diffusion from the surfaces of the particles, and the following increase of concentration was, on the average, 8 times slower, which in turn was related to the internal diffusion from the volume of particles until, already after 10 min, the equilibrium concentration or saturation - 13.9 g/L - was achieved (Figure 12). As the saturated solution was cooled to room temperature (20°C), 81.5 wt% precipitated from the total amount of extractives, and the remaining concentration of extractives in mother liquor at room temperature was 2.7 g/L.

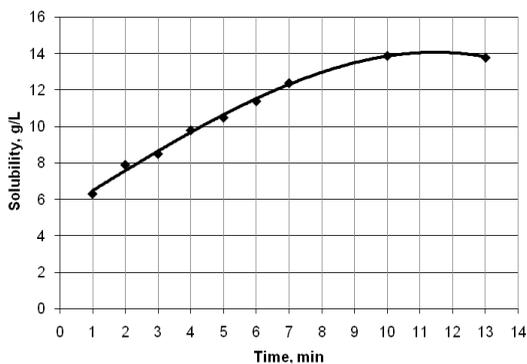


Fig. 12. Equilibration of BOB extractives' solubility at the boiling point of PE140 depending on time

The main indicator characterising the intensive extraction unit in the optimal extraction conditions are the yield and basic composition of extractives from three basic stages of the intensive extraction unit – from the intermediate crystalliser, the evaporator and the extractor.

Using 210 g ($w_{rel.}=3wt\%$) of industrial BOB and PE140, at the flow rate of the fresh solvent 2 L/h, in the 7 h extraction process and in 3 replicates, fractions of extractives' crystals was obtained from separate stages of the unit. During the extraction process, crystals were isolated from the intermediate crystalliser, but after extraction – from the evaporator and the extractor.

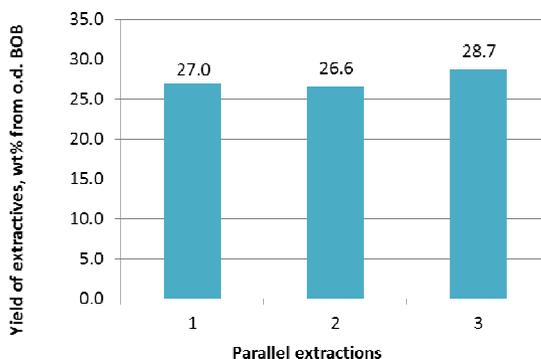


Fig. 13. Total amount of extractives from the intensive extraction unit in 3 equal replicates

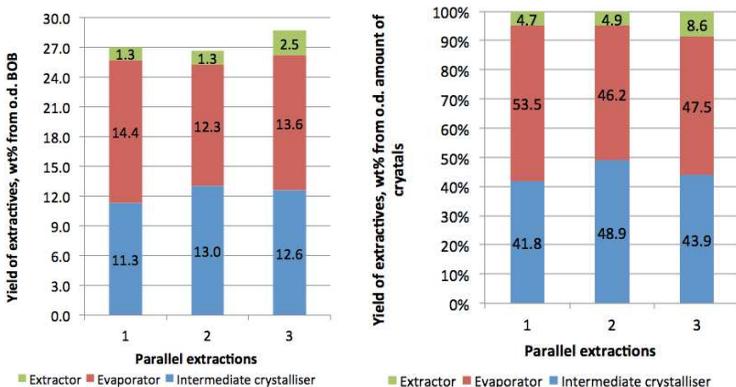


Fig. 14. Yield of extractives from three basic stages of the intensive extraction unit in 3 equal replicates

On the average, a total of 27.4 ± 1.1 wt% of extractives, calculated from o.d. BOB or 56.3 ± 2.3 g extractives were obtained in the intensive extraction unit (Figure 13).

In turn, separately investigating the yield of extractives from each stage of the extraction unit, it turned out that, using an intermediate crystalliser, it was possible to separate an average of 44.9 ± 3.6 wt% of extractives from the total amount of extractives; 49.1 ± 3.9 wt% accumulated in the evaporator, but in the extractor, a small amount of extractives remained at the end of the process, which accounted for 6.1 ± 2.2 wt% (Figure 14).

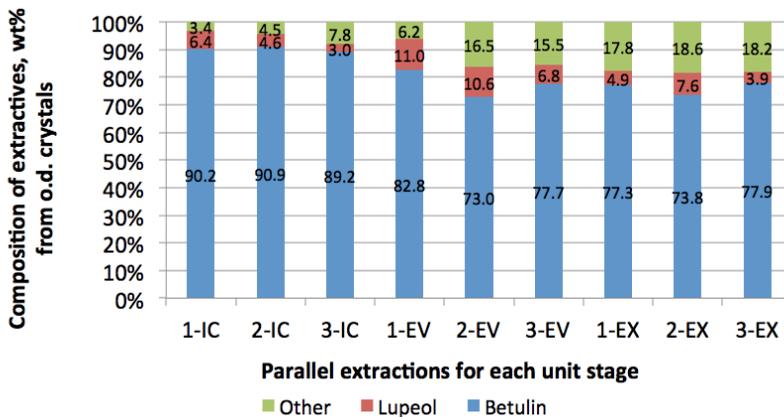


Fig. 15. Basic composition of extractives from three basic stages (intermediate crystalliser – IC, evaporator – EV and extractor – EX) of the intensive extraction unit in 3 equal replicates

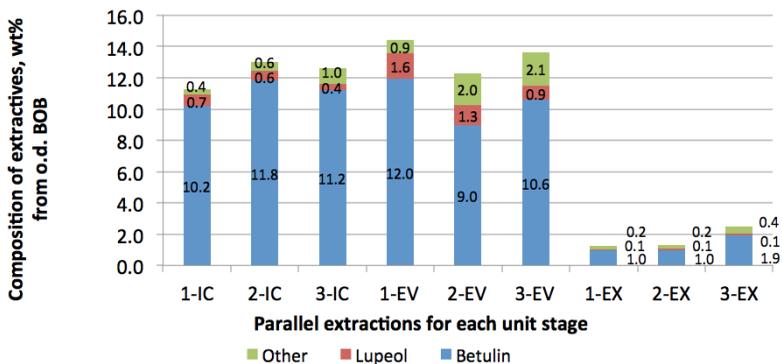


Fig. 16. Basic composition of extractives from three basic stages (intermediate crystalliser – IC, evaporator – EV and extractor – EX) of the intensive extraction unit in 3 equal replicates

With the intermediate crystalliser, crystals were separated, which consisted of 90.1 ± 0.9 wt% of betulin and 4.7 ± 1.7 wt% of lupeol. In the evaporator, crystals accumulated, which after evaporating of the extract, consisted of 77.8 ± 4.9 wt% of betulin and 9.5 ± 2.3 wt% of lupeol. In turn, a solution of extractives remained in the extractor, which consisted crystals with 76.3 ± 2.2 wt% of betulin and 5.5 ± 1.9 wt% of lupeol (Figures 15 and 16).

As a whole, in the 7 h extraction process, it was necessary to replace the intermediate crystalliser only in the first 3 h, because, with these crystal portions, the greatest part was isolated, or 80 wt% from the total extractives isolated in the intermediate crystalliser for 7 h. If the total amount of extractives was, on the average, 13.0 wt% from o.d. BOB, then in the first hour, 2.7 wt% of extractives was isolated, in the second - 4.4 wt%, in the third - 3.3 wt%, but in the next 4 h only 2.7 wt%.

In order to supplement the already existing results and to follow the changes in extractives' components during the extraction process of BOB more detailed, the partially extracted BOB samples together with the hot solvent from the pilot extraction unit each h was sucked out by vacuum pump through a reflux cooler using a specially developed sampling method. Then, the dried samples were extracted for 11-h with EtOH in the Soxhlet apparatus. From the yield of extractives and its composition, compared to untreated BOB (control), content of extractives components in the extraction process depending on process time were calculated. Experimental results are summarised in Figure 17.

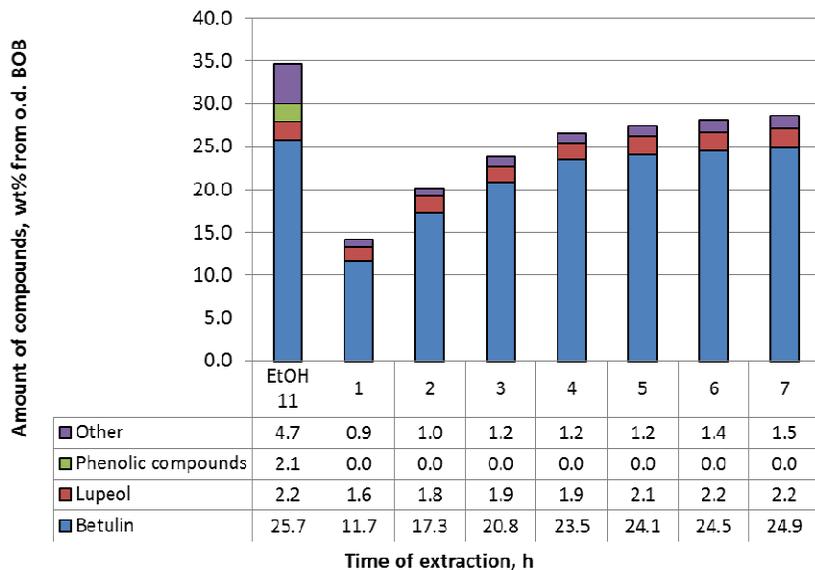


Fig. 17. Changes of the BOB extractives' components using PE140 and compared with the basic components content of untreated BOB EtOH extractives (EtOH 11)

Judging by the results, the 4 h extraction of BOB in unit extractor is sufficient in order to isolate almost the whole amount of triterpenoids.

Attempts of betulin purification and feasibility of its manufacturing in Latvia

As the aim of the study was to obtain betulin with a high purity, then, limited only by the mentioned extraction in a pilot unit, this aim would be attained partly, because during 7-h extraction 49 wt% from the total amount of the isolated extractives accumulated in the evaporator, with only 78 wt% of betulin content. Therefore, an attempt was made to make classic, simple purification operations with crystals from the evaporator. At first crystals were filtered from the mother liquor and washed with a small amount of pure PE140, thereby increasing the content of betulin up to 86.1 wt%. Whereas by evaporating of the separated mother liquor, crystals with an average of 30-40 wt% content of lupeol were obtained, so called "concentrate of lupeol", which was used for the isolation of individual lupeol.

As the crystals filtered from the evaporator contained 10.6 wt% of lupeol, it was decided to recrystallise those crystals in PE140 with the aim to simultaneously purify betulin and to obtain an additional concentrate of lupeol. In this way, it was possible to increase the purity of betulin to 94.3 wt%. Then, the obtained crystals were additionally recrystallised with isopropanol, recommended in the literature, in order to obtain betulin with analytical purity [9]. In cooperation with colleagues of the Latvian Institute of Organic Synthesis, for the obtained crystals after

purification, using 3 different instrumental methods of analysis, the content of betulin was determined, in which the purity varied from 96.6 even up to 102.0 wt% (HPLC – 96.6%, GC/MS – 98.5%, ¹H KMR – 102.0%).

In Latvia, there is a great potential for processing of birch bark, because, judging by the data published in the website of the Latvian Ministry of Agriculture in 2014, the birch plywood export accounted for 240,000 m³. To produce it, such a great amount of bark waste was produced, from which an average of 2,152 tons of betulin and 215 t of lupeol could be obtained [15].

Taking into account the great prospects for birch bark processing in Latvia, using the experimental experience and operating principles of the developed intensive mass exchange extraction pilot unit, a technological proposal has been designed for industrial production of betulin and lupeol using reactors with a volume of 1 m³, in which an average of 90 kg of o.d. ground BOB pellets could be processed in a single pass. The flow diagram of the technological proposal and the description of the manufacturing process are available in the thesis.

Experimentally searching for the rational options for using betulin and other residues of the birch bark processing, it was concluded that:

- betulin can be used as a functional additive in cosmetic creams, simultaneously acting as an emulsifier, an antioxidant and a preservative;
- the lupeol concentrate can be used for isolation of individual lupeol by column chromatography (the study was carried out in cooperation with the Latvian Institute of Organic Synthesis);
- betulin and lupeol can be used for synthesis of betulinic acid and hybrid compounds (the study was carried out in cooperation with the Latvian Institute of Organic Synthesis);
- BOB, extracted with PE140 (in addition, extracted with EtOH), can be used for obtaining antioxidants, and for the production of suberinic acid with a high content of 9,10-epoxy-18-hydroxydodecanoic acid (42-46 wt% from suberinic acids), interesting for organic synthesis, or esterifying with triethanolamine, for preparing polyols, from which rigid polyurethane foams with good closed pores and mechanical characteristics can be obtained;
- extracted BOB can be used as an ecological binder of wood sawdust, which, pressing at 160-200°C, forms a solid, hydrophobic mass with good mechanical properties;
- due to the high content of xylose, birch inner bark can be used as a promising raw material for production of furfural (“platform chemical”) through catalytic hydrothermal pre-treatment; and in a liquefied form (using polyethylene glycol, glycerol and sulfuric acid) as a raw material for making of polyols, which can also be used for the production of rigid polyurethane foams.
- the betulin production residue - extracted BOB - is a good fuel (calorific value - from 27.2 to 28.9 MJ/kg), which is considerably superior to birch wood in terms of combustion heat (calorific value - from 19.3 to 19.7 MJ/kg).

CONCLUSIONS

- 1) Determination of the birch inner bark admixture in BOB from the combustion heat of the sample is a new and prompt method for quality control, in which as high-quality and purified enough outer bark (content of inner bark below 4 wt%) can be considered a sample, the combustion heat of which is above 32-33 MJ/kg;
- 2) In order to obtain a sufficiently pure BOB the duration of ground (≤ 2 mm) birch bark flotation in water is at least 5 h, in which the yield of bark is 39.5 wt% from o.d. bark;
- 3) The crushed pellets' fraction of BOB with the particle size from 0.4 to 2.0 mm is most suitable for extraction studies, which yield after the crushed pellets's screening accounted for 93.2 wt% from the crushed pellets, and bulk density was almost twice higher (500 kg/m^3) than that for raw BOB (263 kg/m^3);
- 4) The optimal extraction time of the pelletised BOB fraction (0.4-2.0 mm) in a Soxhlet apparatus using polar EtOH was 11 h at such an intensity of heating, at which the filling frequency of the Soxhlet apparatus' siphon was 2 times/h;
- 5) The compared species, age and sampling height factors affected relatively little the amount of isolated betulin, but the lupeol content in silver BOB was 2-4 times higher than that in downy BOB, which also, as the specific indicator reagent (2,4-dinitrophenylhydrazine), indicated to the industrial birch bark belonging to the silver birch species;
- 6) With decreasing solvent polarity, solubility of triterpenoids decreases; along with it, also the total yield of extractives decreases, but the selectivity of the solvent relative to triterpenoids increases, as evidenced by up to 5 times lower content of "other" admixtures in non-polar solvent's extractives;
- 7) In the novel intensive mass exchange extraction unit, compared to the Soxhlet apparatus, the intensity of extraction using non-polar hydrocarbons is improved 4-5 times;
- 8) As the best solvents using an intermediate crystalliser, cyclohexane (separated till 46 wt% from the total yield of extractives) and PE140 (59 wt% from the total yield of extractives) stood out;
- 9) PE140 was the most appropriate solvent for the investigation of the intermediate crystallisation method due to the relatively low retail price (5.64 EUR per liter), the high selectivity, and high changes of triterpenoids' solubility with temperature;
- 10) The optimal mass ratio of the BOB pellets' fraction (0.4 to 2.0 mm) and PE140 in the extractor of the pilot unit is 1:8;

- 11) The concentration of extractives in PE140 at the boiling point (104°C) already after 1 min of extraction was 6.3 g/L but after 10 min, an equilibrium concentration or saturation - 13.9 g/L was achieved;
- 12) In the 7 h extraction at the optimal flow rate of fresh PE140 2 L/h, 27.4 wt% of extractives can be obtained, which contain almost all betulin (22.9%) and lupeol (2.0%) of BOB;
- 13) With an intermediate crystalliser, 44.9 wt% (betulin - 90.1 wt%) can be separated from the total amount of extractives, 49.1 wt% (betulin - 77.8 wt%) come into the evaporator, but at the end of the process, 6.1 wt% (betulin - 76.3 wt%) of extractives remain in the extractor after the extraction process;
- 14) In an intensive extraction pilot unit, in optimal conditions, the 4 h extraction of BOB is sufficient in order to isolate almost the whole amount of triterpenoids;
- 15) After extraction in a pilot unit, the betulin crystals isolated by filtration of the mother liquor from the evaporator, containing a high amount (10.6 wt%) of lupeol, can be used for recrystallisation from PE140 to purify betulin (from 86.1 to 94.3 wt%) and to obtain a lupeol concentrate (~30-40 wt%);
- 16) Performing additional recrystallisation in isopropanol of the betulin crystals purified in PE140, analytically pure betulin (96.6-102.0 wt%) can be obtained;
- 17) Betulin, obtained in extraction experiments, can be used as functional additives in cosmetic creams and for organic synthesis; lupeol concentrate – for isolation of individual lupeol; extracted BOB – for preparation of the polyurethane raw material (polyols), for obtaining of suberinic acids with a high content of 9,10-epoxy-18-hydroxydodecanoic acid and as a raw material for obtaining a binder of wood sawdust; birch inner bark - as a promising raw material for obtaining of furfural (“platform chemical”).

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