Available online www.ijpras.com

International Journal of Pharmaceutical Research & Allied Sciences, 2020, 9(3):51-56

Research Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Technology of Production and Recipe of Meat Paste with a Protein Supplement

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ABSTRACT

This article was aimed to develop an antioxidant meat paste using a protein supplement from lupine seeds enriched with selenium and obtained by enzymatic hydrolysis. It was established that the content of selenium, flavonoids, and antioxidant activity in lupine seeds when they are germinated in a solution of sodium selenite with the use of red light after three days is significantly higher. Therefore, technology and recipe of meat paste using a protein preparation has been developed. It has been established that the partial replacement of raw meat in the paste formulation with a protein supplement has a positive effect on the appearance, color, smell, texture, taste, and structure of the product. There is proven feasibility of a separate introduction of a protein supplement and an aromatic additive at the stage of cutting raw meat materials. Based on the studies, the storage periods and storage modes of sterilized canned meat and vegetable pastes were established: 18 months, at a temperature from 0 to 20 °C and $\varphi \leq 75\%$, as well as regulated quality indicators of meat products. 100 g of the product contains up to 15% of the daily requirement of an adult in selenium and up to 10% in flavonoids, which makes it possible to attribute the developed paste to antioxidant functional foods. So, it can be recommended for use in the food industry.

Key words: *Meat Paste, Lupine, Flavonoids, Protein Supplement, Selenium, Enzymatic Hydrolysis, Antioxidants.*

INTRODUCTION

In the contemporary food industry, the prospects for the use of lupine seeds [1] are explained by a high content of protein (30 to 50%), fat (20-25%), and carbohydrates (10-26%) [2-4]. It should be noted that of all plant proteins, lupine is most balanced in amino acid composition, it contains glutamic acid up to 26%, aspartic acid and arginine up to 13%, leucine up to 10% [5-7].

However, the quality of the protein of legumes, including lupine, is determined not only by its amino acid composition but by the number of trypsin inhibitors (alkaloids). Lupine seeds are characterized by a low content of proteolytic enzyme inhibitors (2.5 g/kg), compared with soy (29 g/kg) [8, 9]. In plants, true alkaloids are found in a free state, in the form of water-soluble salts and N-oxides (oxides amines). This group of alkaloids forms due to the decarboxylation of proteinogenic and non-proteinogenic amino acids, followed by condensation with non-

nitrogen-containing structural fragments. The main predecessors of true alkaloids are L-ornithine, L-lysine, L-phenylalanine, L-tyrosine, L-tryptophan, and L-histidine [10]. Protoalkaloids, like the previous group of compounds, are considered the derivatives of amino acids, but their nitrogen atom is located outside the heterocycle in the side chain. The precursors of this group of compounds include the amino acids L-tyrosine and L-tryptophan. Pseudo-alkaloids are a group of compounds that differs from the two described above in that in their molecules amino acids are not a biogenetic source of nitrogen. In the case of the terpenoid or steroid structure of the skeleton, N-atom is usually introduced into the structure of the molecule at rather late stages of synthesis. In the presence of the corresponding carbonyl groups (keto and aldehyde groups), the introduction of nitrogen-containing structures into the molecule occurs with two main reactions - amination and transamination [11].

Among the biologically active substances contained in the seeds of lupine, flavonoids are of particular interest [12]. Flavonoids are a large group of polyphenolic compounds that are widely distributed in nature [13, 14]. These are substances that have antioxidant properties and are synthesized only in the plant cell. The formation of flavonoids is a branch of the large phenylpropanoid biosynthetic pathway of higher plants, during which a wide range of secondary metabolites are formed, such as phenolic acids, lignins, lignans, and stilbenes. Flavonoids are synthesized from an aromatic amino acid, L-phenylalanine, obtained through two alternative metabolic pathways: shikimate or polyketide (acetate - malonate) and substances malonyl - CoA, from the tricarboxylic acid cycle (Krebs cycle) [12].

Flavonoids are synthesized in the cytoplasmic matrix, under the influence of various enzyme complexes contained in it. After formation, the final synthesis products are transported and localized as in subcellular structures, with flavonoids playing the role of pigments mainly located in vacuoles. The activation of the processes of synthesis and accumulation of flavonoids, like alkaloids, occurs during budding and flowering [15]. Flavonoids play the role of interceptors of unstable particles with one or more unpaired electrons, which are formed during the life cycle of the cell. By inhibiting the processes of free radical oxidation, they slow down the mechanisms of cellular aging [16].

In view of the foregoing, the protein preparation from lupine obtained by enzymatic hydrolysis was used as a vegetable protein supplement in a meat paste. Due to this, the **aim** of the research is to develop and estimate the quality of an antioxidant meat paste with the use of a protein supplement from lupine seeds enriched with selenium and obtained by enzymatic hydrolysis.

MATERIALS AND METHODS

The enzyme trypsin, lupine seeds, protein preparation from lupine seeds, and paste were used as research materials. Lupine seeds were enriched with selenium by germination in accordance with the requirement of the Russian State Standard. Two groups of seeds were formed. In the first control group, seed germination was carried out without exposure to light, in the second experimental, during germination, the seeds were irradiated for 8 hours daily with red light with a wavelength of 660 nm and a light flux area of 35 μW/cm^2 using the Avers-San Biolamp manufactured by NPK Avers (Moscow) throughout the entire period of the experiment. All test seeds were pre-washed with running water, then soaked for 6 hours at the temperature of 20-22 ºC in a stainless steel container in a solution of distilled water containing 0.2 g/l sodium selenate, transferred to a baking sheet covered with filter paper soaked in a sodium selenite solution in the concentration mentioned above. During the experiment, the filter paper was moistened. The seeds were germinated until the seedlings reached 3-4 mm, then the seeds were washed with running water.

Antioxidant activity was determined by the potentiometric method. The content of selenium was found by atomic absorption spectrometry according to Russian State Standard. Flavonoids were determined by the chromatographic method.

The amount of protein in pastes is regulated according to RF State Standard 25011-2017 “Meat and meat products. Methods for determination of protein.”, the amount of fat was accorded to Russian State Standard “Meat and meat products. Methods for determination of fat.”

The results were processed by the method of variation statistics using Microsoft Excel, and the confidence level was 0.95. The research results are analyzed with the method of analysis of variance with the use of the Student coefficient.

RESULTS AND DISCUSSION

Studies have established that the shoots of the seeds of the experimental group with the use of red light after two days of germination reached a length of 3-4 mm, while the control samples of seeds had shoots of 3 mm in length on the third day of the experiment. The selenium content in the lupine seeds of the experimental group after three days of germination averaged 1036 μg/kg, in the control group - 812 μg/kg. The data obtained indicates a positive effect of red light on the growth of the seed embryo and the enhancement of metabolic processes in the plant cell. The mechanism of enrichment of lupine seeds with selenium is connected with the diffusion process and can be represented as follows: the proteins that make up the cell membranes of legumes form hydrophilic pores that allow small hydrophilic molecules with a relative molecular mass of Mr up to 600 to pass through. Selenium is embedded in amino acids, in particular methionine forming a selenium-methionine compound and others.

The use of red light when germinating seeds is stemmed from the fact that the metabolism of seeds is associated with the activation of the phytochrome photoreceptor, a chromoprotein having a blue-green color. Its chromophore is an unclosed tetrapyrrole. The protein part of phytochrome consists of two subunits. Phytochrome exists in plants in two forms (F660 and F730) that can pass one into another, changing their physiological activity. When irradiated with red light (KS - 660 nm), the F660 phytochrome transforms into the F730 form. Transformation leads to reversible changes in the configuration of the chromophore and the surface of the protein. Form F730 is physiologically active, controls many reactions and morphogenetic processes in a growing plant, metabolic rate, enzyme activity, growth movements, growth and differentiation rates, etc. Phytochrome is involved in the regulation of many aspects of plant life: germination of photosensitive seeds, opening the hook and lengthening the hypocotyl of seedlings, unfolding of cotyledons, differentiation of the epidermis and stomata, differentiation of tissues and organs, orientation in the cell of chloroplasts, synthesis of anthocyanin and chlorine rofilla. Red light lengthens cells. The content of selenium and antioxidant activity of lupine seeds were studied (Table 1).

Table 1. The content of selenium, flavonoids and antioxidant activity of lupine seeds

Parameter	Germinated lupine seeds	
	Control samples	Experimental samples
Selenium, mkg/g	812±14	1036±11
Flavonoids, mg/kg	1768	1789
Antioxidant activity, mm equivalent	1,23±0,05	2,34±0,04

According to the data from table 1, the germination of seeds in a solution of sodium selenate with exposure to red light increases the antioxidant activity in lupine from 1.23 to 2.34 mm equivalent or by 90.2%. This data is connected to the content of selenium in germinated legume seeds. So, the amount of selenium in the seeds of the lupine of the experimental group is higher by 27.6 in comparison with the content of selenium in the control group. It should be noted that in the germinated lupine seeds it ranges from 1768 to 1789 mg/kg, which also determines their antioxidant activity.

We have developed a technology for a protein preparation from lupine seeds enriched with selenium, which includes the following steps:

- removal of the shell from the germinated lupine seeds was carried out according to the procedure [2], by soaking in water with a temperature of 80℃ and adding sodium chloride in an amount of 35 g for every 1000 ml of water (this technology avoids the ingress of the shell (trypsin inhibitors) into the protein preparation);
- grinding to particle sizes of 500-600 microns;
- flour from lupine was poured with distilled water in a ratio of 1:10;
- the introduction of the alpha-amylase enzyme (amylosubtilin) and glucoamylase (glucavamarine) at a temperature of 37℃ for 3 hours;
- centrifugation;
- autoclaving at a temperature of 120-130ºC at a pressure of 6x105 Pa for 5-6 hours;
- cooling to a temperature of 36º C;
- adding a 0.5% trypsin solution in phosphate-buffered saline with a pH of 7.5 and holding for 50-60 minutes;
- centrifugation;
- drying the centrifugate to inactivate trypsin and obtain a protein preparation with a protein content of at least 41%.

The formulation of sterilized paste canned food from meat and protein supplement from germinated seeds of amaranth and lupine enriched with selenium is presented in Table 2.

Table 2. Recipe for meat paste with a protein supplement.

Raw material, kg/100 g	parameter	
	Control samples	Experimental samples
Broiler chicken meat (thigh), mechanical boning	74	60
Chicken fat	8	8
Fried onions	8	8
Chicken bone bouillon	10	10
Protein supplement	-	14
Food salt, g/100 kg of basic raw materials	1300	1300
Flavoring additive “Rosmix Aroma” (Meat flavors: chicken), g/100 kg of raw meat	200	200

The amount of protein preparation in the formulation was 14%, which allows providing 15% of the daily needs of an adult in selenium when consuming 100 g of the finished product. The developed recipe for the product in accordance with TR TS 034/2013 "On the safety of meat and meat products" corresponds to paste canned food.
To stabilize the functional and technological properties of raw meat, protein preparations of plant and animal origin must be introduced at the first stage of cutting and flavoring food additives at the final stage. In this case, in meat systems in which the formation of protein shells around fat has occurred, the effectiveness of introducing aromatic food additives increases, and the product is obtained with an intense pronounced taste and aroma.
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Grinding of meat raw materials was conducted on a top (diameter 3-4 mm; rotation speed 1000-2000 rpm, 10-15 minutes), the final grinding of meat raw materials was carried out using a cutter. Cutting of meat raw materials, chicken fat, and protein preparation was carried out for 10-12 minutes at a rotation frequency of 1800-2000 vol. min, in the process of chopping, made bone chicken broth, at the final stage of chopping (12-20 minutes) salt, fried onions, and flavoring additive "Rosmix Aroma" (Meat flavors: chicken) were introduced. Then the cans were filled with paste mass using a syringe dispenser, rolled up in cans with a filling factor of cans of 0.85. The reproached tin cans with paste were placed in an autoclave, gradually heated to a sterilization temperature of 120 ° C, sterilized for 30 minutes, and later gradually cooled to 35-40°C.
A tasting assessment of the paste samples of the first group (control), the second group (simultaneous introduction of the protein preparation and aromatic additives in the process of raw meat chopping), the third group (separate introduction of the protein preparation and aromatic additives in the raw meat chopping process) was carried out on a 9-point scale.
It has been established that a partial replacement of raw meat in the paste formulation with a protein preparation has a positive effect on the appearance, color, smell, texture, taste, and structure of the product. The total score of the prototypes of the second and third groups of meat and paste pastes is higher than the control by 1.8 and 3.2 points and is 47.0 and 48.4 points. The data obtained indicate that it is advisable to separately introduce a protein preparation and an aromatic additive at the stage of cutting meat raw materials in the production of pastes. For further comparative studies of quality, samples of the developed paste with the separate introduction of a protein preparation and an aromatic additive were used.
The physicochemical parameters of the control and experimental paste samples were measured (Table 3).

Table 3: Physico-chemical indicators of the control and experimental samples of paste

Parameter	Control samples	Experimental samples
Mass fraction of protein, %	14,5±0,3	19,4±0,7

Mass fraction of fat, %	12,1±0,4	11,2±0,3
Selenium contention, mkg/100g	Traces	16,7±0,1
Flavonoids, mg/100g	22,6±0,2	23,4±0,3
AOA, mol equiv/dm^3	1,4±0,2	3,9±0,1
Mass fraction of sodium chloride, %	1,23±0,04	1,14±0,02

From the data of table 3, it is seen that the introduction of a protein preparation in the formulation increases the protein content by 33% and reduces the amount of fat by 8.0%. The content of selenium and flavonoids in the paste test samples is 16.7 μg/100 g and 23.4 mg/100, respectively, which provides up to 15% and 10% of the recommended intake for an adult [17]. Antioxidant activity (AOA) in the paste test samples at a level of 3.9 mol equiv./dm^3, which is higher than the control by 186%.

All studied microbiological indicators of vegetable and meat pastes met the requirements of the TR TS "On the safety of meat and meat products" after production and during storage for 24 months.

Based on the studies, the storage periods and storage conditions of sterilized canned meat and vegetable pastes were established: 18 months. at T from 0 to 20 0C and φ ≤ 75%, as well as regulated indicators of the quality of meat products (Table 4)

Table 4: Regulated quality indicators of sterilized canned food – antioxidant meat pastes.

Parameter	Characteristic/norm
Appearance	Homogeneous finely divided mass with a small amount of melted fat
Taste and smell	Pleasant, usual for this type of product, with the aroma of spices, without extraneous taste and smell.
Color	Pinkish-gray to brownish-gray
Consistency	Paste-like, uniform throughout the mass
Mass fraction of protein,%	19
Mass fraction of fat,%	15
Selenium contention, mkg/100g	15-18
AOA, mol equiv/dm^3	3,5—5,0
Mass fraction of sodium chloride, %	1,3

CONCLUSION

Therefore, based on the results of the present research, the technology of a protein preparation from lupine seeds enriched with selenium was developed. The production of protein preparation is carried out by enzymatic hydrolysis. It is proved that the use of a protein preparation in the formulation of sterilized meat pastes can improve quality and increase nutritional value. It was found that the protein content and antioxidant activity in the developed pastes are higher, and the amount of fat is lower in comparison with the control samples. When 100 g of product is consumed, the daily requirement of an adult for selenium and flavonoids is provided by 15% and 10%, respectively, which allows us to attribute the developed paste to functional food products. The developed technology and the recipe of meat paste with protein supplements can be recommended for use in the food industry.

ACKNOWLEDGMENTS

This research received no external funding. The authors declare no conflict of interest (Kudryashov L., Diachkova A., Tretyakova I., Tikhonov S., Tikhonova N., Mozzherina I.).

REFERENCES

1. Yousef F M. Antihyperglycemic And Antihyperlipidemic Potential of Aloe Vera Against Streptozotocin-Induced Diabetic Rats. Int. J. Pharm. Phytopharm. Res. 2017; 7(5): 41-46.
2. Kurienko, A.I. Kondrashova, O.N., Improving the technology of separation of the shell of seeds of soy and lupine, Journal of VNIIMZH, 2015, 4 (20), pp. 80-83.

3. Pashchenko, L.P., Antipova, L.V., Pashchenko, V.L, The use of amaranth meal and collagen hydrolyzate in the production of gingerbread, Bread products, 2010, 7, pp. 28-30.
4. Pankina, I.A., Borisova, L.M. The study of alkalinity of lupine seeds, NRU ITMO: Processes and Food Production Equipment, 2015, 4, pp. 80-83.
5. Krasilnikov, V.N., Mehdiyev, V.S., Markina, V.Yu., Tymoshenko, Yu.A. Lupine: the creation of functional food products, a contribution to ensuring the country's food safety, Storage and processing of agricultural raw materials, 2015, 8, pp. 43-49.
6. Kiyabaeva, A.A., Zabodalova, L.A. The use of dry and pasty protein preparation from lupine seeds in the technology of fermented milk products, Mechanics and Technologies, 2014, 3 (45), pp. 57-67.
7. Khrulev, A.A., Beschetnikova, N.A. Protein from lupine: technology, application, prospects, Food industry, 2015, 12, pp. 63-65.
8. Nikonovich, Yu.N., Tarasenko, N.A., Boglova, D.Yu The use of lupine seed processing products in the food industry, News of higher educational institutions, Food technology, 2017, 1 (355), pp. 9-12.
9. Villa, C., Mónica Moura, M. B. M. V., Costa, J. and Mafra I. Immunoreactivity of Lupine and Soybean Allergens in Foods as Affected by Thermal Processing, Foods, 2020, 9(3), 254.
10. Aniszewski,T. Alkaloids - Secrets of Life. Alkaloid Chemistry, Biological Significance, Applications and Ecological Role. Elsevier, 2007, 335 p.
11. Dewick, P. M. Medicinal Natural Products. A Biosyn Edition. Chichester - New York: John Wiley & Sons Ltd., 2002, 515 p.
12. Oyvind M., Andersen K., Markham R. Flavonoids: Chemistry, Biochemistry and Applications, CRC Press Taylor & Francis Group, 2006, 1198 p.
13. Cabrido C, Demayo C G. Antimicrobial and cellular metabolic effects of the ethanolic extract of the dallas red variety of lantana camara. Pharmacophores. 2018; 9(1): 10-18.
14. Qusti S Y, Alseeni M N, Alharbi R A, Balgoon M, Jambi E J, Alotaibi S A. Antibacterial activity of selected plants species extract cited in the holy quran against clinical isolates. Pharmacophores. 2018; 8(5): 18-28.
15. Del Rio, D., Borges G., Crozier A. Berry flavonoids and phenolics: bioavailability and evidence of protective effects, Br.J. Nutr., 2010, 104 Suppl 3, pp.67–90.
16. Huang D., Ou B., Prior R.L. The chemistry behind antioxidant capacity assays, J. Agric. Food Chem, 2005, Vol. 53, pp. 1841-1856.
17. Norms of physiological requirements for energy and nutrients for various groups of the population of the Russian Federation. Methodological recommendations 2.3.12432-08, M.: Federal Center for Hygiene and Epidemiology of Rospotrebnadzor, 2009, 36 p.