Biotechnological aspects of obtaining coagulation structures for functional foods

O V Skripko¹ and S A Kostrykina²

¹Department of Power Engineering, Amur state University, Blagoveshchensk, Amur region, Russia
²Department of Technology Far Eastern State Agrarian University, Blagoveshchensk, Amur region, Russia

E-mail: kostr73@yandex.ru

Abstract. The use of components obtained by the methods of biotechnological transformation of soybeans for the production of innovative products of functional and specialized purpose is an urgent task. Of practical interest is the development and use of technologies that allow the use of traditionally sought-after products, taking into account the principles of safety, functionality and environmental friendliness. The article presents reasonable biotechnological approaches to obtaining coagulation structures containing a complex of valuable nutrients. Biotechnological approaches consist in germinating soybean seeds and coagulating protein substances in soy protein-carbohydrate suspension with special coagulants. The experimental data were processed by methods of mathematical statistics and dependences were obtained to determine the conditions for the formation of coagulation structures - the process of coagulum formation lasts for 2-5 minutes, the coagulation temperature is 72-75 ° C, the mass fraction of the introduced coagulant is 30-40% of the mass fraction of soy protein carbohydrate suspension. Compliance with these parameters makes it possible to get coagulates with high organoleptic, physico-chemical and rheological characteristics. The use of the obtained coagulates will allow obtaining foods with a high content of protein, essential amino acids, minerals and vitamins with a functional orientation.

1. Introduction

In the light of current trends in increasing life expectancy and maximizing able-bodied population maintenance, the prevention of diseases and population nutritional status improvement is an urgent task.

Proteins and lipids have been and remain the most important components of nutrition, necessary not only to compensate for energy costs, but also to ensure many biochemical processes occurring in the human body. Numerous studies have proved the fact that the presence of protein and lipids in a person’s diet affects life expectancy, and the use of animal and vegetable proteins and lipids in a certain ratio is important. It is known that in countries where the population’s diet is balanced for protein, lipids and other nutrients, people's life expectancy is much higher [1, 2]. Therefore, food manufacturers and scientists are directing their joint efforts to develop new technologies and formulations of functional foods for a healthy diet. At the same time, ingredients capable of increasing their nutritional value and improving the chemical composition, physico-chemical characteristics and rheological properties are...
added to food formulations. At the same time, food additives and ingredients obtained from soy and its fractions remain promising.

Soy protein isolates, concentrates, textures, protein fortifiers, soybean oil, soya lecithin, etc. have been used to produce food stuff and are in demand with the different consumer groups of the population – children, sportsmen, vegetarians etc. as well as used in special and functional nutrition for the prevention of cardiovascular, oncological and other diseases [3, 4].

The enrichment of products with soy components has several advantages, but is not exhaustive. A promising area is the production of soy products using the methods of biotechnological transformation of soy raw materials.

2. Materials and methods

2.1. Materials
The objects of research were the main types of the raw materials used: soya grains of the Amur breeding varieties Persona, Alena and Intriga, sour-milk drinks (varenets, kefir, fermented baked milk, ‘Tan’, ‘Ayran’, yogurt, bifidoc, bifilife, immunolact) and products (sour cream, cottage cheese, whey, sour clotted milk), ascorbic and succinic acids, salted, pickled vegetables (cucumbers, cabbage, tomatoes), fruit nectars (pineapple, orange, etc.). All raw materials used met the current regulatory documents requirements in quality and safety.

2.2. Methods
When performing the research, standard and special research methods were used: measuring, instrumental, physicochemical, biochemical, microbiological, organoleptic, statistical and calculated. The study of the chemical composition of soybean seeds was carried out by near infrared spectrometry using an ‘FOSSNIRSystem 5000’ IR scanner. The amino acid composition of products and components was carried out using the AAA 400 amino acid analyzer. Vitamin C content was measured by titrimetric method with potentiometric titration, vitamin B1, B2 content was performed by spectrophotometric method. Determination of moisture was made by drying to constant weight, fat - by infusion with a solvent. Determination of pH was carried out by potentiometric method.

2.3. Research Methodology
Technological experience in obtaining the protein-lycopene component was carried out using soybean varieties Alyona, Intriga, Persona, which are characterized by a high protein content, an optimal ratio of essential amino acids and low trypsin-inhibiting activity.

Soybean seeds were soaked in water t = 18-20 ° C for 24 hours. After that, a 100 mm layer of seeds was placed in a container and germinated without access to light until sprouts 2.0 – 3.0 mm long appeared. Then the seeds were washed and the separated membrane was removed. The cotyledons were crushed in a grinder-extractor with a ratio of seeds: water = 1: 8, in order to obtain a soy protein suspension. The suspension received was heated to t = 60-80 ° C for 2-6 minutes, preliminary adding coagulant solutions to it [5].

In the process of coagulation and sedimentation of protein substances, agglomerated particles of protein were measured. Serum was separated from the obtained protein component by filtration, and the moisture content of the coagulate was adjusted to the required value by self-pressing.

The data of the experiments results were recorded in the planning and experiment results matrix.

The experiment was carried out according to the method proposed by Box and Draper [6], the reliability of the experimental data and the reproducibility of the experiments were evaluated according to the method of E.E. Rafales - Lamarck and V.G. Nikolaev. The experiments reproduced 3 parallel definitions.

The numerical values shown in the tables are arithmetic mean, the reliability of which is P = 0.95, the confidence interval is ± 5.0%.

Experimental studies to substantiate the optimal parameters were carried out on the basis of
techniques proposed by a number of authors. Based on the analysis of the techniques, it was established that the processes under study will be described by a second-order mathematical model [6, 7].

3. Results and discussion

When choosing the basis of the food composition, preference was given to the soy component.

Based on our theoretical and experimental studies, we propose using biotechnological approaches to obtaining protein coagulation structures.

The research analysis and practice demonstrate that biotechnological modification of soy raw materials make it possible to increase its nutritional value, and with subsequent heat treatment carry it out under milder conditions with a significant reduction in the duration of such processing. In this connection, we are proposing to germinate soy grain before using it. The technology is as follows. Soybean seeds are inspected to remove damaged or spoiled specimens, then washed with water at room temperature, then soaked in water with a temperature of 18 ... 20 °C to swell the seeds, soften the shell and accelerate the germination process to achieve a sprout length of 2.0 ... 3.0 mm, to reduce the content of anti-nutrients and, in particular, trypsin inhibitor, and increase the nutritional and digestibility of the finished product [8].

Tables 1 and 2 show the results of the study of the chemical composition and energy value of the germinated soybean.

**Table 1.** Results of the study of the chemical composition and energy value of the germinated soybean.

| Name of raw materials | Protein, g/100 g | Lipids, g/100 g | Carbohydrates, g/100 g | Minerals, g/100 g | The energy value, kcal/100 g | Urease activity, metric unit |
|-----------------------|------------------|-----------------|------------------------|------------------|----------------------------|-----------------------------|
| Soybean variety Persona | 40.0 | 17.7 | 24.3 | 6.0 | 416.5 | 1.01 |
| Soybean variety Alyona | 37.1 | 18.5 | 26.2 | 6.2 | 419.7 | 1.09 |
| Soybean variety Intriga | 39.1 | 18.5 | 24.9 | 5.5 | 422.5 | 1.05 |

It was experimentally established that as a result of germination in the soybean grain, the content of ascorbic acid rises to 25 μg / 100g.

**Table 2.** Minerals and vitamins of germinated soya raw materials, mg / 100 g.

| Name of raw materials | Na | K | Ca | Mg | P | Fe | β-каротин | B1 | B2 | PP | C |
|-----------------------|----|----|----|----|----|----|------------|----|----|----|---|
| Soybean variety Persona | 6.0 | 1607 | 348 | 226 | 603 | 15.0 | 0.07 | 0.94 | 0.22 | 2.2 | 25.1 |
| Soybean variety Alyona | 5.9 | 1608 | 349 | 226 | 605 | 15.0 | 0.06 | 0.94 | 0.21 | 2.2 | 23.4 |
| Soybean variety Intriga | 6.0 | 1600 | 348 | 232 | 600 | 14.9 | 0.07 | 0.95 | 0.22 | 2.3 | 24.8 |

To obtain a soy protein-carbohydrate dispersed suspension, the germinated soybean seeds are crushed while heating to extract soluble substances in eightfold water amount to obtain a thin suspension. The suspension received is separated into a liquid fraction (soy milk) and an insoluble residue. The resulting protein-carbohydrate suspension (soy milk) contains: 3.0% protein, 1.5% fat, 4.0% carbohydrates, 2.1% minerals, vitamins.
Soymilk is essentially a colloidal solution containing bound colloidal particles. In order to form a coagulation structure in such a system, a special effect on colloidal particles is necessary for the chemical bonds between them to appear and a precipitate (clot) to be formed.

An analysis of literary sources revealed the basic conditions for the formation of coagulation structures, where the following is necessary, firstly, a high concentration of the dispersed phase; secondly, the smallest particle size; thirdly, the optimum temperature of coagulation; and, finally, the introduction into the colloidal solution of substances that contribute to the formation of coagulate.

Soy milk, as a colloidal solution, meets the first two conditions. To form a coagulation structure in it, it was necessary to identify possible coagulants, to determine the temperature and duration of the coagulation process.

The second biotechnological technique is the introduction of a coagulant solution.

Given the chemical composition of the obtained protein-carbohydrate disperse system and the need to obtain coagulants suitable for preparing functional food products, the main criteria for choosing coagulants were their acidity, safety, and the presence of nutrients that are beneficial to human health.

In this connection, we used fermented milk products and drinks (containing lactic acid), salted and pickled vegetables - cucumbers, cabbage, tomatoes (containing lactic acid), fruit nectars - pineapple, orange (containing ascorbic acid), food additives - ascorbic and succinic acids as coagulants.

Coagulants were prepared as follows. In fermented milk products and fruit nectars, their quality was monitored in terms of safety, salted and pickled vegetables were crushed to obtain a puree mass, 2% aqueous solutions were prepared from ascorbic and succinic acids.

The prepared coagulants were alternately introduced into the dispersed soy protein-carbohydrate system, heated to a temperature of 60-80 °C, kept at this temperature for 4-6 minutes up to form a coagulate.

The obtained coagulate was separated from the serum by self-pressing, bringing its moisture content to 60-70%. The quality of the finished coagulate was evaluated by a complex of organoleptic, physicochemical indicators and rheological characteristics. Coagulate quality was evaluated on a 5-point scale.

As a result of experimental studies of the coagulation structures formation process, factors such as the mass fraction of the coagulant (M, %), coagulation temperature (t, 0°C) and coagulation duration (T, min) have a significant impact on the quality of the coagulate (N) in the process of its structure formation. Table 3 shows the factors and levels of their variation.

| Factors | M – mass fraction of coagulant, % | t – coagulation temperature, °C | T – coagulation duration, min |
|---------|---------------------------------|---------------------------------|------------------------------|
| M/X_1  | 10                              | 10                              | 1                            |
| t/X_2  | 40                              | 80                              | 6                            |
| T/X_3  | 30                              | 70                              | 5                            |

Regression analysis of the dependence \( Y=f(X_1; X_2; X_3) \to \max \) according to the five investigated options made it possible to obtain mathematical models of the process of obtaining clots and evaluate their adequacy according to the Fisher criterion.

- for coagulates obtained using fermented milk drinks, the mathematical model has the following form:

\[
Y_i = 24.5258 + 0.4872 \cdot X_1 + 0.5231 \cdot X_2 - 1.12775 \cdot X_1^2 - 1.0404 \cdot X_2^2 - 0.5662 \cdot X_3^2 \to \max; \quad (1)
\]

- in decoded form

Table 3. Factors and levels of variation.

Variation range

| Levels     | Designations | M – mass fraction of coagulant, % | t – coagulation temperature, °C | T – coagulation duration, min |
|------------|--------------|---------------------------------|---------------------------------|------------------------------|
| Variation range | E            | 10                              | 10                              | 1                            |
| Top level  | +1           | 40                              | 80                              | 6                            |
| Main level | 0            | 30                              | 70                              | 5                            |
| Lower level | -1           | 20                              | 60                              | 4                            |
\[ N_1 = -48,168 + 0.81519 \cdot M + 1,5088 \cdot t + 3,3971 \cdot T - 0.012775 \cdot M^2 - 0.010404 \cdot t^2 - 0.56618 \cdot T^2 \rightarrow \text{max} \]  
(2)

The optimal values are: \( M = 31.9 \%; t = 72,5 \^\circ \text{C}; T = 3 \text{ min}; N_1 = 24,6 \text{ points}.\)

- for coagulates obtained using fermented milk products, the encoded form of the model has the form:\n
\[ Y_2 = 23,7922 + 0.3092 \cdot X_1 + 0.8368 \cdot X_2 - 0.4950 \cdot X_3 - 0.3125 \cdot X_2 \cdot X_3 - 0.5815 \cdot X_1^2 - 0.8863 \cdot X_2^2 - 0.8863 \cdot X_1^2 \rightarrow \text{max}; \]  
(3)

- decoded form

\[ N_2 = -44,709 + 0.3798 \cdot M + 1,4183 \cdot t + 7,0103 \cdot T - 0.031250 \cdot t \cdot T - 0.0058148 \cdot M^2 - 0.0088630 \cdot t^2 - 0.88631 \cdot T^2 \rightarrow \text{max} \]  
(4)

The optimal values are: \( M = 32,7 \%; t = 75,4 \^\circ \text{C}; T = 2.63 \text{ min}; N_1 = 24,1 \text{ points}.\)

- for coagulates obtained using fruit nectars:

- in coded form

\[ Y_3 = 24,5989 + 0.2074 \cdot X_1 + 0.3875 \cdot X_2 + 0.2414 \cdot X_3 - 0.5625 \cdot X_2 \cdot X_3 - 0.6087 \cdot X_1^2 - 0.6764 \cdot X_2^2 - 0.6764 \cdot X_1^2 \rightarrow \text{max}; \]  
(5)

- in decoded form

\[ N_3 = -55,164 + 0.38596 \cdot M + 1,2670 \cdot t + 10,943 \cdot T - 0.056280 \cdot t \cdot T - 0.0061 \cdot M^2 - 0.0067643 \cdot t^2 - 0.67643 \cdot T^2 \rightarrow \text{max} \]  
(6)

The optimal values are: \( M = 31.7 \%; t = 72,6 \^\circ \text{C}; T = 5.07 \text{ min}; N_1 = 24.7 \text{ points}.\)

- for coagulate obtained using mashed pickled and salted vegetables:

- in coded form

\[ Y_4 = 23,5087 + 0.9282 \cdot X_1 + 0.5714 \cdot X_2 - 0.7750 \cdot X_1 \cdot X_2 - 0.5250 \cdot X_2 \cdot X_3 - 0.9464 \cdot X_1^2 - 0.5741 \cdot X_2^2 - 0.4725 \cdot X_1^2 \rightarrow \text{max}; \]  
(7)

- in decoded form

\[ N_4 = -66,387 + 1,2033 \cdot M + 1,3558 \cdot t + 8,3996 \cdot T - 0.00775 \cdot M \cdot t - 0.0525 \cdot t \cdot T - 0.0094665 \cdot M^2 - 0.0057407 \cdot t^2 - 0.47246 \cdot T^2 \rightarrow \text{max} \]  
(8)

accordingly, the optimal values: \( M = 33.4 \%; t = 73.6 \^\circ \text{C}; T = 4.8 \text{ min}; N_1 = 23.7 \text{ points}.\)

- for coagulate obtained using ascorbic or succinic acid, the mathematical model of the encoded form is:

\[ Y_5 = 23,5063 + 0.5153 \cdot X_1 + 0.3138 \cdot X_2 - 0.4625 \cdot X_1 \cdot X_2 - 0.3625 \cdot X_1 \cdot X_3 - 0.2625 \cdot X_2 \cdot X_3 - 0.4906 \cdot X_1^2 - 0.3213 \cdot X_2^2 \rightarrow \text{max}; \]  
(9)

- in decoded form

\[ N_5 = -24,731 + 0.8509 \cdot M + 0.75115 \cdot t + 2.9250 \cdot T - 0.004625 \cdot m \cdot t - 0.03625 \cdot M \cdot T - 0.02625 \cdot t \cdot T - 0.0049062 \cdot M^2 - 0.0032127 \cdot t^2 \rightarrow \text{max} \]  
(10)

and the optimal values are: \( M = 37.1 \%; t = 73.9 \^\circ \text{C}; T = 4 \text{ min}; N_1 = 23.9 \text{ points}.\)
4. Conclusion

Thus, a series of experiments and mathematical processing of their results made it possible to substantiate the parameters of biotechnology for producing combined coagulation structures. Analysis of the kinetics of structure formation in a soy protein dispersed system using various coagulants shows that the formation of coagulate continues for 2-5 minutes. In this case, the coagulation temperature is 72-75 °C, which provides the necessary heat treatment of the resulting product, by its so-called long-term pasteurization. At the same time, the use of soya grains, various food products and food additives as coagulants allows obtaining formed coagulation structures with a complex of valuable nutrients. The use of the obtained coagulants in food production makes it possible to obtain healthy food products in the form of pastes, sauces, soups, half finished goods, concentrates, food additives, etc.

References

[1] Makarenko V V 2010 Obtaining milk-plant clots for protein-lipid food products Bulletin of the KrasGAU 3 178-82
[2] Skripko O V 2012 Justification of the parameters of the process of obtaining a protein-lycopene product for food concentrates Technique and technology of food production 1(24) 68-73
[3] Petibskaya V S 2012 Soya: Chemical Composition and Use (Maykop: OJSC Polygraph-South/Ruaaia) p 432
[4] Benamouzig R and Tomé D 2003 Postprandial kinetics of dietary amino acids are the main determinant of their metabolism after soy or milk protein ingestion in humans Journal of Nutrition 133 1308–15
[5] Kalenik T K, Costa R, Motkina E V, Kosenko T A, Skripko O V and Kadnikova I A 2017 Technology development of protein rich concentrates for nutrition in extreme conditions using soybean and meat by-products Acta Sci. Pol. Technol. Aliment. 16(3) 255-68
[6] Box G E P and Draper N R 1987 Empirical Model Building and Response Surfaces (New York, NY)
[7] Freedman D A 2005 Statistical Models: Theory and Practice (Cambridge University Press)
[8] Dotsenko S M, Skripko O V and Baykova N S 2009 A method for preparing a milk-protein product Patent No. 2366264 of the Russian Federation (GNU VNII soy)