Investigation of the Composition of Lipids of Siberian Pine Seeds

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(Received March 21, 2002; in revised form October 11, 2002)

Abstract

Intensification of lipid extraction from the seeds of Siberian pine with ethanol by using the electromagnetic field of superhigh frequency in extraction process is investigated. Physicochemical characteristics of the resulting cedar oil are determined, such as refractive index, numbers: acidic, peroxide, iodine, saponification, colour, and mass fraction of non-lipid admixtures. Fractional and fatty acid composition of the oil is investigated.

INTRODUCTION

Development of scientific foundations of the complex processing of renewable natural plant raw material is especially urgent for the Baikal natural territory in which ecological restrictions are valid (the Law of RF on Lake Baikal). A number of valuable products can be obtained from the seeds of Siberian pine (*Pinus sibirica* du Tour), *i. e.*, cedar nuts. The area occupied by commercial forests in the Republic of Buryatia is 1008.8 thousand hectares with total annual resource of the biological yield of the seeds of Siberian pine of 100 kg/ha [1].

The main product manufactured from the seeds of Siberian pine is cedar oil. Being a natural food product of plant origin, cedar oil has no contra-indications for application both for nutrition and for curative or prophylactic purposes [2].

At present, cedar oil is isolated mainly by pressing which does not provide complete removal of oil from seeds. In doing this, the application of high pressure, intensive mechanical, humid and thermal treatment have a negative effect on the protein and lipid complexes and decrease biological value of the oil and oilcake [3]. Extraction method of the isolation of plant oil provides almost complete removal of oil from seeds and ensures obtaining the product of higher quality. The duration of extraction process is 4–6 h. However, solvents used in industrial practice (extraction benzines and hexane [4–7]) are toxic, so it is necessary to separate them carefully from oil and cedar residuum.

The advantage of ethanol as a solvent is its good oil-solving ability at the temperature up to 120 °C, while after cooling to 16–24 °C it gets layered off it [4]. Using this procedure, one can separate oil from alcohol obtaining relatively pure oil without thermal action on miscella. Edible alcohol has no contra-indications for use in food, pharmaceutical, fragrance and

TABLE 1
Levels of factors under investigation

Factor	Level				
Specific power input, W/kg	1125	1375	1625	1875	2125
Duration, min	2	4	6	8	10
Duty	0.75	1.0	1.25	1.5	1.75

cosmetic industry. However, lipid extraction from the seeds of Siberian pine with ethanol in Soxhlet apparatus provides only 45–48 % of absolutely dry kernel mass, which is lower than the level achieved with hexane (63–65 %) [5].

The application of oscillating energy input of superhigh frequency to extraction processes provides an intensification of the process due to superposition of thermo- and barodiffusion on concentration diffusion. It was established [8] that extraction under the electromagnetic field of superhigh frequency (EMF SHF) allows one to increase the yield of extractable components in comparison with classical extraction procedures and is distinguished by simplicity, rapidity and economic efficiency. It was demonstrated in [9] that the application of ethanol for extraction allows achieving the highest effect from the oscillating energy input of superhigh frequency as a result of combination of the spectrum of anomalous dispersion frequencies of the solvent, connected with the framework of the solid, and a shift (or partial shift) of the spectrum of anomalous dispersion frequencies of pure solvent with respect to the spectrum of EMF SHF frequencies.

The goal of the present work is to investigate lipid extraction from kernels of Siberian pine with ethanol in electromagnetic field of SHF, and examination of the composition of resulting oil.

EXPERIMENTAL

Edible ethanol, which relates to low-boiling (78.4 °C) solvents of medium polarity (ε = 25.4) and medium viscosity ($\eta = 2\ 10^{-3}$ Pa s), was used to extract lipids from the seeds of Siberian pine. Lipid content of kernel was determined in agreement with the State Standard GOST 10857–64 "Oil-yielding seeds. Methods of the determination of oil content" (oil content was determined to be 65.7 % of absolutely dry kernel mass). Kernels of the seeds having the humidity 4.0–4.5 % were ground, water was poured on, and treatment with EMF SHF was carried out under periodic stirring in a household microwave oven (Samsung 1714R).

In order to estimate the effect of technological parameters on the yield of lipids and to optimize extraction process, factor experiments were performed. The major factors affecting extraction in EMF SHF are specific power input, determined from the ratio of oscillatory power of the SHF generator to the mass of the product, duration, and the ratio of solvent mass to ground kernel mass (duty). Planning of experiment was performed using Protodyakonov's procedure [10]. The levels of factors under investigation are shown in Table 1.

After extraction, cedar residuum was separated from miscella in filtering module with rotor [11]. Oil was separated from extragent in a separating funnel at the room temperature (18–24 °C). The residual alcohol was removed from oil by washing with water or by evaporation in a rotary vacuum film evaporator [12]. The alcohol was used without rectification for lipid extraction until its concentration decreased to 90 %.

The iodine number was determined according to Kaufman's procedure [13]. Saponification number was determined using the procedure based on the treatment of lipid with a definite amount of 0.5 M solution of alkali till complete Saponification of glycerides; the excess alkali was titrated off with an acid [14]. The acid number was determined by titration of the oil sample with the solution of alkali in the presence of phenolphthalein as indicator [15]. A mixture of alcohol with diethyl ether was used as a solvent. The peroxide number was determined by means of iodometry [16].

TABLE 2

Physicochemical characteristics of cedar oil obtained by different procedures

Characteristics	Extraction	
	with ethanol using SHF	with hexane [5, 6]
Refractive index	1.477–1.479	1.475–1.476
Acid number, mg KOH	0.55–0.60	3.22–3.43
Peroxide number, mmol/kg	3.0–3.5	3.0
Iodine number, g I_2/100 g	158.62–159.12	107.87–113.58
Saponification number, mg KOH	197.15–199.10	186.60–189.98
Colour number, mg I_2	7–8	not determined
Mass fraction of non-lipid impurities, %	0.050–0.055	0.4–1.2
Mass fraction of humidity and volatile substances, %	0.12–0.13	0.10–0.12

Fractional composition of lipids was determined using thin layer chromatography. A Silufol plate was impregnated with 1 % solution of phosphomolybdic acid in chloroform – methanol mixture (2 : 1 vol.). The plates were developed in the system of solvents: hexane – diethyl ether – acetic acid (80 : 30 : 1.5). The number of separate groups of lipids was determined by means of internal normalization of areas.

Methyl esters for the analysis of the composition of fatty acids were obtained by a one-stage method using sodium methylate solution [17]. The analysis was performed by means of chromato-mass spectrometry with Hewlett-Packard 5890-2 gas chromatograph with a quadrupole HP 5971 mass spectrometric detector. A 30 m long HP-5 quartz column (copolymer of 5 % diphenylsiloxane + 95 % dimethylsiloxane) with the inner diameter of 0.25 mm was used. The film thickness of immobile phase was 0.25 μm. The percentage of the mixture was calculated using the areas of gas chromatographic peaks. Qualitative analysis was based on comparing retention times of complete mass spectra of the corresponding pure components represented in the catalogue.

The IR spectra were recorded for the sample without solvent using Specord IR-75 spectrophotometer.

RESULTS AND DISCUSSION

The ground raw material gets heated in EMF SHF rather rapidly; the temperature of inner layers of the material is higher than that of the outer ones. The gradient of total pressure arises inside the material under treatment [9], which intensifies circulation in capillaries; lipids are liberated from the surrounding coatings (spherosomes) and get well dissolved in alcohol during subsequent extraction. The application of SHF to extraction intensifies mass exchange substantially and increases the yield of lipids from the plant material due to profound changes in the structure of its framework [9, 18–20].

On the basis of the data of factor experiment, optimal technological parameters were chosen: specific emissive power, 2000–2500 W/kg; duration, 3–4 min; duty, 1.25–1.5. The yield of cedar oil is 62–64 % of the mass of absolutely dry kernel.

The obtained cedar oil is a transparent light-yellow product possessing a pleasant gentle taste and a weak odour of cedar nuts. Its physicochemical characteristics are shown in Table 2. In comparison with the oil obtained by extraction with hexane, it is characterized by higher iodine number. Low acid number is another evidence of higher quality of the resulting cedar oil. Such a difference in physicochemical characteristics of the indicated samples is likely to be explained by short time of heating in the case of extraction with SHF. Mass fraction of non-lipid admixtures is small, because the substances accompanying lipids (phosphorus-containing, colouring substances, *etc.*) are extracted together with oil by ethanol but those substances do not pass into the lower settled

TABLE 3

Data on the composition of fatty acids in cedar oil

Number of carbon atoms	Fraction, %
$C_{14:0}$	0.31
$C_{16:0}$	6.82
$C_{18:0}$	3.23
$C_{20:0}$	0.31
$C_{16:1}$	0.63
$C_{18:1}$	26.57
$C_{20:1}$	1.08
$C_{18:2}$	40.41
$C_{20:2}$	0.52
$C_{18:3}$	19.65

oil layer when the miscella is cooled; they remain in ethanol [4]. Comparative analysis of literature data provides evidence that the resulting cedar oil corresponds to the edible plant oil in all the normalized characteristics [21].

Investigation of the fractional composition of the resulting cedar oil was performed by means of thin layer chromatography. It was established that the main fraction (99 %) of the oil is represented by triacylglycerides. Free fatty acids and phospholipids are present only in insignificant amounts.

The IR spectra of cedar oil (Fig. 1) exhibit absorption bands at 1377, 1377, 1463, 2855, 2930, 3008 cm^{-1}, indicating the presence of alkyl groups. A weak absorption band at 1652 cm^{-1} is characteristic of vibrations of C=C bond, while the absorption band at 723 cm^{-1} corresponds to the vibrations of $(CH_2)_n$ groups. The presence of intensive absorption band at 1747 cm^{-1}, as well as a band at 1164 cm^{-1}, indicates the presence of ester groups [22].

The composition of the mixture of methyl esters of fatty acids, obtained by treating the cedar oil according to procedure described in [17], is examined by means of gas-liquid chromatography (Table 3). The saturated fatty acids in cedar oil account for 11 %, monounsaturated ones account for 28 %, polyunsaturated for 61 %. The fraction of oleic acid (in the sum of unsaturated acids) is 29.9 %, linoleic acid takes 45.5 %, linolenic acid takes 22.1 %. High content of polyunsaturated acids, including irreplaceable fatty acids, is the evidence of high biological value of cedar oil. Oleic, linoleic and linolenic acids belong to the group of vitamin F, which increases the resistance of an organism toward radioactive rays, provides normal growth and metabolism in an organism, conserves elastic properties of blood vessels, helps removing cholesterol from organism [23].

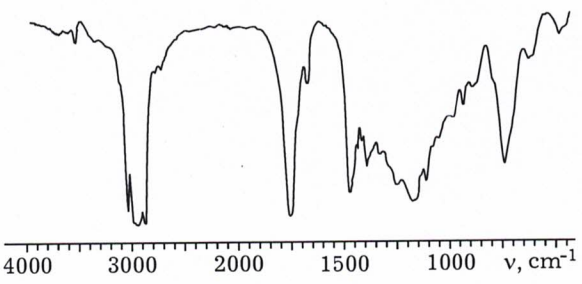

Fig. 1. IR spectrum of cedar oil.

CONCLUSION

The results of investigation showed that extraction of lipids from the seeds of Siberian pine with ethanol using SHF allows one to intensify this process substantially and to increase the yield of lipids. The data of chemical analysis, gas liquid and thin layer chromatography, IR spectroscopy confirm high quality of the resulting cedar oil.

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