







## Prospects for using pine nut products in the dairy industry

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**Abstract:** Functional products are currently attracting a lot of research interest. Modern people's diet does not satisfy their need for nutrients, vitamins and minerals, and functional products can make it more balanced. In particular, our diet is lacking in protein. This paper discusses the prospects for enriching dairy products with plant protein derived from pine nuts and their products. Pine nut paste, fat-free milk and oil cake are a valuable source of fatty acids, vitamins, and microelements. The protein, lipid, vitamin, and mineral content of these products makes them suitable for combining with milk. Their water-holding and fat-emulsifying capacities allow their use as stabilizers and emulsifiers. Siberian pine nuts grow wild in the Kemerovo Region, which makes their use as a raw material economically feasible. The article introduces a number of functional dairy products enriched with pine nut products, such as cheese, ice cream, and cottage cheese. Further, it describes the production process and the products' nutritional value. The chemical composition of new types of dairy products shows that using pine nut oil cake, fat-free flour, paste and oil enriches them with plant proteins, vegetable fats, vitamins as well as macro- and microelements. Replacing dairy raw materials with plants does not reduce the nutritional value of new dairy products. Dairy foods are rich in protein, fat, and minerals. The vitamin content of new dairy products with functional ingredients is similar to that of dairy-based products. Moreover, using functional products (pine nut oil cake, fat-free flour, paste and oil) enriches new types of dairy products with tocopherols.

**Keywords:** Pine nut processing, functional foods, dairy products, dietary supplements, pine nut paste, pine nut oil cake, fat-free pine nut flour

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### INTRODUCTION

The 21<sup>st</sup> century has significantly changed our lifestyle in many ways. Such factors as adverse environmental conditions, physical inactivity, and high mental stress lead to a rapid destruction of vitamins and minerals in the human body. The majority of Russian people suffer from malnutrition for social and economic reasons: most accessible are high-calorie foods with a low nutritional value.

Modern diet is characterised by a high proportion of industrially produced foods. As a result of a curing process, such products lose a considerable part of biologically active components – vitamins, minerals, some amino acids, and other substances that play a significant role in the body's metabolism. The deficiency of such essential nutrients weakens the body's defences against adverse environmental

factors, contributes to chronic fatigue, and decreases mental and physical performance [1].

On the other hand, there is a growing interest in balanced nutrition and an increased demand for products that not only satisfy hunger, but also minimize the harmful effects of the environmental and stress factors. This is due to the rising cost of medical care and raised consumer awareness of the principles of healthy eating. Another reason is a recent increase in life expectancy and the desire of older people to keep healthy [2]. The modern food industry, with its research and production facilities, is able to meet this growing demand for healthy eating and produce a wide range of foods with improved composition, properties, and functions.

Modern principles of healthy eating highlight a need for low-calorie and low-cholesterol foods, as well

as special natural supplements, preferably of plant origin. Functional products are a current global trend, since nutrition problems are common for the whole world, not only Russia. Foreign studies claim that developing new eating habits is a prerequisite for improving people's health [3].

In Russia, providing the population with affordable and high-quality products is part of the state agenda [4]. "The Russian policy of healthy eating" underlines a need to provide various social groups with balanced functional foods. This paper looks at developing functional foods based on raw milk with the use of pine nut products as one of the ways to improve nutrition in Russia.

## STUDY OBJECTS AND METHODS

Our objects of study included:

- Siberian pine seed kernel products, such as pine nut oil, pine nut paste, pine nut oil cake, and fat-free pine nut flour;
- low-fat cottage cheese (GOST R 51074-2003);
- skimmed milk powder (GOST R 52791-2007); and
- dairy prototypes, such as processed cheese, cottage cheese and plant mixture, ice cream, and mayonnaise.

The following studies were conducted with the following methods to determine the nutritional value:

- (1) The fractional composition of proteins – by the Yermakov method, sequential extraction of proteins with a 10% aqueous NaCl solution, a 0.2-normal NaOH solution, and a 70% alcohol solution, followed by albumin and globulin separation by dialysis. To measure the amount of glutelins, a co-extraction of prolamins and glutelins with a 0.2% solution of NaOH was carried out after the extraction of albumins and globulins, followed by precipitation of glutelin at pH 10 in the presence of NaCl.
- (2) The amino acid composition – by ion-exchange chromatography with an automated amino acid analyser AAA-339M after the preliminary hydrolysis of proteins and fractionation of amino acids.
- (3) The fatty acid composition of primary and end products – by chromatography.
- (4) The mass fraction of fat – by extracting it with a mixture of chloroform and ethyl alcohol in the Soxhlet apparatus, followed by removing the solvent, drying at  $103 \pm 2^\circ\text{C}$ , and weighing (GOST 23042).
- (5) The amount of retinol – by a colorimetric method, vitamin B<sub>1</sub> (thiamine) and vitamin B<sub>2</sub> (riboflavin) – by a fluorimetric method, and vitamin B<sub>3</sub> (pantothenic acid) – according to GOST 50929.
- (6) The amount of vitamin E – by a photocolorimetric method.
- (7) The content of mineral substances – according to GOST 26176-84 and MU 01-19/47-92.
- (8) The mass fraction of macro- and microelements – by the atomic absorption spectrophotometry according to GOST 30178-96.
- (9) Functional and technological properties of pine nut oil cake and fat-free flour, namely their water absorption (swelling) and fat absorption capacity as an amount of water (or fat) held by the sample when soaked in a free state without mechanical load (centrifugation) – by weighing the sample before and after watering.

(10) The fat-emulsifying capacity was a relation between the emulsion layer volume and the total mixture volume. The water-holding capacity was a relation between the amount of water bound by the protein product and its weight after drying at  $105^\circ\text{C}$  for 4 hours.

(11) The titratable acidity of the end products was measured according to GOST 3624-92.

(12) The organoleptic evaluation of the end products was conducted according to GOST 8756.1 in the following order:

- appearance: shape, surface, uniformity, absence/presence of impurities;
- colour vs. typical colour of a given product;
- aroma: typical features, aroma balance, a so-called 'bouquet', absence/presence of foreign odours;
- consistency: homogeneity, absence/presence of solid particles;
- taste vs. typical taste of a given product.

(13) The caloric value was based on the actual content of protein, fat, and carbohydrates in the dry protein product, given that 1 g of fat is equivalent to 9.3 kcal, 1 g of protein – 4.1 kcal, and 1 g of carbohydrates – 3.75 kcal.

(14) All the experiments were performed three times. The data were processed by standard methods of mathematical statistics. The homogeneity of the sampling effects was checked using the Student's t-test. The differences between the means were considered significant when the confidence interval was less than 5% ( $p \leq 0.05$ ).

**Functional foods.** In recent years, great efforts have been made to return food its health benefits. Modern nutrition science calls for new types of products or functional foods containing ingredients that benefit human health and increase its resistance to disease. Functional foods can improve many physiological processes in the human body.

One of the first functional food projects started in Japan in 1984, resulting in as many as 100 functional foods produced in 1987. Soon, the concept of foods for specified health use (FOSHU) took off in many other parts of the world. It is estimated that in the next few years the market of functional foods will exceed 30% of all foods sold in Europe.

The problem of functional food production attracts a lot of research efforts both in Russia and abroad [5–8]. The Functional Food Centre (FFC) defines functional foods as natural or specially processed products containing biologically active components in an amount sufficient for preventing disease and improving health [9]. Functional foods are widely used along with ordinary foods by both healthy and sick people [5, 10–12].

The technology for developing functional foods is based on the modification of traditional foods to increase their content of useful ingredients and biologically active components. Functional foods are characterized by a high content of nutrients that our diet is usually lacking in, namely amino acids, fatty acids, vitamins and minerals, as well as dietary fibre.

Functional foods acquire beneficial properties due to their complex composition, which depends on the formulators' ability to adequately combine various ingredients. In other words, the beneficial properties of

one ingredient should not be neutralized by those of another.

The goal is to increase the content of valuable nutrients to a level consistent with the physiological norms of their consumption. The content of enriching ingredients in functional foods ranges from 10 to 50% of the average daily requirement. Their quantity depends on how well they can preserve their properties during production or storage so that it does not get lower than the recommended level at the end of the storage period.

A high content of vital nutrients is not the only benefit of functional foods. They are meant to regulate the proportion of harmful substances in the body and produce a positive effect on the immune system, metabolism, and other physiological processes [6, 13–15]. This role is performed by vitamins, minerals, and antioxidants.

The development of foods containing functionally interrelated nutrients of different nature and structure should be based on reliable information about their physiological effect on the metabolic and regulatory functions of the organism, taking into account their synergistic and integrated action.

Unlike traditional products, functional foods enriched with vital nutrients can meet the body's needs in them without leading to excessive eating.

One of the main stages in the development of a functional product is a justifiable selection of functional ingredients that create new properties related to the product's ability to exert a physiological effect.

The potential ability of functional ingredients to change the taste, aroma and texture of the finished product plays an important role here. However, new foods should be as appealing to the consumer as conventional ones and have the same organoleptic properties. The new taste, aroma, and texture should be consistent with most consumers' cultural traditions and eating habits.

To produce new functional foods with a desired structure, as well as technological and consumer characteristics (including storage stability and affordability), the developers need to use additional ingredients that improve their appearance and flavour. Functional foods should be as good as traditional ones, and this is the task that modern nutrition science is able to fulfil.

The technology for developing functional foods makes it possible to regulate their caloric content, which is a very important issue for those people who have a high calorie intake, with the resulting health implications. As a rule, functional foods have a reduced calorie content alongside an increased content of nutrients that our diet is deficient in.

Functional food production is a science-based sector, and studying the impact of nutrition on human health is currently one of the most promising areas of food science [16]. Experimental research in healthy nutrition confirms the beneficial effect of functional foods on the body. In particular, our diet can affect the following aspects of our health:

- immunity;
- the cardiovascular system;
- blood cholesterol level;

- intestinal microflora and the gastrointestinal tract;
- blood glucose level;
- arterial pressure;
- minerals assimilation degree; and
- osteogenesis [17].

Functional foods have a number of major functions, in particular they 1) make up for the deficiency of biologically active components identified as a result of studying the actual diet that people in a particular region live on; 2) help our organs and systems maintain their normal functions due to a high content of essential vitamins and minerals; 3) reduce the risk of disease by making the diet more balanced; and 4) create a favourable dietary background by enhancing beneficial microflora to ensure sufficient absorption of nutrients ingested with food.

Researchers divide functional ingredients into the following main groups: dietary fibre, vitamins, antioxidants (tocopherols), minerals, and polyunsaturated fatty acids [13, 14]. Below are their key characteristics.

**Dietary fibres** are polysaccharides that can be structural (cellulose, hemi-cellulose, and pectin substances) and non-structural (gums, mucus). Not digested or absorbed by the body, they regulate the physiological and biochemical processes in the digestive organs and improve the digestibility of food nutrients [18, 19]. The most common food fibres are cellulose, pectins (glycanogalacturonans), slimes, and gums.

Cellulose is the most common plant homopolysaccharide and the main structural component of the plant cell membrane. Its major physiological effect lies in its ability to bind water (up to 0.4 g water per 1 kg fibre). Cellulose largely texturizes food and is almost indigestible in the intestine: its digestibility ranges from 6 to 23%, depending on its origin, content in the diet, and the nature of processing.

Microcrystalline cellulose, carboxymethylcellulose, and methylcellulose are widely used in food production. Hemicelluloses are a group of high-molecular polysaccharides which, unlike cellulose, are easily dissolvable in water and aqueous solutions of alkalis, and are able to hydrolyse in aqueous solutions of acids. They are the second largest component of plant cell walls, after cellulose. One of their most important functions in food is that they are able to retain water and bind cations. Also, as dietary fibres, they are part of the indigestible bulk, which is extremely important for intestinal motility. Finally, hemicelluloses help to remove bile acids and lower blood cholesterol.

Pectic substances (glycanogalacturonans) are biopolymers that make up the cell wall and act as a structuring agent. Pectin is often used as a gelling agent and a stabilizer. One of the most important properties of pectic substances is their ability to interact with heavy metal ions and radionuclides, which makes them a valuable additive in food production for health-promoting purposes. The prophylactic dose of pectin, recommended by the Food and Agriculture Organization (FAO) and the World Health

Organization (WHO), is 2–4 g per day or 8–10 g per day for those working in adverse conditions.

Slimes and gums are a group of complex unstructured polysaccharides, including water-soluble carbohydrates forming viscous and sticky solutions. Gum substances have a structure similar to hemicelluloses, but a lower molecular weight. Gums are widely used in food production as binders, thickeners, emulsifiers, and stabilizers owing to their valuable properties of swelling, increased viscosity and stickiness. Arabic gum and locust bean gum are among the most commonly used gums in the food industry.

It is a well-known fact that dietary fibre deficiency is a risk factor for a number of diseases, including colon cancer, irritable bowel syndrome, hypomotor dyskinesia of the colon, diverticula, cholelithiasis, obesity, atherosclerosis, ischemic heart disease, diabetes, varicose dilatation of veins, etc. The FAO and the WHO point to a low consumption of dietary fibre almost in all the countries.

**Vitamins** are low-molecular biologically active compounds that take part in all essential functions of the body and are therefore vital to man. They are involved in metabolism and provide protection against harmful environmental factors and diseases. Since the human body is not able to synthesize vitamins or store them for future use, they must be regularly supplied from outside. However, the standard daily diet cannot provide the content and variety of essential vitamins that meet the physiological needs.

Many vitamins combine with proteins and form enzymes, which makes them especially important in nutrition. As a rule, vitamins are coenzymes, i.e. active groups of enzyme systems. Lack of vitamins can delay the formation of enzymes and cause impaired digestion, which in turn leads to metabolic disorders.

There are a number of natural compounds that can be converted to vitamins by metabolic processes. Such substances are called provitamins. For example, carotenoids are provitamins of vitamin A, sterols are provitamins of the D group, and nicotinic acid is a provitamin of nicotinamide (vitamin B<sub>3</sub>).

Vitamin B<sub>1</sub> (thiamine), a coenzyme of several enzymes, is involved in metabolic processes and energy metabolism, necessary for the normal functioning of the nervous system. Vitamin B<sub>1</sub> is found in baker's and brewer's yeast, soybean, rice bran, pine nuts, and buckwheat. Liver and kidneys are the sources of vitamin B<sub>1</sub> among animal products. In addition, this vitamin is synthesized by certain types of bacteria. Vitamin B<sub>2</sub> (riboflavin) is involved in the metabolism of carbohydrates, fats and proteins, and is necessary for the normal functioning of the visual organs. Pine nut kernels are rich in vitamin B<sub>2</sub>.

**Antioxidants.** Tocopherol (vitamin E) is the most important intracellular antioxidant that protects fats and other easily oxidizable compounds from oxidation. Also, vitamin E is a carrier of electrons in redox reactions and is necessary for normal metabolism in the muscle tissue. Lack of this vitamin leads to muscle atrophy and also affects the vascular and nervous tissues. Approximately 20–30 mg of tocopherol comes with food; however, only 50% or less is absorbed in the intestine [14]. Pine nut products are a possible source

of tocopherol: 30 g of pine nut kernels can satisfy a daily human need for this substance.

**Minerals.** The human body contains about 3 kg of various mineral substances, including 81 out of 92 naturally occurring chemical elements. Many elements are contained in a bound state, in the form of mineral salts, complex compounds ions and organic substances. Twelve elements (C, O, H, N, Ca, Mg, Na, K, S, P, F, and Cl) are structural, since they constitute 99% of the body's elemental composition. Fifteen microelements (such as Fe, J, Cu, Zn, Cr, Mo, Ni, V, Se, etc) are essential for vital activities, despite their low content.

Minerals are constituent elements of proteins; they are involved in the biosynthesis of nucleic acids as well as carbohydrate and lipid metabolisms. They also perform a catalytic function, activate and regulate enzymes; they are involved in the biosynthesis of vitamins and in the synthesis of hormones, affecting their activity. Although minerals do not have the energy value (like proteins, fats or carbohydrate), they need to come in regularly. Their main source is food, although the standard daily diet does not contain all the necessary minerals.

Calcium is the most abundant of all the minerals in the human body (1–2 kg), 98–99% of it contained in the bone and cartilage tissues. Also, calcium participates in the transmission of nerve impulses, promotes blood clotting, and affects the work of the heart muscle. Dietary calcium mainly comes from dairy products, of which there should be plenty. However, there may be certain problems with its absorption, e.g. in a diet rich in fats.

Phosphorus is a biological companion of calcium. The human body contains 600–900 g of this element, of which 90% is found in the bone tissue. As part of adenosine triphosphoric acid (ATP), phosphorus is involved in different metabolic processes, including energy metabolism. Phosphorus metabolism is closely related to calcium metabolism; therefore, the consumption of these two substances must be balanced.

Magnesium is involved in enzymatic, carbohydrate and phosphorus metabolism. This element is important for the normal functioning of the nervous and muscular tissues. The need for magnesium increases in older people who are prone to hypertension and have elevated levels of cholesterol in the blood.

Magnesium enters the body mainly with food. The richest source of magnesium is plant products, such as cereals, legumes, bran, pine kernels, and vegetables. Magnesium is a calcium antagonist; therefore, it is important to consume them separately and maintain their balance in the diet.

Sodium is an important extracellular and intracellular element. It is involved in maintaining a constant volume of fluid in the body and in maintaining an acid-base balance. Sodium is important for the nerve reactions, and its content directly affects the state of the nervous system. The main source of sodium is table salt, whose consumption rate is 8–12 g per person. Excessive salt intake, however, is harmful to the body.

Potassium is an intracellular element that regulates the acid-base balance in blood. It participates in the transmission of nerve impulses and regulates certain

enzymes. The human body contains 160–250 g of potassium, of which 98% is intracellular. One of its most important functions is to maintain the potential forming on the cell membrane and regulate osmotic pressure in the cells. In some physiological processes, potassium acts as a sodium antagonist, and its increased concentration leads to the release of sodium from the body.

Potassium deficiency impairs the functioning of the neuromuscular and cardiovascular systems and manifests itself in depression. Its excess, however, can cause sudden cardiac arrest. The daily need for potassium ions ranges from 2.5 to 5.0 g. Normal metabolism requires the 1:2 ratio of potassium and sodium in the diet.

In addition to the main “structural” elements, all organisms contain microelements (or trace elements), whose concentration is 0.001–0.00001%, and ultramicroelements, with a concentration of 0.00001% or less. Fifteen of those elements (iron, iodine, copper, zinc, cobalt, chromium, molybdenum, nickel, vanadium, selenium, manganese, arsenic, fluorine, silicon, and lithium) are considered essential, or vital.

Trace elements play a very important role in the human body. They perform a structural function, as components of hard and soft tissues (silicon, strontium, fluorine, and aluminium). However, their main role is to contribute to all the physiological functions of the body.

Trace elements ensure the most important functions of proteins. As structural elements of protein substances, they take part in the biosynthesis of nucleic acids and thus affect the functioning of the genetic apparatus, and cell growth and division. Also, they are involved in the formation of high-molecular compounds (nucleoproteins and lipoproteins) and enhance phospholipids, which are important for nervous activity.

Trace elements are involved in carbohydrate metabolism, affecting insulin activity, and in lipid metabolism, which is important in the prevention of obesity and fatty infiltration of internal organs.

Additionally, trace elements perform a catalytic function as metalloenzyme components or enzyme activators and regulators, and participate in the biosynthesis of vitamins, contributing to their absorption and use by the body.

Further, microelements are involved in the synthesis of hormones and other biologically active substances (kinins, serotonin and vasopressin) and mediators, affecting their activity.

It is worth noting the relation between microelements and malignant growth processes. On the one hand, an excess of some microelements and a deficiency of others can predispose the organism to the development of tumours; on the other hand, some microelements have a strong antitumor effect. Moreover, some trace elements (silver, mercury and manganese) have antimicrobial action and others have an anti-stress effect.

However, industrial development and the use of fertilizers contribute to an excessive intake of various trace elements, which can harm the human body. At the same time, trace elements can also have an antitoxic

effect by increasing the immunobiological reactivity of the body.

To sum up, both the deficiency and excess of some trace elements can affect the vital functions of the body.

Iron is necessary for the formation of haemoglobin and a number of enzymes, taking part in the transport of oxygen: 80% of iron contained in the human body is a component of haemoglobin. Iron deficiency leads to anaemia and a number of other diseases. Due to unbalanced nutrition, every fifth person in the world suffers from iron deficiency.

Zinc is predominantly found in the muscles, red blood cells, plasma, prostate gland, spermatozoa, and is a component of the insulin hormone. Also, zinc is a part of metalloenzymes involved in various metabolic processes, including the synthesis and breakdown of carbohydrates and fats. It is important for the functioning of a number of enzymes that ensure the normal operation of the pituitary gland and the pancreas. Zinc takes part in the synthesis of proteins and nucleic acids; it is important for stabilizing the structure of DNA, RNA and ribosomes and is involved in the translation process. Thus, zinc affects the functioning of the genetic apparatus, cell growth and division, keratogenesis, osteogenesis, and reproductive function, and contributes to the immune response and wound healing. Finally, zinc affects the production of behavioural reflexes, brain development, and flavour perception.

Manganese is mainly found in bones, liver and kidneys. Its major biological property is in its influence on the bone tissue. Further, manganese has a significant effect on growth, reproduction, immunity, and metabolism. Its biological role is also associated with osteogenesis processes and the metabolism of proteins, carbohydrates, and mineral salts. Manganese activates redox processes and has a positive effect on the blood-forming organs. It takes part in carbohydrate metabolism (ensuring normal insulin secretion and increasing glycolytic activity), lipid metabolism (preventing the deposition of fat in the liver) and in the synthesis of cholesterol. Finally, it is a component of a number of enzymes.

**Lipids containing polyunsaturated higher fatty acids.** The recommended fat content in the diet is 90–100 g per day, with 30% of this intake coming from vegetable oils. Polyunsaturated fatty acids should comprise about 10% of the total fat content. If there is a pathology of lipid metabolism, the ratio of polyunsaturated to saturated acids should be 5:1. In the nutrition of elderly people and patients with cardiovascular diseases, this ratio should be 2:1 [20]. Polyunsaturated fatty acids are components of cell membranes and other structural elements of tissues. They contribute to the regulation of cell metabolism and platelet aggregation, affect the exchange of cholesterol, participate in the metabolism of group B vitamins, and increase the body's resistance. A deficiency of polyunsaturated fatty acids increases the risk of cardiovascular diseases, leads to the disruption of normal kidney function, and reduces the body's resistance.

According to the monitoring data, Russian people consume plenty of oils containing  $\omega$ -6 fatty acids (sunflower, soybean and corn oils) and almost no oils rich in  $\omega$ -3 fatty acids (linseed, rapeseed, camelina, mustard, and hemp oils).

Obviously, enriching traditional foods with functional ingredients has a direct impact on human health. It should be noted that functional foods are not drugs: they are products of mass consumption and often part of a regular diet. In the structure of modern nutrition, functional foods occupy an intermediate position between ordinary and medical foods. Ordinary foods are chosen according to one's eating habits and income to satisfy hunger. Medical foods are prescribed by the doctor as part of a therapeutic diet to be consumed during the period of therapy or medication. Functional foods agree with standard eating habits and, at the same time, help maintain health. Unlike drugs, they have no contraindications for prolonged use.

Healthy people use functional foods to prevent disease. Groups of people in need of functional foods include children of different ages, elderly people, workers in hazardous industries, polar explorers, people living in environmentally unfriendly regions, etc. The composition of multicomponent functional foods may vary according to consumers' differentiated needs. By changing the formula, we can develop specialized baby foods or dietary foods.

Functional foods must meet the following requirements:

- have scientifically-proven beneficial properties;
- have a daily intake rate established by experts;
- possess specific physico-chemical characteristics and precise methods for their measurement;
- have no negative impact on the nutritional value;
- be used as ordinary foods and have similar consumer characteristics; and
- contain natural ingredients [9, 13, 21].

Functional food production requires compliance with hygienic requirements established for each ingredient, as well as the finished product. In terms of quality and safety, functional foods must:

- guarantee safety for prolonged use;
- produce a physiological effect on the body, when consumed daily in moderate amounts;
- be easily and fully digestible; and
- have their biochemical composition and biomedical properties complying with the so-called 'life formula', in terms of both essential substances and their balanced content.

Functional products must be adapted to the Russian food market and be compatible with the local staple foods. It is expected that their organoleptic properties will be what local consumers are accustomed to. In the long term, regular use of functional foods should lead to a remarkable improvement in the structure of everyday nutrition.

The Kemerovo Technological Institute of Food Industry (Kemerovo State University) has developed a concept of producing new generation foods from complex dairy-based raw materials with the use of Siberian pine nuts and their products to give them functional properties.

According to the concept's biomedical aspects, functional foods will:

- make up for the deficiency of essential nutrients in people's diet and meet their physiological needs for nutrients;
- increase the body's resistance to adverse environmental conditions with biologically active substances contained in Siberian pine seeds; and
- contribute to the regulation of the body's organs and systems.

The development of foods with Siberian pine nut products is governed by the following principles:

- natural origin, diversity, availability, stability of physico-chemical properties, and environmental safety of ingredients;
- compliance of the composition with the requirements of modern nutrition science;
- ensuring consumer appeal through flavour, colour, texture, and other properties;
- simplicity of production process without long setup times or changeovers;
- improved efficiency of enterprises processing Siberian pine seeds; and
- diversification of functional dairy products [17].

**Protein content in the diet.** According to the principle of rational nutrition, the ratio of proteins, fats and carbohydrates in the human diet should be 1:1.2:4. Proteins that enter the body with food are broken down into amino acids, essential and non-essential, during the digestion process. To provide the body with a sufficient amount of essential and non-essential amino acids, the diet should include both animal and plant proteins, whose most beneficial ratio is 1:1 [22].

Protein is an indispensable nutrient and a component of all our organs. Proteins make up 15–20% of human body weight, while the normal proportion of fats and carbohydrates is 5–6% or less [18]. The need for proteins lies in their functions: in particular, they are involved in metabolic processes and are the basis of such vital substances as enzymes, hormones, and antibodies.

The human need for protein can vary under a number of factors, but its average adult intake should be 1 g per 1 kg of body weight to ensure the normal functioning of all vital systems. Protein deficiency disrupts metabolism and lowers immunity. Since proteins are part of enzymes, their deficiency reduces enzymatic activity. Also, lack of protein affects the functioning of the musculoskeletal, cardiovascular, respiratory and other systems.

The biological value of protein coming with food depends on the amount and balance of amino acids contained in it. Although it used to be thought that amino acids were only important for the synthesis of proteins, and their ratio had to correspond to DNA and RNA codes, now researchers are increasingly discovering new amino acid functions and their special influence on certain types of the body's vital activities. Almost all amino acids are transformed into essential biochemical substances that have an important effect on human health.

Amino acids have a beneficial effect on the brain, restore the liver and kidneys functions, and are highly

effective in parenteral nutrition, especially during intensive care. Also, they improve the cardiovascular system and the hematopoietic function of the bone marrow. By accelerating the adaptation of the heart muscles to increasing loads, amino acids contribute to better sports performance.

The most important amino acids for human health are arginine, aspartic acid, glutamine, proline, threonine, lysine, cystine, methionine, valine, leucine, and isoleucine. The best source of essential amino acids are products of animal origin (eggs, milk, meat, and fish). Non-essential amino acids are contained in vegetable proteins coming from legumes, grains, cereals, and oil plants.

Nutrition studies have revealed a deficiency of plant and animal proteins in the Russian diet and a general imbalance between them. Therefore, one of the priorities of the Russian food industry is to enrich people's diet with essential proteins and enhance the nutritional and physiological value of foods.

Making up the deficiency requires two simultaneous measures: 1) increase total protein intake and 2) improve the quality of proteins consumed by the population. It is also important to combine animal and plant proteins in such everyday foods as milk, bread, sour cream, and others. This is one of the most promising directions of the Russian food industry and the development of functional foods [23, 24].

To compensate for the lack of protein, functional products are based on milk, dairy whey, or eggs. They may also use plant proteins from secondary raw materials (e.g. oil cake). Plant proteins have a high nutritional value and are therefore used in the development of combined animal- and plant-based functional foods.

There are four main groups of functional foods currently popular in different parts of the world: grain-based, dairy-based and fat-based foods, as well as non-alcoholic drinks. These four groups can become a good foundation for functional nutrition [25].

Dairy products account for 65% of the modern functional foods market. The Russian market of functional dairy products is growing by 25–30% per year, three times as fast as the entire dairy market in Russia. However, this is not the only reason why functional dairy products play such an important role in the human diet.

Nutritionists recommend daily consumption of dairy products, as they contain most of the nutrients we need and are a valuable source of proteins, fats, carbohydrates, calcium, phosphorus, and other minerals. Also, dairy products contain a number of vitamins and trace elements that are well balanced and easily digestible [23].

However, the fatty acid composition of milk fat, despite the presence of arachidonic and linoleic essential acids, is not well balanced for adults. The content of linoleic acid in it is too low against a high content of saturated fatty acids. It is a fact that a low content of linoleic acid is not good for the body's metabolic processes [25]. Therefore, the dairy industry is actively developing new foods with a complex composition of fats combining milk fat with vegetable oils and their modifications.

Quite promising is a combination of dairy products with plant-based ingredients, such as cereals, vegetables, fruits, and berries. They are a valuable source of dietary fibre (cellulose and pectin), vitamins and minerals. This opens up broad possibilities for the regulation of the amino acid and vitamin composition of the combined foods.

**Plant raw materials.** Using plant materials in the technology of dairy products is already a steady trend. Plant-based components can be rich in not only protein, but also in other functional ingredients, including polyunsaturated fatty acids with high biological activity. Functional foods enriched with polyunsaturated fatty acids have a beneficial effect on the nervous, immune and cardiovascular systems, helping maintain normal levels of cholesterol and triacylglycerols in the blood.

Plant ingredients have a multifunctional nature: they contain a wide range of biologically active substances, often in high concentrations. These include amino acids, polyunsaturated fatty acids, vitamins, minerals, dietary fibre, etc. Using plant materials makes new products highly balanced in terms of amino acids, fatty acids, minerals, and vitamins. Cereals stand out among other plant ingredients because of a high content of insoluble dietary fibre, various vitamins, and calcium.

According to published research, the most promising types of protein-containing raw materials in Russia are legumes: lentils, peas, beans, and chickpeas. Also popular are grains, mainly in the form of extruded products, and secondary raw materials, including bran. Finally, plant protein comes from oil-bearing plants of the legume family (soybean, peanut), Asteraceae (sunflower, safflower), Malvaceae (cotton), and Cruciferae families (rapeseed, mustard, winter cress, and sesame) [26, 27].

Another promising source of proteins might be unconventional types of oil-containing raw materials, such as tomato pomace (canning industry waste), grape seeds (winemaking industry waste), and corn germ (flour-milling and starch production waste). Grape seeds, for example, contain up to 10–12% of oil, 21% of protein, 45% of carbohydrates, and 12% of tonic substances. There is also increased interest in some species of plants, in particular amaranth, lupine, and flax, whose industrial use is expected in the near future [26]. Grain crops and wild plants play a major role in solving the problem of food protein deficiency.

Nuts, especially pine nuts, are another valuable source of plant materials. A distinctive feature of pine nut kernels is that they are highly variable in composition depending on the geochemical conditions of Siberian pine habitat, environmental factors, the quality and degree of maturity, their storage conditions, method of shelling, and other factors.

Until recently, only oil was considered the main component of Siberian pine seeds and it was mainly used in the cosmetic industry, but also in medicinal and preventative nutrition. However, Siberian pine nuts are also a source of valuable nutrients and therefore they can be successfully used in the food industry.

Pine nut oil cake and flour are rich in plant protein and have a balanced composition of amino acid, which

makes them a good alternative to soy protein. Pine nuts also contain significant amounts of polyunsaturated fatty acids whose content in the finished product is regulated by pine nut oil used as a functional ingredient.

Pine nuts have certain advantages over other types of plant materials. For example, oilseeds and their products (oil cake and oil meal) contain not only a large amount of plant protein, but also some harmful substances. Sunflower seeds contain a lot of polyphenolic compounds (chlorogenic acid). Rapeseed, mustard and other plants of the Cruciferae family have a high content of phytic and erucic acids. Proteins obtained by processing pine nuts, however, do not contain any undesirable substances and can be used in food production.

An important factor is that the proteins of milk and pine nut kernels are complementary in terms of limiting amino acids. Their compatibility has great advantages for enriching the diet.

Pine nut proteins are characterized by relatively high solubility: an average content of soluble proteins is 71.5%. Also, they contain all essential amino acids, including isoleucine, lysine, and tryptophan, and some non-essential amino acids, such as arginine, proline, glutamic, and aspartic acids. Siberian pine nut proteins are of exceptional biological value and, due to their amino acid composition, they can be successfully used in food enrichment.

This combination of amino acids suggests cholesterol-lowering properties; therefore, pine nuts could be recommended as a medicinal or preventative product in the treatment of cardiovascular diseases.

The lipid composition of pine nuts is characterized by a high content of linoleic and  $\gamma$ -linolenic acids. Linoleic acid plays an important role in lipid metabolism. It is converted into arachidonic acid in the presence of group B vitamins and tocopherols, which also come from pine nuts. The physiological effect of  $\gamma$ -linolenic acid is determined by its transformation into prostaglandins of the first type, which regulate the body's numerous cellular and tissue functions. In particular, they contribute to platelet concentration, vessels contraction and expansion, improved immune system, and increased endurance [15].

Pine nut kernels contain significant amounts of minerals, such as phosphorus, potassium, magnesium, and iron. Like any nuts, pine nuts have a low content of calcium; however, this deficiency is easy to compensate for by using pine nuts as an ingredient in multicomponent functional foods. It seems quite promising to combine pine nuts with foods rich in calcium, such as dairy products.

Pine kernels are richer in vitamins than any other nuts. Also, they have a high content of tocopherols, which makes them biologically active and easy to store.

The concentration of toxic substances in pine nuts does not exceed the permissible level, which makes them absolutely safe for consumption. Pine nuts can be used fresh or processed, with no restrictions as to processing methods.

Research evidence suggests that pine nuts can be classified as a multifunctional food material with a high content of technologically significant components [15].

Given their high nutritional value, pine nut products can be used by all people regardless of their age or physiological state, as well as in treatment and prevention of disease. This type of wild plant is optimal for the production of functional foods of high nutritional value.

**Economic aspects.** Modern food industry has an increased interest in the use of biologically active substances from natural plant materials, including wild plants. Scientists are seeking new sources of raw materials to satisfy a growing demand for multi-component functional products. To ensure their biological value, raw materials should have a good capacity to synthesise biologically active substances.

Siberia is home to a large number of plants with beneficial properties. Many of them have a high content of biologically active substances. Coniferous species are of remarkable value for the medical and cosmetic industries, while Siberian pine is of great interest to the food industry. Siberian pine nuts are popular all over the world due to their nutritional value, and their gathering has been a common trade in Russia for a long time.

Siberian pine, which plays an important role in the plant community, has an enormous habitat, stretching from the north-east of the European part of Russia to Eastern Siberia. Most of the habitat is taiga, located far from large cities. This area is not treated with pesticides, herbicides or chemical fertilizers and is least affected by dust and gas emissions from industrial enterprises. Pine nut harvesting areas are geographically isolated from the sources of technogenic pollution and have a relatively low level of toxic elements, radionuclides and pesticides, ensuring the safety of raw materials and foods [28, 29].

It should be noted that most of the pine forests in Kuzbass (Kemerovo Region) are located in remote mountainous and lowland areas, away from transport routes and other infrastructure facilities. Pine nuts harvesting covers a relatively small part of the forests spread out near the villages along the river banks and some other accessible areas. Altogether, the harvesting area accounts for no more than 3.9% of all Siberian pine forests, or approximately 0.11% of the region's total forestry [30].

According to the statistics, the Kemerovo Region harvested 143 tons of Siberian pine seeds in 2006, which is only 5.8% of the potential yield [30]. The nut harvesting area in Kuzbass covers 7,100 hectares, which is less than 3.8% of the total pine forestry in the region.

Biologically, however, Kuzbass is one of the most promising regions for the nut industry [29]. It is a sustainable area of Siberian pine productivity.

Siberian pine nut crops are known to be uneven, with high-yielding years alternating with low-yielding years, which has certain implications for harvesting and production planning. However, Kuzbass does not have such dramatic leaps in seed productivity as some other regions; moreover, it has the most frequent increased yields – one in every 3–5 years. This can be explained by the optimal correspondence between the rhythm of physiological and biochemical processes in Siberian pine, on the one hand, and external conditions, on the other. The average annual biological yield of Siberian pine nuts in the Kemerovo Region is 50 kg per



hectare. To sum up, the region has good forest resources to develop the nut industry and organize the production of pine nut oil and other useful products.

On the other hand, in view of a difficult environmental situation in the Kemerovo Region, the task of providing the population with balanced nutritional foods acquires a special importance. In this regard, the use of unconventional plant materials, in particular Siberian pine seeds, seems to be quite a promising direction for the food industry. Firstly, it ensures sustainable use of natural resources. Secondly, it is a new source of food raw materials. Finally, it expands the range of foods for general and functional purposes.

The fact that Siberian pine grows wild in remote areas makes pine nuts quite an expensive product. Therefore, it is important to ensure the maximum yield of high quality products. The technological properties of Siberian pine seeds depend not only on their genetic features, as well as the climatic and geographic conditions of their habitat, but also on the conditions of their post-harvest treatment and storage.

Siberian pine seeds are biologically unequal. Small seeds (with a diameter of 7 mm or less) have a lower technological quality (high acid number, low content of crude lipids and protein), compared to large seeds.

The post-harvest ripening of pine seeds also depends on their size. Large seeds display an increased oil content, a decreased acid number with a relatively high lipase activity, and a reduced peroxide number with a stable lipoxigenase activity. Large seeds ripen longer than small ones, which makes it possible to control this process by technological means in order to increase the oil yield from the seeds and improve its quality.

At constant temperature and relative humidity, different seed tissues absorb a different amount of moisture from the air. Nut shells have a higher water-absorbing capacity than kernels or seeds. This creates favourable conditions for the development of microorganisms on the seed surface.

Because of sorption hysteresis, seeds may retain a different content of moisture even after prolonged storage in a dry state, which must be taken into account when establishing the optimal conditions for post-harvest seed treatment, drying and storage.

Drying significantly reduces the content of mould and bacterial flora. Dry seeds display a poor development of microflora and a limited number and variety of microorganisms, which contributes to longer storage. When stored in favourable conditions, the content of oil in the kernel, as well as its acid and peroxide numbers, remains almost unchanged.

Observing the optimal storage conditions for dried seeds (temperature:  $4 \pm 2^\circ\text{C}$ ; relative humidity of air:  $70 \pm 5\%$ ) maintains their quality throughout the year. Also, optimal storage contributes to nuts maturation, increasing the content of oil in the kernels and thus improving their quality [17].

## RESULTS AND DISCUSSION

Pine nut kernels and their products are used as ingredients in the production of pine nut milk, dairy products, bakery and confectionery, alcoholic drinks, medicinal and preventative drinks, dietary supplements, hygiene products, soap, toothpaste, and cosmetic creams.

Pine nut oil is the main end-product of processed kernels. It has a mild nutty aroma, light amber colour, and a slightly bitter taste. The quality of pine nut oil depends on the quality of raw materials, post-harvest treatment and storage, as well as methods and modes of extraction. Cold pressed oil has the finest flavour and nutritional qualities. The combination of essential and biologically active substances in pine nut oil opens up good prospects for its use as a dietary oil and as a biologically active additive in dairy products with a pronounced therapeutic effect.

Pine nut oil is produced by pressing or extraction. Pressed oil is used for dietary, cosmetic, medicinal, and preventative purposes. Extracted oil has a dietary use after refining.

Modern technologies allow us to diversify and increase the output of target products. They help make pine kernels processing more cost-effective and create new ingredients for functional foods, for example, pine nut paste, oil cake and fat-free flour.

Cakes and meals made from oil plants, which are classified as secondary raw materials, are important additional ingredients in food production. They are a source of high-grade protein, easily digestible carbohydrates, vitamins, and minerals. Flour obtained by extracting lipids from fat-containing material (cake or meal) is the cheapest and most accessible form of plant protein.

Pine nut products are recognized as safe and easily producible materials that can be used as complex enriching agents in the production of functional foods due to their nutritional value and functional properties.

**Pine nut paste.** Siberian pine seeds are introduced into complex dairy products in the ground form. A disintegrator is used as a fine grinder to produce a homogeneous, plastic and viscous paste, resistant to separating. The product has a light cream colour, a sweet and slightly astringent taste, and an intense nutty aroma.

Pine nut paste has the same organoleptic characteristics as its raw material, Siberian pine seed kernels, but a more intense taste and aroma. Its chemical composition is almost identical to that of the raw material. However, it has a lower amount of fibre and ash and a higher content of protein and fat due to the partial separation of the kernel shells.

Mechanical treatment does not affect the amino acid composition of pine nuts. However, the destruction of the kernel cellular structures and a high degree of fineness increase the protein digestibility coefficient, making proteins more accessible to digestive enzymes. The high fineness of pine nut paste also contributes to its uniform distribution in the product enriched.

**Pine nut oil cake.** Pine nut oil cake is a light cream-coloured powder without any foreign inclusions that has a light aroma, characteristic of pine nut kernels, and a sweetish taste. Its main components are proteins, lipids, carbohydrates, and minerals. The oil cake protein includes 19 amino acids, at least 40% of which are essential. Among the deficient amino acids are leucine, methionine and phenylalanine.

Oil cake carbohydrates, which are of great nutritional and functional importance, affect the taste and the

digestibility of end-products. The vitamin and mineral value of pine oil cake depends both on the chemical composition of the processed pine nuts and on the residual oil content after pressing. Pine oil cake has an increased content of phosphorus and magnesium. Like pine kernels, it is characterized by a low calcium content and a pronounced predominance of potassium over sodium. Calcium deficiency can be compensated by combining pine oil cake with calcium-rich dairy products.

The vitamin value of pine nut oil cake is in its content of water-soluble B vitamins (B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>5</sub>, and B<sub>6</sub>), and tocopherols. Vitamins B<sub>1</sub>, B<sub>3</sub>, and B<sub>5</sub> are present in significant amounts, while the content of thiamine and tocopherols is lower than in the nut kernels. Tocopherols perform an antioxidant function, contributing to the product preservation. However, it is recommended to store both the oil cake and the products containing it at a low temperature to ensure minimal loss of vitamin E.

**Pine nut flour.** Pine nut fat-free flour is a light cream-coloured powder with a subtle aroma, characteristic of pine nut kernels, and a sweetish taste. Its major chemical components are proteins and carbohydrates, resulting in its classification as protein- and carbohydrate-rich raw material with a high content of mineral substances. Table 1 shows the amino acid composition of pine nut flour.

Fat-free pine nut flour has at least 40% of essential amino acids. Its well-balanced protein has a high content of lysine, which is deficient in cereals, and arginine, as well as glutamic acid. However, we should also note the presence of a limiting amino acid, leucine.

The protein digestibility coefficient of pine nut flour averages 78.5%, which is common for plant materials. However, it is a few times lower than that of pine nuts, possibly due to the protein denaturation as a result of heating when extracting oil. The carbohydrate composition of pine nut flour is characterized by a high content of sucrose and starch. In addition, the product is rich in dietary fibres, mainly cellulose and pentosans. Fat-free pine nut flour is also rich in B vitamins and minerals (excluding calcium).

The most important functional and technological properties of plant-based supplements are those resulting from the interaction of proteins and water, namely, hydration, swelling, solubility, viscosity, thickening, water- and fat-holding capacity, as well as emulsifying and foaming capacities. Good swelling capacity of pine nut flour and oil cake, and their ability to absorb and retain moisture, are important for the product consistency.

Fig. 1 shows the degree of increase in the volume of pine nut oil cake and fat-free flour during hydration depending on temperature.

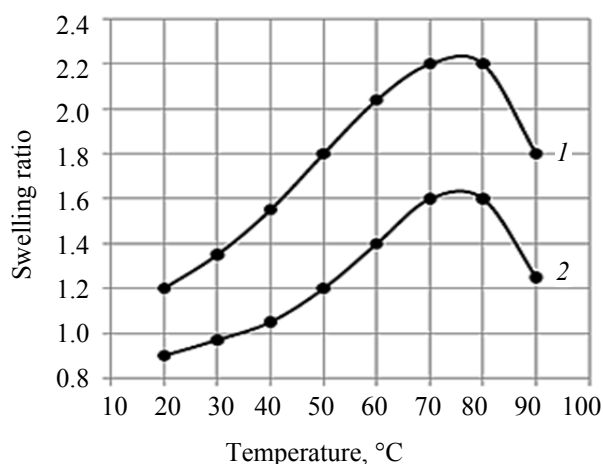
Both products are characterized by the maximum degree of swelling at a temperature of 75–80°C. Pine nut flour has a greater swelling capacity and is more hydrophilic than pine nut oil cake.

The ability to bind and firmly hold fat is another important characteristic of a raw material intended as a protein fortifier in the production of fat-containing dairy and emulsion products.

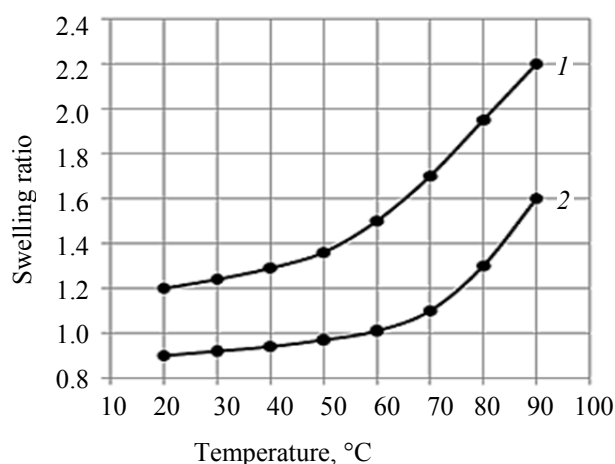
Pine nut oil cake and fat-free flour have good fat-holding and fat-emulsifying capacities, which can be maximized by regulating the time of mixing, as well as the temperature and duration of emulsification.

**Table 1.** The amino acid composition of pine nut flour compared to ideal protein

Amino acid	Ideal protein (FAO/WHO)		Pine nut flour	
	g/100 g protein	Score, %	g/100 g protein	Score, %
Valin	5.0	100	5.20	104.00
Isoleucine	4.0	100	5.00	125.00
Leucine	7.0	100	5.86	83.70
Lysine	5.5	100	6.40	116.36
Methionine + Cystine	3.5	100	3.50	100.00
Threonine	4.0	100	4.00	100.00
Tryptophan	1.0	100	2.40	240.00
Phenylalanine + Tyrosine	6.0	100	7.90	131.66
Total	36.0	100	40.26	



(a)



(b)

**Fig. 1.** The degree of increase in the volume of fat-free pine nut flour 1 – and oil cake 2 – when swelling in water (a) and milk (b).

**Table 2.** The fat-holding capacity of pine nut oil cake and fat-free flour depending on temperature

Type of additive	Temperature, °C			
	20 ± 2	40 ± 2	60 ± 2	80 ± 2
Pine nut oil cake	150 ± 3	160 ± 3	175 ± 3	193 ± 2
Fat-free pine nut flour	205 ± 2	240 ± 5	252 ± 3	285 ± 3

According to a study of their functional properties, pine nut oil cake and fat-free flour can be used as emulsifiers and stabilizers. Therefore, they can be used in the production of emulsion products, such as cheeses, sauces, paste-like dairy products, and creams. In particular, we could recommend their use in the production of foods whose technological properties can be improved by binding fat or water.

The chemical composition of pine nut oil cake and fat-free flour and their functional and technological properties justify their wide use as a protein and mineral supplement to increase the nutritional and biological value of functional foods. Adding fat-free pine nut flour and pine nut oil to dairy products (for example, ice cream) creates a high-quality functional product that contains easily digestible proteins (of milk and flour) with a balanced amino acid composition, essential fatty acids, important bio-elements (magnesium, zinc, iron, and iodine), and vitamins (E and B group).

It is possible to add a small amount of pine nut oil to butter to balance the lipid composition of the end-product by changing the proportion of saturated, monounsaturated and polyunsaturated fatty acids. Butter in this case is produced in the traditional way.

In general, pine nut oil cake and fat-free flour are well compatible with food materials, including dairy products, and can be used as a stabilizer of protein-fat emulsions (sauces, pastes, and creams).

A study of cultivating lactic acid microorganisms on the basis of milk and pine nut material revealed good prospects for combining pine nut oil cake with dairy materials. Such a combination is expected to expand the range of low-calorie dairy products with an increased nutritional and biological value.

Adding pine nut oil cake or fat-free flour to dairy materials significantly changes the content of proteins, fats and minerals. Introducing oil cake increases their content by 10.0%, 8.0%, and 7.4% respectively per each percent of the added plant component. When pine nut flour is added, the protein content increases by 13.3% and minerals by 9.0%, but the mass fraction of

fat, on the contrary, decreases on average by 1.2% per each percent of the added component.

Changes in the composition affect the properties of milk. In particular, titratable acidity of milk and plant mixtures is higher than that of milk and is directly proportional to the mass fraction of the plant component. Active acidity of milk and plant mixtures is weakly dependent on the amount of the protein component. However, it decreases with an increase in the amount of pine nut oil cake or fat-free flour. This process can be explained by the ongoing restructuring of proteins and accumulation of alkaline groups [31].

The process of ripening in milk and plant mixtures is different from ripening in the milk medium. Adding pine nut products to milk activates the lactic acid process, reducing the time of ripening and increasing the rate of lactic acid accumulation. The reproduction of microorganisms becomes more intense, which affects the ripening process. Active reproduction of lactic acid bacteria changes the properties of the product, increasing the average size of casein particles and the number of peptides and free amino acids [32].

A combination of pine nut oil cake with a dairy base is also used to produce cottage cheese and yogurt. The mixture is pasteurized, cooled and fermented with lactic acid bacteria. The use of oil cake as an active additive intensifies the process of ripening, reducing its length and improving the quality of curd.

Pine nut meal is used in the production of fermented milk products. Due to a significant content of poly- and oligosaccharides, it is used as a prebiotic in the production of bifidus-containing fermented milk products. Pine nut milk can be used as a base for preparing a fermented product obtained either from pine nuts or from pine nut oil cake and water or buttermilk in the ratio of 1:15 to 15:1. A small amount of skimmed milk powder can be added to the formulation, from 0.5 to 3.0% of the mixture weight [17]. The use of pine nut products can significantly reduce the amount of expensive raw milk and produce foods that are rich in plant proteins and have good consumer appeal.

In general, pine nut oil cake and fat-free flour are dry, light, compact and easily stored products with low transportation costs. When used in traditional food production, they do not require any special equipment or capital investment. Thus, the use of these ingredients does not increase production costs.

To sum up, the benefits listed above make the use of pine nut paste, oil, oil cake and fat-free flour highly desirable in the production of functional dairy products, especially fermented milk products.

**Table 3.** The composition and properties of milk and plant mixtures with pine nut oil cake

Indicator	Cow milk	Mass fraction of pine nut oil cake, %				
		1	2	3	4	5
Mass fraction of dry substances, % including:	11.00 ± 0.10 <sup>a</sup>	11.70 ± 0.10 <sup>a</sup>	12.36 ± 0.10 <sup>a</sup>	13.51 ± 0.10 <sup>a</sup>	14.30 ± 0.10 <sup>a</sup>	15.12 ± 0.10 <sup>a</sup>
protein	3.0 ± 0.1 <sup>b</sup>	3.3 ± 0.1 <sup>b</sup>	3.6 ± 0.1 <sup>b</sup>	3.9 ± 0.1 <sup>b</sup>	4.2 ± 0.1 <sup>b</sup>	4.5 ± 0.1 <sup>b</sup>
fat	2.50 ± 0.05 <sup>c</sup>	2.70 ± 0.05 <sup>c</sup>	2.90 ± 0.05 <sup>c</sup>	3.10 ± 0.05 <sup>c</sup>	3.40 ± 0.05 <sup>c</sup>	3.60 ± 0.05 <sup>c</sup>
carbohydrates	4.70 ± 0.10 <sup>b</sup>	4.98 ± 0.10 <sup>b</sup>	5.27 ± 0.10 <sup>b</sup>	5.55 ± 0.10 <sup>b</sup>	5.84 ± 0.10 <sup>b</sup>	6.12 ± 0.10 <sup>b</sup>
ash	0.67 ± 0.02 <sup>d</sup>	0.72 ± 0.02 <sup>d</sup>	0.76 ± 0.02 <sup>d</sup>	0.81 ± 0.02 <sup>d</sup>	0.85 ± 0.02 <sup>d</sup>	0.9 ± 0.02 <sup>d</sup>
Titratable acidity, °T	18.0 ± 0.3 <sup>a</sup>	18.5 ± 0.2 <sup>a</sup>	19.0 ± 0.3 <sup>a</sup>	21.0 ± 0.3 <sup>a</sup>	23.0 ± 0.3 <sup>a</sup>	25.0 ± 0.2 <sup>a</sup>
Active acidity, pH unit	6.60	6.63	6.68	6.72	6.75	6.80

Note. The values followed by the same letter in the table do not differ significantly (p-value < 0.05).

At the next stage, we studied possible uses of pine nut products in dairy products.

**Processed cheese.** Recent years have seen intensified research efforts to develop new types of soft cheese products, since they are more cost-effective in production than hard cheeses, they do not require maturation and have a high nutritional value. Processed cheese is a very popular product, relatively inexpensive but highly nutritional. Its production process allows producers to satisfy various tastes, even when there is a shortage of raw materials.

Processed cheese production technology offers ample opportunities for diversifying its composition. Functional additives in processed cheese production include fruits, berries, vegetables, marine products, vegetable fats, and other components [33, 34].

Production of dairy products using pine nut oil cake and fat-free flour is based on the joint precipitation of animal and plant proteins. Introducing pine nut products into milk leads to changes in the protein and fatty phases, carbohydrate and salt composition, significantly increasing the content of proteins and minerals. Pine nut oil cake increases the content of lipids, while pine nut flour produces the opposite effect.

The titratable acidity of milk and plant mixtures is higher than that of milk, and is directly proportional to the mass fraction of the plant component. The active acidity, on the contrary, is inversely proportional to the amount of pine nut oil cake and fat-free flour.

Introduction of protein supplements (pine nut oil cake and fat-free flour) into milk changes its organoleptic and technological properties. In particular, milk acquires a nutty aroma and taste which intensify as the amount of the protein component increases. The colour of the milk changes from white to light cream.

An increase in the protein component (pine nut oil cake and fat-free flour) reduces rennet coagulability and curd synergetic ability, prolonging the duration of renneting. Titratable acidity of whey decreases, while its active acidity increases, changing the quality of curd. Less whey is released and the curd becomes moister. When a milk and plant mixture is used, the curds are softer than those obtained by coagulating milk. This effect is produced by the hydration properties of pine nut oil cake and fat-free flour.

The release of whey during curd syneresis decreases to a greater extent when pine nut oil is introduced, rather than fat-free flour. Unlike the latter, pine nut oil cake contains lipids, which leads to an increased amount of fat in the milk and plant mixture. Fats slow down the release of whey. In general, larger amounts of pine nut oil cake or fat-free flour in milk and plant mixtures contribute to increased curd yield. The dependence is almost linear: the more oil cake (or fat-free flour) is used, the greater the curd yield. This occurs due to increased protein content and curd moisture, lowering the cost of production.

The ripening of dairy and plant mixtures containing pine nut oil cake or fat-free flour is accompanied by an increased number of microorganisms, higher titratable acidity and lower active acidity, as well as improved coagulability and syneresis capacity. The clotting time gets shorter. Thus, the use of pine nut products not only

enriches the end-product with useful substances, but also optimizes the production process.

The process of making a cheese product is also influenced by a number of technological factors, such as the temperature of curd processing and cheese forming, the duration of self-pressing, and the method of salting, maturation and storage. These factors were studied to determine their optimal values [17].

It was established that raising the curd-processing temperature decreases the mass fraction of moisture and salt in the cheese and increases the active acidity of the cheese mass. Changes in the cheese composition caused by the differences in the curd processing temperature from 35 to 39°C affect the organoleptic properties of the product. The optimal curd-processing temperature, which significantly improves the organoleptic properties, is  $37 \pm 1^\circ\text{C}$ .

The optimal duration of self-pressing was established as 16 hours. A series of experiments with varying methods of salting confirmed that the best method was curd salting.

The optimal duration of cheese product ripening was established as 24 hours. This period ensures good organoleptic characteristics of the cheese product and makes it ready for consumption. A longer period of ripening only leads to insignificant changes in organoleptic characteristics [17].

Pine nut paste is also used in the production of processed cheese. Replacing milk with pine nut paste is not equivalent in terms of protein content, since the mass fraction of protein in pine nut paste is lower than in animal products. However, pine nut paste has a balanced amino acid composition with all the essential amino acids. Thus, using pine nut paste to replace only part of dairy ingredients can ensure a similar biological value in processed cheese products.

The main component of pine nut paste are lipids, in particular, triacylglycerols. Compared to milk fat, pine nut oil contains 10 times as much linoleic acid and more than 20% of biologically valuable  $\gamma$ -linolenic acid, which is completely absent in animal fats. The use of pine nut paste in processed cheese production can significantly change the fatty acid composition of the product's fat phase and increase its biological value.

Experimental data show that the complete replacement of animal fats with pine nut products has a negative effect on the organoleptic indicators. The optimal amount is 5 to 10% pine nut paste replacing some part of butter and skimmed milk powder.

Processed cheese has a moderate cheesy flavour and a light nutty aroma, a moderately firm texture and a light cream colour.

Several studies were conducted to assess the influence of major process factors (such as melting temperature and a mass fraction of paste and fat) on the organoleptic, structural and technical properties of processed cheeses with pine nut paste, and to identify optimal indicators. They showed that the best flavour and aroma were achieved at a melting temperature of 75–85°C, a paste mass fraction of 8.75 to 10.0%, and a fat mass fraction of 40 to 45%.

**Table 4.** The organoleptic indicators of processed cheese

Amount of additive	Flavour and aroma		Texture		Colour		Total score
	Characteristic	Score	Characteristic	Score	Characteristic	Score	
Control	Moderate cheesy	14	Moderately firm	8	Light cream	2	28
5.0	Moderate cheesy	14	Moderately firm	8	Light cream	2	28
10.0	Moderate cheesy and nutty	15	Moderately firm	8	Light cream	2	29
15.0	Moderate cheesy, nutty, and slightly bitter	13	Slightly sticky	7	Light cream	2	26

**Table 5.** The organoleptic properties of ice-cream with fat-free pine nut flour

Number of sample	Degree of replacement, %	Organoleptic assessment, score				Total score
		Colour and appearance	Flavour and aroma	Texture	Total score	
1	1.0	5.0	5.0	3.0	13.0	
2	1.5	5.0	4.5	3.0	12.5	
3	2.0	5.0	5.9	3.0	13.9	
4	2.5	5.0	6.0	2.9	13.9	
5	3.0	5.0	5.8	2.8	13.6	
6	3.5	4.8	5.8	2.6	13.2	
7	4.0	4.0	5.3	2.4	11.7	

A study of the temperature-texture dependency established the optimal temperature as 75°C. Increasing a mass fraction of pine nut paste up to 7.5–12.5% made the texture more plastic and softer. Its fraction of 8.75–10.0% added a light pine nut flavour to the product, improving its organoleptic properties compared to the control cheese.

Finally, the optimal dosage of pine nut paste was established as 8.75–10.0%, both in terms of the quality and biological value.

The stage of entering pine nut paste into the cheese base affects the fatty acid composition of the end product. Prolonged heat exposure leads to the formation of free fatty acids as a result of partial hydrolysis. At the same time, low molecular weight fatty acids are partially distilled off with steam. Therefore, it is recommended to minimize the heating time of the fat phase, which includes pine nut paste, and introduce the latter 5–7 minutes before the end of melting. In this case, the product texture will meet all consumer requirements.

**Cottage cheese and plant products.** Pine nut paste is also used in the production of cottage cheese and plant foods based on low-fat cottage cheese. Functionally, pine nut paste enriches the product with polyunsaturated fatty acids, plant protein, minerals (Mg, Fe, and Zn), vitamins, and antioxidants.

Pine nut paste affects the rheological characteristics of the end-product. First, cottage cheese has a coagulation and condensation structure with a high moisture content, both in the free and in the bound state. Free moisture affects the mobility of macromolecular protein bodies and dissolves low molecular weight compounds. Ultimately, free moisture affects the ductility. Pine nut paste reduces the moisture content of the cottage cheese and plant product and, as a result, increases its viscosity.

In the experimental study, the mass fraction of pine nut paste in the cottage cheese and plant mixture ranged from 5 to 30%. The samples with a paste content of 15 and 20% showed the best organoleptic characteristics.

The cottage cheese and plant foods made with pine nut products contain 5–8 times as much linoleic acid and 10–20 times as many polyunsaturated fatty acids as ordinary 9%–fat cottage cheese.

Pine nut paste enriches cottage cheese with manganese, which the latter contains in negligible amounts, as well as potassium, magnesium, phosphorus, iron, and zinc.

**Ice cream.** Ice cream is less often modified to improve its nutritional properties, compared to other dairy products. Nevertheless, there is a need for ice cream consistent with the principles of healthy eating. For this, ice cream needs to have a reduced calorie content, a lower fat and sugar content, and be enriched with vitamins, minerals, and biologically active substances [7, 8]. Such functional ingredients as pine nut oil and fat-free flour can increase the nutritional and biological value of ice cream by enriching it with essential amino acids, polyunsaturated fatty acids, vitamins, and mineral elements. Fat-free pine nut flour also has good stabilizing and emulsifying properties and can improve the texture and appearance of ice cream.

Due to its high nutritional and biological value, fat-free pine nut flour can be used instead of skimmed milk powder. It can also improve ice cream overrun. The optimum mass fraction of fat-free pine nut flour is 2.5 to 3.5%, while pine nut oil can replace 10–30% of cream butter. These amounts of pine nut products give ice cream a pleasant milky and nutty flavour and aroma.

The use of pine nut oil and fat-free flour in ice cream production does not impair the quality of the end-product, but it allows producers to use less raw milk and reduce production costs. The combination of animal and plant proteins makes ice cream more resistant to melting and improves its structure and texture. New types of ice cream enriched with pine nut oil and fat-free flour are characterized by high organoleptic scores.

It was established that homogenized cream mixtures where milk fat was replaced with pine nut oil had 1.3 times as many fat globules up to 1.5 µm in

size, compared to milk fat mixtures. Ice cream mixtures with smaller fat droplets had a larger number of small bubbles forming during the freezing process that are resistant to mechanical stress and do not burst during mechanical processing in the freezer, compared to large ones.

Replacing milk fat with plant oil (pine nut oil) in the range of 10–30% of the total fat phase has a positive effect on the organoleptic characteristics of ice cream, giving it a pine nut flavour and aroma.

After studying the effect of fat-free pine nut flour, pine nut oil and stabilizer doses on ice cream flavour, aroma and overrun, we established their optimum dosage as 2.8–3.4%, 20–25%, and 1.5% respectively, ensuring good organoleptic, structural, and mechanical properties of the end-product.

The use of unconventional raw materials in ice cream production (namely fat-free pine nut flour and pine nut oil) affects the quality and technological properties of liquid cream mixtures. A study of their homogenisation modes showed that the process of homogenisation changed the structure and properties of protein substances. In particular, the size of casein micelles decreased, and some of them broke up into submicelles, which were adsorbed on the surface of fat globules. A higher degree of the protein phase dispersion (total surface) led to an increase in surface charge and enhanced the hydration properties. Milk proteins spread on the surface of fat globules and protected them from sticking, which prevented fat from settling in the process of mixture maturation and storage and improved overrun, with no grains of butter forming during freezing.

The homogenization of liquid ice cream mixtures was carried out in laboratory conditions at different temperatures – 70, 80 and 90°C – and a constant pressure of 12.5 MPa. It was found that introducing 2.8–3.4% of fat-free pine nut flour instead of skimmed milk powder did not reduce the quality of the homogenized mixtures.

Since the use of pine nut flour increased the mass fraction of protein in the mixture, its dispersion rose from 76.4 to 89%, while the degree of homogenization decreased from 9.5 to 6.5%. The presence of plant proteins contributed to the formation of strong and stable fat globule membranes.

According to the experiments, 80°C was the optimum temperature of mixture homogenization that ensured a high-quality product. At this temperature, the average size of fat globules was no more than 1.5 µm, and there was an increase in the kinetic stability of the fat phase.

The pressure of homogenization is another factor affecting the emulsion stability. Studies showed that 12.5 MPa was the optimum pressure [17].

A study was conducted to measure the impact of freezing modes on the quality of ice cream containing pine nut flour and oil. Freezing leads to the formation of ice crystals and a structure of the product. The size and shape of ice crystals depends on the freezing rate, the mixture composition, the mass fraction of bound moisture, overrun, and the size of air bubbles. The presence of fat-free pine nut flour has a positive effect on the overrun. An increase in the mass fraction of pine

nut flour from 3.0 to 3.4 improves the overrun by 12.0%. Moreover, a combination of animal and plant proteins makes ice cream more resistant to melting.

In addition, when using pine nut flour and oil, producers can economise on milk raw materials (skimmed milk powder and butter) and therefore reduce production costs.

**Mayonnaise.** A current trend is to produce medium- and low-calorie mayonnaise that does not contain cholesterol and is enriched with vitamins and biologically active substances [12, 35–38].

Unlike high-calorie mayonnaise, medium- and low-calorie mayonnaise needs emulsifiers and stabilizers. However, the choice of emulsifying and stabilizing agents should provide a high physiological value of the product. Natural plant-based emulsifiers are the safest and the most active biologically. Partial or complete replacement of egg powder, the traditional emulsifier, with plant materials will also reduce the cholesterol level in the end-product.

In particular, mayonnaise emulsions can be based on a combination of skimmed milk powder and fat-free pine nut flour in the ratio of 2.6:1.0, respectively. This optimum ratio ensures a high content of protein and essential amino acids, including limiting amino acids, methionine and cystine. Adding fat-free pine nut flour to skimmed milk powder enriches the milk and plant mixture with potassium, magnesium, phosphorus, iron, zinc, and manganese.

The milk and plant mixture has a high solubility and a fat emulsifying capacity. According to the equations analysis, the maximum stability of the emulsion, high viscosity and maximum organoleptic scores were observed in those mayonnaise samples which had the following composition: 30.0–35% fat, 1.5–2.0% egg powder, 12–14% dry milk and plant mixture (3.4–3.8% fat-free pine nut flour) [17].

The final stage of the study aimed to determine the nutritional value of dairy foods with pine nut products, which is the key quality criterium.

The chemical composition analysis of the new dairy products containing wild plant materials (Siberian pine seeds and their products) showed that pine nut oil cake, fat-free flour, paste, and pine nut oil enriched the products with plant protein, vegetable oil, polysaccharides (starch, fibre, and pentosans), vitamins, and macro- and microelements. Replacing raw milk with plant materials does not reduce the nutritional value of new dairy products, which have a high content of protein, fat and minerals.

The use of pine nut oil cake, fat-free flour and paste as a component of fermented milk products supplements animal proteins with plant proteins.

A comparative analysis of the amino acid composition showed that adding pine nut oil cake or fat-free flour to soft cheese products did not reduce their nutritional value. These plant ingredients led to a slight decrease in essential amino acids, compared to the control sample, but increased the amount of sulphur-containing amino acid, which limits dairy product proteins, and tryptophan.

According to the quality assessment, the proteins of processed cheese with pine nut paste have a well-balanced content of essential amino acids and a high

biological value. The enrichment of cottage cheese with pine nut paste leads to a slight decrease in essential amino acids and, at the same time, an increase in isoleucine and leucine. Also, the product acquires some non-essential amino acids (arginine, aspartic and glutamic acids), compared to the control sample.

Ice cream and dietetic mayonnaise enriched with fat-free pine nut flour have a well-balanced content of essential amino acids (over 100% score). Cottage cheese enriched with pine nut paste acquires a few times as much vitamin E, 5–8 times as much linoleic acid, and 10–20 times as much polyunsaturated fatty acids as ordinary 9%-fat cottage cheese.

Further, functional ingredients (pine nut oil cake, fat-free flour, paste and oil) enrich new types of dairy products with tocopherols.

All the dairy products with pine nut additives described in the paper have a biologically balanced amino acid composition. Combinations of animal and plant proteins are easily digestible and have a beneficial effect on the maintenance of human nitrogen balance. The products are adapted to the Russian food market; they are compatible with the main foods and do not change their organoleptic properties, familiar to every person.

It was also established that pine nut products did not reduce the shelf life of new dairy foods. The use of pine nut oil cake and fat-free flour did not affect the microbiological indicators during storage. Storing processed cheese at a temperature of  $(4 \pm 2)^{\circ}\text{C}$  for 7 days led to no changes in organoleptic characteristics (taste, aroma, texture, and appearance). Storing a 9%-fat cottage cheese and plant product at a temperature of  $(4 \pm 2)^{\circ}\text{C}$  maintained its organoleptic characteristics for 15 days. The microbiological indicators of liquid ice cream mixtures remained within permissible limits for 48 hours at a temperature of  $(4 \pm 2)^{\circ}\text{C}$ .

Thus, pine nut products used as biologically active ingredients in functional foods do not reduce their shelf life, compared to traditional dairy products.

## CONCLUSIONS

Siberian pine seeds and their products are promising plant raw materials, with a wide range of physiological properties, a unique biochemical composition and a set of biologically active substances. Always comprising a large proportion of the Siberian food balance, pine nuts have not lost their value or local appeal.

Modern processing technologies are able to increase the output of pine nut oil and expand the range of by-products. Pine nuts are a raw material for producing pine nut paste, oil cake and fat-free flour, which are used as supplements in the food industry.

Siberian pine seeds (pine nuts) contain extremely valuable substances, such as fats rich in polyunsaturated fatty acids, proteins with a well-balanced amino acid composition, soluble and insoluble carbohydrates, minerals (phosphorus, magnesium, iron, zinc, and iodine), B vitamins, and tocopherols. Their high content of functional proteins, polyunsaturated fatty acids, vitamins, and biocomponents makes them a good ingredient for dairy products. Siberian pine seeds and their products (pine

nut oil, oil cake and meal) are widely used in the production of dairy and fermented milk products, namely, cottage cheese, sour cream, kefir, yogurt, and desserts, including whipped milk drinks, confectionery creams, cheese and others.

Dairy products enriched with Siberian pine nut ingredients have a high nutritional and biological value due to their unique biochemical composition. The synergistic effect of dairy and plant raw materials is manifested in increased digestibility of new dairy products, improved physico-chemical and organoleptic indicators, inhibited development of undesirable microflora, and a longer shelf life.

The nutritional and biological value of dairy products containing pine nut oil, oil cake or meal improves as a result of enriching the finished product with plant proteins with a balanced amino acid composition, polyunsaturated fatty acids, minerals (magnesium, iron, and iodine), vitamins, and ballast substances (cellulose, hemicellulose, and pentosans).

Adding a plant component containing over 60% of oil changes the ratio between monounsaturated and polyunsaturated fats and enriches the dairy product with vitamin E, essential amino acids, and minerals.

Given their valuable properties and accessibility, Siberian pine nuts and their products are used to create a variety of new functional foods. The development of competitive multi-component foods with beneficial functional properties seems to be a promising direction in the current environmental, social and economic situation.

The chemical analysis of new dairy products showed that adding pine nut oil cake, fat-free flour, paste or oil to their formula enriched them with plant protein, vegetable oil, polysaccharides (starch, fibre, and pentosans), vitamins, and macro- and microelements. Replacing raw milk with plant materials did not reduce the nutritional value of new dairy products. They had a high proportion of protein, fat and minerals and were as rich in vitamins as their dairy-based counterparts. Also, the use of functional ingredients (pine nut oil cake, fat-free flour, paste and oil) enriched new dairy foods with tocopherols.

Thus, including functional dairy foods enriched with Siberian pine nut products in the daily diet ensures efficient nutrition of cells and has a comprehensive therapeutic and health-promoting effect on the human body in the long run. Finally, the development of functional dairy-based products with pine nut ingredients is worthwhile both technologically and economically.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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