Technology for reducing urease activity in soybeans

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Abstract. The main biochemical component of soy is protein, but it contains quite active anti-nutritional substances, such as proteolytic enzyme inhibitors, urease, lectins and others that reduce its nutritional value. This is the main deterrent to the use of soy in raw form for feeding farm animals and widespread use in the food industry. The article deals with the issues of inactivation of anti-nutrient substances using low-frequency ultrasound in water with hydrogen peroxide.

1. Introduction
Soy, depending on varietal characteristics and growing conditions, may contain 27 - 50% protein, 15 - 28 % oil, 14 - 33.2 % carbohydrates. Also, soy contains the optimal amount of such useful substances as: mineral salts from 3.2 to 4.2 %; calcium from 320 to 350 mg; iron from 9.2 to 14.9 %; phosphorus; in significant amounts of vitamins P, C, PP, E, and small A, B1, B2, B3, B6, K - all this indicates a high potential nutritional potential of soy [1].

A negative factor is that soy proteins contain inhibitors of proteolytic enzymes. Moreover, soy has two trypsin inhibitors that bind cystine and methionine. The most negative effect is provided by trypsin inhibitors-water-soluble Kunitza and alcohol-soluble Bauman-Birka [2].

Kunitz inhibitors are water-soluble proteins with a molecular weight of 20000-25000 Da that bind a single trypsin molecule to a small number of disulfide bridges, with an isoelectric point of 4.5. Bauman-Birka inhibitors are alcohol-soluble proteins with a molecular weight of 6000-10000 Da and with a small number of disulfide bridges that can inhibit trypsin and chymotrypsin with an isoelectric point of 4.0-4.2 [3].

Once in the stomach, only a part of soy inhibitors loses up to 30-40 % of their activity [4]. The remaining part, getting into the digestive system in an active form, inhibits the enzymes produced by the pancreas; this leads to its hypertrophy and sharply reduces the digestibility of soy protein [5]. This explains the relevance of research aimed at creating technologies that reduce the inhibitory activity of soy during its processing.

There are known technologies for inactivating harmful soy substances by water washing, soaking in a solution of salts, alkalis and acids [8, 9, 10]. However, chemical methods of processing soybeans are associated with high rates of process duration and the cost of wastewater treatment and waste disposal.

There are known thermal methods of processing soy, when the grain is fried, boiled, steamed, subjected to infrared or microwave processing [11, 12, 13]. The considered methods act selectively
and to a greater extent destroy urease, and the activity of inhibitors is preserved. Prolonged heat treatment leads to the destruction (denaturation) of the grain structure and the formation of porridge. During heat treatment in autoclaves, urease is destroyed, and the trypsin inhibitor is destroyed only by 45%. With microwave treatment, the trypsin inhibitor is destroyed, and the urease is only 40% destroyed [14]. All of the above are significant disadvantages of technology.

Micronization in soybean processing is used both as a separate technological operation and in combination with other technological operations [15]. The disadvantage of this technology is uneven heating in the volume of the entire grain [16]. During infrared processing, the grain is heated for a short period to 140-200°C, which leads to an instant increase in the stress state of the structure and, as a result, the appearance of micro-cracks, denaturation of inhibitors, and part of the starch is converted into dextrin. This treatment leads to overheating of the grain mass.

Methods of extrusion and expansion are also used, soya is processed under high pressure and at temperatures above 1000C [17]. However, the disadvantage of these methods is a high degree of protein denaturation and energy consumption.

Technologies that reduce the anti-nutrient substances of legumes without exposure to high temperatures are based on hydrodynamic extraction of the inhibitor in a liquid medium. In the production of food products from soy, such as tofu, it is soaked from 4 to 16 hours, which is undoubtedly unproductive and costly [18].

However, heat treatment, extrusion, simple soaking and other methods, as shown by patent and literary analyses, cannot completely rid soy of all anti-nutritional substances – this was the justification for the relevance of the topic and the feasibility of improving soybean processing technologies.

2. Materials and methods

The objects of research were zoned in the Saratov region varieties of soy «Zlata», «Bara», «Soer-4», «Soer-5» with different indicators of activity of the trypsin inhibitor and urease. The degree of urease activity was determined according to GOST 13979.9-69 Oilcakes and oilmeals. The degree of soybean grinding, the concentration of the oxidizer in the working solution, its consumption, and the parameters of ultrasonic radiation were studied. The experiment matrix is presented in table 1.

| Processing time, minutes | 10 | 20 | 30 | 40 | 50 |
|--------------------------|----|----|----|----|----|
| Concentration of hydrogen peroxide, % | 3 | 6 | 9 | 12 | 3 | 6 | 9 | 12 | 3 | 6 | 9 | 12 |
| Ultrasound frequency, kHz | 18 | + | + | + | + | + | + | + | + | + | + | + |
|                           | 20 | + | + | + | + | + | + | + | + | + | + | + |

3. Results of grain analysis

The share of water-soluble compounds in soy protein accounts for up to 90% of inhibitory activity and due to the fact that the mass ratio of water - and alcohol-soluble groups is approximately 2.3:1 for the removal of anti-nutrients, their extraction into a solvent is possible [20].

The purpose of this work was to develop technology and technical means for maximum removal of anti-nutritional substances in soy without affecting the useful high-performance nutritional components.

To intensify the process of inactivation of inhibitors, the mechanism of oxidation of soy protein in water with hydrogen peroxide under the action of low-frequency ultrasound was studied.
From the amino acid composition of soy protein, shown in figure 1, it follows that it consists of 9 interchangeable amino acids, which include aspartic and glutamic acids in an amount of 10.9% and 17.3%, respectively, of the total protein content [21]. They are dicarboxylic acids and have negatively charged radical groups. These amino acids have two carboxyl groups that dissociate, giving away two protons, but since they only have one amino group that takes one proton, these amino acids behave like acids and their solution has an acid reaction.

**Figure 1.** Forms of amino acids.

The amino acid ion itself is negatively charged. Thus, the reaction is accompanied by the formation of free-radical oxidation products, which leads to the formation of reactive oxygen species (figure 2).

![Chemical reaction](image)

**Figure 2.** The formation of free oxygen species.

On this basis, it follows that the action of hydrogen peroxide $H_2O_2$ on soy protein, in accordance with the amino acid composition, it must be oxidized. The targets of hydrogen peroxide are well-defined molecular structures, namely the sulphydryl, or thiol, SH-groups of a protein molecule belonging to the side chains of cesteine residues.

Thiol groups of proteins are subjected to mild oxidation with the formation of sulfonic acid (SOH), as well as between (or) intramolecular disulfide bonds, which are accompanied by conformational changes in the protein molecule. Redox transformations of SH-groups of essential cysteine signaling proteins to sulfonates and disulfides and, moreover, to sulfinites.
The rate and nature of oxidation of SH-groups depend on the concentration of reagents, pH acidity, temperature, as well as the spatial location of SH-groups in the protein. It is known that SH-groups play the role of a sensor of hydrogen peroxide concentration. In this case, many redox-sensitive proteins are activated as a result of oligomerization, which is induced by the oxidation of thiol groups, followed by the formation of intermolecular or intramolecular disulfides. Thus, the superimposition of the action of collapsing vapor-gas cavitation bubbles formed from ultrasonic acoustic waves of low frequencies (18-20 kHz) on the presented nonlinear system can be described by a nonlinear resistance or capacitance. Since the neutral amino acids that are part of the soy protein (valine, arginine, isoleucine, leucine, methionine, treoin, tryptophan, phenylalanine, alanine, glycine, Proline, serine, tyrosine, cysteine), do not have a charge, but given the fact that in an acidic environment with an increase in the number of protons (acid) increases the number of NH\text{3}^+ groups, hydrogen peroxide, showing weak acid properties (K = 1,4\cdot10^{-12}), dissociates in two stages:

\begin{align}
\text{H}_2\text{O}_2 \rightarrow & \text{H}^+ + \text{HO}_2^- \\
\text{H}_2\text{O}_2 \rightarrow & \text{H}^+ + \text{O}_2^{2-}
\end{align}

Hydrogen peroxide molecules, having a strong polarity, recharge soy protein molecules from "-" to "+" charge, thereby suppressing the reactionary chemical activity of oxidized soy protein urease enzymes, which contributes to faster protein breakdown by enzymes of the gastrointestinal tract.

Based on the above, according to the classification of solubility of D. N. Pryanishnikov, inhibitors manifest albumins that perform plastic functions in cell tissues. The composition of albumins from the total amount of soy protein includes: leucine-7.6%; aspartic acid-10.7%; and glutamic acid-17.2 %, having a negative charge of the radical group. The percentage of the amino acid composition of soy protein (34.9% total) and the classification by radical polarity are shown in table 2.

| Amino acid                  | % contents | Polarity\textsuperscript{a} |
|-----------------------------|------------|-----------------------------|
| Arginine                    | 6,6        | «+» positively charged R-groups |
| Valine                      | 5,8        | nonpolar R groups           |
| Histidine                   | 2,6        | «+» positively charged R-groups |
| Isoleucine                  | 5,1        | nonpolar R groups           |
| Leucine                     | 7,6        | nonpolar R groups           |
| Lysine                      | 5,8        | «+» positively charged R-groups |
| Methionine+Cysteine         | 3          | polar uncharged R groups    |
| Threonine                   | 3,8        | polar uncharged R groups    |
| Tryptophan                  | 1,1        | aromatic R groups           |
| Phenylalanine + Tyrosine    | 7,4        | aromatic R groups           |
| Aspartic acid               | 10,7       | «-» negatively charged R groups |
| Alanine                     | 4,2        | nonpolar R groups           |
| Glycine                     | 4          | nonpolar R groups           |
| Glutamic acid               | 17,2       | «-» negatively charged R groups |
| Proline                     | 5,2        | nonpolar R groups           |
| Serine                      | 5,6        | polar uncharged R groups    |
| Tyrosine                    | 3          | aromatic R groups           |
| Cysteine                    | 1,3        | polar uncharged R groups    |

\textsuperscript{a} - Classification of amino acids by radical polarity (by Leninger)

The result of the conversion of proteins soy based acoustic microflows in the presence of hydrogen peroxide is the change in the isoelectric point of a protein with the change of the negative charge "-" positive "+". Thus, disulfides are reorganized into two inter-chain disulfide bonds, the formation of which activates the protein, changing its physical and chemical properties, leading it to interact. It is
experimentally confirmed that when the environment changes, the macromolecules of proteins become charged, their ability to attach water is activated and they become able to form complex mixtures.

Thus, the oxidized soy protein without free radical oxidation is not adapted to destruction by tryptophan and chymotrypsin, since its chemical compound has a peptide bond. Oxidized proteins in the ultrasound medium intensively attach water, showing hydrophilic properties, swell, increase the mass and volume.

The normalized indicators of anti-nutritional substances include the content of urease from 0.1 to 0.3 units of pH (GOST 27149-95 Soy-bean oilcake as live-stock feed. Specifications) or 0.02-0.2 units of pH (GOST R 53799-2010 Toasted soybean meal as livestock feed. Specifications). The activity of urease before treatment was determined in the studied soy samples (table 3).

**Table 3. The content of anti-nutritional substances in soy.**

| The indicator of the grade | The name of the variety |
|---------------------------|------------------------|
| Urease activity, [pH units] | Zlata | Bara | Soer-4 | Soer-5 |
| 2.33 | 2.26 | 2.21 | 2.31 |

Treatment of crushed soy with hydrogen peroxide with a minimum concentration of 3 % showed that the activity of urease decreases with increasing degree of grinding (figure 3).

![Figure 3. Urease activity, pH units.](image)

The best results were achieved with fine grinding of soy, when the particle size reached 0.25 mm. Soybean treatment was performed at different concentrations of hydrogen peroxide under the influence of ultrasound 18 and 20 kHz, which were selected in the experiments (table 1). Experimentally, the optimal concentration of the solution was established, which was 9% and the ultrasound frequency was 20 kHz [22].

Figure 4 shows data on the decrease in urease activity during ultrasound treatment with a frequency of 20 kHz in an oxidizing solution of hydrogen peroxide, a concentration of 9% for 30 minutes.
4. Conclusion
Low-frequency ultrasound combined with the oxidizing effect of hydrogen peroxide allowed to reduce the activity of urease to the normalized values, while the most technologically advanced crop was soy grade "Zlata".

Experimental studies have shown that the effect of low-frequency ultrasound on soy in an oxidizing solution of hydrogen peroxide reduces the activity of urease to normalized values. Oxidized soy protein acquires a reactive chemical activity, for faster cleavage by enzymes.

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