The research of changes in biochemical parameters extracts of sprouting soybean and rapeseed seeds

L A Samofalova¹, N A Berezina², O V Safronova³ and T O Kunitsyna³

¹Federal Scientific Center of Legumes and Groat Crops, 10, building 1, Molodezhnaya street, pos. Streletsky, Orel district, Orel region, 302502, Russia
²Orel State Agrarian University named after N V Parakhin, 69, Generala Rodina street, Orel, 302019, Russia
³Orel State University named after I S Turgenev, 95, Komsomol’skaya street, Orel, 302026, Russia

⁴E-mail: oksana-orel@mail.ru

Abstract. At different periods of swelling and incubation (12, 24, 36, 48, 60 h) soybean seeds were selected: swollen (after reaching the critical humidity of soy 58.6 %; rapeseed 43.6 %); sprouted “spouts”, with sprouts ≥1 cm. It was found that the protein content in seedlings gradually decreases from 37.0–41.3% in dormant seeds, to 18.6–20.3% in sprouts after 48 hours in rapeseeds and 60 hours of incubation in soybeans. The developed method of differentiation of germinating seeds by physiological state made it possible to register step-by-step changes in extracts according to pH and buffer capacity (BC). This rapid analysis is applicable in the development of technology for protein complex extraction and preparation of protein preparations from non-traditional sources.

1. Introduction
The priority of the state policy in the field of healthy nutrition of the population is related to the elimination of the deficit of high-grade protein and micronutrients. The strategy for achieving this goal is not only to increase the productivity of crop production and animal husbandry based on the technology of cultivation of legumes, oilseeds and cereals, but also to provide scientific support for the latest technologies for deep processing of food raw materials and food production, increasing protein production from non-traditional sources. The focus is on the processes of enzymatic and chemical modification of plant raw materials, which increase the efficiency of processing, contribute to improving the quality and nutritional properties of final products.

The work was carried out with the financial support of the Ministry of Science and Education in the framework of the State Task of the Federal Scientific Center of Legumes and Groat Crops under the item 0636-2019-0008 "Mobilization of genetic resources of legumes and groat crops for use in breeding process".

Spare proteins from legumes and oilseeds are important raw materials for the food industry. Their behavior in food systems is determined by their functional properties, which can be regulated by chemical and biotechnological methods that ensure the flow of hydrolytic processes [1]. The use of seed endoenzymes activated during germination in biotechnology is very promising.
A number of researchers [2, 3] believe that at the initial stages of germination, after water absorption during soaking, enzymes are activated that ensure the formation of an amino acid pool necessary for the synthesis of hydrolases, which in turn catalyze the mobilization of reserve substances [4]. It is especially important that during the proteolytic decomposition of food proteins, a large number of physiologically active peptides are absorbed, which are more functional compared to the reserve proteins of dormant seeds [5, 6, 7].

With limited hydrolysis of seed spare proteins, a high-molecular component (modified protein) is formed, as well as polypeptides with a significantly lower molecular weight. Depending on the requirements imposed on the properties of the protein component, the problem arises of obtaining in the process of hydrolysis either a predominantly modified protein, or polypeptides, or an amino acid pool. To do this, it is necessary to record the stages of hydrolysis. In our study, we consider the possibility of recording changes in the electrochemical characteristics of water extracts of germinating seeds.

2. Materials and methods
Zoned varieties of dicotyledonous agricultural plants were chosen as the objects of research, the main share in the protein complex of which is made up of globulins. The structural relationship of dicotyledonous globulins is expressed in a uniform type of spatial configuration, combined by the formula 11-13S. Based on the similarity of quaternary structures, it is possible to draw a parallel not only between soybean and oilseed rape, but also between such phylogenetically distant crops as buckwheat and hemp. This also suggests the same type of dissociation of globulins under the influence of modifying factors (in particular, during natural fermentation during germination) and the possibility of solving the problem of their isolation into solutions.

Changes in the electrochemical properties and, consequently, the functionality of proteins can be judged by biochemical parameters – the activity of hydrogen ions in the pH medium and buffer capacity. The concentration of hydrogen ions in different plant species and varieties varies significantly. In biological studies, an accurate method for determining the concentration of hydrogen ions is used not only to characterize crops and varieties, but also to control metabolic processes, including during germination [8].

The method of setting up the experiment was as follows: starting germination according to the author's method described [9]; then sorting seeds by physiological state, we covered all the stages indicated above. The process was stopped after 60 h of incubation, since seeds with sprouts of more than 1 cm passed into the plant formation phase. Hoods with a 1:10 hydro module were prepared. Changes in the protein complex were analyzed by biochemical, electrochemical, and physico-chemical parameters. Rapeseed seeds, due to their physiological characteristics, were incubated for 0, 12, 24, 36, 48 hours.

The moisture content by drying and bringing to a constant mass, the determination of total conditionally soluble nitrogen was carried out in an extract from fallen seeds. Before preparing the extracts, dry seeds were degreased.

To determine the active acidity (pH) and buffer capacity of the protein-salt complex, dry, swollen, hatched seeds were dried on a filter, ground on a rotary-type grinder to flour, a weight of flour of 10 g was taken, mixed with 100 cm³ of hot distilled water and infused for 1 h, extracts were prepared, filtered. pH and BC were determined in the obtained extract according to the procedure described in [8]. The BC titration curve was plotted based on the volume of 0.1 M alkali (acid) solution cm³, which is required to be added to 100 cm³ of the sample in order to change its pH by one.

3. Results
The method of starting germination and differentiating the seeds of two crops by physiological state allowed us to register changes in the protein complex from swelling-hydration, to hydrolysis-peptization, as well as the appearance of an amino acid pool. Registration of states was carried out using indicators of the active acidity of the medium pH and the buffer capacity of BC. The duration of swelling was determined until the seeds reached critical humidity. According to the authors data [10, 11] this indicator is different for different seeds: legumes account for 47-59% of the raw weight, while oilseeds
account for 44-62%. In our studies, the critical moisture content of soybean seeds was established – m=58.6%; rapeseed 43.6% (table 1).

Table 1. Determination of the range of critical humidity in sprouted seeds, %.

| Swelling-germination, h | soybean | rapeseed |
|------------------------|---------|----------|
| 12-18                  | -       | 44.8     |
| 20-24                  | -       | 45.2     |
| 30-36                  | 57.6    | 42.5     |
| 42-48                  | 59.8    | 44.2     |
| 60                     | 61.3    | 44.8     |
| 72                     | 62.7    | -        |

* - a dash indicates that there are no sprouted seeds.

As can be seen from the table, the protein content in seedlings gradually decreases from 37.0-41.3% in dormant seeds, to 18.6-20.3% in sprouts after 48 hours in rapeseed and 60 hours of incubation in soy (table 2). At the same time, in swollen seeds, the decrease is least significant m=2.3%. More significant changes occur during the transition to the formation of sprouts - 16.7%.

Table 2. Average total protein content of germinating soybean seeds in physiological phases.

| Physiological state of seeds, incubation time, h | Grade, protein content, % |
|------------------------------------------------|---------------------------|
| resting                                        | Lanceolate, Swapa         |
| swollen, 24 h                                  | 37.0, 41.3                |
| "spouts", 48 h                                 | 35.3, 38.5                |
| sprouts 1 cm, 48 h                             | 35.0, 36.9                |
| sprouts >1 cm, 60 h>                           | 24.5, 26.2                |
|                                               | 18.6, 20.3                |

The active acidity of the medium (pH) is created in cells as a result of the intake and formation of various substances - mineral salts, organic acids, and more complex hydrolysis products-the synthesis of cellular polymers. A significant role is played by proteins that have electrochemical properties that manifest themselves in metabolic processes. Due to the natural mechanisms of metabolism in plant tissue, extracts obtained from seeds have a certain pH and BC level, i.e. the ability to maintain a given concentration of hydrogen ions (H+) in the system, despite its dilution or addition of substances with variable pH values. The difference of 0.06 units established by us at the stage of swelling is 10% of the decrease in the concentration of hydrogen ions from dry seeds, which, apparently, is associated with the consumption of pre-existing enzymes at the earliest stages. In the future, the observed acidic pH shift by 48 h of incubation is 10% of the increase in the concentration of hydrogen ions in the medium, which indicates the beginning of hydrolysis of cellular polymers. A hydrogen ion activity value of 6.56 for hatched seeds and seeds with sprouts means an increase in the concentration of hydrogen ions of about 100% compared to the initial value for dry seeds.

According to [8], buffer systems in living organisms and biological objects determine the constancy of the acid content of various protein fluids. pH constancy is also maintained by the cell buffer system. Thus, a certain increase in BC found at the beginning of incubation is gradually replaced by a gradual decrease towards the period of active growth, i.e., the appearance of new proteins. It was also found that in soybean seeds, a drop in pH and BC coincides with the appearance of large sprouts after incubation for more than 48-60 hours, when the formation of a new protein becomes obvious. A similar pattern is seen in the case of germinating rapeseed seeds, which also contain a significant amount of protein (figures 1-4).
4. Discussion
In a complex polydisperse system, which consists of extracts from sprouting soybean seeds, prepared by us according to the method as dilute systems, the source of hydrogen ions is the polyionic structure of the protein complex, protein breakdown products, organic acids, their salts, and mineral compounds in ionic form. At the same time, it should be taken into account that the overall picture is a consequence of changes in osmotic conditions in the cells of the embryo and cotyledons, due to water absorption during swelling and additional dilution during the preparation of extracts, according to the 1:10 method, i.e. the buffer content in the solution is reduced.

It is known that cellular proteins generally have a negative charge, which is balanced by inorganic kathions [12]. The results of studies of the activity of hydrogen ions and the buffer capacity of soybean seeds are shown in figures 1 and 2, which show that there is a relationship between the physiological state of seeds and electrochemical indicators.

![Figure 1. Hydrogen ion activity pH of soybean seeds in different physiological phases: 0-dormant; 1-4-swollen (12 h, 24 h, 36 h, 48 h); 5-6-hatched (36 h, 48 h); 7-sprouts (48 h); 8-sprouts >1 cm (60 h).](image)

The graphs show that in the initial period of 12 hours, the pH of swollen seeds shifts to the neutral side, protonation of the complex is observed, which is replaced by gradual deprotonation and a shift to the acidic side, especially noticeable during pecking and growth. Such a picture may be the result of a change in the total electric charge of the surface of a protein molecule, due to the destruction of chemical bonds and the decay of the quaternary structure.

Dissociation of 11SS proteins is three-step, following the scheme: 11SS → 2∙7S → 6∙3S → 12∙2S [13, 14]. This is due to the fact that the structure of spare globulin seed proteins is organized in such a way that hydrophilic N-terminal amino acid groups are located on the surface of the molecules, while hydrophobic ones are located 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 pre-existing endoenzymes, which is accompanied by the appearance of subunits, large polypeptides, peptides and peptones, and surface-active properties of proteins are manifested. Further, the synthesis of intermediate exchange enzymes and their activity lead to the accumulation of the albumin fraction and peptide residues, which easily pass into solution and also participate in the structure formation of the polydisperse system.

Thus, the changes detected in swollen seeds after 12 hours from the start of germination are associated with dissociation of 11SS proteins into semi molecules, destruction of subunits, and partial hydrolysis associated with the launch of pre-existing end enzymes. The beginning of hydrolysis of cellular polymers is indicated by a pH shift to 48 h of incubation. A hydrogen ion activity value of 6.56 for hatched seeds and seeds with sprouts means an increase in the concentration of hydrogen ions of about 100% compared to the initial value for dry seeds.
Figure 2. Buffer capacity of soybean seeds in different physiological phases: 0 - dormant; 1-4 swollen (12 h, 24 h, 36 h, 48 h); 5-6 hatched (36 h, 48 h); 7-48 h sprouts; 8-60 h, sprouts.

The buffer capacity shows that the ratio of acidic and alkaline active groups changes. It was also found that in soybean seeds, a drop in pH and buffer capacity coincides with the appearance of large sprouts after incubation for more than 48-60 hours, when the formation of a new protein becomes obvious. At the same time, as shown in table 2, the total protein content is reduced by 1.5-2 times.

Figure 3. Changes in the activity of hydrogen ions - pH and buffer capacity-BC in the general population of germinating soybean seeds: incubation time (12±2 °C); 0, 12 h, 24 h, 36 h, 48 h, 60 h.

It is noteworthy that the seeds that are in different incubation periods coincide in the physiological phase. This is due to the fact that in the total volume of any batch of seeds, individual specimens are physically, biochemically and physiologically heterogeneous. This is an objective reality caused by many biological, soil-climatic and agrotechnical factors. For such reasons, it was advisable to register electrochemical parameters in the general population of germinating seeds.

Figure 4 shows that the pH constancy is maintained by the buffer system of cells: a certain increase in buffer capacity at the beginning of incubation is gradually replaced by a gradual decrease by the period of active growth, i.e., the appearance of new proteins.

The obtained results of changes in the electrochemical properties of germinating soybean seeds were compared with rapeseed seeds also in the general population (figure 4).

The conditions of the experiment did not provide for incubation for more than 48 hours, i.e. the transition to the formation of a new plant. In contrast to soybean seeds, the revealed dynamics of the buffer activity of proteins of germinating rapeseed seeds indicates the activation of the protein complex and the salt composition of protoplasm, much earlier in 16-32 hours before hatching.

Studies show that the drop in buffer capacity coincides with the seeds reaching critical humidity, i.e. it can be considered the result of dilution of the cell contents. The subsequent increase in indicators can be explained by the activation of hydrolysis and the increase in synthetic processes in the protein complex. The pH level of rapeseed extracts is 6.5–7.0.
5. Conclusion

Thus, using the example of soybean and rapeseed seeds, which belong to dicotyledonous plants by the structure of a protein complex with the same subunit composition, it is shown that the registration of changes in physiological phases during germination can be carried out by electrochemical parameters of pH and buffer capacity. The indicators reflect the stages of hydrolysis of the protein complex from the destruction of subunits into semimolecules to the formation of peptides and an amino acid pool, when the protein content decreases markedly.

The results obtained make it possible to control the course of hydrolytic processes in the protein complex, including globulins, using rapid analysis and solve problems of increasing the extraction of proteins from non-traditional sources.

References

[1] Braudo E E, Danilenko, A N Dianova V T et al. 2000 Plant protein: new perspectives: collection under the editorship ed E E Braudo (Moscow: Pishchepromizdat) 6-23
[2] Kulioli J 1991 Vegetable protein (Moscow: Arponpromizdat) 527
[3] Bau H M, Villaume C, Nicolas J P and Mejean L 1997 Effect of germination on chemical composition, biochemical constituents and antinutritional factors of soya bean (Glycine max) J. Sci. Food Agric 73.1
[4] Dalling M J and Bhala P L 1984 ed D R Murrey Academic Press: Sidney et al. 2 163
[5] Samofalova L A and Donskaya M V 2021 IOP Conference Series Earth and Environmental Science 640(2) 022076
[6] Filippova M M, Karelina A A and Ivanov V T 1997 Bioorganic Chemistry 23 88
[7] Grace Oluwatoyin Oguntakin, Sogo James Olatunde, James Abiodun Adeyanju and Olanike Oyindamola Leke 2015 Journal of Chemical and Pharmaceutical Research 7(11) 494-8
[8] Ermakov A I Arasimovich V V Yarosh N P et al. 1987 Methods of biochemical research of plants (Leningrad: Agropromizdat. Leningrad department) 430
[9] Samofalova L A and Safronova O V 2017 Legumes and cereals 3(23) 68-74
[10] Golovina E 2020 Legumes and cereals 1(33) 45-9
[11] Askochenskaya N A, Ermakova A I, Arasimovich V V et al. 1987 Physiology and biochemistry of dormancy and seed germination (Leningrad: Agropromizdat. Leningrad department) 430
[12] Nechaev A P, Traubenberg S E, Kochetkova A A et al. 2003 Food chemistry (St. Petersburg: GIORD) 640

Figure 4 - Buffer capacity of germinating rapeseed seeds, incubation: 1-0 h, 2-12 h, 3-24 h, 4-36 h, 5-48 h.