Agronomy Research **13**(4), 979–991, 2015

Study of functional and technological characteristics of protein concentrates from lupin seeds

L. Kuznetsova[1,*], M. Domoroshchenkova[2] and L. Zabodalova[1]

[1]Institute of Refrigeration and Biotechnologies, ITMO University, Lomonosova street 9, 191002, Saint-Petersburg, Russia
[2]All-Russia Scientific Research Institute of Fats, 10, Chernyakhovsky Street, 191119, Saint-Petersburg, Russia
[*]Correspondence: mamaeva.ludmila@mail.ru

Abstract. The purpose of the research was to study functional and technological characteristics of concentrated protein preparations produced from lupin seeds and to demonstrate a feasibility of their usage as functional ingredients in food products. The method of production of concentrated protein preparations from seeds of *Lupinus angustifolius* was developed based on an acidic water extraction of non-protein compounds of lupin flour in presence of an optimized multi-enzyme composition composed of 1.1 ± 0.2 unit g^{-1} of Celluclast and 5.2 ± 0.4 units g^{-1} of Pentopan Mono and 2.5 ± 0.2 units g^{-1} of Amilosubtilin. It was shown that crude protein content of protein concentrate preparations obtained with enzymatic treatment of polysaccharides increased to (63.2 ± 1.3) % on m.f.b. versus (50.4 ± 1.3) % on m. f. b. in the control preparation produced without an enzymatic treatment. Process parameters of infrared drying of lupin protein concentrate wet residues in the short wavelength range up to 2.5 μm were studied. The comparative analysis of functional and technological characteristics of lupin protein preparations, lupin flour and soy flour was carried out. Water-holding capacity, fat-holding capacity and emulsifying capacity of lupin protein concentrates produced via enzymatic treatment have been increased versus the control sample. The process and the formulation of fermented dairy product with inclusion of lupin protein additives in a range of 0.5% to 2.0% (% w w^{-1}) were developed. Sensory, physico-chemical and structural-mechanical properties of a dairy product enriched with lupin protein were analyzed. It was shown that usage of lupin protein preparations as nutritional supplements was beneficial for human gastrointestinal tract. 1.5% (% w w^{-1}) inclusion level of the lupin preparation in the food formulation provided the best consumer properties of finished products. It was shown that the lupin protein concentrate preparation can be used as a functional ingredient in fermented dairy products.

Key words: lupin protein concentrate, multi-enzyme composition, functional and technological properties, enriched dairy products, functional food ingredients.

INTRODUCTION

Production of vegetable proteins from non-traditional plant sources is one of perspective directions of increase of assortment and volumes of production of valuable protein ingredients for the food industry in Russia. Protein-based preparations can be used in formulations of sports nutrition products, specialized dry mixes, in meat and milk production and in bakery and in confectionery industry. Imported soybeans are used

often for industrial production of protein products. It could result in a high cost of finished products and besides soy protein preparations could be obtained from genetically modified plants (Papavergou et al., 1999; Sipsas, 2003).

In recent years many technological solutions have been developed. It allows to produce quality high-protein products from non-traditional vegetable raw materials (Ocheme and Chinma, 2008; Domoroshchenkova et al., 2009; Iakovchenko and Silantjeva, 2014). In particular, a new technology of isolated vegetable protein has been proposed (Lqari et al., 2002). Studies of the chemical composition and properties of flax seeds for development of functional foods have been conducted (Minevich, 2009).

Usage of additives produced from narrow-leaf lupin seeds in formulations of products from blended raw materials and in bakery, meat and dairy products analogues could be regarded as an innovative direction in food industry (Krasilnikov et al., 2010; Osman and Razig, 2010; Vij et al., 2011).

Study of functional and rheological properties of lupin proteins such as solubility, gel-forming capability, water-holding capacity, fat-holding capacity, foaming capacity and stabilizing properties etc. has shown that lupin proteins are very similar to soy proteins (Hojilla-Evangelistaa et al., 2004; Khalil et al., 2006). The rheological properties of the defatted white lupin flour suspension and viscoelastic properties of lupin proteins obtained by ultrafiltration have been studied at the Illinois National Center for Agricultural Utilization Research. The behavioristic characteristics of these systems during deformation are similar to the behavior of wheat gluten proteins under similar conditions (Xu and Mohamed, 2003; Xu et al., 2006).

However, lupin protein isolate (LPI) has formed weaker and more deformable gels versus gels formed by soy protein isolate presumably due to a large amount of free sulfhydryl groups which were increased upon heated (Berghout, J.A.M. et al., 2015).

This study consideres usage of additives produced from food grade lupin seeds as an import nutritional and functional substitute due to an increase of protein content and improvement of functional and technological properties of final protein preparations and due to an increase of nutritional and biological value of enriched products as well as on basis of an improvement of structural and mechanical properties of enriched dairy products.

The presence of some anti-nutritional components and a high level of structural and storage polysaccharides in lupin seeds necessitate the use of methods that reduce its amount in final products (Ferket and Middleton, 1998, Lott J et al., 2000; Yuliarti et al., 2011). This problem is solved by the use of enzyme preparations with different substrate specificities that ensure a higher solubility of lupin polysaccharide complex.

The enzymatic degradation of polysaccharides causes a modification of functional and technological properties of protein preparations, such as water-holding capacity, fat-holding capacity, fat emulsifying capacity and gel-forming capability etc., which provides an opportunity to use them as a functional agent in food media (Damodaran et al., 1989; Enujiugha et al., 2003).

The objective of the study is to develop a method of production of lupin protein concentrate including a stage of an enzymatic degradation of polysaccharides, to demonstrate possibilities of lupin protein concentrate usage as a food additive and to develop a process and a formulation of enriched fermented dairy product.

MATERIALS AND METHODS

Materials

Lupin flour was kindly provided by the Russian Research Institute of Lupin, Brynsk. It was produced by grinding of dehulled lupin seeds of *Lupinus angustifolius* of 'Snezhet' variety planted in Brynsk origin (Russia). It contained 46% of crude protein on moisture free basis (m.f.b.) and 7% of crude fat on m.f.b.

For enzymatic treatment the following enzyme preparations have been used:

- Cellolux-F – an enzyme preparation that contains complexes of cellulase with activity of 2,000 ± 200 units g^{-1}, xylanase with activity up to 8,000 units g^{-1}, glucanase with activity up to 1,500 units g^{-1}. The preparation catalyzes the breakage of cellulose, xylans, β-glucans of plant cells up to sugars. The enzyme preparation was supplied by 'Sibbiopharm', Russia;
- Amilosubtilin – an enzyme preparation containing a complex of amylolytic enzymes, as well as related enzymes: β-glucanase, xylanase, glucoamylase, protease. Activity: 1,500 units g^{-1}. The enzyme preparation was supplied by 'Sibbiopharm', Russia;
- Celluclast BG – cellulase preparation made by submerged fermentation of the selected strain of fungus *Trichoderma reesei*, containing 3,500 endoglucanase units $gram^{-1}$. Preparation was provided by Novozymes, Denmark;
- Pentopan Mono BG – xylanase preparation from fungi *Aspergillus oryzae*, containing 2,500 fungal xylanase units $gram^{-1}$, Novozymes, Denmark.

A fullfat soybean flour sample for comparative studies was provided by the All-Russia Scientific Research Institute of Fats. It contained 42% of crude protein on m.f.b., 18% crude fat on m.f.b.

The yogurt starter culture of *Streptococcus salivarius ssp. thermophilus and Lactobacillus delbrueckii ssp. bulgaricus* were provided by the All-Russia Scientific Research Institute of Fats, Russia.

Preparation of the lupin concentrates

A method of soy protein concentrate production via acid leaching of non-protein substances at isoelectrical pH value of proteins (Sair, L. 1959) from soybean meal or flour has been used on an industrial scale. At the first stage of the study a similar technique has been used and adjusted for obtaining of protein concentrates from lupin flour and optimal conditions of extraction of non-protein compounds of lupin flour in acidic medium have been investigated (Kuznetsova et al., 2014).

A sample of lupin flour was mixed with water in ratio of 1:15. pH of this mixture was adjusted to 4.5 with 5% HCL solution at constant stirring at temperature 55°C. Then a multi-enzyme composition composed of (1.1 ± 0.2) units g^{-1} of Celluclast + (5.2 ± 0.4) units g^{-1} of Pentopan Mono + (2.5 ± 0.2) units g^{-1} of Amilosubtilin was added. The suspension was stirred at constant temperature and pH in the range 4.4-4.5 for 40 minutes. The resulted suspension was centrifuged at 3,500x *g* for 15 min. and separated into two phases- a solid residue and whey. Crude protein was analyzed in a solid phase and it was used as an indicator of an efficiency of bioconversion of carbohydrates of initial lupin flour. Total sugar content was determined in lupin whey.

The wet residue of lupin protein concentrate was subjected to infrared radiation at short wavelengths from 2.0 microns to 2.5 microns at the density of IR emitter heat flow of 2.84 kW $(m^2)^{-1}$ (Demidov et al., 2011). The width of a layer of the wet lupin concentrate residue was 4–5 mm. The distance from the IR emitter to the layer varied in the range of 60 to 80 mm. The drying was performed to reach up to 10% moisture content of the product. A fine powder of lupin protein concentrate passing through a sieve No.80 (0.178 mm) was obtained. It was used in subsequent experiments as a functional ingredient.

Preparation of the enriched fermented product

The study of dairy products technology with use of lupin protein concentrate as a functional ingredient was based on a method of industrial production of dairy products.

Samples of dairy products were obtained by water dilution of powered skimmed milk up to 3% dry matter content at 45°C and at constant stirring. Then the resulted milk was cooled up to 6°C and left for three hours in order to restore the natural structure of milk (normal density to achieve a due protein swelling and to eliminate a watery taste). Then the milk was pasteurized and cooled up to the fermentation temperature. Then 0.5% to 2% w w^{-1} additives of dry lupin protein preparation to the weight of the mixture were added with an interval of 0.5% w w^{-1}. The mixture was thoroughly stirred for 10–15 minutes to obtain a uniform distribution of particles. At the next stage a yogurt starter was added and the mixture was fermented in a thermostat. Active and titratable acidity of the mixture as well as organoleptic and structural and mechanical properties of the system were monitored every hour. When the clot achieved a well-formed structure (and the value of titratable acidity ranged from 75°T to 80°T), the fermentation process was stopped. Samples of the enriched fermented product were cooled up to 4–6°C. The water holding capacity of samples was measured and the viscosity and organoleptic properties were evaluated.

Methods

Moisture content in the lupin protein concentrate was determined by the gravimetric method (AOAC, 1998). Crude protein content was analyzed by Kjeldahl method at the automated analyzer Kjeltec Auto 1030, Sweden, according to the standard protocol of the equipment vendor. The conversion factor used to estimate protein content was N x 6.25. Changes in pH were measured with Orion 920A pH-meter (Russia). Analysis of the total content of water-soluble carbohydrates in lupin whey was conducted by the Bertran method.

An IR dryer (Vympel, Russia) was used to dry the lupin protein preparation.

For determination of the water-holding capacity the sample of protein preparation was weighted and stirred with distilled water (1 g per 30 ml of water) at 1,000 rpm then the suspension was centrifuged at 3,000 rpm. A supernatant was decanted and a tube with a wet residue was weighed. Water holding capacity (WHC) was determined by the following formula (1):

$$WHC = (C - B)\ 100/A\ , \quad (1)$$

where: W - amount of water retained by 1 g of the sample, %; A - weight of sample, g; B - weight of centrifuge tube with a dry sample, g; C - weight of the centrifuge tube with a wet residue, g.

For determination of the fat-holding capacity (FHC) the sample of protein preparation was weighted and stirred with refined bleached deodorized sunflower oil (5 g per 30 ml of oil) at 1,000 rpm for one minute and then the mixture was left for 30 minutes. Then the suspension was centrifuged for 25 minutes at 3,200 rpm. Free oil was carefully decanted and a tube with a residue was weighed. The fat holding capacity was determined by the formula (2):

$$FHC = (A–B)\ 100/C\ , \quad (2)$$

where: A - weight of centrifuge tube with sample and retained oil, g; B - weight of centrifuge tube with sample, g; C - weight of sample, g.

For determination of the fat emulsifying capacity (FEC) the sample was weighted and stirred with distilled water (7 g per 100 ml of water) at 4,000 rpm for one minute. Then 100 ml of refined bleached deodorized sunflower oil were added to the mixture and stirred in a high-speed mixer at 8,000 rpm for 5 minutes. The final mixture was poured into calibrated centrifuge tubes and centrifuged for 5 minutes at 2,000 rpm. The volume of an emulsified layer and total volume of a mixture were measured. The fat emulsifying capacity was determined by the formula (3):

$$FEC = V_e\ 100/V_o\ , \quad (3)$$

where: V_e - volume of the emulsified layer, cm^3; V_o - total volume of the mixture, cm^3.

For determination of the critical gel concentration (CGC) 10 homogeneous suspensions of the protein preparation in distilled water were prepared with concentration of 30%, 31%, etc. at intervals of 1%. Suspensions were transferred into 10 g centrifuge tubes and placed in a water bath and incubated for 30 minutes at temperature of 90 ± 2°C. Then the mixtures were rapidly cooled with a tap water to temperature 25°C, placed in a refrigerator and kept for 16 hours at temperature 4–6°C. The lead ball (m = 0.5 g) was placed on the surface of the suspension and left for 2 hours at temperature 4–6°C (Mahotina, 2009). The concentration of the sample when there was no destruction of gel structure under the pressure of the lead ball was considered as CGC.

The dynamics of acid accumulation during fermentation milk product was determined based on the values of titratable acidity-TTA (IDF Standard 86:1981 ISO 6091:1980).

Water holding capacity of coagulates was determined by centrifuging of samples for 30 min and determining the precipitated whey volume every 5 min.

Determination of structural and mechanical properties of clots was carried out on the ‘Rheotest-2’ rotational viscometer using a measuring cylinder N. To determine the

viscosity loss coefficients (Lv), the degree of recoverability of the structure (Rs), and the coefficient of mechanical stability (MS), the following formulas were used (4; 5):

$$Lv = 100(\eta_{first}-\eta_{last})/\eta_{first}, \quad (4)$$

where η_{first} - initial viscosity of the intact structure (viscosity at the time of the device switching on); η_{last} - viscosity of the most broken structure (the last measurement);

$$MS = \eta_{first}/\eta_{last}; \; Rs = 100\,\eta_r/\eta_{first}, \quad (5)$$

where: η_r – viscosity measured after structure recovery for 15 min.

Statistical evaluation of the data

All experiments were performed in with three replicates at least; data were processed by methods of mathematical statistics at theoretical frequency 0.95. Statistical processing of data was carried out using computer programs Microsoft Office Excel 2010 and Mathcad 15.0.

RESULTS AND DISCUSSION

Preparation of lupin protein concentrates using multi-enzyme composition

The objective of this stage of the study was to prove the working hypothesis that the enzymatic degradation of lupin polysaccharides would result in an increased concentration of proteins in a solid phase as well as in a more dynamic transition of soluble substances into whey.

First of all, a technology was developed that allowed to produce the lupin protein concentrate using an alternative multi-enzyme composition in optimum conditions for extraction of non-protein compounds (Kuznetsova and Zabodalova, 2013). In order to produce the alternative multi-enzyme composition specific to polysaccharides of the original lupin flour individual enzyme preparations were used such as Celluclast BG, Pentopan Mono BG and Amilosubtilin. The experiment results were compared with those obtained using the Cellolux-F complex cytolytic system (Table 1).

Table 1. Characteristics of lupin concentrate and lupin whey

The name of the enzyme	Crude protein content of concentrate, % on m.f.b.	Moisture content of concentrate, %	Dry matter content of whey, %	Total sugars content of whey, % on m.f.b.
Negative control sample	50.40 ± 1.30	75.22 ± 1.06	1.30 ± 0.36	10.10 ± 0.31
Cellolux-F	54.12 ± 1.10	78.31 ± 0.42	1.90 ± 0.22	27.00 ± 0.20
Multi-enzyme composition	63.17 ± 1.30	77.48 ± 1.10	2.20 ± 0.23	29.00 ± 0.25

At addition of the multi-enzyme composition the total sugar content of the lupin whey has reached maximum content of 29% on m.f.b. The crude protein content of the protein concentrate was increased to (63.2 ± 1.3) % on m.f.b., i.e. 9% higher than in case of usage of the Cellolux-F complex cytolytic system.

Protein concentrates drying

Wet paste of lupin protein concentrate was placed into the dryer chamber, applied in a thin (4–5 mm) layer on the base plate and exposed to the short-wave (2.0–2.5 microns) infrared radiation (Fig. 1).

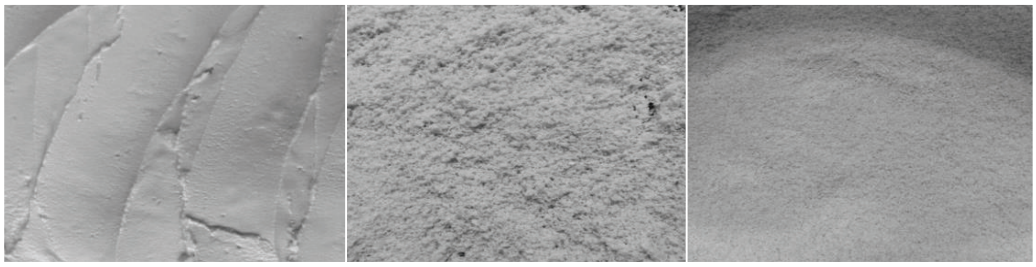

Figure 1. Drying of lupin protein concentrate. *a* – a wet paste immediately after placing in the IR-dryer camera, *b* – after 4 h of drying, *c* – after 5 h of drying.

It was important to find an appropriate balance between the drying time and the distance from the IR emitter to a layer of the product preventing a thermal denaturation of proteins.

The temperature on the surface layer was held at 42°C. The drying process was stopped at an average moisture content of the product of 10%. The drying time depended on the distance between an infrared emitter to the layer of protein concentrate. It was adjusted within the range of 60–80 mm. The final product drying time was 5 hours for the selected distance from the IR-emitter to the product layer of 60 mm. In case of increasing of the distance up to 80 mm the drying time was increased up to 6.5 hours.

Study of functional and technological characteristics of lupin protein preparation (LPP)

The functional and technological properties the following protein additives were analyzed: lupin flour, fullfat soybean flour, lupin protein preparation obtained without enzymatic treatment (negative control) and lupin protein preparation obtained with enzymatic degradation of polysaccharides (Table 2).

Table 2. Functional and technological characteristics of protein concentrates, lupin flour and soybean flour

Sample	Type of treatment	WHC, %	FHC, %	FEC, %	CGC, %
Soybean flour	-	171 ± 20	75 ± 5	52 ± 1	39 ± 1
Lupin flour	-	210 ± 15	97 ± 5	65 ± 1	35 ± 1
LPP	negative control sample	317 ± 15	209 ± 5	87 ± 2	35 ± 1
LPP	cellolux-F	353 ± 20	267 ± 7	92 ± 2	37 ± 1
LPP	multi-enzyme composition	360 ± 17	312 ± 7	97 ± 1	39 ± 1

According to Table 2 functional and technological properties of the tested sample of fullfat soybean flour were worse than of the samples of lupin flour and lupin protein preparations. On the other hand WHC of industrial samples of soy protein isolates could much higher than of lupin preparations and reach up to 500–600% (M. Domoroshchenkova et al, 2007).

WHC, FEC and FHC of the tested protein preparations were consistently increased in the following sequence: lupin flour < LPP (control) < LPP (produced with the Cellolux-F enzymic preparation) < LPP (produced with multi-enzyme composition). This may be explained by a gradual increase of crude protein content in the protein preparations according to the mentioned sequence and due to modification of spatial structure of proteins (alteration of the charge of protein globules, bounds release, hydrophobic properties modification, α-helix unfolding etc.) which leads to improvement of functional and technological properties of the preparations. However the gel-forming capability was lower for enzyme treated lupin samples which could be related with a breakage of protein-polysaccharide bonds, partial proteolysis and polysaccharide complex degradation caused by action of enzymes. As known the structural modification of proteins leads to the decrease in the ability to form the gel spatial grid (Schwenke et al., 1998). CGC values of lupin protein concentrate in the range of concentrations of 30–40% are characteristic for a weak gelling agent.

As a result of enzymatic degradation of polysaccharide complex at the stage of extraction of protein-free compounds from lupin flour the functional and technological properties of the finished protein preparation were improved as compared to those of the raw material sample as well as to those of the control sample. WHC of the concentrate preparation obtained with multi-enzyme treatment was 43% higher, FHC – 103% and FEC – 10% higher compared to corresponding functional characteristics of the control concentrate sample.

Analysis of organoleptic and physico-chemical parameters of enriched fermented foods

Values of active and titratable acidity (TTA) of samples of fermented milk products with the additives of the lupin protein preparation were assessed immediately after addition of the starter population (0 hr), after 2 hours and thereafter every hour of the experiment. Dynamics of acid accumulation of samples of dairy products enriched with lupin protein concentrate is given in Table 3.

Table 3. The effect of LPP mass fraction on the acid accumulation dynamics

LPP, %	Time, h					
	0	2	3	4	5	6
	Titratable acidity , °T					
-	21 ± 1	42 ± 1	55 ± 1	70 ± 1	83 ± 1	85 ± 2
0.5	21 ± 1	42 ± 1	55 ± 2	71 ± 1	82 ± 1	85 ± 2
1.0	22 ± 2	44 ± 1	58 ± 2	73 ± 2	85 ± 1	87 ± 1
1.5	23 ± 1	48 ± 2	60 ± 2	78 ± 2	86 ± 1	90 ± 1
2.0	25 ± 1	50 ± 2	62 ± 1	80 ± 1	90 ± 2	90 ± 1

Introduction of the protein preparation in amount of 1% and above slightly increased the initial value of TTA of the mixture (at 0 hours) due to the acidic pH value of the initial protein preparation associated with the isoelectrical processing technique.

It was used the water extraction of non-protein compounds, which led to pH in an acidic range in finish product.

The total fermentation time was 6 hours, and the control production of TTA (without LPP) reached 85°T. As the concentration of LPP in the mixture increased, the value of TTA increased at the respective stages of the experiment. In the case of sample with 2% of LPP value of TTA increased to 90°T (at 6 h).

At selected stages of the experiment, organoleptic characteristics of mixtures were assessed. Addition of 0.5% of LPP did not lead to any noticeable change of values. Enriched dairy products were homogeneous of viscous consistency. But upon increasing of LPP up to 2% the whey was separated and the product color was changed from milky white to creamy. Samples of products with LPP of 1.5% and 2.0% had a slight beany flavor.

The ability of milk clots to retain moisture could provide indirect evidence of structure formation processes in the system. Thus samples with a fairly solid clot had a minimum amount of separated whey and respectively a high WHC. Control samples had the lower WHC (Fig. 2).

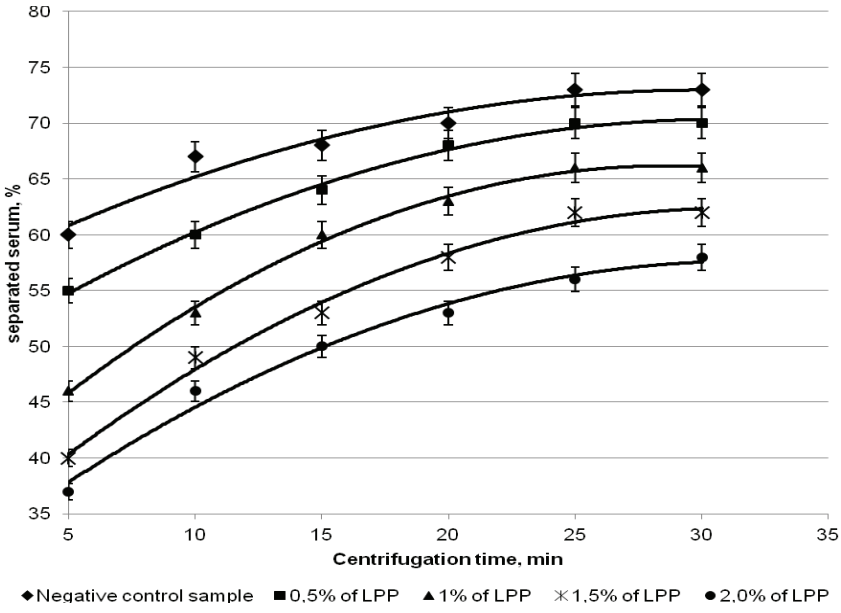

Figure 2. Characteristics of water holding capacity of clots.

A gradual increase of the content of LPP in the mixture resulted in a corresponding increase in water holding properties of clots. This is associated with the growing concentration of plant proteins in the systems which are characterized by high WHC.

Study of rheological properties of enriched fermented food samples

The rheometry of these food media was performed at room temperature using the 'Rheotest 2' rotational viscometer. On selected stages of the fermentation process (at 0, 2, 3 hours, etc.) the flow properties of fluids were measured in strictly defined conditions provided by the universal viscometer construction. An examined food medium was placed in an annular gap between the rotating inner cylinder and the outer cylinder remaining stationery. Measurements of viscosity expressed by the effective viscosity coefficient were carried out at constant shear rate of 27 s^{-1} (Fig. 3).

The initial viscosity of the samples with LPP dosage of 0.5%; 1.0% to 2.0% were measured immediately after inoculation of the starter (0 h). Then the samples were placed into a thermostatic chamber for fermenting. Spatial network of gel formed by dairy and plant proteins gradually began to develop. After 2 h of fermentation, it was possible to measure the first visible changes in the structural and mechanical properties of samples. For this purpose the samples were taken out from the thermostat and their effective viscosity at the selected constant shear rate was measured. Shear rate of 27 s^{-1} was selected from the range of available shear rates on the device (from 3 to 1312 s^{-1}), which allowed to measure the viscosity in the range required for monitoring of structure formation of clots.

Alongside with the increase of the duration of the fermentation process complex formation of clots structure took place in the samples of enriched fermented milk products together with a growth of viscosity.

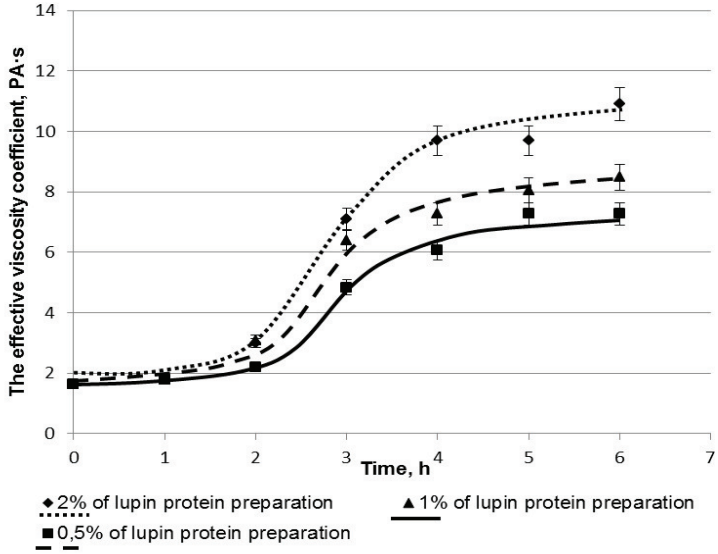

Figure 3. Modification of viscous properties of samples during fermentation process.

In diagrams we can identify the main phases of the formation of spatial structure of clots: induction phase, coagulation phase and metastable equilibrium phase. Upon increasing of lupin protein concentrate dosage in the composition of clots up to 2% their density and viscosity increased as well.

After completion of the fermentation process milk products were cooled up to 4–6°C and properties of finished products were evaluated. The viscosity properties of the products were evaluated by the hysteresis loop method. For that purpose the samples were tested under different shear rates in the range from 3 to 1312 s^{-1} with a gradually increasing and decreasing velocity gradient. Addition of 0.5% of LPP didn't influence the rheological characteristics of the product. The sample with 2% LPP had a more viscous structure; however the thixotropic recovery capacity was slightly worse than of the control sample, which was probably due to increased number of fragile irreversibly destroyed bonds.

The studied food systems have expressed thixotropic properties, for numerical expressions the estimated coefficients were used. Rheometry was performed at a uniform shear area during 2 minutes at intervals of 15 s at a shear rate of 27 s^{-1} (Table 4).

Table 4. The structural and mechanical properties of enriched dairy products

LPP, %	Coefficients		
	Lv, %	MS	Rs, %
-	54.7 ± 1.4	2.20 ± 0.15	70.1 ± 3.1
0.5	54.9 ± 1.3	2.20 ± 0.11	68.5 ± 3.2
1.0	54.9 ± 1.1	2.20 ± 0.10	62.0 ± 3.6
1.5	55.2 ± 2.1	2.21 ± 0.13	59.5 ± 3.2
2.0	61.5 ± 2.3	2.60 ± 0.14	57.7 ± 3.1

According to Table 4 the structural and mechanical properties of systems with LPP concentrations of 0.5%, 1.0% and 1.5% were the same with the properties of the control sample. However in case of the increased LPP concentrations the clots have lost the structure recovery ability. This is proved by the increase of the coefficient of mechanical stability (MS) up to 2.60 for the sample with 2.0% LPP.

The sample of fermented dairy product with 2.0% LPP had the worst structural and mechanical properties, its viscosity loss had reached 6.8% compared to the control product, while MS had increased by 18% indicating the change of the ratio of coagulation-condensation bonds (more elastic) and condensation-crystallization bonds (more fragile due to the formation of chemical bonds) in the clots with the growth of the last-mentioned ones.

Based on a series of experiments carried out we can conclude that the maximum amount of the protein concentrate that can be used is 1.5%, which will provide a fermented dairy product with good organoleptic and structural and mechanical characteristics, with a total protein content of 4% inclusive 1% of plant proteins.

CONCLUSIONS

A process of the effective concentration of LPP using a multi-enzyme composition was investigated. Crude protein content of the obtained lupin protein concentrate has reached (63.2 ± 1.3)% on m.f.b. while crude protein content of the control product was (50.4 ± 1.3)% on m.f.b.

The infrared drying parameters for the wet paste of LPP have been set within shortwave range up to 2.5 microns. As a result, a fine beige powder has been obtained which can be used without limitations for various applications in food industry.

Functional and technological properties of the preparation have been examined and it has been shown that the process of enzymatic degradation of polysaccharides leads to

a modification of spatial structure of protein molecules accompanied by an increased ability of protein to manifest the water holding capacity, fat holding capacity and ability to emulsify fats. The critical gelling concentration of the lupin protein preparation has been determined. The functional and technological properties of the preparation have been compared with the samples of fullfat soybean flour and of lupin flour.

The technology of enriched dairy products has been proposed. The organoleptic, physico-chemical, structural and mechanical properties of clots produced with 0.5%, 1.0%, 1.5% and 2.0% LPP additives have been examined. The effective LPP concentration which ensures better organoleptic and structural and mechanical properties of the products has been determined.

Thus LPP used as a functional ingredient is able to increase the biological value of feedstock, as well as to improve its composition. Total protein content in the final fermented dairy product is increased up to 4 g per 100 g of product; fat content reaches 0.4 g fat per 100 g, carbohydrates content reaches 5.1 g per 100 g, including fiber content up to 0.1 g per 100 g. The energy value of the new developed product is increased by 26% compared to a non-enriched dairy product. At a regular use of this product dietary fibers added to a daily diet will contribute to a beneficial effect on the gastrointestinal tract of a man.

ACKNOWLEDGEMENTS. This work was partially financially supported by Government of Russian Federation, Grant 074-U01.

REFERENCES

AOAC. 1998. *Official Method of Analysis*. Association of Official Analytical Chemists (16th Edn), Washington DC, USA.

Berghout, J.A.M.; Boom, R.M.; Goot, A.J. van der. 2015. Understanding the differences in gelling properties between lupin protein isolate and soy protein isolate. *Food Hydrocolloids* **43**, 465–472.

Bertrand., G. & Thomas, P. 1910. *Guide pour les manipulations de chimie biologie*, Dunod et Pinat, Paris, 468 pp.

Damodaran S. J. Kinsella, W. Soucie. 1989. Interrelationship of molecular and functional properties of food proteins. *Food Proteins. Am. Oil Chemists' Soc.,* 21–51.

Demidov A.C., Voronenko B. A., Demidov, S. F. 2011. Drying sunflower seeds with the help of infrared radiation. *New technologies* **3**, 25–30.

Domoroshchenkova M.L, Demyanenko T. F., Kamysheva I. M., Spetsakova I. D., Stoykova V. Y., Kuznetsova O. I. 2007. The study of functional and technological properties of isolates of soy proteins. *Oil and fat industry* **4**, 24–28.

Domoroshchenkova M.L., Eggi E.E., Mekhtiev V.S., Demyanenko T.F. 2009. Narrow-leaf lupin as a perspective source of food protein. *Storage and Processing of Agricultural Raw Materials* **10**, 53–56.

Enujiugha V.N., Badejo A.A., Iyiola S.O., Oluwamukomi M.O. 2003. Effect of germination on the nutritional and functional properties of African oil bean (Pentaclethra macrophylla Benth) seed flour. *Food agriculture and environmental* **1**(3-4), 72–75.

Ferket, P. R. and Middleton, T. 1998. Antinutrients in poultry feeds. *Proc. World Poult. Sci. Assoc. 10th Europe Poultry Conference.* Jerusalem, Israel, 43–52.

Hojilla-Evangelistaa, M.P., Sessa, D.J. & Mohamed, A. 2004. Functional Properties of Soybean and Lupin Protein Concentrates Produced by Ultrafiltration-Diafiltration. *JAOCS* **81**, 1153–1157.

Iakovchenko, N., Silantjeva, L. 2014. Vegetable ingredients in soft cheese made from concentrated skim milk by ultrafiltration. *Agronomy Research* **12**(3), 717–726.

IDF Standard 86:1981 ISO 6091:1980.

Khalil, A.A., Mohamed, S.S., Taha, F.S. & Karlsson, E.N. 2006. Production of functional protein hydrolysates from Egyptian breeds of soybean and lupin seeds. *African Journal of Biotechnology* **5** (10), 907–916.

Krasilnikov, V.N., Mehtiev V.S., Domoroshchenkova, M.L., Demyanenko T.F., Gavrilova I.P., Kuznetsova L.I. 2010. Prospects for the use of protein from the seeds of blue lupine domestic breeding in the gluten-free pastry products. *Food Industry* **2**, 40–43 (in Russian).

Kuznetsova, L., Zabodalova, L., Baranenko, D. 2014. On the potential of lupin protein concentrate made by enzymatic hydrolysis of carbohydrates in dairy-like applications. *Agronomy Research* **12** (3), 727–736.

Kuznetsova, L., Zabodalova, L. 2013. Study of high-protein products of a complex raw material composition technology on the lupin basis. *Chinese-Russian conference Nutrition and special foods in extreme conditions* **9**, 31–36 (in Russian).

Lott, J.N.A., Ockenden, I., Raboy, V. & Batten, D. 2000. Phytic acid and phosphorus in crop seeds and fruits: a global estimate seed. *Science Research* **10**, 11–33.

Lqari, H., Vioque, J., Pedroche, J. & Millán, F. 2002. Lupinus angustifolius protein isolates: chemical composition, functional properties and protein characterization. *Food Chemistry* **76**, 349–356.

Mahotina, I.A. 2009. The formation of consumer properties of lupin protein preparation. *The abstract of dissertation*, *Moscow*, 7–10.

Minevich, I.E. 2009. The technological solutions for processing flax seed to create functional foods. *The abstract of dissertation*, *Moscow*, 2–5

Ocheme, O., Chinma C. 2008. Effects of soaking and germination on some physicochemical properties of millet flour for porridge production. *Journal of Food Technology* **6**(5), 185–188.

Osman, M.M.D., & Razig, K.A.A. 2010. Quality Attributes of Soy-yoghurt During Storage Period. *Pakistan Journal of Nutrition* **9** (11), 1088–1093.

Papavergou, E. J., Bloukas, J. G. & Doxastakis, G. 1999. Effect of lupin seed proteins on quality characteristics of fermented sausages. *Meat Science* **52**, 421–427.

Sair, L. 1959. *Proteinaceous soy composition and method of preparing*. US Patent 2,881,076.

Schwenke, K., Dahme, A., Wolter, Th. 1998. Heat-induced gelation of rapeseed proteins: effect of protein interaction and acetylation. *J. Am. Oil Chem. Soc* **75**, 83–87.

Sipsas, S. 2003. Protein concentrates and isolates. *Seeding a Future for Grains in Aquaculture Feeds* **28**, 7–9.

Vij, S., Hati, S. & Yadav, D. 2011. Biofunctionality of Probiotic Soy Yoghurt. *Food and Nutrition Sciences* **2***,* 502–509.

Xu, J., & Mohamed, A.A. 2003. Thermal and rheological properties of Lupinus albus flour. *Journal of the American Oil Chemists Society* **80**(8), 763–766.

Xu, J., Mohamed, A.A., Hojilla-Evangelista, M. P., & Sessa, D. J. 2006. Viscoelastic properties of lupin proteins produced by ultrafiltration-diafiltration. *Journal of the American Oil Chemists' Society* **83**(6), 553–558.

Yuliarti, O., Matia-Merino, L., Goh, K.K., Mawson, J.A. & Brennan, C.S. 2011. Effect of Celluclast 1.5L on the Physicochemical Characterization of Gold Kiwifruit Pectin. *Int. J. Mol. Sci.***12**, 6407–6417.