Study of the effect of soybean and chickpea seeds watering on protein complex solubility

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Abstract. The functional properties of the protein complex of soybean and chickpea seeds during germination have been studied due to the growing interest in hypoallergenic foods and the search for new sources of plant proteins in the technologies of innovative products. Methodology and technique of germination start, developed earlier, allowed to reveal peculiarities of the process in chickpea seeds. Critical points of successful swelling of chickpea seeds with protein content of 22.5±1.8 at 22 °C: the duration of soaking 6 - 7 h, critical humidity 58.7% in the north-east, inclination from 28 h. Ability to germinate 94%. Comparison with soybean seeds revealed a close nature of the water absorption rate. Conditions for obtaining a stable water polydisperse system from swollen seeds: degree of crushing <0.5 mm; extraction temperature 50 - 60 °C. Dimensions of peeling phase - stability index in systems from swollen seeds 8-30 %, from dry seeds 40-60 %. Correlation dependence between technological factors and physiological state is 0.84-0.98. Electronic microscopy of repentant and swollen seed cells clearly confirms the start of hydrolysis processes.

1. Introduction

Due to its high yield and protein content of up to 50% in beans, soybean is a key agricultural crop in solving food problems and stands out as a basis for agro-food transformations in the Russian Federation. Soybean is distinguished among leguminous crops not only by completeness of chemical composition, but, first of all, by content of essential amino acids in protein, which is alternative to animal proteins.

In 2018 the Oryol region was included in TOP-10 regions by soybean areas and develops this direction. District varieties created by breeders of the Federal Scientific Center for Grain Legumes show high yields of 17.2 centners/ha (with a national average of 14.5 centners/ha) [1].

Along with soya, chickpea culture has attracted breeders' attention in recent years. Its production is developing very dynamically: from 150 thousand ha in 2010 to 850 thousand ha in 2018. Chickpea crop is characterized by high drought and heat resistance, and the seedlings can withstand short-term frosts up to -6 0C. These features are attractive due to climatic changes observed in recent years on the territory of Russia (increased average daily temperatures, more frequent droughts). Nut and its products are used as a functional additive in a number of food products, complete proteins, balanced amino acids, the presence of minerals and vitamins make it attractive. It is an excellent product for dietetic nutrition [2]. The advantages of the crop are low exposure to pests and diseases of major leguminous crops, high symbiotic efficiency in the application of specific strains of nitrogen fixing bacteria. As a result of many years of research, the specialists of FNC ZBK have created a new variety...
of chickpea Avatar, characterized by high indicators of seed quality: protein content is 22.3%, weight of 1000 seeds is 249 g, digestibility factor is 2.2, cooking time is 131 minutes. [3].

In connection with the growing interest to hypoallergenic foodstuffs and the search for new sources of plant proteins in the technologies of innovative products, the research of biochemical characteristics and functional properties of protein complex of soybean and chickpea seeds at the start of germination (change of physiological state) are especially relevant.

It is known that the spare proteins of chickpea and soybean 11-13S combines phylogenetic relation, they are full on the content of essential amino acids and close in fractional composition. Solubility, functionality of such proteins determines the success of their processing into drugs or natural products.

All known technological methods of protein separation from legumes are based on chemical and physical influences, leading to the change of protein complexes nature and, practically, to the loss of functional properties, and only biotechnological approaches allow to gently separate them into solutions [4, 5]. The change of functional properties of protein complex at change of physiological state, connected with the start of germination, is of considerable interest.

The aim of the conducted researches was to study the solubility of protein complex of soybean and chickpea seeds in the state of rest and germination; to determine the conditions of obtaining the stable water polydisperse system from swollen seeds. Water extracts from dry seeds were used as a control.

2. Materials and methods

On the one hand, the species difference between soybean and chickpea seeds, and the matrix heterogeneity of the seed mass in each batch, on the other hand, we considered it necessary to specify germination conditions and develop methodological approaches. Justification of methodological approaches and methods to start germination for soybean seeds and some other dicotyledons, united by phylogenetic affinity of protein complex is presented in our publications [6].

At the first stage of the work the tasks of the research included: in laboratory conditions to test the process of germination of chickpea seeds: to determine critical points (degree of watering of seeds, critical humidity), to identify the stage of swelling, which borders with the start of germination, to compare with soybean seeds.

At the second stage, we studied the impact on the aggregate stability of the polydisperse system from swollen seeds of the degree of crushing and extraction temperature. Dispersions from dry seeds were prepared as a control in the same conditions.

For this purpose, dry and swollen to critical humidity chickpea and soybean seeds were dried to 22 - 25% moisture, grinded in a colloid mill to particle size 1 - (<0,5) mm, poured water at a temperature of 40-75 °C, in a ratio of 1:6-8, insisted, filtered.

The research material was soybean seeds of Mezenka and chickpea varieties of Avatar I reproduction, at rest and swelling, and water dispersions from them.

The activity of seeds under study was determined according to GOST 10968 - 88 "Germination energy and ability to germinate"; the degree of seed swelling was determined by the ratio: the difference in the mass of seeds before and after swelling, to the mass before swelling, in recalculation for 1 g of dry matter, the content of Lowry protein.

The microstructure of the endosperm of dry and swollen soybean seeds was studied using the electron scanning microscope YSM-6390 Japan. The authors express their gratitude to the specialists of FSBNU VNII of fruit crop selection (Eagle) for providing equipment and assistance in conducting research.

Aggregate stability of polydisperse systems was estimated by stability index, sample volume n=12. Experimental data were processed by methods of mathematical statistics in STATISTICA v.10 (StatSoft, Inc.) and Microsoft Office Excel 2010.

3. Results

Researches have established that energy of germination on experimental batches of seeds of soya and chickpea makes 80-85 %, ability to germination 94-96 %.
In vitro testing of the germination process of chickpea seeds showed that the process of swelling continues during the whole period of soaking. Soybean and chickpea seeds after 12 hours are on average 2.3 to 2.6 times heavier than their original weight. In soybean seeds, the intensity of swelling decreases after 8 hours, and in chickpea seeds it decreases by 6-7 hours.

It was found that critical humidity of seeds of both crops is from 56.8 to 59.5 % per raw mass, time of single specimens swelling is from 28 to 34 hours depending on water absorption temperature. The assumption that in swollen seeds the cell content is hydrolysed and present in dissolved state was confirmed on soybean seeds with an electron microscope.

Aqueous extracts were then prepared from the dry and swollen seeds, representing polydisperse systems.

A positive effect on the stability of the system of seed grinding and temperature increase up to 60 °C and the negative effect of temperature above 60 °C have been revealed. Correlation dependence 0.84-0.98. Dry seeds have 40-60% peeling phase size. In swollen seeds - 8 - 30 %. Verification tests of swollen seeds showed considerably higher stability of the system - 3-8% of the peeling phase.

4. Discussion

Pre-selection of seeds of both crops according to germination energy and germination ability allowed to choose the lots with high activity.

Water absorption, swelling, and germination are the essence of various physiological states of germinating seeds, and the main driving role belongs to the protein complex, which consists of preexisting endoferments (albumin fraction) and, mainly, spare globulins [7, 8]. At the same time, it should be taken into account that the majority of specialists do not separate the phases of swelling and sticking, while the determining function is performed by the achievement of critical seeds' humidity, after which germination starts.

The place of deposit of spare proteins in leguminous seeds are intracellular formations - protein bodies, or aileron grains. By their structure aleyron grains are represented by two types: simple, containing no foreign inclusions, they are typical for soya and other leguminous seeds; complex, containing inclusions, typical for oil seeds [9]. The chemical composition of soybean seeds also contains a significant amount of fat (15-18%) enclosed in spherosomes.

On the electronic photo of opened cells of dry soybean seeds, in the state of physiological rest, protein and fat inclusions are clearly visible (Figure 1).

Figure 1. Electronic photograph of soybean seed cell structures at physiological rest. Large spherosomes and aileron grains with packed proteins are clearly visible

Depending on the botanical seed type and chemical composition, different amounts of water are required to swell and start germination. The trigger, as mentioned above, is for the seeds to reach critical humidity. The conditions for regulating the process in the laboratory are temperature, sufficient
aeration and seed immersion time. Compliance with these conditions prevents rotting, loss of weight and nutrients [10].

How the process of soaking the seeds affects the endosperm content is shown in the electronic photo of the cells of swollen soybean seeds (picture below 2).

![Electronic photo of endosperm of swollen soybean seeds. It shows an open cell dissolved content. Mineral inclusions can be seen on the cell surface.](image)

It is known that when living seeds are swollen, in addition to physical processes, biochemical ones are included - proteins-enzymes are activated, at the initial stages of germination start hydrolysis processes prevail, but the stage of swelling is not considered. The photos below show the inclusion of these processes. Thus, swollen cells are filled with biologically active substances in dissolved form, and proteins are already at this stage of hydrolysis.

Figure 3 shows the process of chickpea seed swelling in comparison with soya, from which it is seen that the set of moisture continues the whole period of soaking. After 12 hours, the seeds of both crops are on average 2.3-2.6 times heavier than their original weight.

![Swelling curves of chickpea seeds Avatar and soybean varieties Mezenka at water temperature 22 °C.](image)
At the same time, when germination starts, there is always a danger of soaking the seeds and loss of activity, so the attention was focused on the critical points of decreasing the activity of swelling - 8 hours in soybeans and 6-7 hours in chickpea.

The difference between cultures is explained by the different protein content in their composition. Mezenka soybean seeds contain 38±2.5% of soybean seed and Avatar chickpea seeds 22±1.8%.

Proteins exhibit hydrophilic properties and can absorb up to 180% of water from the mass of dry matter. At the same time, not only proteins have affinity to water, but also starch, which in this case is present in chickpea seeds (carbohydrate complex makes more than 50% on er.), which absorbing ability makes up to 70%, and fibers up to 30% [11]. This explains the duration of moisture absorption by seeds of chickpea and soybean up to 12 hours.

In this graph, the phases of water absorption and swelling are not separated, while the start of enzyme activity and the beginning of hydrolytic processes start from reaching critical humidity. In a sense, it can be assumed that endo-enzymes are activated at the points of decreasing swelling intensity, as can be seen from the figure.

In this connection, we have carried out experiments to determine the critical humidity and studied the effect of two temperature regimes at the set duration of moisture absorption (Table 1).

| Table 1. Soybean and chickpea seed germination activity, average values |
|---------------------------------------------------------------|
| Indeces | Moisture Absorption, ч |
|         | Soybeans, 8 | Chickpea, 7 |
|         | 10 °С | 22 °С | 10 °С | 22 °С |
| Germination energy, % | 80 – 85 | 94 – 96 |
| Ability to germinate, % | 34 | 28 | 32 | 28 |
| Beginning of the tickering of single specimens, h; | 57.6 | 59.5 | 56.8 | 58.7 |
| moisture, % | 39.9±1.5 | 22.5±1.8 |
| Protein content, % p.v. (hours) | 1.39 (72) | 2.08 (48) | 1.58 (60) | 2.38 (48) |

Researches have shown that critical humidity of seeds of both cultures makes from 56.8 to 59.5% on raw weight, time of activation of single copies from 28 to 34 h depending on temperature of key water that coincides with data of theoretical sources [9, 11]. Taking into account the data obtained, soybean seeds from 8 to 10 hours of soaking and chickpea seeds from 7 to 9 hours were considered swollen, and the start of soybean germination and biochemical processes was assumed to occur within the same period.

Then polydisperse systems were prepared by the above method.

Comparison on stability of systems from dry seeds and swollen ones allowed to draw conclusions on start of hydrolytic processes at achievement of critical humidity, so from dry seeds sizes of peeled phase made 40-60%, from swollen 8-30 %.

In determining the influence of technological factors - the degree of seed crushing and extraction temperature, statistical treatment allowed to reveal the presence of trends and non-linear character of the connection (Figures 4, 5).

Thus, in dispersions made of dry seeds there is a correlation relationship between the degree of grinding and temperature increase up to 75 °C.

In dispersions from swollen seeds there is a nonlinear relationship between two factors: the minimum stratification is observed when the seeds are finely crushed to 60 °C, the temperature increases to 75 °C. The correlation dependence decreases with increasing particle size.
Figure 4. Dependence of mean values of stability index on extraction temperature at different degrees of dry seeds grinding.

Figure 5. Dependence of average stability index values on extraction temperature at different degrees of swollen seeds grinding.

In our opinion, this is due to the fact that the structure of spare proteins in globulin seeds is organized in such a way that hydrophilic N-terminal amino acid groups are located on the surface of the molecules, while hydrophobic groups are inside the molecules. The formation of a hydrate shell in protein globules promotes the unwinding of the quaternary structure and the launch of hydrolytic processes under the control of preexisting endoenzymes, which is accompanied by the appearance of subunits, large polypeptides, peptides and peptones, surface-active properties of proteins. In addition, the synthesis of intermediate exchange enzymes and their activity lead to the accumulation of albumin fraction and peptide residues, which easily transfer to the solution and also participate in the structuring of the polydisperse system. However, in the swollen state when starting hydrolytic processes in cell structures, protein fragments of chains are more sensitive to the temperature factor due to open N-terminal groups and the temperature above 60 °C leads to deposition of some of them.

Pilot tests on the stability of dispersions from sunken seeds showed that the peeling phase size was 3-8 %. These results can be considered as confirming the assumption that the change of physiological state is accompanied by the activation of the protein complex FTS.
5. Conclusion
The application of the previously developed method to start soybean seeds germination, to study chickpea seeds has shown:
1. Critical points of successful swelling of chickpea seeds with protein content 22.5±1.8 at the temperature 22 °C: the duration of soaking 6 - 7 hours, critical humidity 58.7% in the north-eastern part of the country, inclination from 28 h. Ability to germinate 94%. Comparison with soybean seeds revealed a similar swelling rate.
2. Conditions for obtaining a stable water polydisperse system from swollen seeds: degree of grinding <0.5 mm; extraction temperature 50 - 60 °C. Dimensions of peeling phase - stability index in systems from swollen seeds 8-30 %, from dry seeds 40-60 %.
3. The obtained results allow to expand the possibilities of processing enterprises for obtaining innovative products from chickpea seeds.

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