



**Bochkarev S.,
Krichkovska L.,
Petrova I.,
Petrov S.,
Varankina O.,
Belinska A.**

RESEARCH OF INFLUENCE OF TECHNOLOGICAL PROCESSING PARAMETERS OF PROTEIN-FAT BASE FOR SUPPLY OF SPORTSMEN ON ACTIVITY OF PROTEASE INHIBITORS

Вивчено закономірність впливу параметрів технологічної обробки білково-жирової основи для харчування спортсменів на її біологічну цінність. Визначено залежність часу попередньої обробки білково-жирової основи надвисокочастотним випромінюванням та її вихідної вологості на ступінь подальшого гідролізу білка. Доведено, що в обраному діапазоні величин вищеназаних чинників можливо максимальне збільшення біологічної цінності продукту.

Ключові слова: олійне насіння, інгібітори протеаз, надвисокочастотне випромінювання, ферментативний гідроліз білка.

1. Introduction

Inhibitors of proteinases are present in representatives of many groups of plants, including oilseeds. Their characteristic feature is the formation of stable compounds, devoid of enzymatic activity, with proteolytic enzymes. Depending on the chemical structure, localization in the plant, the level of activity, substrate specificity, protease inhibitors are grouped into three classes: Kunitz inhibitors, Baumann-Birk's trypsin and chymotrypsin inhibitors, microorganism serine proteinase inhibitors that do not act on trypsin and chymotrypsin [1].

Inhibitors of proteases are a protective factor of plants from adverse environmental factors, including the effects of insects, viruses, bacteria, etc. They are contained in seeds of cereals, legumes, oilseeds and other crops: in maize, peanuts, potatoes, etc. Soybean beans contain five or more trypsin inhibitors in an amount of 5–10 % of the total protein content [2].

The inhibitory activity of these compounds ceases when it is possible to disrupt the disulfide bonds of the molecule. But because of the extreme strength of the disulfide bond, considerable energy from 20 to 20 000 kcal is needed to break it (1.5 kcal is enough to break the hydrogen bond) [1]. For example, treatment of soybean seeds, even at the temperature of 232 °C for 8 minutes, does not completely destroy protease inhibitors [3].

Incoming with food protease inhibitors in a human organism interfere with the proteins assimilation, suppressing the activity of digestive enzymes. The maximum allowable amount of trypsin inhibitors in food products should not exceed 0.5 % according to [4].

Due to the lack of food protein in the world there is a problem of increasing the biological value of vegetable protein, in particular, the oilseeds protein. A protein-fat base, enriched with essential amino acids and polyunsaturated fatty acids of the ω -3 group, was developed in studies [5, 6] in accordance with the physiological needs of athletes, workers of heavy physical labor, military

personnel. This protein-fat base contained in justified proportions the following components: dried ground seeds of sunflower, sesame and flax. The protease inhibitors presence in its composition, which reduce the digestion of protein by the body, significantly limits its use in the food industry.

Neither inhibitors of proteases of sunflower, sesame and flax seeds, nor their effects on proteases and the protein complex of the protein-fat base have not been sufficiently investigated. Thus, the study and justification of ways to regulate their activity are relevant and have important theoretical and applied importance for the oilseeds processing efficiency increasing.

2. The object of research and its technological analysis

The object of this research is the biological value of protein-fat base depending on its preliminary processing conditions. A technological audit was conducted to reveal the peculiarities of this dependence. The purpose of which was to determine the change in the amino acid content in samples of protein-fat base, which underwent various technological treatments, after enzymatic hydrolysis.

The principal possibility of protein splitting depends significantly on a time of preliminary treatment by microwave radiation, as well as on an initial moisture content of the raw material. Therefore, the main direction of improving the processing of the protein-fat base is to determine the optimal process parameters. This will increase the biological value of the protein-fat base.

3. The aim and objectives of the study

The aim of research is perfection of technological processing of protein-fat base for nutrition of athletes on the basis of modeling and optimization of physico-chemical processes of denaturation of enzyme inhibitors. This will increase the biological value of the protein-fat base,

i. e., the degree of digestion and digestibility of proteins in the gastrointestinal tract.

For effective achievement of this aim it is necessary to solve the following:

1. To study the regularities of the influence of the pretreatment time of the protein-fat base by microwave radiation and its initial moisture on the degree of subsequent enzymatic hydrolysis of the protein.

2. To establish the optimum range of values of selected pre-treatment factors for maximizing the biological value of the protein-fat base.

4. Research of the existing solutions

Among the main directions of the oil seeds proteinase inhibitors activity decrease, identified in the resources of the world scientific periodicals, the following can be singled out:

- «dry» and «wet» thermal denaturation of proteins [7–11];
- germination of seeds [12];
- processing in an electromagnetic field of various frequencies [13–15];
- heat treatment in the presence of chemical denaturing agents (ethanol, acetic acid, ammonia, hydrochloric acid) [16].

In particular, the advantages and disadvantages of «dry» and «wet» thermodenaturations of soybean proteinase inhibitors were presented in [7–9]. It was shown that the thermal denaturation of inhibitor molecules in moistened raw materials is most effective. But there were such parallel processes as irreversible denaturation of soluble fractions of albumins and globulins, which entailed a loss of biological value of the product. A similar drawback was noted in the work [10]. In studies it was shown that the ratio of water-, salt- and alkali-soluble proteins in fat-free oil seeds (for example, soybean and cotton seeds) varied with the intensity of heat treatment – by converting water-soluble proteins into salt-, alkali- and water-insoluble proteins [17]. It is known that processing at relatively low temperatures entails protein denaturation, which increases nutritional value, because of the proteinase inhibitors inactivation and the increasing of proteins availability for hydrolysis. But in parallel, there are reactions of reducing sugars of plant raw materials with amino acids. These reactions are initially reversible, because of Schiff bases available for the action of digestive enzymes are formed. However, these products quickly turn into Amadori compounds, which are practically not digested.

An alternative solution to this issue was set out in the [11, 12]. It was determined that during the germination of seeds the activity of proteinases increased, and the activity of their inhibitors decreased. The weak side of this approach is associated with a change in the organoleptic indicators of finished products (the appearance of a bitter taste), as well as with significant time-consuming for processing.

Thermal, acoustic and electromagnetic effects on activity of the enzymes in the oilseeds were analyzed [13–15]. In particular, the treatment with ultrasound and an electromagnetic field of different frequencies during sunflower seeds ripening had proven effect [13]. The results of the works are of interest [14, 15]. But their object was the activity of hydrolases, in particular, lipases – not activity of inhibitors of hydrolytic enzymes.

A definite solution of this problem was shown in [16], but its drawback lies in the fact that raw materials for the inactivation of protease inhibitors were treated with chemical reagents, the use of most of which is unacceptable in food technologies.

As can be seen from the review, the inhibitors of soy proteinases were the most profoundly studied [7, 10, 11, 16]. In particular, it was shown that their presence in animal feeds caused growth retardation, pancreatic hypertrophy and an abnormally large lack of sulfur-containing amino acids.

Thus, the results of the analysis lead to the conclusion that there is no consensus according to the possibility of reducing of the activity of protease inhibitors in oilseeds. The choice of the most suitable technical solution for each individual object can be based on the results obtained in the model systems. However, processing in the electromagnetic field of the low-frequency range is promising in terms of biochemical, technological and economic aspects [12–14].

5. Methods of research and experimental part

The following materials were used for research:

- sunflower seeds according to DSTU 7011:2009;
- flax seeds according to GOST 4967:2008;
- sesame seeds according to GOST 7012:2009;
- *Distizym Protacid Extra* enzyme preparation according to the current regulatory documentation.

The protein-fat base was a mixture of dried shredded sunflower seeds – 20 ± 5 %, flax seeds – 50 ± 5 % and sesame seeds – 30 ± 5 % of the seeds mixture weight. The initial protein content in the base was determined by the Kjeldahl method.

The initial moisture content of the protein-fat base was corrected by moistening and determined thermogravimetrically. Pre-treatment by microwave radiation was carried out at a frequency of 2450 MHz. Then, all samples of the protein-fat base were dried to moisture of 8 %.

The dried samples were subjected to the enzymatic hydrolysis. The *Distizym Protacid Extra* enzyme preparation was used as a source of proteolytic enzymes (production by *Erbloeh Geisenheim AG*, Germany). Proteolytic activity of the preparation is due to the action of acid fungal peptidase, which destroys proteins to amino acids. The preparation is effective at the temperature of 50–58 °C and pH of 2.0–6.0. The activity of *Distizym Protacid Extra* is 350 units/ml.

The effectiveness of the pretreatment of the protein-fat base by microwave radiation (i. e., the biological value increasing) was evaluated by the amount of α -amino nitrogen after the enzymatic hydrolysis. An amino nitrogen – the nitrogen of free amino groups of amino acids – was determined by the Sorensen formol titration method.

Mathematical methods were applied for the experiment planning and data processing using the *Microsoft Office Excel 2003 (USA)* and *Stat Soft Statistica v6.0 (USA)* software packages. The three-level plan for the two-factor response function was applied to study the dependence of the amino nitrogen content in the protein-fat base versus the time of its preliminary treatment by microwave radiation and its initial moisture content. The studies were carried out in triplicate. For a given degree of probability ($P=95$ %), the relative error did not exceed: 2 % for amino nitrogen content determination; 2 % for raw materials initial moisture determination.

6. Research results

Data on the effect of time of the protein-fat base preliminary processing by microwave radiation and its initial moisture on the content of amino nitrogen after enzymatic hydrolysis of the protein are shown in Fig. 1. It can be seen from the figure that the content of amino nitrogen increases from 2.1 % to 8.3 % after hydrolysis of the protein with an increase of time of the pretreatment by microwave radiation from 100 seconds to 280 seconds and with initial moisture content of the raw material from 10 % to 12 %.

This indicates about a process of thermal denaturation of inhibitors of proteinases of moistened oilseeds treated by ultrahigh-frequency radiation. Attention is drawn to the fact that the content of amino nitrogen after enzymatic hydrolysis of the protein increases from 8.3 % to 4.0 % with the time of preliminary treatment by microwave radiation from 280 seconds to 360 seconds and decreases from 12 % to 15 % with an increase of initial moisture of the base. Obviously, this is due to the fact that an irreversible denaturation of proteins occurs under more stringent processing conditions, which reduces their availability for proteinases.

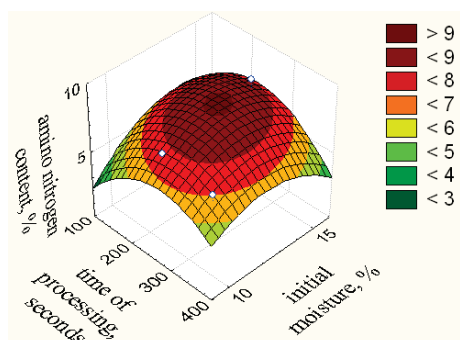


Fig. 1. Dependence of the amino nitrogen content in the protein-fat base after enzymatic protein hydrolysis versus the time of preliminary treatment by microwave radiation and the initial moisture content of raw material

The regression equation, which is the dependence of amino nitrogen content in the base after hydrolysis of protein versus the time of preliminary treatment by microwave radiation and initial moisture content of the raw material, is shown:

$$AN = -30,7821 + 0,0787 \cdot \tau + 4,4993 \cdot \omega - 0,0001 \cdot \tau^2 - 0,002 \cdot \tau \cdot \omega - 0,1538 \cdot \omega^2, \quad (1)$$

where AN – amino nitrogen content (*amino nitrogen*), % from the content of the original protein in the base; τ – time of processing of raw materials by microwave radiation, seconds; ω – initial moisture of raw materials, %.

The verification of the coefficients significance, which was carried out by the Student's test, showed the significance of the coefficients presented in the polynomial (1). Based on the conducted experiments, the regression equation was tested for adequacy by the Fisher criterion (at significance level $\alpha = 0.05$)

Based on the analysis of the equation (1) and the graphical dependence, optimal conditions of pretreatment of the protein-fat base were investigated: 250–350 seconds of microwave treatment time and its hydration to the 12–14 % of

moisture content. This will increase the biological value of the protein-fat base, namely, increase the degree of digestion and digestibility of proteins in gastrointestinal tract.

7. SWOT analysis of research results

Strengths. Among the strengths of this research, it is necessary to note the results obtained for the optimal ranges of protein-fat base processing parameters – the time of preliminary processing by microwave radiation and the initial moisture. According to the results of analysis of modern scientific literature to date, such results are absent. It is difficult to select a priority method for reducing the activity of proteinase inhibitors in the technology of oilseed processing due to this reason. The use of the obtained data allows to solve the problem of choosing a method of pretreatment of the protein-fat base for the biological value increase. It is useful to note the economic attractiveness of the selected method of pretreatment for food technology.

Weaknesses. The weak side of this research is related to the fact that a choice of optimal parameters of preliminary processing of the protein-fat base depends on a number of characteristics of a raw material (for example, oilseed variety, degree of their maturity) and, accordingly, will vary with a change in these characteristics. Therefore, in order to prevent this drawback, special attention should be given to quality of raw materials, which imposes special obligations on a producer.

Opportunities. Research of the preliminary treatment of oilseeds in the electromagnetic field of the low-frequency range can be developed in such branches of food industry as a confectionery, as well as in technology of processing plant materials into protein products (isolates, concentrates, fat-free flour, textured proteins). The problem of increasing the biological value of vegetable protein products can be solved by this way.

Threats. Difficulties in the introduction of the obtained research results can be associated with such factor as management of food industry enterprises. The investment of additional funds, even insignificant ones, in acquisition of necessary equipment and absence of tangible results influence on the position of decision-makers. There is every reason for this risk, since the obtained mathematical model of the process, as it was mentioned above, requires standardization of a number of indicators of oilseeds.

Thus, SWOT analysis of research results allows to determine the main directions for the successful achievement of the stated aim of research, namely:

- to develop of scientifically grounded recommendations for standardization of indicators of oilseeds raw materials for production of the protein-fat base in accordance with physiological needs of athletes, workers of heavy physical labor, military personnel;
- to evaluate the effectiveness of pretreatment of oil seeds in the electromagnetic field of low-frequency range under industrial conditions;
- to develop the technological solutions for obtaining the protein-fat base with increased biological value.

8. Conclusions

1. The regularities of the influence of the time of preliminary treatment of the protein-fat base by microwave radiation and its initial moisture on the degree of

subsequent enzymatic hydrolysis of the protein has been studied. This dependence is a quadratic function that predicts an increase in the content of amino nitrogen after hydrolysis of the protein with an increase in the time of pretreatment by microwave radiation and the initial moisture content of the raw material to a certain limit (from 2.1 % to 8.3 %). Then, the content of amino nitrogen decreases again (from 8.3 % to 4.0 %) with increasing of pretreatment time by microwave radiation and with the base initial moisture increasing.

2. An optimum range of values of the selected factors of preliminary processing for the maximum possible increase in the biological value of the protein-fat base has been established. The optimal time of pretreatment of the protein-fat base by ultra-high-frequency radiation is 250–350 seconds, the initial moisture content of the raw material is 12–14 %. Obtaining narrower ranges is not guaranteed due to unstable parameters of plant raw materials.

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ИССЛЕДОВАНИЕ ВЛИЯНИЯ ПАРАМЕТРОВ ТЕХНОЛОГИЧЕСКОЙ ОБРАБОТКИ БЕЛКОВО-ЖИРОВОЙ ОСНОВЫ ДЛЯ ПИТАНИЯ СПОРТСМЕНОВ НА АКТИВНОСТЬ ИНГИБИТОРОВ ПРОТЕАЗ

Изучена закономерность влияния параметров технологической обработки белково-жировой основы для питания спортсменов на ее биологическую ценность. Определена зависимость времени предварительной обработки белково-жировой основы сверхвысокочастотным излучением и ее исходной влажности на степень последующего ферментативного гидролиза белка. Доказано, что в выбранном диапазоне величин вышеназванных факторов возможно максимальное увеличение биологической ценности продукта.

Ключевые слова: масличные семена, ингибиторы протеаз, сверхвысокочастотное излучение, ферментативный гидролиз белка.

Bochkarev Sergiy, Senior Lecturer, Department of Physical Education, National Technical University «Kharkiv Polytechnic Institute», Ukraine, e-mail: bochkarev.s.v@gmail.com, ORCID: <http://orcid.org/0000-0003-4399-7907>

Krichkovska Lidiya, Doctor of Biological Sciences, Professor, Head of the Department of Organic Synthesis and Nanotechnology, National Technical University «Kharkiv Polytechnic Institute», Ukraine, ORCID: <http://orcid.org/0000-0001-8197-9801>

Petrova Iryna, PhD, Doctor of Juridical Science, Professor, Department of Information Security, Kharkiv National University of Internal Affairs, Ukraine, e-mail: ekobezpeka.ira@gmail.com, ORCID: <http://orcid.org/0000-0002-1652-6715>

Petrov Sergey, PhD, Associate Professor, Department of Organic Synthesis and Nanotechnology, National Technical University «Kharkiv Polytechnic Institute», Ukraine, e-mail: petrowsa@gmail.com, ORCID: <http://orcid.org/0000-0001-6500-5310>

Varankina Oleksandra, PhD, Associate Professor, Department of Biotechnology, Biophysics and Analytical Chemistry, National Technical University «Kharkiv Polytechnic Institute», Ukraine, e-mail: avarankina@gmail.com, ORCID: <http://orcid.org/0000-0002-6117-7091>

Belinska Anna, PhD, Associate Professor, Department of Organic Synthesis and Nanotechnology, National Technical University «Kharkiv Polytechnic Institute», Ukraine, e-mail: belinskaja.a.p@gmail.com, ORCID: <http://orcid.org/0000-0001-5795-2799>