The Reduction Of Activity Of Antialimentary Compounds Of Vegetable Raw Materials Under Exposure To Electric Discharges

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Abstract: The article deals with the issues related to the harmful effects on the human body of anti-nutritional components (anti-nutritional factors of natural origin) that can block active enzymes that break down protein compounds. A comparative analysis of anti-nartative components of protein-containing plant origin under the influence of thermal nature and parameters of electric-discharge treatment has been carried out.

1. Introduction

The main biochemical component of soy is protein. Soy is one of the most high-protein crops cultivated in the world. According to different authors, the seeds of this culture can accumulate an average of 38-42% of the protein, varying this indicator from 30 to 50%.

Soy proteins are heterogeneous in structure and function. Among them there are substances that are considered to be antialimentary (anti-nutritional) components of food. These are inhibitors of proteolytic enzymes, lectins, urease, lipoygenase and others. Most of soy protein (about 70%) is made up of storage proteins of the class 7% (β-conglycinins) and 11% (glycinins), which are quite normally absorbed by mammals. Soy flour is the most widely used source of protein in the creation of balanced feed, and a food protein ingredient, however, in the process of obtaining it is necessary to inactivate anti-nutritional (anti-nutritional) components [1,4,6,7,8].

Soy beans and soy products (soy milk, okara, etc.) contain more than 5 trypsin inhibitors (5-10% of the total protein content). Their activity ranges from 7 to 38 mg / g. A distinctive feature of these substances is that, interacting with enzymes designed to break down proteins (proteases, etc.), they form stable complexes, devoid of both inhibitory and enzymatic activity. The result of this blockade is a decrease in the absorption of protein substances. Once in the stomach, some of the inhibitors (30-40%) lose their activity, and the most stable ones reach the duodenum in active form and inhibit the enzymes produced by the pancreas. As a result, the pancreas is forced to produce them more intensively, which can ultimately cause its hypertrophy with further development of disorders of carbohydrate-lipid metabolism. In terms of chemical structure, properties and substrate specificity, soybean inhibitors mainly belong to two families: Kunitz inhibitors - water-soluble proteins with a molecular weight of 20,000-25,000 Da, binding one trypsin molecule; Bauman-Birk inhibitors are alcohol-soluble proteins with a molecular weight of 6000-10000 Da and a small number of disulfide bridges that can inhibit both trypsin and chymotrypsin [2,3].

The most common method for reducing the activity of trypsin inhibitors is thermal treatment in the presence of alkali. But this method is not always effective, about 20% of soybean trypsin inhibitors have thermal stability.
Pamirsky I.E. [1,2] using the Internet server BLAST (Basic local Alignment Searcy Tool) and the Bio Edit 5.0.9 program, the high structural homology of the protein pancreatic protease inhibitor aprotinin and the plant analogue of soybean trypsin inhibitor was calculated (Fig. 1).

Figure 1 - Electronic three-dimensional models of the tertiary structures of aprotinin and soybean inhibitor [1,2]

Modern studies of foreign and domestic colleagues in in vitro experiments have shown that aprotinin prevents fibrinolysis, and an inhibitor of soy isolate has a similar effect [3,4,5].

Scientific research of Solomentsev M. showed that the trypsin-inhibiting activity is influenced by the temperature regimes of exposure to table 1 [3,4].

Table 1 - Trypsin-inhibiting activity (Kunitz inhibitor) of thermally exposed soy products, (mg / g) [1]

| Sample name                  | Exposure temperature $^{\circ}$C | 60   | 80   | 100  |
|------------------------------|----------------------------------|------|------|------|
| Soy milk (soy emulsion)      |                                  | 0.071±0.007 | 0.0542±0.011 | 0.0170±0.10 |
| Protein-carbohydrate mass (okara) |                                | 1.227±0.01 | 1.123±0.014 | 0.740±0.11 |

Table 1 shows that heat treatment reduces the activity of trypsin inhibitors by almost 98.59% for both soy milk and protein-carbohydrate mass (okara).

Note also that microwave heating of soy protein extracts for 1.5–2 min completely destroys trypsin inhibitors [4].

But the impact of temperatures above 80 $^{\circ}$C, and even more so microwave - heating, is associated with the destruction of protein and other biologically active compounds.

2. Main part

In order to obtain an environmentally friendly target product of soy milk and reduce the activity of anti-nutrients, soybeans and soybean meal were treated with rectangular electric discharges with the following processing parameters: $U = 27$ kV, $C = 0.4$ μF, interelectrode gap length 1.5 mm, the ratio "solid: liquid" $S: W = 1: 10$, the range of change in the number of applied pulses from 150 to 600 imp / l in the electric discharge chamber "sharp - plane".
Table 2 - The yield of protein compounds (in %) and trypsin and antitrypsin (in mU/ml or γ/ml)

| Material          | Humidity, % | Protein, % | Trypsin, mU/ml | Antitrypsin mU/ml | Antitrypsin γ/ml |
|-------------------|-------------|------------|----------------|------------------|-----------------|
| Soy beans         | 5.12±0.01   | 44.7±0.13  | 5.78±0.02      | 23.67±0.01       | 5.29±0.01       |
| Soybean meal      | 4.46±0.03   | 50.4±0.014 | 0.48±0.12      | 10.24±0.11       | 2.03±0.03       |

The protein yield from soybeans changes nonlinearly with an increase in the number of pulses applied (Table 3).

Table 3 - Protein yield from soybeans depending on the number of pulses

| Sample number | Number of pulses | Dry residue, % | Protein, mg / ml |
|---------------|-----------------|----------------|------------------|
| 1             | 1500            | 3.96           | 11.00            |
| 2             | 3000            | 6.33           | 17.70            |
| 3             | 4000            | 7.99           | 21.10            |
| 4             | 5000            | 7.22           | 31.15            |
| 5             | 6000            | 7.96           | 33.15            |

The extraction efficiency is ~ 90% of the initial content of water-soluble complexes. In the range of 100-300 imp / L, the trypsin concentration increases ~ 8 times, while the protein concentration increases ~ 3 times (Fig. 2).

Analysis of water extracts obtained by electric discharge processing of soybeans showed the presence of the main product - protein, as well as enzymes - trypsin.

To assess the integrity of protein molecules in the extracts, proteins were separated by gel filtration with Toyopearl-55 on a 2.6 x 40 cm column, and standard spectra and spectra of products obtained as a result of electric discharge treatment were compared.

Figure 2. Protein yield mg/ml; trypsin, mg/g, antitrypsin; mg/g, depending on the number of pulses.
Proteins from the centrifugates of suspensions obtained at 200 and 600 imp / L, as well as products after mechanical treatment of the soy suspension (in an agate mortar), were eluted in 4 stages of 25 ml of Tris-HCl buffer with pH = 7.5. The eluate was collected in 4 ml fractions. The protein in each fraction was determined spectrophotometrically at a wavelength of 280 nm. The wavelength range was determined according to the standard method [11,12].

The spectra of proteins eluted from the column are shown in Fig. 3 a. The abscissa shows the values of the total volumes of the eluate, and the ordinate shows the optical density of the fractions.

For all compared samples, the soy protein eluted from the column in two peaks. The first peak in the region of 20-25 ml corresponds to the molecular mass of the protein of 550 KD, the second peak is found in the region of 50-60 ml and corresponds to the molecular mass of the protein of 200 KD.

Shown in fig. 3b, the dependences are similar in the shape of peaks and the time of protein release, and it can be concluded that the proteins included in the compared samples are identical. In the course of electric discharge processing, the main product - vegetable proteins - retain their structure [7,8,9,10].

Figure 3. Protein spectrum of soy milk obtained by mechanical method (A), and electric discharge processing (B) - 2000 impulses, (C) - 6000 impulses

The analysis of the activity of inhibitors was determined in accordance with the recommendations of OSP TU 431.1418.990.02-92 and OSK TU 431.1419.990.01-92 in the laboratory of the Pyatigorsk Research Institute of Balneology and Physiotherapy. Samples for analysis were taken during the processing of the soy-water suspension at the hydromodule (S: W = 1: 10) through the number of pulses 100 imp / l, 200 imp / l, 300 imp / l.

In the pulse range of 100-300 imp / l, the antitrypsin content in the liquid phase of the suspension first increased to 10.6 γ / ml, and then decreased, reaching 1.65 γ / ml at 300 imp / l, i.e. when more than 300 imp / l is supplied, antitrypsin is cleaved (Kazub V T, Orobinskaya V N, Konovalov D A, 2007).
The phenomenon of the cleavage of antitrypsin, an enzyme that impairs the assimilation of proteins, is of independent scientific interest and requires further study.

For the purity of the experiment, experiments were carried out to reduce the activity of trypsin inhibitors in other types of organic plant raw materials and to obtain protein from new raw materials sources, the burdock variety "Tohako Rio" was used as an experimental object. The study of the chemical composition of this type of organic raw material showed that this variety is rich in proteins, the total protein content in % for air-dry raw materials is 7.55 ± 0.13, shoots of 1 year were used for the study. Comparative analysis of the effect of electrical discharges on the decrease in the activity of anti-nutritional factors: 1) soy isolate trypsin inhibitor; and 2) an antitrypsin inhibitor contained in burdock variety “Tohako Rio” was determined in accordance with the recommendations of OSP TU 431.1418.990.02-92 and OSK TU 431.1419.990.01-92. The soy-water suspension, “burdock extract containing a trypsin inhibitor”, was treated with electric discharges at a hydromodule of 1:10, the antitrypsin content in the liquid phase of the soy isolate suspension first increases to 15.6 mg / g and then decreases to 1.65 mg / g (under the influence, the splitting of antitrypsin occurs).

![Figure 4](image-url)

**Figure 4.** Change in trypsin inhibitory activity in mg / g depending on the number of pulses

A decrease in antitrypsin containing in burdock extract occurs in the range from 300 - 500 imp / l and the residual trypsin inhibitory activity is 1.23 mg / g per air-dry raw material.

The phenomenon of the cleavage of antitrypsin, an enzyme that impairs the assimilation of proteins, is of scientific interest and requires special additional study.

3. Conclusions
When processing soybean suspension with electrical impulse discharges, the yield of protein and trypsin, an enzyme that ensures the assimilation of soy proteins by the body, increases, which improves the quality of the ingredients obtained. With an increase in the number of electrical impulses of 300 imp / l, the Kunitz inhibitors are degraded, which reduces their activity and increases the absorption of protein in the body.
A comparative study of the effect of electric discharge processing on the anti-nutritional factors of soybeans and burdock varieties "Tohako Rio" showed that when exposed to electric discharge processing, protein destruction does not occur, and the activity of trypsin inhibitors and their content decreases, which improves the quality of the products obtained.

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