Scientific justification for the composition and technology of a phospholipid-plant food complex

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Abstract. One of the promising and effective ways to make food products functional today is to use complex functional food ingredients (FFI), in particular, a phospholipid-plant food complex. Scientifically-based selection of ingredients provides balanced in terms of vitamin and mineral composition, capable of providing a corrective effect on the physiological systems of the body. FFI technology has been developed, consisting of fat-free rapeseed phospholipids, dry extracts of germinated seeds of naked oats and Melissa officinalis, succinic acid. The global trend in the food industry is the conversion of biologically active substances into a nanoform the main advantage of which is an increase in their digestibility. Amphiphilic molecules of long-chain phospholipids in an aqueous medium can spontaneously form liposomes that have prospects for use in food technologies as stabilizers of labile biologically active substances. Naked oats are well balanced in the composition of essential amino acids. The maximum number of free amino acids and short-chain peptides was found with a shorter seed germination time (up to 12 hours). The nutrient content of extracts of germinated seeds of naked oats and Melissa officinalis was determined. A range of concentration of ingredients at which antioxidant and immunomodulating properties of the complex are manifested is proposed. It is recommended to include in the daily food ration 40-60 g of a complex, both independently and as a part of foods. The purpose of the complex is to increase the body's resistance to stress, primarily for people performing prolonged physical exertion in difficult climatic or environmental situations.

1. Introduction
For a modern person, along with the emotional pressure and stress due to the high pace of life, the influence of adverse environmental factors and the use of refined foods are common. These factors, as well as the significant costs of medical care, force a large part of the population to revise the criteria for food choices, focusing on the ability to ensure the preservation of their own health.

The promotion of healthy nutrition in the media and the aggressive marketing support of manufacturers provide consumers with a wide selection of food products with a physiological focus. The consumer market offers food products of a given chemical composition and energy value: balanced for macro- and micronutrients deficient for the body, reducing the risks of acute and chronic diseases, including increasing stress resistance [1].
However, taking into account the dynamically developing and changing needs of the population, food producers are faced with the task of finding and introducing new raw materials, ingredients (including functional ones) and innovative technologies.

A promising direction in the development of functional and specialized food products is the use of combined functional food ingredients. We should say that today complexes containing phospholipids and plant extracts, including medicinal ones, deserve special attention. Scientifically-based selection of ingredients provides a phospholipid-plant food complex, balanced for the main nutrients: proteins, fats, carbohydrates, as well as vitamins and minerals, the content of which will provide a corrective effect on the physiological systems of the body [2].

2. Purpose of the study
In this regard, a relevant area is the development of technology for the production of complex biologically active food systems, the physiological and technological effects of which may be more significant in comparison with their separate use, due to the synergistic effect of the ingredients. The purpose of this study is to develop a formulation and technology of a phospholipid-plant complex using local Siberian medicinal plant materials – germinated seeds of naked oats, Melissa officinalis and rapeseed phospholipids.

3. Objects of the study
As the objects of study, new varieties of oats, naked (Aldan) and membranous (Phobos), were used. Samples of oats were germinated under standard conditions for 6, 12, 18, 24 hours. To obtain a phospholipid-plant food complex along with germinated seeds of naked oat we used: concentrate from Melissa officinalis leaves, succinic acid and rapeseed phospholipids – liquid rapeseed lecithin (an experimental batch manufactured by Zavodoukovsky oil mill, LLC), the quality and safety of which were previously studied by the authors [3].

4. Materials and methods
Quantitative determination of the free forms of amino acids in deproteinized extracts of germinated seeds was carried out by standard methods according to GOST 32195-2013 (ISO 13903:2005). Derivatization was carried out with phenylisothiocyanate in order to identify amino acids, the resulting derivatives were separated on a ReproSil-Pur C-18 AQ column (4.6x250 mm) in a solvent system. As a control, standard solutions of L-amino acids were used. The elution time was determined by absorbing light with a wavelength of 254 nm. Quantitative determination of vitamins was carried out by standard methods: β-carotene – according to GOST R 54058-2010, vitamin C – according to 24556-89, vitamin B1 – according to GOST 25999-83, vitamin E – according to GOST R 54634-2011, polyphenolic substances – according to R 4.1.1672-03.

5. Results and discussion
A distinctive feature of oat protein is a higher content of essential amino acids, %: lysine – 50, valine – 20, leucine – 20, isoleucine – 13. The fractional composition of proteins of oat grain protein also differs, %: glutelins – 36, prolamins – 28 and globulins – 21. Polysaturated fatty acids of naked oat are in a balanced ratio - low content of linolenic and high oleic and linoleic acids. Polysaccharides are starch (36.3 %), cellulose (10.5%), hemicellulose (10.2%), which is 88% β-glucan, which can actively remove cholesterol from the body. Also, oat grain contains vitamins and minerals [4].

So, a distinctive feature of oat grains is the presence of selenium (19.0 µg/100 g, in combination with vitamins E and C, almost completely absorbed by the body), manganese (4.92 pg/100 g), silicon, dietary fiber (cholesterol-binding) and mucilaginous substances (normalizing digestion). In addition, according to EU regulation, oats are considered gluten-free [5, 6].

Recently, nutritionists have preferred germinated oats, the optimal germination time for seeds of grain crops (from one to three days) is determined by the length of the seedlings (no more than 2 mm), as it is proved that the sprouted seeds of grain crops such as wheat, barley, rye, especially seeds of
naked oats are characterized by more balanced chemical composition. For example, the content of vitamins B, E and Si increases by 1.5 times, macro- and microelements (Ca, Na, Cu, Fe and Zn) – by 2 times [7, 8].

Such a natural product requires significantly less energy to be digested in the gastrointestinal tract. The introduction of extracts of germinated oat seeds into a food product helps to increase metabolism, the body's immune responses, increases the mineral and vitamin status, and promotes detoxification [9].

In the available literature, there are no data on the amino acid composition that determines the germination time for specific crops, which would give a more complete characterization of the biological activity of foods obtained from germinated seeds.

The results of the study showed that acid extracts of non-germinated samples of oats and ones germinated according for 18 and 24 hours do not contain free amino acids. It can be assumed that protein hydrolysis is inhibited in non-germinated oat seeds, which explains the absence of free amino acids. The free amino acid elution profile of germinated samples is characterized by a single peak with a varying height that does not correspond to any of the standard L-amino acids. Considering the characteristics of the elution gradient and the conditions of chromatography, it is highly likely that the appearance of the peak is due to the presence of small concentrations of low molecular weight proteins or peptides not precipitated by sulfosalicylic acid. Similar results were obtained during deproteinization of extracts by other methods - boiling and precipitation with acetone, which is in favour of the assumption of the peptide nature of the compounds remaining in the extract. These results may indicate active protein hydrolysis by the end of the first day, the resulting amount of amino acids has a maximum value, then they are assimilated during the growth of sprout tissues.

Since no free amino acids were detected during the analysis, acid hydrolysis of the presented samples was carried out according to the method described in GOST 32195-2013. The resulting hydrolysates were subjected to derivatization, followed by chromatography. Comparison of the chromatograms of samples with the elution profile of phenylisothiocyanate derivatives of standard samples of L-amino acids makes it possible to identify the amino acid residues contained in the hydrolysate. The greatest number of amino acids can be identified in samples of naked oats germinated for 12 hours (Figure 1) and membranous oats germinated for 24 hours of the day (Figure 2).

![Figure 1. Amino acid elution profile of hydrolysed extract of naked oat germinated for 12 hours](image)

The concentration of amino acids released during the hydrolysis of samples varies in the range of 0.2 - 1 mmol/l for naked oats and 0.1 - 0.5 mmol/l for membranous oats. Studies have determined the
time of germination of naked oats – 12 hours, which allows obtaining a highly valuable product with a maximum content of free amino acids and other biologically active compounds.

Phospholipids are a necessary component of the cell and play an important role in the metabolism of the human body, as they perform a number of functions: they are part of cell membranes, deactivate radical processes on the surface of the membrane, thereby supporting its physiological activity [10, 11]. Not having antioxidant properties, phospholipids activate the body's antioxidant system due to synergistic interactions with the fatty acid components of triglycerides, as well as tocopherols and flavonoids, and are able to penetrate cell barriers [12]. They also promote better absorption of fat-soluble vitamins A, D, E and K; carotenoids, ubiquinones and a number of other biologically active substances [2].

Such properties of phospholipids as emulsifying and water-holding capacity are widely known and used in food technology. These technological properties of phospholipids increase the organoleptic and physicochemical indicators of the quality of products [13].

![Amino acid elution profile of hydrolysed extract of membranous oat germinated for 24 hours](image)

**Figure 2.** Amino acid elution profile of hydrolysed extract of membranous oat germinated for 24 hours

It is known that the physiologically and technologically functional properties of lecithins are determined by the group composition of the phospholipids contained in them. In this regard, the group composition of the phospholipids of the rapeseed lecithin used was studied (Table 1).

**Table 1.** Group composition of phospholipids of rapeseed lecithin samples

| Phospholipid group                        | Mass fraction of phospholipids in rapeseed lecithin, % | To the sum of phospholipids, % |
|------------------------------------------|------------------------------------------------------|-------------------------------|
|                                          | Experimental data | Literature data | %                       |
|------------------------------------------|-------------------|-----------------|-------------------------|
| Phosphatidylcholines                     | 38.4±1.4          | 25.3            | 38.3                    |
| Lysophosphatidylcholines                 | 6.3±0.6           | –               | 6.3                     |
| Phosphatidylethanolamine                 | 21.3±1.2          | 13.9            | 21.2                    |
| Phosphatidylserines + Sphingomyelins     | 24.5±1.4          | 10.8            | 24.4                    |
| Phosphatidic and polyolphosphatidic acids | 9.8±1.1           | 23.8            | 9.8                     |
The group and fatty acid composition of rapeseed lecithins is advantageous due to the higher content of phosphatidylcholines and phosphatidylethanolamines, which are the most physiologically and technologically functional groups of phospholipids, have a significant amount of oleic acid, and also a lower amount of saturated fatty acids [15]. Phosphatidylcholines and phosphatidylethanolamines are the main structure-forming components of all biological membranes of the cell, which largely determines their manifestation of physiologically active properties [16].

Currently, the main global trend in areas related to human life is the transfer of active substances into nanoforms, which is most clearly manifested in areas such as medicine, veterinary medicine, food industry, the production of biologically active additives, and cosmetic products [17].

Nanosystems have several advantages over traditional methods of delivery of effective and biologically active substances, the main of which is an increase in their bioavailability and, as a consequence, better digestibility. Such nanostructured carriers as liposomes, micellar systems, nanoemulsions, and other nanoscale materials are currently of greatest interest [18].

It is known that amphiphilic molecules of long-chain phospholipids in an aqueous medium are able to spontaneously form associates - liposomal systems, which have broad prospects for use in food technology as food additives and stabilizers of labile biologically active substances.

Melissa officinalis is a pharmacopoeial plant in many countries of the world, including the Russian Federation since 1996. A wide range of physiological effects of Melissa officinalis - sedative, antidepressant, antispasmodic, immunomodulating, antiviral, antioxidant, etc., is determined by the content of biologically active compounds. The experiment investigated dry powdered concentrate of Melissa officinalis leaves obtained by electrodialysis, followed by freeze-drying [19]. The content was established, %: extractives - 92.6±4.4, flavonoids - 2.13±0.20, tannins - 3.18±0.55, essential oils - 2.30±0.15, vitamin C - 18.6±1.2. Melissa officinalis contains macroelements (K, Ca, Mg, Fe) and microelements (Mn, Cu, Zn, Cr, Se etc.). Thus, due to the established composition, the Melissa officinalis concentrate is able to exhibit antioxidant, immunomodulating and anti-inflammatory effects [20].

Succinic acid has the antioxidant activity due to deactivation of metal cations by changing valence which initiate the processes of radical oxidation of lipids, nucleic acids, proteins. In food technology, succinic acid is used to regulate the acidic environment in a product that is conducive to the development of lactic acid bacteria; succinic acid has been found to promote better preservation of vitamins C, P and PP and maintain a high level of activity of hydrolytic and proteolytic enzymes in fruit and vegetable juices.

In the technology of the phospholipid-plant food complex, dry concentrates of germinated naked oat seeds (4.2% humidity) and Melissa officinalis leaves (3.8% humidity), succinic acid and fat-free rapeseed phospholipids were used.

At the first stage of obtaining a phospholipid-plant complex, phospholipids were heated to a temperature of 65-67 °C at 2500 rpm for 10-12 minutes, then an aqueous solution of succinic acid and Melissa officinalis leaves were added to the melt of phospholipids. The resulting complex was cooled to a temperature of 35-37 °C and an extract of germinated seeds of naked oat was added. At all technological stages, mixing was performed at 2500 rpm, the duration of the process depends on the total mass of the components. The ratio of ingredients in % of the total mass was used, the mass of the individual ingredient was correlated with the weighted average daily intake of nutrients for an adult [22].

The cooled phospholipid-plant food complex was subjected to freeze-drying, then the resulting product was crushed to a particle size of 13-15 microns. The resulting phospholipid-plant food complex is a powdered product and has a uniform consistency, light brown colour, the smell of lemon balm, slightly sour taste without an aftertaste.

A single dose of the phospholipid-plant food complex in the composition of the food product is 40-60 g, which allows you to replenish the diet with physiologically active functional ingredients. Table 2 presents the range of use of nutrients of the phospholipid-plant food complex.
Table 2. The recommended ratio of ingredients in the phospholipid-plant food complex, in % of the total mass

| Ingredient                   | Mass fraction, % |
|------------------------------|------------------|
| Rapeseed phospholipids       | 40–46            |
| Germinated naked oats seed   | 40–45            |
| extract                      |                  |
| Melissa officinalis leaves   | 17–4             |
| extract                      |                  |
| Succinic acid                | 3–5              |

Table 3 presents the content of functional ingredients in the phospholipid-plant food complex, the daily consumption rate and % of the rate, the content of which is more than 15%.

Table 3. The content of functional ingredients in the phospholipid-plant food complex, mg/100 g

| Nutrients     | Rapeseed phospholipids | Germinated naked oats seed extract | Melissa officinalis leaves extract | Succinic acid | Total Consumption rate [21] | % of the rate |
|---------------|-------------------------|-----------------------------------|----------------------------------|--------------|-----------------------------|--------------|
| Content, %    | 45.0                    | 40.0                              | 10.0                             | 5.0          | 100.0                       |              |
| β-carotene    | –                       | 0.7                               | 0.5                              | –            | 1.2                         | 4.0          | 30.0          |
| Vitamin B₁    | –                       | 0.8                               | 0.1                              | –            | 0.9                         | 15.0         | 60.0          |
| Vitamin B₂    | –                       | 1.3                               | 0.3                              | –            | 1.6                         | 2.0          | 80.0          |
| Vitamin C     | –                       | 23.0                              | 18.6                             | –            | 41.6                        | 70.0         | 59.0          |
| Vitamin E     | –                       | 2.3                               | –                                | –            | 2.3                         | 10.0         | 23.0          |
| Phospholipids | 4500.0                  | 1.3                               | –                                | –            | 4501.3                      | 7000.0       | 64.3          |
| Succinic acid | 5.0                     | –                                 | –                                | –            | 5.0                         | 7.0          | 71.4          |

6. Conclusions
The prospect of using germinated naked oat seeds, phospholipids, Melissa officinalis and succinic acid in the technology of phospholipid-plant food complex is shown. Ionic interactions between the ingredients allow obtaining a powdery homogeneous product with a given chemical composition, which determines the antioxidant hours (which in turn activates an increase in stress resistance during prolonged physical exertion and in difficult environmental situations, for example, at low temperatures) and immunomodulating properties. The optimal duration of germination of naked oats by the content of free amino acids and short-chain peptides is determined - 12 hours. The content of biologically active substances in extracts of germinated naked oat seeds and Melissa officinalis is determined. A range of concentration of ingredients is proposed at which antioxidant and immunomodulating properties of the phospholipid-plant food complex are manifested. It is recommended to include 40-60 g of phospholipid-plant food complex in the food ration, both independently and as part of bakery and meat foods to increase the body's resistance to stress, in difficult environmental situations, with prolonged physical exertion.
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