Agronomy Research **13**(4), 1010–1019, 2015

Development of cheese product with hydrolyzed soybean emulsion

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Abstract. The expediency of production of food products based on complex raw material compositions is currently proved. According to the modern concept of a healthy nutrition it is important to optimize the composition of the product considering the content of biologically valuable substances in raw materials during a development of such products. This paper deals with the studies demonstrating an option of usage of soybean emulsion as a raw material of plant origin for development of a high-protein food product similar to a soft cheese. Soybean food emulsion (SFE) was developed and produced by All-Russian Scientific Research Institute of Fats. For minimizing activity of antigenic factors of soybeans (such as glycinin and β-conglycinin) SFE was subjected to partial proteolysis by a complex enzyme preparation. At the Department of Milk Technology and Food Biotechnology of ITMO University recipes and technology of a cheese product with HSFE were developed. Experimental samples were prepared with different doses of HSFE in recipes. The degree of hydrolysis of the HSFE was varied from 6% to 12% with intervals of 2%. Amino acid score for essential amino acid was calculated, the absence of limiting amino acids in developed products confirms their high biological value.

Key words: cheese product, soybean emulsion, hydrolyzed soybean emulsion, degree of hydrolysis, biological value of protein, amino acids.

INTRODUCTION

Nowadays more attention is paid to development and production of food products based on scientific requirements to human diets. However a balanced diet of a modern man is a compromise between limited possibilities of a society and/or a person in the supply of food and of the theory based optimal nutrition. Most often it could be explained by a limited availability of some foods or their high cost. It's especially true for proteins which demand could be satisfied by consumption of meat, fish and in many cases–of dairy products.

Special attention should be paid to high-protein dairy products–cheeses. According to the recommendations of the Ministry of Health and Social Development of the Russian Federation (August 2, 2010, N 593n) per capita cheese intake should be 6 kg per annum (for all types of cheeses including processed cheeses and cheese products). The cheese demand in the RF is 876,000 tons (based on the country's population in 2014). Russian producers release to the market up to 350,000 tons of cheeses per year

(data of 2014). Therefore physiologically recommended level of cheese consumption in Russia could be satisfied up to 40% only by the domestic cheese production not taking into account export volumes of these products. Thus the share of imported cheese exceeds 50%.

Currently volumes of production of soft cheeses in Russia are higher than volumes of hard cheeses. This is connected with a lower dosage of milk per unit of finished product and an absence of a long cheese ripening period which results in an accelerated turnover of funds and in a reduction of labor expenditure while lowering production expenses. Soft cheeses can be sold without ripening and they have a high biological value (Yakovchenko & Silantjeva, 2014).

However it is known that in any cheese manufacturing process the smaller part of the initial raw material mixture (about 20% of milk) is converted to a cheese and the majority of mixture (about 80%) is converted to byproducts–namely a whey. Thus raw materials expenses for the production of high-protein foods are essential. Therefore the purpose of this study was to select a combination of raw materials of animal or vegetable origin for development of a cheese product. A process of selection of perspective ingredients was associated with a number of objectives including the optimization of the biological value of the product and reaching of the economic efficiency of its industrial processing.

Soybeans are reaching in proteins and contain a number of biologically active compounds which could contribute to nutritional value and health benefits of finished products (Medic et al., 2014). It encourages food manufacturers to develop new soy products and to incorporate more soy additives in food formulations. Over the years the All-Russian Scientific Research Institute of Fats was conducting complex studies on development of processing technologies of various soy products in particular of soybean emulsions for edible applications (TS 9146–166–00334534–97) (Domoroshchenkova et al., 2006).

Soybean food emulsion (SFE) is a product of processing of whole soybeans. The quality parameters of the SFE must meet the following requirements according to TS:

- dry matter content min. 15.0%;
- crude protein content min. 6.0%;
- crude fat content min. 2.3%;
- pH 6.5–7.0.

SFE is rich in vitamins and minerals as it contains in average: 41.55 mg calcium, 105.60 mg phosphorus, 0.27 mg potassium, 0.74 mg zinc, 0.26 mg ascorbic acid, 0.24 mg niacin, 0.13 mg thiamine, 0.13 mg riboflavin, 0.06 mg vitamin B6 per 100g of SFE et al. Besides, SFE contains other important dietary components such as dietary fibre in amount of 0.9 g per 100g product. SFE also contains isoflavones which are known to have anti-cancer properties.

Oligosaccharides of soybeans causing flatulence (raffinose, stachyose, verbascose) are removed in process of soaking and draining of soybeans. Trypsin inhibitors detrimental for protein digestion are inactivated by a heat treatment in process of manufacturing of SFE. Lipoxygenase and lipase enzymes causing lipids oxidation and hydrolysis are heat inactivated as well too.

Hydrolyzed soybean food emulsion (HSFE) is a product of enzymatic hydrolysis of SFE. The studies of All-Russian Research Institute of Fats report on the increase of biological value of HSFE as a result of enzymatic treatment. This is explained by a generation of low molecular weight peptides during the hydrolysis which are easily absorbed by the body and by a decrease of total antigenicity of HSFE (Domoroshchenkova et al., 2003; Domoroshchenkova et al., 2014). The use of hydrolysed soybean food emulsion (HSFE) with a high degree of hydrolysis in food formulations is particularly important as hydrolysed proteins improve the digestibility of the product. HSFE is characterized by a high biological value due to the inactivation of anti-nutritional factors of soybeans and removal of physiologically undesirable components.

MATERIALS AND METHODS

The test samples were prepared by combining vegetable and animal raw materials. The control sample was manufactured from fresh cow milk meeting the requirements of the Russian State Standard GOST R 52054–2003 and of Technical Regulations of the Customs Union 'On the safety of milk and dairy product' (TR CU 033/2013).

Production of HSFE

SFE with 12% dry matter content meeting requirements of TS 9146–166–00334534–97 was a used as a substrate for enzyme treatment. SFE was hydrolysed in a pilot reactor equipped with automatic titration unit and temperature control at agitation rate 150 rpm. The initial SFE was treated with proteolytic enzyme preparation Flavourzyme TM type A (Novo Nordisk A/S, Bagsvaerd, Denmark) with an activity of 1,000 Leucine Aminopeptidase Units (LAPU) g-1at E/S–40 LAPU/g, t–50°C, pH–6.5. The degree of hydrolysis (DH) of hydrolysed soybean emulsion was varied from 6% to 12% with an interval of 2%. DH was determined by changes of amino nitrogen content (Novo Nordisk's Analytical Method AF 298/1). The reaction was stopped by elevation of reaction mixture temperature to 85–900°C.

Production of clots

Coagulation of test samples was performed with the following reagents: dairy starter cultures of *Lc. lactis*, *Lc. cremoris*, *Lc. diacetilactis* (TS 49559); rennet powder (TS 10–02–824); calcium chloride (TS 6–09–4711).

In experiment with the acid coagulation method of cheese production a bacterial starter was used as a coagulant, in case of the acid-rennet coagulation method of cheese production – a rennet and a bacterial starter, in case of the rennet coagulation method of cheese production – a rennet. To optimize the coagulation process calcium chloride solution was added.

The reagents were added to the mixture in following quantities: starter – 2% v v^{-1}; rennet powder – on the basis of calculation of the clotting mixture at 32–35°C for 30–35 min; solution of calcium chloride on the basis of addition of 40 g of anhydrous salt per 100 kg of mixture.

The samples were kept at the same conditions during the process of study.

Complex analysis of raw materials, coagulates and finished products were performed according to the following procedures.

Sensory evaluation

Organoleptic characteristics of the product were examined according to ISO 22935–3:2009 Milk and milk products – Sensory analysis – Part 3: Guidance on a method for evaluation of compliance with product specifications for sensory properties by scoring (IDT). The samples were evaluated by a trained panel of 12 members. Twelve panellists (age 22–38 years) qualified for sensory evaluation techniques and regular consumers of products estimated the sensory properties of the samples.

Quality parameters analysis

The titratable acidity was analysed according to AOAC (1998).

Crude protein content was analysed by Kjeldahl method at the automated analyser Kijltec Auto 1030, Sweden, according to the standard protocol of the equipment vendor (GOST R 54662–Cheeses and processed cheeses).

Aminoacid content of proteins was analysed by HPLC method at the analyser DJEOL, Japan, at the department of biochemistry and molecular biology of All-Russian Institute of Plants, St. Petersburg. Samples were hydrolysed by 6N hydrochloric acid during 24 hours at temperature of 110°C in a nitrogen atmosphere in sealed glass tubes and then subjected to HPLC analysis.

All experiments were performed with at least three replicates; data was processed by methods of mathematical statistics at theoretical frequency 0.95.

RESULTS AND DISCUSSION

The previous research has demonstrated positive results of development of fermentation process of dairy-soy mixtures and of coagulating of proteins with a mixed composition. Therefore a possibility of usage of the known methods of coagulation of proteins of a dairy-soy mixture in production of cheese product was proposed.

At the initial stage of the current research we've studied the process of the joint coagulation of proteins at 2:1 ratio of cow milk and SFE in the initial mixture (based on the data of preliminary studies).

Organoleptic characteristics of samples obtained by different coagulation methods were analysed. In studies of coagulation process of combined protein mixtures the best results were achieved for the acid and acid-rennet coagulation methods. In the experiment with the rennet coagulation of proteins of dairy-soybean mixture the small-size flocculated clot was formed and the resulted whey was poorly separated. It is known that milk coagulation ability caused by the rennet and the quality of the clot are affected by several factors such as a ripeness of milk, a pasteurization temperature, a temperature of coagulation and clot processing. But in our case usage of the higher ripeness milk (22–25°T), of the optimal temperature of mixture pasteurization (70–72°C), of the optimal temperature of coagulation (33–35°C) and processing of clot (40–45°C) in order to reach a firmer clot haven't resulted in the formation of a clot of an acceptable quality. It could be caused by a lack of casein level required for the rennet coagulation. Thus samples obtained by the rennet coagulation were not used in further studies.

The study showed that it was possible to receive a plastic and high quality clot with a homogenous consistency in process of production of the acid-rennet coagulated cheese product with SFE additive apparently due to the presence of fibre in SFE.

In experiments with 1/3 ratio substitution of milk by SFE the resulted clots were characterized by a weak taste of soybeans. Combined clots had a white colour with a creamy shade inherent to soy products.

One of the main reasons limiting the widespread usage of soyfoods and soy additives in European diets is its low consumer appeal. Soy products often have specific odour and flavour which are unusual for a Russian consumer. Usage of soy additives by the Russian dairy industry is relatively limited (Domoroshchenkova et al., 2006; Domoroshchenkova & Lishayeva, 2010). Therefore there is an objective to develop new food products containing dairy and plant components with consumer properties equal or close to the properties of traditional foods. In the circumstances a serious attention was paid to the evaluation of organoleptic characteristics of cheese products with a different composition.

Sensory tests were performed with 3 samples with a different ratio of dairy and plant components in the mixture: 2:1 (sample 1), 1:1 (sample 2), and 1:2 (sample 3). Organoleptic characteristics of acid coagulated samples are presented in Fig. 1. Acid-rennet coagulated samples showed a similar dynamics in organoleptic score. The studied characteristics of sample 1 are the most close to the control.

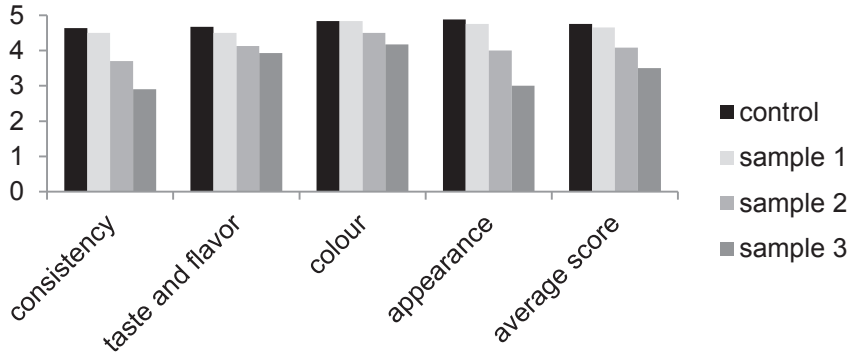

Figure 1. Organoleptic parameters of acid coagulated samples.

Thus the positive indicators of clots were achieved for the acid and acid-rennet method of coagulation of proteins for milk and soy mixture with ratio 2:1.

A positive effect of partial controlled hydrolysis of soybean food emulsions for usage in formulations of cheese products was proposed. The influence of the degree of hydrolysis (DH) of soybean emulsion used as a part of the initial mixture on fermentation of samples was studied. Control samples were prepared from milk and from milk and SFE mixture. On the basis of preliminary results of the study the ratio of dairy and plant components in the starting mixture was adjusted to 2:1.

The coagulation process of the mixture is one of the most important processes in manufacturing of cheeses. Its success depends on a number of factors which are described by many authors (Smirnova & Ostroumova, 2006). The most indicative effect of influence of DH of HSFE on an acid accumulation process of samples could be demonstrated for an acid coagulation method of dairy-soybean mixture. The titratable

acidity of the mixture, the duration of the fermentation process and the ability of a clot to separate whey were used as evaluation criteria.

Fig. 2 shows changes of the titratable acidity of samples with different DH during the fermentation process. The increase in acidity during fermentation of the mixtures of different composition was uneven. Changes of the acidity of the control sample were small during the first four hours, then after 6 hours of fermentation process the acidity started to increase quickly and after 14 hours it reached the acidity of the finished clot (90°T), and after 24 hours - the maximum 120°T . The increase of the titratable acidity of the combined mixture was more intensive at the beginning of fermentation process. It was observed that for the higher DH of HSFE in the starting mixture the fermentation process proceeded more intensively. However the intensity of the fermentation process of dairy-soy mixture dropped greatly after 10 hours of fermentation time versus a control sample. The clot with HSFE with 6% DH was ripened in 16 hours of maturation of the mixture, with 8% DH- in 12 hours, with 10% DH- in 10 hours and with 12% DH- in 8 hours.

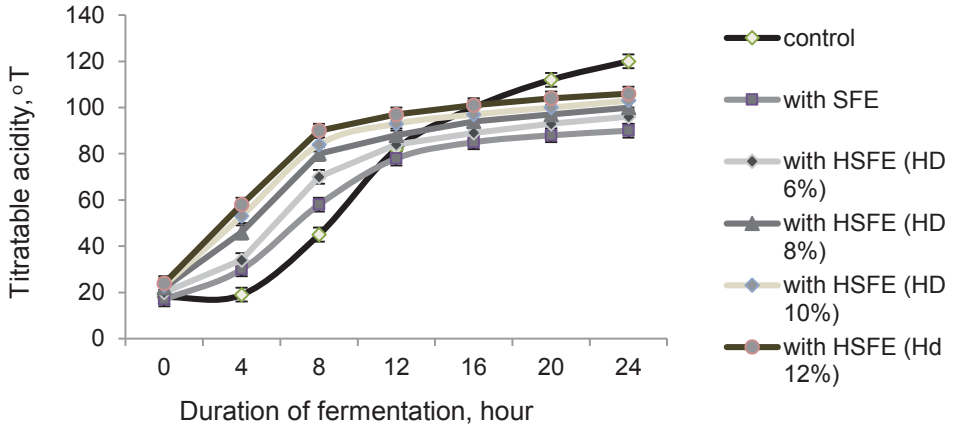

Figure 2. Changes of titratable acidity of the samples during fermentation process.

This can be explained by the positive influence of protease treatment of soybean emulsion associated with an increase of hydrophobicity of proteins and their ability to emulsify fats.

The ability of clots to separate whey during self-pressing process is equally important in the production of soft cheeses. Fig. 3 shows the influence of DH of HSFE in the dairy-soy raw materials mixture on the syneresis properties of the test clots obtained by acid coagulation. It is obvious that the control samples had a higher ability to separate whey. It was observed that the clots with HSFE have separated whey better than the samples with SFE. The samples with HSFE with DH of 12% were more similar to the control clots by the ability to separate the whey. The volume of the separated whey during self-pressing was decreased by 2–2.5% with a decrease of DH of HSFE per each 2%.

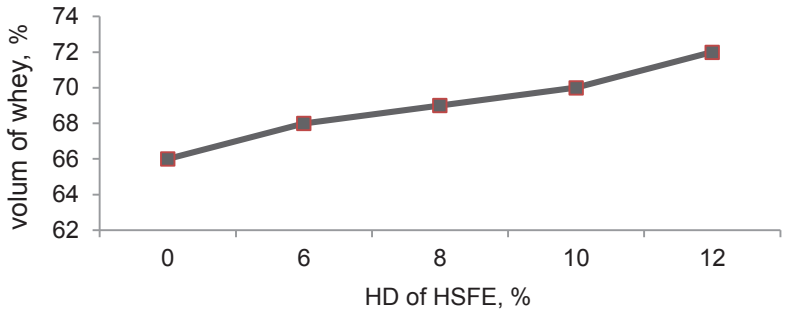

Figure 3. The influence of DH of HSFE on the ability of clots to separate whey during self-pressing process (by acid coagulation of samples).

The varying water-holding capacities of clots have affected quality of finished products mainly the consistency of cheeses. A total score of products with HSFE with different DH was evaluated (Fig. 4).

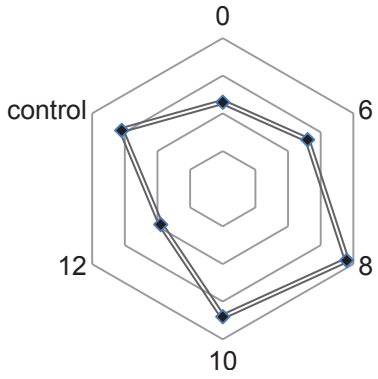

Figure 4. Total score of organoleptic evaluation of samples with varying DH of HSFE.

Many authors consider the issues of improving of the nutritional value of foods in particular by an increase of a biological value of protein components (Orlova & Nasonova, 2014; Zabodalova et al., 2014; Nadtochii & Koryagina, 2014). For evaluation of biological value of protein components of products the content of amino acids should be determined. The amino acid composition of cheese products based on a raw material mixture of milk and SFE in a ratio of 2:1 is presented in Tables 1 and 2.

The physiologically approved protein average daily requirements are systematically studied and analysed in the decisions of FAO/WHO and of national organizations of different countries including the Russian Federation (FAO 1970; FAO/WHO 1991, 2007, 2011) (Baranenko et al., 2014).

Table 1. Amino acid content of cheese products

Amino acids	acid-rennet cheese product with SFE		acid cheese product with SFE	
	g per 100 g of product	% to protein	g per 100 g of product	% to protein
Essential amino acids, total incuding:	3.110	41.4	7.170	43.0
Histidine	0.250	3.3	0.470	2.8
Lysine	0.453	6.0	0.994	6.0
Threonine	0.258	3.4	0.657	3.9
Valine	0.431	5.7	0.952	5.7
Methionine	0.163	2.2	0.569	3.4
Isoleucine	0.315	4.2	0.636	3.8
Leucine	0.623	8.3	1.288	7.7
Tryptophan	0.112	1.5	0.232	1.4
Tyrosine	0.307	4.1	0.647	3.9
Phenylalanine	0.448	6.0	1.195	7.2
Non-essential amino acids, total incuding:	4.416	58.6	9.509	57.0
Alanine	0.345	4.6	0.650	3.9
Arginine	0.186	2.5	0.332	2.0
Aspartic acid	0.642	8.5	1.509	9.0
Glycine	0.258	3.4	0.453	2.7
Glutamic acid	1.216	16.1	2.811	16.9
Proline	1.105	14.7	2.276	13.6
Serine	0.315	4.2	0.780	4.7
Cysteine	0.099	1.3	0.228	1.4
Total	7.526	100	16.679	100

Table 2. Amino acid score of the cheeses with SFE

Essential amino acids	FAO/WHO, 2007, g 100 g^{-1} of protein	Amino acid content of cheese products , % to protein		Amino acid score of cheese products, %	
		by acid-rennet coagulation	by acid coagulation	by acid-rennet coagulation	by acid coagulation
Histidine	1.5	3.3	2.8	220	190
Isoleucine	3.0	4.2	3.8	140	127
Leucine	5.9	8.3	7.7	141	131
Lysine	4.5	6.0	6.0	133	133
Methionine+ Cysteine	2.2	3.5	4.8	159	218
Phenylalanine + tyrosine	3.8	10.1	11.1	266	292
Threonine	2.3	3.4	3.9	148	170
Valine	3.9	5.7	5.7	146	146

Amino acid score of essential amino acids was calculated by a comparison of the of the amino acid content of the test protein with its recommended pattern:

$$Amino\ acid\ score = \frac{g\ of\ amino\ acid\ in\ 100\ g\ test\ protein}{g\ of\ amino\ acid\ in\ 100g\ protein\ FAO/WHO,2007} \quad (1)$$

To refine the indicators of biological value of protein component we used basic indicators and criteria: such as biological value of protein component (BV) and coefficient of differences of amino-acid scores (CDAAS) (Lipatov, 1995).

CDAAS shows the average differences of essential amino acids score (DAAS) as compared to the minimum level of an essential amino acid. The coefficient of differences of amino-acid scores (CDAAS) is calculated as follows:

$$CDAAS = \frac{\sum \Delta DAAS}{n}, \quad (2)$$

where: *DAAS* – difference of amino-acid score of an essential amino acid and a minimum amino acid score; *n* – amount (8) of essential amino acids.

$$\Delta DAAS = Ci - Cmin, \quad (3)$$

where: C_i – score of *i*-essential amino acid; C_{min} – minimum amino-acid score.

$$BV = 100 - CDAAS, \%, \quad (4)$$

Calculated quality indicators of protein component of cheese products are presented in Table 3.

Table 3. Quality indicators of the protein component of the cheese products

Quality indicators	cheese product by acid-rennet coagulation	cheese product by acid coagulation
Minimum amino acid score, %	133	127
ΔDAAS, %	286	390
CDAAS, %	36	49
BV, %	64	51

Quality indicators confirm a relatively high BV of protein component of cheese products. Moreover, estimate indicators of cheese product by acid-rennet coagulation slightly higher on indicators of cheese product by acid coagulation.

CONCLUSIONS

On the basis of the conducted studies we've reached the following conclusions:

1. The possibility of production of cheese products by acid and acid-rennet coagulation of mixture of cow's milk and soybean food emulsion was demonstrated.

2. The ratio of milk and SFE of 2:1 in raw materials mixture allowed obtaining the product with acceptable quality.
3. DH of HSFE influenced the clot formation process and its quality. The best results were obtained for HSFE with DH 8–10%.
4. Finished cheese products obtained by acid and acid-rennet coagulation were characterized by a relatively high BV of protein component.

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

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