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SPECTROSCOPIC CHARACTERISATION OF CHAGA (*Inonotus obliquus*) PREPARATIONS: A CONTRIBUTION OF PHENOLICS AND POLYSACCHARIDES

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Abstract

Chaga is wood-decay fungus (*Inonotus obliquus*) widely used in traditional medicine due to high amount of biologically active compounds including phenolics and polysaccharides. The aim of this study was spectroscopic analysis of food supplements containing chaga, evaluation of their quality and composition based on characteristic bands and multivariate analysis. The topological parts of the chaga sclerotium were also analysed.

Introduction

Chaga (*Inonotus obliquus*) is wood-decay fungus that causes white rot of birch wood. It first produces a white decay of the wood in living trees, which is bounded by a red-brown stripe to healthy wood. After the death of the tree, the wood is discoloured, softened, broken and cracked¹. Chaga needs to be harvested only from live or freshly cut birch trees. Chaga growing on dry and dead trees has a significantly lower content of bioactive substances². Traditionally chaga has been used in Russia to treat gastrointestinal disorders, cardiovascular diseases, diabetes. Chaga helps to alleviate the symptoms of cancer. For example, you can use chaga to prepare tea. This tea increases overall stamina, relieves pain and is used to treat heart, stomach and liver diseases². The most common chaga is sold as a 20–30 % concentrated aqueous extract, which is obtained from the crushed fruiting body and the tumour parts of the birch on which it grows. These product can treat some stomach ulcers, purulent wounds and swelling³. Like other wood-decay fungi, chaga produces many secondary metabolites with biological effects⁴.

Experimental

Materials

Specification of the chaga preparations are shown in Table I. Ten food supplements containing chaga residues, i.e. eight water extracts and two pulps (milled sclerotia), as assigned by sellers, were obtained from trading companies. Wild growing sclerotium of chaga was obtained from Siberia forests, three different part were used for analyses: an outside part, an inside part and a part proximal to tree.

Table I
Specification of the chaga preparations

Title	Description	Origin
1	Water extracts	Russia
2		Czech Republic
3		Finland
4–6		Russia
7		Czech Republic
8		China
9	Pulp	Germany
10		Germany
11	Sclerotium	Outer part
12		Inner part
13		Proximal to tree

Spectroscopic methods

FT-IR ATR (4000–650 cm⁻¹) and FT-NIR (10 000–4000 cm⁻¹) spectra were recorded on Nicolet 6700 spectrophotometer (Thermo Scientific, USA) using appropriate beam-splitters, smart holders and Omnic 8.0 (Thermo Scientific, USA) software.

Diffuse reflectance UV-VIS spectra were measured on a two-beam UV-VIS spectrophotometer UV4 (UNICAM, Great Britain) using Vision 3.0 software.

Results and discussion

FT-IR ATR analysis

The FT-IR spectra of food supplements 1–10 and sclerotium 11–13 are shown in Fig. 1. The spectral differences reflect specificities of the complex pigment according to the number of characteristic groups. A broad band centred at 3405 cm^{-1} was found in all the spectra. This band was assigned to OH stretching vibration in water, phenolic and alcoholic hydroxyls. Weak bands near 2927 and 2854 cm^{-1} arose from antisymmetric and symmetric vibrations of CH_2 groups. The other CH stretching vibrations are located in the region of $2800\text{--}3000\text{ cm}^{-1}$. A very strong band near 1595 cm^{-1} was assigned to C=C stretching in aromatic compounds. This band was absent only for sample 8. Several shoulders at higher wavenumbers corresponded to C=O stretching in ketones or carboxylic groups and their derivatives (amides, esters etc.). The scissoring vibration of CH_2 groups was found as a shoulder near 1460 cm^{-1} overlapped by another band at 1452 cm^{-1} . The latter band has a contribution from antisymmetric C-H bending in OCH_3 groups. Several strong to medium bands at 1309 , 768 and 526 cm^{-1} were found in most of the spectra except samples 3 and 8. The two latter bands were assigned to all-in-phase out-of-plane =CH bending and out-of-plane deformation of aromatic ring; the former band was assigned to COC vibration of aromatic esters. The bands at 1124 and 1227 cm^{-1} were assigned to COC stretching in saturated ethers and esters, respectively. Two bands at 1038 and 1080 cm^{-1} arose from CO and CC stretching vibrations in polyphenols. The spectra of samples 6, 9 and 10 showed several intensive overlapping bands in the $950\text{--}1200\text{ cm}^{-1}$ region, mainly CO and CC stretching vibrations in pyranoid rings, demonstrating the presence of polysaccharides as the main component of insoluble sclerotium pulp. Sample 6 should thus obtain an insoluble part containing polysaccharide together with the extract. By contrast to the other samples, the spectra of samples 3 and 8 demonstrated very similar profiles in the region of $1200\text{--}500\text{ cm}^{-1}$ characteristic for starch. These two samples are not chaga extract but mixtures of starch with pigments. Therefore, significant difference in characteristic IR bands confirmed that these two preparations were falsified by producers and unjustly labelled as chaga extracts. The FT-IR spectra of different parts of chaga sclerotium (Fig. 1 C) showed marked differences in the regions characteristic for vibrations of aromatics and carbohydrates. The spectrum of outer black part 11 has the most intense band at 1593 cm^{-1} (C=C stretching) but the weakest one at 1078 and 1046 cm^{-1} (CO and CC stretching). By contrast, the bands of yellow inner part 12 at 1595 cm^{-1} was less intense, and those at 1077 and 1039 cm^{-1} were more pronounced than the corresponding bands of 11. The FT-IR spectrum of part proximal to tree 13 showed the most intense bands of polysaccharides at 1077 and 1039 cm^{-1} , but the region of C=C stretching vibrations in aromatics ($1800\text{--}1500\text{ cm}^{-1}$) is overlapped by two IR bands at 1633 and 1558 cm^{-1} that may have contribution from amide vibrations in proteins.

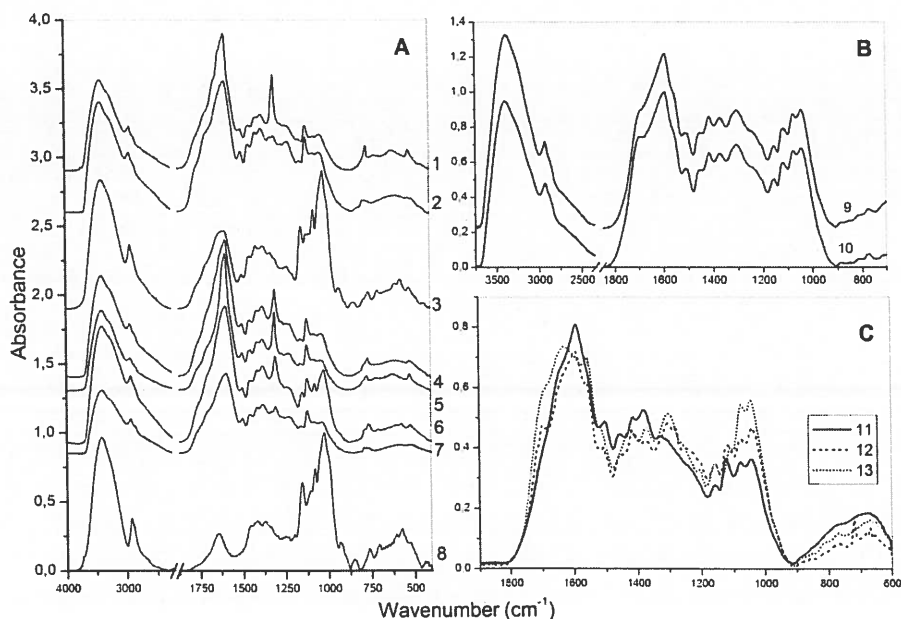


Fig. 1. FT-IR ATR spectra of chaga extracts (A), pulps (B) and sclerotium (C)

Diffuse reflectance FT-NIR analysis

The normalised FT-NIR spectra of food supplements 1–10 and sclerotium 11–13 are shown in Fig. 2A. These spectra were used for multivariate analyses to discriminate the samples according to their colour and composition. Resulting HCA dendrogram and PCA component score graph PC1 versus PC2 are shown in Fig. 2B and 2C, respectively. The main clusters in HCA dendrogram (Fig. 2B) were assigned to the most of extracts (samples 1, 2, 4, 6 and 7), pulps and sclerotia (samples 3, 8, 9, 10, 12 and 13), starch (sample 8) and extremely coloured samples 5 (extract) and 11 (outer part of sclerotium). The PCA score graph (Fig. 2C) showed similar clusters of extracts, pulps and sclerotium. Samples 5 (extremely coloured extract), 6 (pulp + extract) and 8 (starch) were located out of the main clusters. Sample 11 (outer part of sclerotium) is located in proximity to sample 5; both are highly coloured. Starch containing sample 3 is situated between samples 8, 9 and 10.

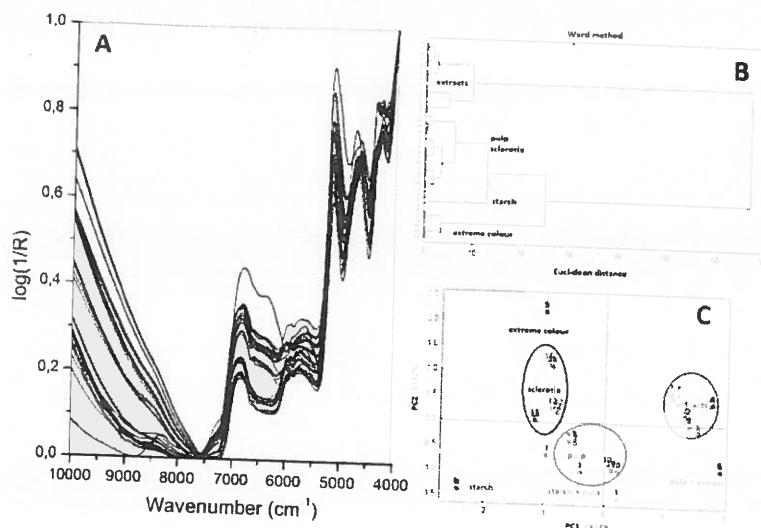


Fig. 2. FT-NIR spectra of chaga extracts, pulps and sclerotium (A) and resulting graphs of multivariate statistical analyses of NIR data: HCA dendrogram (B) and PCA score graph PC1 versus PC2 (C)

Diffuse reflectance VIS spectroscopic analysis

The diffuse reflectance Vis spectra of food supplements 1–10 and chaga sclerotium 11–13 are shown in Fig. 3. The spectra showed broad bands characteristic for unsaturated and aromatic brown coloured pigments typical for chaga sclerotium. It is evident that the spectra of samples containing starch (3, 8) or pulp polysaccharides (6, 9 and 10) showed weaker bands above 600 nm. The Vis spectra of sclerotium also showed significant differences: only the spectrum of highly pigmented outer part have strong bands at 600–750 nm.

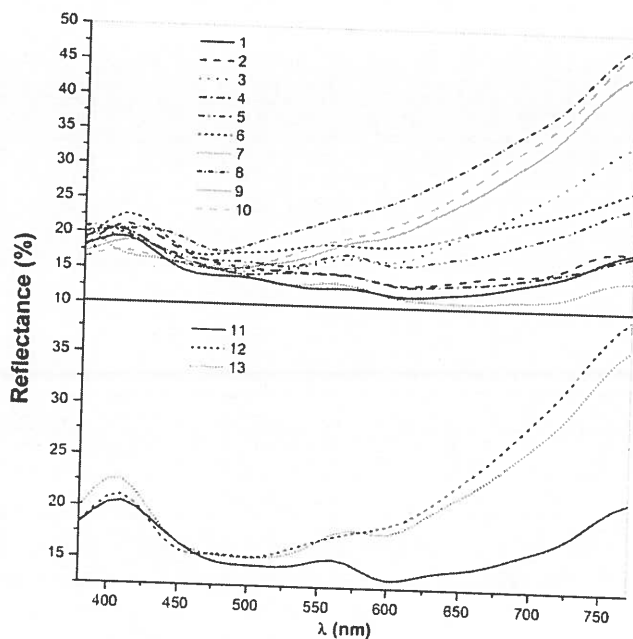


Fig. 3. Diffuse reflectance Vis spectra of food supplements 1-10 (top) and chaga sclerotium 11-13 (bottom)

L a* b* colour model*

The colour model of samples 1–13 is shown in Fig. 4. The samples containing starch (3, 8) or pulp polysaccharides (6, 9 and 10) are characterised by higher L^* and positive a^* and b^* values, so the inner and proximal to tree parts of sclerotium (12, 13). By contrast, most of pure extracts and outer part of sclerotium 11 have lower L^* and negative a^* and b^* . All the samples demonstrated strong correlation between the chromatic parameters probably because of one type of pigment predominating in all samples at different concentrations.

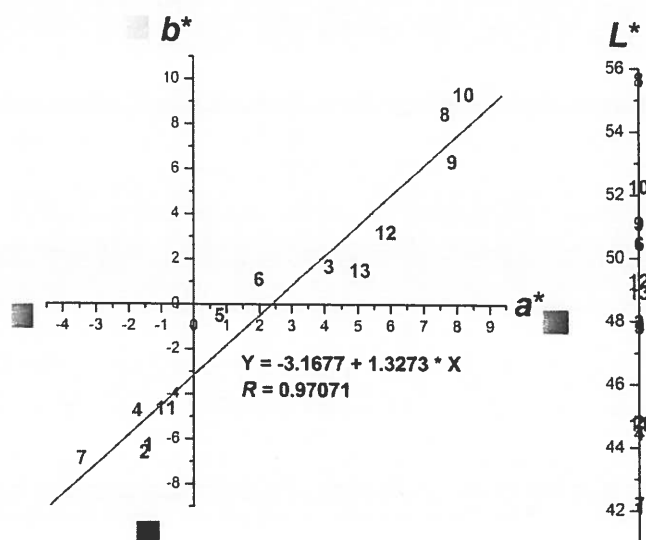


Fig. 4. CIE L*a*b* colour diagram for samples 1–13

Conclusions

Spectroscopic analyses of food supplements proved that (i) samples 1, 2, 4, 5 and 7 are clean aqueous extract from chaga sclerotium, (ii) samples 9 and 10 are milled sclerotia (pulp) of the same source, and (iii) sample 6 may contain both extract and solids from chaga. Samples 3 and 8 contained undeclared starch and thus are falsified by producers. Diffuse reflectance Vis spectra and CIE L*a*b* parameters confirmed that one type of pigments predominated in all the samples, but its concentration highly varied. Samples containing starch and/or pulp polysaccharides demonstrated high lightness and positive a* and b*.

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FORMULAS BASED ON THERMOPLASTIC STARCH WITH APPLICATION OF MONOGLYCERIDES FOR BIODEGRADABLE POLYMERIC PRODUCTS

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Abstract

The aim of research is to expand the range of modifying components for biodegradable polymeric products based on polyethylene with monoglycerides as a softener. We investigated the influence of the ratio of components added to polymeric formulas with monoglycerides and their physico-mechanical characteristics with the use of ultrasound. It was found that it was possible to replace 100 % of sorbitol alcohol in thermoplastic starch by monoglycerides and decrease glycerin amount by 16.7–33 %, in comparison with a ratio already known.

Keywords: biodegradable polymers, compositions, thermoplastic starch, monoglycerides, physico-mechanical properties

Introduction

Due to the increase in production of polymeric materials, the environmental problems connected with a rational use of natural resources and the questions about the use of plastics as waste products are considered important, sharp and relevant in our current world. The problem has economic and ecological aspects, as it is interdependent on the increasing need for environmental protection and reduction in costs for polymeric products utilization. Earlier we did the research on the development of biodegradable hybrid compositions (BHC) and film products on their basis with the use of thermoplastic starch (TPS) and polyethylene of low density (PELD)¹⁻³. BHC filled with starch with assimilated microorganisms and polymeric matrixes showed a reduced decomposition time in bio humus in the environment. The improvement of physico-mechanical characteristics of BHC and film products was achieved by application of ultrasonic during their manufacture when using glycerin and sorbitol as softeners⁴. The presented research is aimed at the expansion of the range of modifying components for biodegradable polymeric products by using ultrasound. Products of the lipid nature, such as monoglycerides, which act as softeners of a polymeric matrix instead of sorbitol, were used.

Materials and methods

Following chemicals were used: corn starch used as a raw material in accordance with State standard of Russian Federation distilled glycerin the PK-94 brand (GOST 6824-96); crystal sorbitol by regulatory documentation; polyethylene of low density (high pressure) of brand 11503-070 and monoglyceride distilled (MGD)⁵ 10-1197-95. Raw materials and subsidiary products met the requirements of Technical Regulations of the Customs Union 021/2011 (Ref.⁶).

BHC was produced on the base of TPS. For this purpose TPS granules were mixed with polyethylene at a ratio 30:70 (control option). TPS granules were added into a receiving bin of the extruder brand KED 03-088 (Arsenal Ltd, Russia) with following temperature distribution zones: 1 – 110 °C, 2 – 150 °C, 3 and 4 – 165 – 190 °C, the head – 17 °C. A screw rotation frequency was up to 100 rotation per minute and that resulted in BHC plaits. Initial TPS and BHC prepared on its basis were granulated with a crushing assembly to the average size of about 2 mm. Film samples from polyethylene-starch formulas were obtained by a flat-crack extrusion in a laboratory apparatus with a barrier screw. The film was fed to a metal shaft of the receiving device and the side of films which was not in a contact with the cooling shaft was investigated. For the ultrasound processing of BHC, the attachment (Mashplast Ltd, Russia) was used generating a power of 1.5 kW. The ultrasonic attachment was put between the third and the fourth zone of the extruder, between the cylinder and the flat-slot head. The wave guide of the ultrasonic attachment was immersed directly into a melt of BHC. Ready sample of films were gained in the receiving device with rotating shafts. A film sample had the width of 25 mm, the thickness of 216–246 μm, and the length was 50 mm.

Breaking tensile strain and the specific break extension were tested at 23±2 °C, the relative humidity of 50±5 % using a tension testing machine RM-50 (Mashplast Ltd, Russia) with computer interface. The limit of an allowed error for loading while measuring at a forward stroke did not exceed ± 1 %. The maximum deviations of a sample diameter was ±0.2 mm.

Results and discussion

At the first stage, films were made with TPS obtained at various temperatures without ultrasound processing of a polymer melt and with ultrasound processing. Then the physico-mechanical properties of these films were analyzed. Temperature in the extruder changed from 130 to 190 °C and solution containing polyethylene: glycerin: sorbitol at a ratio of 60:30:10, was introduced at the same time as a softener into the TPS structure. Values of straining and strength characteristics of films with TPS made at 130 and 150 °C are given in Fig. 1 and Tab. I. It is obvious that the films with TPS made at 130 °C were unstable both with and without ultrasound applied.