Study of the calcium and phosphorus exchange dynamics in germinating oilseeds

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Abstract. The article is aimed at unifying technological approaches to production of functional drinks from hemp and soybean seeds. We studied the change in chemical states of calcium and phosphorus during swelling and germination, as one of the factors that enable the osmoregulation of the protein complex and increase stability of plant dispersions from bioactivated seeds. Seeds at rest, swelling seeds and germinating seeds and aqueous dispersions based on them were studied. The sources of insoluble and soluble forms of macronutrients in the cellular structures of bioactivated seeds were studied. It has been established that swelling forms of calcium included in the general metabolism become mobile. A further increase in calcium content in the aqueous dispersion up to 98.5% is accompanied by a rapid increase in conditionally soluble calcium (14.6 times) and an increase in stability of the system. Calcium metabolism in seeds is accompanied by changes in the phosphorus-salt composition. Within 48 hours, with an insignificant (4.7%) decrease in total phosphorus, an increase in soluble inorganic phosphorus (67.5%) and a decrease in organic phosphorus (51.9%) were observed. The appearance of inorganic phosphates and calcium ions in cell juice can be associated with the hydrolysis of phytin. In such forms, digestibility of phosphorus and calcium increases. Similar results were obtained for dispersions from germinating soybean seeds.

1. Introduction. Problem statement
The improvement of modern food production technologies is related to the development of specialized functional products. Herbal analogues of milk are close to cow milk in their basic chemical composition and contain a number of indispensable components in an easily digestible form. Functional drinks based on germinating oilseeds are highly digestible and contain biologically active protein, peptides, free amino acids, lecithin, soluble sugars, dietary fiber, biogenic macro- and micronutrients, vitamins, phytohormones and other valuable components [1, 2].

The content of calcium and phosphorus in the seeds of different crops is quite high; however, oilseeds are most rich in these elements (Table 1).
Table 1. Content of calcium, phosphorus and magnesium, mg% in ash [3, 4, 5, 6]

| Element  | oil plants, mg / 100g | grain plants, mg/100 g |
|----------|-----------------------|-------------------------|
| Ash, %   | soybeans              | cannabis                | oat         | barley     | buckwheat |
| calcium  | 348                   | 505                     | 117         | 93         | 20         |
| phosphorus | 603                  | 480                     | 361         | 353        | 298        |
| magnesium| 226                   | 184                     | 135         | 150        | 200        |

According to [7, 8, 11], calcium determines colloidal properties of the cytoplasm in the cells of oilseeds in the form of oxalates; it is part of enzymes. Calcium enters the human body from food, however, it cannot be properly absorbed by the body without phosphorus. Calcium is presented by carbonates, phosphates, oxalates and other sparingly soluble salts. An adult needs 0.8 g of calcium per day. Calcium ingested with food is insoluble. In the alkaline environment of the small intestine, it forms sparingly soluble compounds [8, 9]. The absorption of calcium depends on its ratio to fats, magnesium, phosphorus, and proteins. The most favorable ratio of calcium and phosphorus in food is 1: 1.2–1.5. Excess phosphorus disrupts the absorption of both calcium and iron [10, 11, 14].

Oilseeds and legumes contain more calcium than most whole-milk products, however, its absorption is blocked by phytin (calcium-magnesium salt of inositol phosphoric acid) and oxalic acid. Phytin is an undesirable component; however, it can be found in any plant cell and is a reserve of organic phosphorus in plants released during germination. [3, 12, 13].

Phosphorus in seeds is part of lipid and non-lipid inorganic and organic compounds and their biochemical role is extremely important. Phosphorus is an essential component of a number of vitamins and enzymes. Phospholipids are a structural element of cell biomembranes. Phosphoric acid is bound to proteins in nucleoproteins; phosphoric esters are involved in the metabolism of carbohydrates. Finally, with the participation of phosphorus, high-energy phosphate bonds are formed in ATP type compounds (adenosine triphosphate) due to which energy is stored and transferred in living systems [7, 15, 16].

Chemical forms of calcium and phosphorus compounds are the most important factors determining the functionality of proteins in dispersed systems and the most effective at pH in the neutral and alkaline environments [17, 18].

The aim of this study: using the example of hemp and soybean oil seeds whose 11-13S reserve proteins are phylogenetically related, to study the change in the chemical states of calcium and phosphorus during swelling and germination, as one of the factors that enable osmoregulation of the protein complex and increase stability of plant dispersions from bioactivated seeds.

2. Materials and methods
The objects: soybean and hemp seeds of recognized varieties of the first storage year in the period after the end of physiological maturation, that is, from November to August in the at rest, swelling, and germination phases and aqueous dispersions based on them.

The method involved the following stages: watering seeds and subsequent incubation in a humid environment until “nibbling”, selection according to physiological phases, drying in mild conditions to a moisture content of 25 ± 1.5%, crushing, grinding, mixing crushed raw materials with hot water 60 ± 2°C at a hydraulic module of 1: 8, infusion, filtering.

When preparing soybean dispersions, the temperature regime of extraction varied from 40 to 75 °C with an interval of 5 °C. The aggregate stability of soybean dispersions was evaluated by the stability index.

Chemical forms of calcium were determined in dispersions by trilonometric and ion-selective methods using a Ca-selective electrode (GOST 26570-95). When working with dry seeds, the trilonometric method is used after ashing the sample and dissolving ash in hydrochloric acid according to A. Ya. Dundenkov. Using this method, it is possible to control the mass fraction of total calcium in solutions and the content of conditionally soluble calcium, which remains in serum after protein
precipitation with trichloroacetic acid.

Total calcium content, soluble content after 6 hours of sedimentation, bound calcium in the sediment were determined. After settling with a TCA solution in serum, calcium content associated with proteins was determined; in the supernatant, content of ionized Ca2+; the difference between protein and ionized were determined by the content of colloidal calcium.

The mass concentration of ash was determined by dry ashing by burning in a muffle furnace at 500 °C according to GOST 13979.6-69 “Cake, meal and mustard powder”.

The determination of the mass fraction of calcium ions is based on the formation of a stable complexon Trilon B (disodium salt of ethylenediaminetetraacetic acid) with divalent calcium. The complex compound of Trilon B binds calcium cations so firmly that when it is added to milk, poorly soluble calcium compounds with phosphorus and proteins dissolve. As a metal indicator in the method by A.Ya. Dudenkova murexide is used. In the alkaline environment, in the absence of calcium ions it becomes blue-violet, and if they are present, it becomes red-pink.

The determination of inorganic and acid-soluble phosphorus according to GOST 26657-97. The method for determining inorganic phosphorus is based on the reaction of inorganic phosphorus with ammonium molybdenum acid, a complex compound (phosphor-molybdenum acid) is formed. It is restored by a mixture of hydroquinone and sodium sulfite. The resulting blue color is colorimetered.

3. Discussion

When the seed swells, water triggers enzymatic processes. The transition of enzymes from a bound state to a free active state is accompanied by conformational changes in biopolymers of cell structures, preparation of organelles for activation of metabolic processes [16].

Salts of phosphoric acid in the germinating seeds can be in the form of Ca3(PO4)2 phosphate, CaHPO4 hydrophosphate, Ca dihydrogen phosphate (H2PO4) 2. It is known that part of calcium phosphate is a true solution, the other part is colloidal; an equilibrium is established between them:

\[ n \text{CaHPO}_4 \rightleftharpoons (\text{CaHPO}_4)_n \]

True solution  Colloidal solution

The shift of salt equilibrium depends on the pH of the protoplasm, the temperature, and other factors. In amphoteric electrolytes, which are proteins, the degree of swelling and solubility depends on the degree of dissociation of functional groups of particles in the system and the pH of the solution and the interaction with ionic groups [2]. In the dispersions, the pH was 6.5-6.65.

In cow milk, the calcium content is 100-140 mg; it is present in three forms: free or ionized calcium - 11% of all calcium (8.4–11.6 mg); calcium phosphates and citrates - about 66%; casein - about 23%. Calcium phosphate, calcium hydrogen phosphate, calcium dihydroxophosphate and more complex compounds are in the colloidal state and a small part (20-30%) of it is in the form of true solutions. Thus, the bulk of calcium is found in a bound form, and experts disagree about its digestibility [18].

The results of our studies are presented in Table 2. It can be seen that from dry seeds at rest only 14.8% of calcium turns into the extract, the content of conditionally soluble calcium is 12.3% under the observed complete stratification of the dispersion within 6 hours of settling.
Table 2. Dynamics of calcium content in dispersions (1: 8) from hemp seeds

| Physiological condition | Total content | Conditionally soluble in serum after sedimentation | B bound form (organic polymers, weakly dissociating colloids, enzymes, phytin) | Note |
|------------------------|--------------|----------------------------------------------------|--------------------------------------------------------------------------------|------|
| in seeds, mg           | 437.0        | -                                                  | -                                                                               | -    |
| in a dry seed extract, g/ml | 0.065       | 0.008                                              | 0.054                                                                           | complete separation after 3 hours of settling |
| in an extract after swelling, g / ml, 12 h | 0.078       | 0.062                                              | 0.014                                                                           | no visible separation, sediment slight sediment |
| 24 h                   | 0.105        | 0.094                                              | 0.009                                                                           | light sediment |
| 36 h                   | 0.118        | 0.102                                              | 0.008 traces                                                                    |         |
| 48 h                   | 0.129        | no sediment                                        | no sediment                                                                     | uniform stable dispersion |

In swollen seeds, calcium becomes mobile and is included in general metabolism (Figure 1). Conditionally soluble calcium accumulates during the swelling (the at rest phase) - 11.7 times to the original one, the polydisperse system becomes stable, the amount of sediment decreases. An increase in calcium content in the dispersion up to 98.5% compared to the initial one is observed by the 4th 8 hour. At the same time, conditionally soluble calcium increases by 14.6 times and the content of bound calcium decreases, the system becomes stable.

Figure 1. Dynamics of calcium in the dispersion of germinating hemp seeds, mg / ml: 1 - total content; 2 - content of "conditionally soluble" in serum; 3 - content of bound in the sediment.
More detailed studies of chemical forms of calcium were carried out in “serum” after 6 hours of settling dispersions (“conditionally soluble” Ca, table 3, figure 2). It has been established that conditionally soluble calcium is represented by three chemical forms whose content relative to each other is: colloidal - 61.5-71.9%; as part of proteins 20.8-28.5%; ionized - 7.2-9.9%.

Table 3. Dynamics of calcium solubility when changing physiological phases, mg / ml

| Swelling and incubation, h | As part of proteins | In the supernatant "serum" |
|---------------------------|----------------------|-----------------------------|
|                           | ionized              | colloidal                   |
| 12                        | 0.013                | 0.005                       | 0.043                       |
| 24                        | 0.021                | 0.008                       | 0.064                       |
| 36                        | 0.026                | 0.009                       | 0.067                       |
| 48                        | 0.033                | 0.011                       | 0.072                       |

During the at rest phase (the lag phase lasts for 12-24 hours), content of calcium associated with proteins increases 1.6 times in swollen seeds, 1.6 times in ionized seeds, and 1.5 times in colloidal seeds.

As enzymatic processes develop, all forms are characterized by a general positive dynamics in the transition to an aqueous solution. At the same time, the relative content of colloidal calcium is reduced by 10.4%. Thus, the most significant changes are recorded at the stage of swelling.

Further studies established that calcium metabolism is accompanied by a change in the phosphorus-salt composition (Table 4, Figure 3).

Table 4. Dynamics of phosphorus forms during hemp seed germination

| Swelling, hour | Content of P₂O₅, g/100g |
|---------------|--------------------------|
|               | total inorganic acid soluble / organic / |
| 0             | 0.4685 0.1928 0.2547      |
| 24            | 0.4558 0.2995 0.1554      |
| 48            | 0.4462 0.3230 0.1224      |

Within 48 hours with an insignificant (4.7%) decrease in total phosphorus, an increase in soluble inorganic phosphorus (67.5%) and a decrease in organic bound phosphorus (51.9%) are observed. On the first day of swelling, a decrease in total phosphorus by 2.7%, in organic phosphorus by 38.9%, an increase in inorganic phosphorus by 55.3% were revealed. These data can be considered as indirect confirmation of the destruction of phytin, which blocks the absorption of calcium [3]. Thus, according to [15], the hydrolysis of phytin during seed germination is accompanied by the release of inorganic phosphates and the appearance of calcium and magnesium ions in the cell sap. In such forms,
digestibility of phosphorus, calcium, and magnesium increases [11].

Thus, using hemp seeds as an example, it was established that in the early phases of swelling and germination, there is a significant increase in mobile forms of calcium and phosphorus, indicating the osmoregulation process. A change in the functional and technological characteristics of the protein complex is indicated by an increase in the stability of seed dispersions.

![Figure 3. Dynamics of the forms of phosphorus in germinating seeds: 1 – quiet; 2 - after 24 hours, lag phase; 3 - after 48 hours, germination](image)

Using the example of germinating soybean seeds, statistical studies of structural changes in the protein complex were carried out. During treatment of seeds at rest, stability of the system is positively affected by a decrease in particle sizes and negatively affected by the temperature above 60°C. However, it is inappropriate to talk about the aggregation stability of the system, since the size of the exfoliated phase is 40-60%.

Under equal degrees of grinding and temperatures, sizes of the exfoliated phase of the bioactivated soybean seeds are 8-30% in swollen seeds, and 3-8% - in germinating ones.

4. Conclusion

Thus, it was found that cellular metabolism during the germination of oilseeds using hemp and soya causes ionization of macrocells of calcium, phosphorus, decomposition of the phytic complex, osmoregulation of proteins and ensures stability of aqueous dispersions. The appearance of inorganic phosphates and calcium ions in cell juice can be due to the hydrolysis of phytin. Digestibility of phosphorus and calcium increases.

The results were taken into account when developing technologies for new types of functional drinks for milk substitutes.

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