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Peculiarities of Chemical Composition of Sainfoin Seeds Powder

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This paper is devoted to studying chemical composition of the powder of the seeds of non-traditional legume, sainfoin. The experimental studies showed that crushed seeds of sainfoin make a flowing fine powder of light brown color with a pleasant unpronounced specific smell with floral notes. The taste is grassy with the after-taste typical for legumes. The chemical composition of sainfoin seeds is dominated by proteins and fiber, and fat content does not exceed 8%. The total content of amino-acids is 26.94/100 g of the product, with the share of indispensable ones being 37.85%. The limiting amino acid is tryptophan (48.0 %). By the composition of essential amino acids, proteins of sainfoin seeds are slightly inferior to the proteins of soybean seeds, but are better than the proteins of peanut seeds. The composition of fatty acid of the lipid complex of sainfoin seeds is dominated by (over 40% of the total) linolenic ù-3 acid with sufficiently low (less than 20% of the total) content of linoleic ù-6 acid. The lipid composition of sainfoin seeds, along with triacylglycerols, contains about 40% of related lipids, which are dominated by sterols, aliphatic alcohols, phospholipids and tocopherols. All this makes the lipid complex of sainfoin seed a promising means of adjusting fatty acids composition in food products of functional and specialized purpose, dietary supplements, and a valuable raw material for creating pharmaceutical substances and preparations. Adding sainfoin seeds powder into the nutritive medium has no inhibitory effect on development of the tested organism. At the same time, 58% of the organism's physiological need for protein is satifsied, as compared to caseine.

Key words: sainfoin, chemical composition, biological value.

INTRODUCTION

Place of sainfoin in the legumes family

Perennial grasses are the most efficient and least energy-consuming forage crops. They allow to solve the problem of obtaining balanced protein forage, ensure preservation of soil fertility, enhance productivity and environmental safety of crop production, and assist in establishing grassland phytocenoses and landscape areas, reclamation of contaminated and eroded lands, and construction of roads slopes and forest-park objects[1].

In addition to ensuring high yield of biomass with high nitrogen content, perennial

grasses of the legume family are good predecessors, since they enrich soil with various mineral substances in the form of connections available for most plants[2]. According to data [3], introduction and increasing the share of legumes in the structure of field rotations is a stabilizing factor for quantitative and qualitative characteristics of soil fertility. Thus, besides increasing availability mineral forms of nutrients for plants, the use of legumes in crop rotation helps to reduce losses of humus and to optimize of its composition, and therefore has a positive effect on stabilization of environmental stress in field crops.

With this regard, and with regard to the problem of protein deficiency, the study of non-traditional legumes focused at expanding the scope of their usage is of high interest.

Among perennial legumes, sainfoin is a promising but insufficiently used crop.

Sainfoin (genus Onobrýchis Gaerth, family Fabaceae (Legumes)) includes 133 wild species that are native to Central and Southern Europe, North Africa and Western Asia [4]. It is a perennial herbaceous plant that reaches the height of 30-70 cm. Sainfoin features a tap root system penetrating the soil to the depth of 3 m. The stem is straight, slightly branching on the top. The leaves are odd pinnate, compound, formed from 15 to 25 small leaves. The flowers are purple, pink, whitish or yellowish, collected into alar racemes, or into spikes. Sainfoin fruit is a tapered, hemispherical bean that may rarely be rolled into a spiral, leathery, wrinkled, not dehiscent, containing one or two seeds[5, 6]. The seeds are rather small, the weight of 1,000 seeds ranges between 18 and 20 g.

Sainfoin provides highly nutritious forage with protein content of up to 16% in the flowering phase, and up to 23% in the after-grass phase. It is characterized by the average exchange energy of about 10 MJ/kg. Its hay is eaten almost completely. High content of tannin in the leaves of sainfoin has a beneficial effect on digestibility of crude protein in the rumen of ruminants and prevents bloat accompanied by formation and excessive accumulation of gases[7]. Besides, the plant is rich in flavones, nitrogen-free compounds, ascorbic acid, and rutin. Largely because of this fact, sainfoin introduction into the diet increases animals' resistance to various diseases, and has anthelminthic and anticoccidial effect.[7-10].

The use of sainfoin in folk medicine in form of infusions and decoctions made of the green part of the plant and root[11] is also known.

In the Russian Federation, three types of sainfoin are of industrial importance: Onobrychis transcaucasica Grossh., Onobrychis viciaefolia and Onobrychis arenaria DC.[4].

Onobrýchis arenaria is a valuable forage plant that deserves a wider use in crop rotation; it belongs to leies – plants that improve soil composition and structure. Onobrychis arenaria was named so for its ability to grow on any soil, including sandy soils. It features high resistance to droughts and frosts[4]. Onobrychis arenaria's disadvantages are its rough stem and small quantity of leaves. In modern culture, the hybrid varieties of this plant are popular.

According to data[4], due to its powerful elongated root system that reaches the depth of over 2.5 meters, Onobrychis arenaria DC. is capable of assimilating calciferous and phosphorus compounds that are hardly accessible for other plants, which then enrich the soil in available form due to the root remnants, by the number of which sainfoin is superior to alfalfa[10].

Using sainfoin

In the Russian Federation, sainfoin is mostly cultivated as a fodder crop, the green mass of which is used in the form of hay or grass meal. For the production of green fodder, sainfoin is mowed before or during the flowering period, which considerably reduces the possibility of implementing other promising ways of using this raw material.

One of such ways is the use of sainfoin as a bee plant, high efficiency of which has been proven in practice in a number of foreign, including European, countries, where sainfoin honey is highly valued. Sainfoin honey productivity can reach 400 kg/ha. Sainfoin honey features light amber color,

transparency, rich taste and fine aroma. The honey does not crystallize for a long time, and after crystallization it is a homogeneous white creamy mass with a cream tint, consisting of very finely dispersed crystals.

Along with high organoleptic properties, the sainfoin honey is characterized by high physiological value. It contains large quantities of vitamins, valuable minerals, enzymes and other physiologically functional nutritional ingredients[12 - 14].

Another area of using sainfoin is related to obtaining seeds and their subsequent use as raw food material. For the implementation of such usage, Onobrychis arenaria DC. is most promising, due to the combination of nectar-bearing properties and relatively low green mass and larger seeds.

According to data[13], the combination of targeting sainfoin as a bee plant and a raw material for obtaining seeds with proper agrotechnics of cultivation results in the average yield of 126 kg/ha for nectar and 7 kg/ha for seeds.

Thus, the listed features of sainfoin ensure increased attention of scientists of developed countries to this culture, which more and more frequently becomes the subject of research programs for creating the scientific basis for transition to organic agriculture[7, 13, 14].

The results of analyzing the literature data, which describe features of complex processing of sainfoin seeds, their chemical composition and pharmacological properties, testify that this plant might be used as an alternative source of vegetable protein, dietary fiber and physiologically valuable lipids[8, 11, 12, 15]. This, in turn, shows feasibility of performing research for studying possible use of sainfoin seeds and products of their processing in food technology, including candy manufacture.

This paper presents the results of studying sainfoin seeds powder (SSP) obtained from hulled seeds of arenaria 1251 sainfoin breed, afterwards ground in a laboratory mill (passing through a 1 mm sieve should be 90 %).

METHODS

Mass fraction of protein was determined using the N2/protein DKL8 quantitative identifying system manufactured by VELP SCIENTIFICA, Italy. The biological value of sainfoin seed powder was studied by experimental determination of amino acid composition with the use of the "KAPEL-105M" system of capillary electrophoresis manufactured by Lumex, Russia[16]. The relative biological value (RBV) of protein was determined using the rapid test method with the use of tetrahymena pyriformis ciliates in accordance with the recommendations of A. D. Ignatiev et al.[17].

Mass fraction of fiber was determined on an installation for fiber analysis FIBRETHERM FT12 manufactured by Gerhardt, Germany, in accordance with GOST 10846-91. Mass fraction of fat was determined on an automatic installation for solid-and-liquid extraction SOXTHERM SOX414à manufactured by Gerhardt, Germany.

The lipid complex was separated by extraction with diethyl ether at 20 °C with the use of laboratory reactor LR-2ST manufactured by IKA, Germany. The composition of fatty acids in the lipid complex was determined according to GOST R 51486-99 with the use of gas-liquid chromatograph Crystal – 5000 and the chromatographic column SOLGEL-WAX 30m×0.32 mm ID SOLGEL-WAX×0.5 µm. The mass fraction of phosphorus in the lipid complex was determined using the calorimetric method in accordance with GOST 31753-2012.

The composition of associated lipids was studied by the TLC method, followed by densitometry [18] and HPLC with the use of a high performance liquid chromatograph Agilent 1260 Infinity manufactured by Agilent Technology, USA.

The results of experiments were assessed with the use of modern methods of calculating static accuracy with the use of the Statistica 6.0, Microsoft Office Excel 2007 and Mathcad software applications.

All studies were performed on the equipment of SUC "Research Center for Food and Chemical Technology" of the Federal State-Funded

Educational Institution of Higher Vocational Education "The Kuban State Technological University".

Results

Milled sainfoin seeds are a bulk of fine powder of light brown color with a pleasant unexpressed specific odor with floral notes. The taste is grassy with the after-taste typical for legumes.

Chemical composition of sainfoin seeds powder is shown in Table 1. Data for soybean seeds that are most widely used in processing for food technology are presented for comparison[19].

It has been shown that the chemical composition of sainfoin seeds is dominated by proteins and fiber. Protein content in sainfoin seeds corresponds to the lower limit of the range of values

Table 1: Sainfoin seeds chemical composition

Indicator name	Indicator value	
	Sainfoin seeds	Soybean seeds [19]
Mass share, %:		
crude protein	28.7	28.0-50.0
crude fat	7.3	17.5-23.0
moisture and volatile matter	8.5	5.6-7.9
crude fiber	19.4	5.1-10.2
minerals	5.4	3.8-4.8

Table 2: Composition of amino acids

Name of the amino acid	"FAO" protein, % to protein	Sainfoin seeds powder		Wheat flour [5]	
		g/100 g of product	% to protein	g/100 g of product	% to protein
Indispensable:					
lysine	5.5	1.59	5.90	0.2	2.54
phenylalanine+tyrosine	6.0	1.98	7.35	0.88	10.92
leucine+isoleucine	11.0	2.97	11.02	1.34	12.87
methionine+cystine	3.5	1.09	4.04	0.40	5.27
valine	5.0	1.28	4.75	0.51	4.88
threonine	4.0	1.16	4.31	0.32	3.05
tryptophan	1.0	0.13	0.48	0.12	1.15
Replaceable:					
asparagine		2.93	10.88	0.41	3.94
alanine		1.22	4.53	0.36	3.44
arginine		3.18	11.80	0.50	4.79
histidine		<0.25	0.93	0.22	2.10
glycine		1.47	5.46	0.38	3.68
glutamine		4.66	17.30	3.22	30.86
serine		1.66	6.16	0.45	4.35
proline		1.37	5.09	1.05	10.06
Total amount of amino acids		26.94	100	10.43	100
First limiting amino acid		tryptophan – 48.0 %		lysine – 46.2 %	

Table 3: Lipid complexes fatty acid composition

Fatty acid name	Lipid complexes fatty acid composition, % of the amount		
	Sainfoin	Soybeans[27]	Sunflower[28]
$C_{14:0}$ Myristic	absence	Up to 0.2	Up to 0.2
$C_{16:0}$ Palmitic	7.43	8.0-13.0	5.6-7.6
$C_{18:0}$ Stearic	3.06	2.4-5.4	2.7-6.3
$C_{20:0}$ Arachidic	0.55	0.1-0.6	0.2-0.4
$C_{22:0}$ Behenic	0.80	absence	0.5-1.3
$C_{24:0}$ Lignoceric	0.29	Up to 0.4	0.2-0.3
Σ SFA	12.13	13.9-16.2	11.5-13.8
$C_{16:1}$ Palmitoleic	0.07	Up to 0.2	Up to 0.3
$C_{20:1}$ Eicosenoic	0.47	Up to 0.3	Up to 0.2
$C_{22:1}$ Erucic	0.24	Up to 0.3	Up to 0.2
$C_{18:1}$ Oleic	24.95	17.7-26.1	14.0-39.4
Σ MUFA	25.73	18.2-26.4	14.5-39.6
$C_{18:2}$ Linoleic	18.77	49.8-57.1	50.0-75.0
$C_{18:3}$ Linolenic	41.31	5.5-9.5	Up to 0.2
Σ PUFA	60.08	59.3-62.6	50.2-75.0

Table 4: Chemical composition of the sainfoin seeds lipid complex

Name of the component	Component content, % of the total
Triacylglycerols	62
Mono and diacylglycerols	4
Phospholipids	6
Sterols	8
Aliphatic alcohols	7
Free fatty acids	4
Tocopherols	6
Hydrocarbons, carotenoids, waxes	3

variability interval of this indicator for soybean seeds. Fat content is not high, and does not exceed 8%, which does not allow to consider sainfoin seeds as a promising source of oil, similar to soybeans.

The biological value of the protein complex was assessed by studying the composition of amino acids. Taking into account that one of the alleged uses of sainfoin seeds powder is the production of flour confectionery products, a comparison was made with the use of the literature data that describes amino-acids composition in wheat protein[5]. The data is presented in Table 2.

Table 5: Relative biological value

No.	Sample	Number of ciliates 1×104 in 1 cm^3				RBV, %
		Flask No. 1	Flask No. 2	Flask No. 3	Average value	nitrogen
1	Edible casein	47	48	46	47	100
2	Grape seeds	21	20	22	21	45
3	Spelt seeds	23	24	20	22	47
4	Sainfoin seeds	26	28	28	27	58

Analysis of the presented data shows that the amino acid score for protein in sainfoin seeds powder for threonine is 107.8%, for lysine - 107.3 % (for wheat flour, this score does not exceed 76.3 % and 46.2%, respectively).

The total amount of amino acids in sainfoin seeds powder is 26.94 g/100 of the product, of which the share of the indispensable ones is 37.85%. The limiting amino acid for the protein complex of sainfoin seeds powder is tryptophan (48.0 %).

It is known that among vegetable proteins, soy and peanut proteins are the closest to animal proteins in terms of amino acid composition. In this regard, it was of interest to compare the composition of essential amino acids in sainfoin, soybean and peanuts (Figure 1).

The diagram in Figure 1 shows that by the composition of indispensable amino acids, proteins in sainfoin seeds are somewhat inferior to proteins content in soybean seeds, but superior to proteins content in peanuts.

For example, lysine content in the protein in sainfoin seeds is over 1.7 times higher than that in peanuts. As it is known [21], lysine is one of the most valuable indispensable amino acids, since its deficiency in the diet disrupts blood formation, reduces the number of red blood cells and hemoglobin level, leads to atrophy of muscles and impaired calcium absorption. Lysine deficiency may slow down protein synthesis in muscles and connective tissues. Interaction of lysine and vitamin C results in formation of L-carnitine, the substance that helps muscles to use oxygen more efficiently, increases endurance, promotes bone growth, and promotes production of collagen, a fibrous protein that is part of bones, gristles and other connective tissues.

Threonine, the content of which in sainfoin exceeds that in peanuts, neutralizes toxins and helps to prevent fat accumulation in the liver, being an important component of collagen[22, 23].

Amino acids, being the “building blocks” of the organism, help people to rehabilitate after diseases, recover after severe physical exercise, and are useful for athletes who consume a lot of energy in the period of training[23].

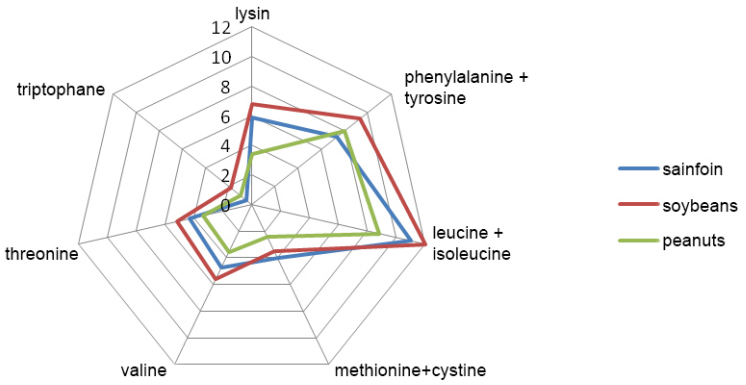

Fig. 1: Composition of indispensable amino acids in seeds of sainfoin, soybean [20], and peanuts [20]

DISCUSSION

Thus, sainfoin seeds powder has a more balanced amino acid composition, as compared to wheat flour and peanuts, so it can be considered quite a promising food ingredient, the use of which for food helps to adjust the balance of limiting amino acids.

In spite of relatively low oil content in sainfoin seeds, studying the composition of the lipid complex is of great interest, due significant role of lipids in human physiology.

It is known that the lack of polyunsaturated fatty acids (PUFA) in the organism leads to growth impairment, necrotic skin lesions, changes in

capillaries permeability and other pathological disorders[23, 24]. Polyunsaturated fatty acids are precursors of hormone-like substances, prostaglandins, which feature antiatherogenic, immunomodulating, geroprotective and other physiologically functional properties. Essential fatty acids that are not synthesized in the organism include linoleic and linolenic acids, which, in the process of biosynthesis with participation of vitamins B_1 (thiamine) and B_6 (pyridoxine), form arachidonic acid that features the highest biological efficiency[24-26].

Fatty acid composition in the lipid complexes of sainfoin seeds powder

Biological efficiency of sainfoin seeds lipid complex was assessed by analyzing the composition of fatty acids that form its triacylglycerols. The comparison is based on the data for lipid complexes of soybean and sunflower seeds, as most widely used for vegetable oils production[27]. The results of the analysis are shown in Table 3.

As one can see from the data in Table 3, unlike lipid complexes of sunflower and soybean seeds, where the composition of triacylglycerols is dominated by linoleic (ω-6) and oleic acid (ω-9) acids, lipid composition of sainfoin seeds is dominated by linolenic (ω-3) and oleic (ω-9) acids. For the analyzed lipid complexes, the qualitative and quantitative composition of other acids is similar.

It should be noted that from the standpoint of modern nutrition science, quantitative composition of ω-6, ω-3 and ω-9 fatty acids characterizes to a certain extent the physiologically functional properties of vegetable oils[25]. The results of modern medico-biological and clinical studies indicate that polyunsaturated fatty acids of group ù-3, and the monoene fatty acids of group ω-9 have hypocholesterolemic, hypolipidemic and membrane-protective properties, prevent thrombosis and have other physiologically active properties[22 - 25].

According to data[29], the use of products or preparations containing linolenic acid in the diet for the patients with alimentary-dependent pathologies, which are characterized by reduced activity of the "6-desaturase enzyme that is engaged in the metabolism of linoleic acid, ensures the so-called "biological bypass", since it makes it possible to bypass the obstructed section of the metabolic chain of transformations.

Therefore, oils with predominant content of ù-3 fatty acids are used to create functional products, biologically active food additives (BAA) and / or pharmaceutical preparations to be used in complex therapy of alimentary-dependent diseases, including obesity, atherosclerosis, arrhythmia, hypertension, thrombosis, arthritis, and diabetes[25]. On the contrary, in the scientific literature one can find evidences that excessive consumption of oils with a predominance of the ù-6 family essential fatty acids may trigger development of atherosclerosis, cardiovascular system diseases, etc.[30].

The ratio of polyunsaturated fatty acids of ω-6 and ω-3 families in the fat diet of a healthy person should be (5-10):1, in clinical nutrition - (3-5):1 [31], as recommended by the Institute of Nutrition. The main sources of fatty acids of group ù-3 fats are fish and animals (up to 30% in the total fatty acid composition) and flax (30-60% in the total fatty acid composition) and to a lesser extent - rape seed and false flax vegetable oil (7.0-15.0 and 20.0-39.0% in total fatty acid composition, respectively)[32, 33].

With the regard to the above, high (over 40%) content of linolenic ω-3 acid in the oil of sainfoin seeds, with sufficiently low (less than 20%) content of linoleic ω-6 acid, makes it a promising tool for adjusting fatty acid composition of functional and specialized food products, dietary supplements, and a valuable raw material for pharmaceutical substances and preparations.

Physiological value of lipid complexes of plant origin are not limited to their biological effectiveness, since along with triacylglycerols, they contain a number of related lipids with independent physiologically functional properties.

Chemical composition of the sainfoin seeds lipid complex

Chemical composition of the sainfoin seeds lipid complex is shown in Table 4. It is shown

that sainfoin seeds lipid composition, along with triacylglycerols, contains about 40% related lipids, which are dominated by sterols, aliphatic alcohols, phospholipids and tocopherols. These substances are physiologically functional food ingredients, the level of their presence in food is a criterion of functionality of the latter.

Thus, the studies performed show that the sainfoin seeds powder contains valuable food nutrients consisting of the protein and the lipid complexes. Meanwhile, it is known that plant raw materials, along with nutritional substances, may contain hazardous and potentially hazardous substances. For example, seeds of the Legumes family feature high (about 6%) content of trypsin and chymotrypsin inhibitors, active lipoxygenase and urease, soybeans glycoprotein, which have a negative effect on human body [20]. In this regard, in developing recommendations on using the new non-traditional raw materials for food purposes, it is necessary to conduct detailed studies of their chemical and biochemical composition, which will be the subject of our further research.

Relative biological value of sainfoin seeds

However, in the initial stage, the physiological impact of a product may be assessed by determining its relative biological value (RBV) in experiments *in vitro*, for example, using tested organisms. RBV is an integral indicator of product's physiological effects on a living organism, since it takes into account safety, digestibility, digestibility, the use of metabolism in the processes. The RBV indicator is expressed in percent from standard protein, casein.

RBV of sainfoin seeds powder was assessed by using the test organisms - Tetrahymena pyriformis ciliates, in accordance with the recommendations of A. D. Ignatiev et al.[17]. For comparison, we performed similar studies with grape and spelt seeds.

The results of the research are shown in Table 5.

It has been found that adding sainfoin seeds powder to the nutritional medium has no inhibitory effect on the development of the test organism. With that, as can be seen from Table 5, sainfoin seeds powder is able to satisfy the physiological needs of the protozoa organisms in protein for 58 %, as compared to casein, which is, as compared to other studied samples, higher by a mean of 10%.

CONCLUSION

Analysis of the literature data showed feasibility of research aimed at studying the possibility to use sainfoin seeds and products of their processing in food technology, including confectionery.

As a result of organoleptic assessment, it has been found that milled sainfoin seeds are a light brown flowing fine powder with a pleasant unexpressed specific odor with floral notes. The taste is grassy with the after-taste typical for legumes.

It has been shown that the chemical composition of sainfoin seeds is dominated by proteins (28.7%) and fiber (19.4%). The content of protein in sainfoin seeds corresponds to the lower limit of the variation range for this indicator for soybean seeds (28.0 to 50.0). The fat content is not high, and does not exceed 8%.

The total amount of amino acids in sainfoin seeds powder is 26.94 g/100 g of the product, with the share of the indispensable ones being 37.85%. The limiting amino acid for the protein complex of sainfoin seeds powder is tryptophan (48.0 %).

It has been experimentally shown that sainfoin seeds powder has a more balanced amino acid composition, as compared to wheat flour and peanuts, so it can be considered quite a promising food ingredient, the use of which in food composition helps to adjust the balance of limiting amino acids. Unlike lipid complexes of sunflower seeds and soybeans, where the composition of triacylglycerols is dominated by linoleic (ω-6) and oleic (ω-9) acids, the lipid composition of sainfoin seeds is dominated by linolenic (ω-3) and oleic (ω-9) acids. High (over 40%) content of linolenic omega-3 acid in the oil of sainfoin seeds with sufficiently low (less than 20%) content of linoleic acid ω-6 makes it a promising means of correcting the fatty acid composition of

functional and specialized food products, dietary supplements, and is a valuable raw material for creating pharmaceutical substances and preparations.

It is shown that sainfoin seeds lipid composition, along with triacylglycerols, contains about 40% of related lipids, which are dominated by sterols, aliphatic alcohols, phospholipids and tocopherols.

Using the method of biotesting with the use of Tetrahymena pyriformis ciliates as test organisms, it has been proven that adding sainfoin seeds powder to the nutritional medium has no inhibitory effect on the development of the test organism. The tested organism's physiological need for protein is satisfied for 58 %, as compared to casein.

In general, the studies performed show that the sainfoin seeds powder contains valuable food nutrients consisting of the protein and the lipid complexes, which makes it a promising ingredient for using in food technology, including confectionery.

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