STUDY OF COMPOSITION AND BIOLOGICAL VALUE OF PINON KERNEL OF SIBERIAN PINE

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Abstract: The problem of rational use of natural resources and raw materials, and providing the population with nutritious foods is particularly important in regions that are characterized by bad environment. In this regard, unconventional plant material, in particular pinons of *Pinus sibirica* for the needs of the food industry will not only solve the problem of rational usage of natural resources and the creation of an additional amount of raw food, but also contribute to the extension of the range of general and functional food products. The purpose of study was to investigate protein, lipid, and mineral-vitamin complexes of pinon kernels. Molecular weight distribution of peptides and proteins was assessed by polyacrylamide-gel electrophoresis (PAGE) with Laemmli method. The quantitative content of water-soluble vitamins was determined using capillary electrophoresis system with the device Kapel-105", fat soluble vitamins - by high-performance reversed-phase liquid chromatography, mass fraction of macro- and microelements - by atomic absorption spectrophotometry. The relative content of individual fractions in the lipid complex was as follows: phospholipids - 7.9% mono- and diacylglycerols - 6.1%, respectively, sterols 2.7% - carotenoids - 0.9%, triterpene alcohols - 1.8%, free fatty acids - 1.1%, triacylglycerols - 71.2%. Proteins of pinon kernels are heterogeneous in composition, contain seventeen fractions, which have molecular weight ranging from 66.85 to 13.33 kDa. Carbohydrate complex consists essentially of digestible carbohydrates and has a high content of hydrolysable sugars from 4.6 to 5.0%, starch - 4.7±0.2%, pentosans - 1.8 ± 0.2%, and fiber - 2.3 ± 0.1%. In the mineral complex phosphorus, potassium and magnesium, iron, zinc, manganese, and copper are the predominant elements. The structure of vitamin complex includes tocopherols from 30.5 to 32.0 mg, vitamin A - 0.02 mg, and carotenoids - 0.8 mg per 100 g of lipids. The degree of meeting the daily need of human body in vitamins and minerals, when taking pinon kernels in food, is calculated. Research results allow us to recommend pinon kernels as poly-functional food raw materials with high content of technologically important components for the production of original premium products.

Key words: Pinon kernel, lipids, protein, mineral and vitamin complex, fatty acid composition, amino acid composition, polyunsaturated fatty acid of ω-6 family, linoleic acid, γ-linolenic acid

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INTRODUCTION

Climatic extremes and harsh working conditions, as well as high degree of anthropogenic and technogenic pollution have a significant impact on the health of the population of the Siberian region, contribute to the increased risk of formation of occupational and environmentally caused diseases. The negative effects of production and environmental factors are aggravated by improper nutrition. The nutritional status of the population of the Siberian region is characterized by insufficient in-take of proteins, dietary fiber, vitamins and minerals. The deficiency of vitamins B_1, B_2, B_6, C, E and minerals Ca, K, Zn and others was also revealed. In order to reduce the influence of physical factors of production and the effects of xenobiotics on the body it is necessary to include foods containing biologically important components of natural origin in the daily diet. When choosing such products, people should give preference to those that are made from local raw materials, most similar to geo- and biochemical composition of the human body, living in the particular climatic zone, and therefore more useful [1].

One of the most promising and innovative kinds of wild plant raw materials in the Siberian region are the seeds of Siberian pine (pinons). Pinons are widely known thanks to the unique food and biological value of their components. One of the tendencies of pinon kernels usage is their processing to produce cedar oil. Cake and hull remaining after oil extraction are secondary raw resources. From them you can get a diverse range of protein products both for direct consumption in human nutrition, and obtaining various types of food products.

In order to expand the areas of pinon kernel usage and their products in the food industry in the development of commercial, special, therapeutic and prophylactic foods, a deeper study of their chemical composition is necessary.

The chemical composition of the seeds of Siberian pine depends on the area of growth. The study of the composition and biological value of the seeds of Siberian pine (*Pinus sibirica Du Tour*) is going on to the present. It is high variability in composition and biological value of seeds of Siberian pine depending on

the zone of growth, which brings about an increased interest of scientists to their research. The knowledge of the composition and biological value of pinons kernel allows us to combine it with other plant and animal materials, and thus, approximate the contents of main nutrients in new products to the norms of physiological needs for the population of the Siberian region.

The purpose was to study the protein, lipid and mineral-vitamin complexes of pinon kernel of Siberian pine growing in the Kemerovo region.

OBJECTS AND METHODS

The object of study is the kernel extracted from different batches of seeds of Siberian pine growing in the Kemerovo region.

Mass fraction was determined by extracting them with the mixture of chloroform and ethanol in a Soxhlet apparatus, followed by the removal of the solvent, pre-drying at 103 ±2°C and weighing (GOST 23042).

The protein content was determined on the total nitrogen (protein) analyzer RAPID N ELEMENTAR, working according to Dumas techniques, the sample was burnt with the registration of total nitrogen in the thermal conductivity detector. To determine protein on the analyzer the sample was encapsulated, wherein the analysis accuracy was 0.5%.

For qualitative and quantitative determination of amino acid composition the system based on high performance liquid chromatography (amino acid analyzer) Aracus PMA GmbH was used. The method is based on post-column derivatization and consists in the separation of amino acids on the ion exchange column with the step gradient of pH and subsequent reaction with ninhydrin in the reactor. Detection of colored derivatives of amino acids is carried out using spectrophotometric detector with wavelengths 570 and 440 nm.

Molecular weight distribution of peptides and proteins was assessed by electrophoretic method in polyacrylamide gel (PAAG) by Laemmli techniques [2].

Special plates were prepared for PAAG polymerization, and chamber reservoirs for electrophoresis were filled with electrode buffering solution (0.066 M Tris, 0.19 M glycine, 0.1% SDS). Previously prepared sample analyzed was added to each well of the new-formed gel.

Sample preparation was as follows: 20 mkl of protein, 10 mkl of buffer for samples, and 10 mkl of distilled water were added to tubes of epindorf type. After the sample has been stirred and boiled for 5 minutes the device was turned on, and the separation of proteins was observed. Electrophoresis was performed at the current of 50 ± 0.1 mA and 75 ± 0.2 mA.

After electrophoretic separation the gel was washed and stained with the three reagents: first, with fixing solution, then with the solution of "washing" and only after that with the staining solution. Gel washing with each of the reagents was carried out for 10 minutes at the temperature of 80 ± 2°C. At the last stage gel bleaching was performed in distilled water at the temperature 25 ± 2°C.

Gels viewing and photographing was performed on UV transilluminator TCP 20M («Viber Lourmat»,) at the wavelength of radiation - 312 nm. Data storage and processing was carried out by gel-documenting system Vitran-Photo.

The obtained amino acid sequence of peptides was defined by automatic sequencer (Edman techniques). The method is based on the treatment of test phenyl isothiocyanate sample, which leads to the cleavage of one amino acid from the N-terminus of the sequence and its subsequent identification by liquid chromatography under pressure.

The separation of individual lipid fractions was performed by thin-layer chromatography. The system: hexane-diethyl ether-acetic acid in the ratio 80:20:1 was used as an eluent. Analysis was performed by ascending chromatography in a sealed chamber on the plates «Silufol». As the developer we used a 10% alcoholic solution of phosphomolybdic acid.

The quantitative content of water-soluble vitamins was determined by capillary electrophoresis on the device “Kapel 5”. This device is equipped with liquid capillary cooling system, and autosampler. Indications are determined off-line using spectrophotometrical detector based on the deuterium lamp and monochromator with the diffraction grating, the working range of wavelengths being from 190 to 400 nm.

Determination of fat-soluble vitamins was performed by reversed-phase high-performance liquid chromatography on chromatograph "Milichrom" with spectrophotometric detector in the spectral range 190-360 nm. The essence of the method lies in the extraction of vitamins from the sample under analysis by an extractant.

The mass fraction of macro- and microelements was determined by atomic absorption spectrophotometry on the device “Hitachi”.

RESULTS AND DISCUSSION

A distinctive feature of pinon kernels of Siberian pine is the variability of their composition, due to geologic and geographical, soil and climatic conditions of pine growth, pinon maturity, storage conditions, method of kernel separation and other factors [3, 4].

The chemical composition of pinon kernels of Siberian pine growing on the territory of the Kemerovo region is presented in Table 1.

Table 1. The chemical composition of pinon kernels of Siberian pine

Components	Mass fraction, % for absolutely dry matter		
	District		
	Yashkinsky	Tashtagolsky	Tisulsky
Protein	19.2±0.2	18.0±0.2	17.6±0.2
Lipids	60.5±0.5	62.5±0.5	62.6±0.5
Carbohydrates, including:	18.3±0.2	17.1±0.1	17.4±0.2
- hydrolysable sugars	5.2±0.2	4.8±0.5	5.0±0.3
- polysaccharides	13.4±0.2	12.3±0.4	12.4±0.2
Ash	2.5±0.1	2.4±0.1	2.4±0.1

The main component of the pinion kernels are lipids, the average content of which in studied raw materials was 62.5 ± 0.5% (based on absolutely dry matter). The absolute maximum of fat in the studied samples of pinon kernels of Siberian pine growing on the territory of the Kemerovo Region, amounted to 64.4% (pinons from Tisulsky District) and minimum to 60.5% (Yashkinsky district).

Lipid biological properties depend on the structure of triacylglycerols, as well as the presence of biologically active compounds, namely phospholipids and sterols. Phospholipids are involved in the construction of all cell membranes and cell organelles. Sterols and their esters are used for the synthesis of different physiologically active substances [5, 6].

Lipid complex of pinon kernels of Siberian pine appeared to be presented by spare and structural lipids. The relative content of individual lipid fractions in the complex was as follows: phospholipids - 7.9%, mono- and diacylglycerols - 6.1%, respectively, sterols - 2.7%, carotenoids - 0.9%, triterpene alcohols - 1.8%, free fatty acid - 1.1%, and triacylglycerols - 71.2%.

The biological efficiency of lipid complex of pinon kernels of Siberian pine is determined by the composition and amount of fatty acids (Table 2). The composition and the ratio of fatty acids in triacylglycerols of cedar oil define its physical-chemical, physiological properties, and digestibility.

Table 2. Fat - acid composition of cedar oil

Name of acids	Conditional Designation	Mass fraction,%	
		Average value	GOST 30623-98
Saturated, including:	-	7.7	-
- palmitic	$C_{16:0}$	4.4	3.0–3.9
- stearic	$C_{18:0}$	2.7	3.4–4.1
Monounsaturated, including:	-	24.1	
- oleic	$C_{18:1}$, ω-9	23.1	22.1–36.0
Polyunsaturated including:	-	67.9	-
- linoleic	$C_{18:2}$, ω-6	46.7	36.0–69.0
- α-linolenic	$C_{18:3}$, ω-3	0.3	0.3–0.4
- γ-linolenic	$C_{18:3}$, ω-6	20.1	18.0–24.3

Cedar oil extracted from the pinon kernels of Siberian pine contains acids of all groups - saturated, monounsaturated and polyunsaturated with a number of carbon atoms from 14 to 22. Palmitic acid prevails over other saturated ones in quantity, its maximum content in the studied samples of cedar oil does not exceed 4.4%. The ratio of stearic acid is almost two times smaller and ranged from 2.7%. Side by side with these saturated fatty acids in the cedar oil, there were identified myristic (0.2%), pektadecanoic (0.08%), arachic (0.2%), and behenic (0.1%) acids.

Oleic acid, the content of which was 23.1%, predominates over monounsaturated fatty acids in cedar oil. Oleic acid is involved in the synthesis of spare and structural lipids. Oils characterized by a high content of oleic acid contribute to the reduction of cholesterol and low density lipoproteins in blood [6]. Alongside with the cis-isomers of fatty acids cedar oil has in its composition a small amount of vaccenic acid (trans isomer) in an amount of 0.33 ± 0.02%.

Polyunsaturated fatty acids are contained in cedar oil mainly as those of acid ω-6 family: linoleic and γ-linolenic acid with high biological activity and are its essential nutrition factor. These acids are involved in the formation of structural lipids and various physiologically active substances. They are precursors of long-chain polyunsaturated fatty acids, the main natural source of which is the fish oil alone.

Since gamma-linolenic acid is associated with the increase in cholecystokynin and glucagon-like peptide hormone production, it causes the feeling of satiety and reducing food intake. Hypotensive, hypocholesterolemic, hypolipidemic, choleretic, wound healing action of cedar oil has also been associated with the activity of γ-linolenic acid. Despite the fact that the gamma-linolenic acid may be formed in the body from linoleic acid, its dietary intake is very important because the activity of enzymes (desaturases) converting linoleic to gamma-linolenic acid may be reduced with age under the influence of a number of factors [3, 7].

Phospholipids constitute the most complex and valuable group of structural lipids contained in vegetable oils. In lipid the complex of studied samples of pinon kernels, the amount of substances containing phosphorus compared to traditionally used in food oils is rather high and amounted to an average of 1.1 ± 0.1% in recalculation on stearooleolecitin. Furthermore, the unsaponifiable fraction of lipids of pinons kernel contains sterols (0.18%) and squalene (0.1%).

A relatively high protein content determines the nutritional value of the kernel of pinons. Protein content in pinon kernels is subjected to fluctuations and depends on the climatic conditions of growth and maturation [3, 4]. Within the Kemerovo region protein mass fraction averaged 18.2 ± 0.2% in the samples of pinon kernel. Protein fluctuations, depending on the zone of growth, ranged from 19.2 ± 0.2% (Yashkinsky district of the Kemerovo region) to 17.6 ± 0.2% (Tisulsky District of the Kemerovo Region).

Proteins of pinon kernels are spare proteins, and this determines their physical-chemical properties and the ability to be dissolved in various solvents. Qualitative and quantitative fractional composition of protein determines on the one hand the nutritional value and on the other, its functional properties.

The amount of extractable protein in the protein complex of pinon kernel is on average 71.5%, while the share of easily extractable proteins account for only 31.5% of the total protein. Salt-soluble protein fraction averaged 16.8%, water-soluble - no more than 14.7%, alkali-soluble - 40.0%. The amount of protein in the insoluble precipitate makes up 28.5%. This suggests that the consumption of pinon kernel as food protein will not be absorbed by more than 3/4.

Fractional composition of soluble proteins was evaluated using the method of Laemmli protein electrophoresis (Fig. 1).

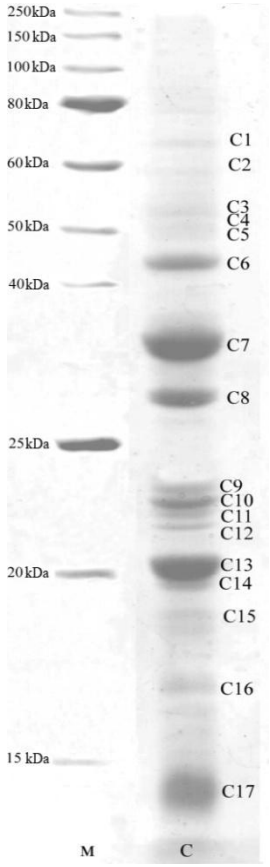

Fig. 1. Electrophoregram of aqueous extract by polyacrylamide gel electrophoresis (12% separating gel, 4% stacking gel): M - marker; C - in the rigging of low-fat kernels of pinons.

Electrophoretic studies of protein complex by method of Laemmli showed that proteins of pinons are heterogeneous in composition, contain seventeen fractions, the molecular weight of which fluctuates from 66.85 to 13.33 kDa (Fig. 1). The major proteins presented on electrophoretogram are proteins with the molecular weight of 32.16 kDa, 27.6 kDa, 20.14 kDa, 14.29 kDa. The amount of these protein fractions amounts to 49.76%. B6 fractions (molecular weight of 44.23 kDa) and B10 (molecular weight of 22.52 kDa) can also be attributed to the major proteins, as their percentage of the total amount is that of 8.70% and 6.88% respectively. According to their characteristics, they can be attributed to the hydrolysis products of larger protein fractions. Other proteins, whose content varies from 3.68 to 2.80%, have the molecular weight from about 13.33 to 66.85 kDa.

Nutritional value of pinon kernel is determined by its amino acid composition. The determination of the qualitative and quantitative composition of the amino acid protein complex of pinon kernel (Table 3) show that it contains all the essential amino acids, which accounted for not less than 36% and is characterized by high content of arginine, proline, aspartic acid and glutamic acid.

Table 3. The content of amino acids in the protein of pinons

Amino acid	Content, g / 100 g of skim dry matter	
	Average content	Fluctuation range
Essential amino acids:	36.14	36.1–36.25
valine	5.15	5.00–5.30
isoleucine	4.84	4.74–4.95
leucine	6.41	6.07–6.75
lysine	6.16	6.05–6.28
methionine	1.99	1.90–2.09
threonine	4.07	4.05–4.10
tryptophan	2.47	2.45–2.50
phenylalanine	5.05	4.95–5.16
nonessential amino acids	63.80	63.46–63.75
alanine	3.75	3.67–3.84
arginine	13.32	13.15–13.49
aspartic acid	13.04	12.98–13.11
histidine	2.68	2.65–2.72
glycine	4.42	4.40–4.45
glutamic acid	11.47	10.40–12.55
proline	7.50	6.74–8.26
serine	3.58	3.56–3.60
tyrosine	2.71	2.63–2.80
cysteine	1.33	1.15–1.51

Protein of pinon kernel surpasses wheat protein in the content of essential amino acids such as lysine, threonine, valine, methionine, tryptophan, and arginine, aspartic acid, and alanine as well. It is rather close to soybean protein in the content of valine, isoleucine, threonine, phenylalanine, and surpasses it in the content of tryptophan and arginine, but is inferior to soy protein in the content of leucine and glutamic acid from 1.2 to 1.5 times. Comparison of pinon kernel protein with animal proteins shows that the former like the majority of the vegetable proteins, is inferior to animal protein in the content of essential amino acids, but exceeds them in the content of arginine almost 2 (chicken egg) and 3 (cow's milk) times, being not inferior to the content of histidine and glutamic acid.

Carbohydrate complex of pinon kernel of Siberian pine growing on the territory of the Kemerovo Region, is characterized by high content of hydrolysable sugars and starches. In the group of sugars under consideration sucrose prevails from 4.6 to 5.0%. The amount of starch was 4.7% on average (the range of fluctuations is from 4.2 to 5.3%).

The content of individual fractions of dietary fiber, particularly fiber and swelling non-starch poly-

saccharides - pentosans. Pentosans are the building blocks of ribonucleic acids, which are concentrated in the kernel bud of pinons and ensure its special physiological function. According to the information received, the amount of pentosans averaged 1.8 ± 0.2%, fiber - 2.3 ± 0.1%. Thus, carbohydrate complex of pinon kernel consists essentially of digestible carbohydrates.

Phosphorus, potassium and magnesium are the predominant elements in the mineral complex of pinon kernel. High concentrations of these elements, as well as those of iron, zinc, manganese and copper favor the biological value of this kind of raw material. The mineral composition of pinon kernel is particularly low in calcium, potassium being predominant over sodium (Table 4).

Table 4. Mineral and vitamin complex of pinon kernel

Food substance	Average value of the food, mg / 100g	The degree of satisfaction of daily need of the human body, %
Macronutrients		
potassium	631.2 ± 0.2	25
calcium	26.6 ± 1.2	3
magnesium	306.0 ± 0.2	76
sodium	10.5 ± 0.7	1
phosphorus	986.7 ± 11.3	123
Trace elements		
iron	6.75 ± 0.8	67 – for males 37 – for females
manganese	8.8 ± 0.9	440
copper	1.3 ± 0.12	130
zinc	10.6 ± 0.9	88
Vitamins		
B_1	0.34 ± 0.02	23
B_2	1.07 ± 0.02	59
B_3	0.80 ± 0.05	16
B_5	2.50 ± 0.10	12
B_6	0.15 ± 0.05	7
E	30.20 ± 1.10	200

The data show that the degree of satisfaction of the daily needs of healthy adult male and female population in micronutrients is sufficient: the content of manganese and copper in 100 grams of kernels exceeds the needs of the individual and the content of zinc and iron is close to the standards. These elements possess important physiological functions: they have antioxidant properties and are involved in regulating the activity of several enzymes. Pinon kernels almost satisfy the daily needs in the content of magnesium and phosphorus being in excess quantity. Phosphorus is involved in many physiological processes, in cell regulation and regulation of acid-base balance. It is a part of phospholipids, nucleotides and nucleic acids and is required for the mineralization of bones and teeth. Magnesium is a cofactor of many enzymes involved in the synthesis of proteins and nucleic acids. It has the stabilizing effect for membranes and is required for calcium, potassium and sodium homeostasis support.

As food raw materials, pinon kernels are of interest in terms of their content of vitamins. By B-vitamin activity pinon kernels do not concede to such oilseeds as those of sunflower and soybeans, and exceed nut-like ones: walnuts, almonds, pistachios. It should be noted that in pinon kernel of Siberian pine in comparison with the above nut-like plants and oilseeds, vitamin B_2 (riboflavin) is 3 times more than thiamine. Vitamin B_2 is involved in redox reactions, increases the susceptibility of color visual analyzer and darkness adaptation [8, 9].

The consumption of 100 g of pinon kernels will satisfy the daily needs of human body per 59% in riboflavin, and per 23% in thiamine.

The structure of vitamin complex of pinon kernel include tocopherols from 30.5 to 32.0 mg per 100 g of product. Alongside with the total content the composition of tocopherol isomers is an important characteristic of the tocopherol isomers. In lipid complex of pinon kernels α-tocopherol share is on average of 52.2%, γ - 11.3%, δ - 36.5% of the total tocopherol amount. This ratio of various isomeric forms of tocopherols that differ between themselves by their biological and antioxidant activity, brings about adequate stand-bone lipid oxidation during storage and high biological activity of the product. The body can satisfy the daily consumption of vitamin E taking in 30-50 g of pinon kernels.

The content of vitamin A and carotenoids is insignificant - 0.8 and 0.02 mg, respectively per 100 g of lipids extracted from pinon kernels.

Research results allow us to recommend pinon kernels as multifunctional food raw materials with a high content of technologically important components for the production of original premium products.

Using seeds of Siberian cedar pine as a natural resource of many biologically and physiologically active components opens up great food industry perspectives to expand the range of products of high nutritional value and functionality.

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