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The Phenomenon Of Pectin And Its Use In The Dairy Industry.

AN Fedosova[1]*, MV Kaledina[2], NP Shevchenko[2], LV Voloshchenko[3], and IA Baydina[4].

[1]Candidate of Biological Sciences (Ph.D.),Technological Faculty, Belgorod State Agrarian University named after V.Ya. Gorin, Russia.
[2]Candidate of Engineering Sciences (Ph.D.), Technological Faculty, Belgorod State Agrarian University named after V.Ya. Gorin, Russia.
[3]Candidate of Agricultural Sciences (Ph.D.), Technological Faculty, Belgorod State Agrarian University named after V.Ya. Gorin, Russia.
[4]Technological Faculty, Belgorod State Agrarian University named after V.Ya. Gorin, Russia.

ABSTRACT

The article summarizes the research results having been obtained for several years. The substantiation of theoretical and practical aspects of the process of pectin exposure to dairy raw materials in a wide range of its concentration is given. The process of milk fractionation with pectin in order to use fractions for the development of functional productswas of particular interest.The division of milk into fractions occurs at a pectin content of 0.6-0.8% (recount to dry) to the raw materials mass. Under the influence of pectin, two layers are formed: (above) diluted whey-polysaccharide solution and (below) concentrated protein or protein-lipid solution, in the case of whole milk use), with a clear line of fractions separation. There are a number of hypotheses that explain the ability of pectin at its content of 0.6-0.8% (recount to dry) to fractionate milk, but the hypotheses do not affect or explain other pectin behavior at its content beyond the above limits.The article proposes to discuss the ion-osmotic theory of milk fractionwith pectin, which, understandably, explains the mechanism of the milk fractionation at the content of pectin 0.6-0.8% and the processesoccurring outside the area of concentration.Studies are conducted to argue the theoretical aspects of the impact of pectin on dairy raw materials.In the direction of practical use of the theory of milk fractionation with pectin method of normalization of fat content in milk is proposed, designed for fractionation, the possibility of enrichment of fractions with functional ingredients due to the selective redistribution of milk components under the influence of the proposed mechanism of the process at a dry pectin content of 0.65% to the mass of milk is reflected.For the protein-lipid fraction, a study on theminerals content (Ca, P, Mg, Zn, Fe) and vitamins (A, E, C) was performed, their share in 100 g of raw materials relative to the daily consumption of adults was estimated.

Keywords: pectin, fractionation, flocculation, ion-osmotic processes, whey- polysaccharide fraction (WPF), protein-lipid fraction (PLF)

***Corresponding author**

INTRODUCTION

The connection between the structure of the molecule and the unique propeties of pectin

Pectin substances, including pectin, play the role of a binding component of cells in the structure of plant tissues and perform a number of other life-supporting functions of plants. Modern ideas about pectin substances are given in the work of Yu.S. Ovodov [1]. Pectin soluble parts consisting of partially or fully methoxylamine remnants of ά-D-galactopyranoside acids bound in positions 1-4are called pectin. Pectin is widely used in various fields of human activity [2].

The uniqueness of the pectin properties is determined with the combination of molecule properties of carboxylic acids, polyatomic alcohols, esters and conformational form of the Piran cycle of galacturonic acid.

The Piran cycle of galacturonic acid is capable to be in three spatial conformational forms: “armchair”, “bath” and “curved bath”, but the conformation of the “armchair” cycle is more energy stable. This fact for pectin was experimentally proved[3].

Conformational features of the structural parts of the molecule form the availability of active centers, providing high adsorption properties of pectins, enhanced by reactive capable functional groups.

Carboxyl groups provide pectin ability to form salts basicallywith any metals, but the biological value in human life has the ability of pectin to bind toxic and radioactive metals. Reliability of binding of polyvalent metals is provided by chelated structure of salts stabilized by donor-acceptor bonds of galacturonic acidalcohol groups. Pectin is widely used for detoxification of the human body when working with toxic metals and in areas of high radiation [4, 5].

Pectin shows experimentally proved antimicrobial activity to pathogens of acute intestinal infections (Salmonella, Shigella, cholera vibrioand other pathogens). There are various explanations of the mechanisms of pectin antimicrobial action on these microorganisms, including the role of galactose in this process, capable of complementarity (spatial compliance) of the ligand-receptor type to bind these microbial cells, not allowing them attaching to the intestinal wall [6, 7].

The literature data and our own studies confirm the absence of pectin inhibitory effect on lactic acid bacteria and the presence of prebiotic properties to the probiotic microflora [8, 9].

Pectin is a polar biopolymer of polysaccharide nature with typical properties of hydrophilic colloids. Under certain conditions, pectin has the ability to self-association of molecules, leading to the formation of gel. The use of pectin as a thickener, stabilizer and gelling agent of various food and non-food products is a well-known fact and does not require literary evidence.

In the dairy industry, from our point of view, the greatest interest is the reverse ability of pectin to be a destabilizer of milk. Destabilization leads to the division of milk into two homogeneous fractions with no signs of coagulation: whey-polysaccharide (WPF) and protein, and when using fat-containing raw milk – protein-lipid fraction (PLF). Both fractions have high biological value and are an excellent basis for the development of functional products. The condition for a process is a narrow zone of concentration of pectin in the range of 0.6-0.8% by weight of raw materials calculated on the dry pectin [10, 11].

The aim of the researchis to study and substantiate the theoretical and practical aspects of the process of pectin exposure to dairy raw materials in a wide range of its concentration for the development of functional products that meet the requirements of the state policy in the field of healthy nutrition and national priorities.

Research problems:

- generalization of research results having been obtained for several years;
- additional research aimed at the argumentation of theoretical aspects of the impact of pectin on dairy raw materials;

- practical implementation of the proposed theory of milk fractionation with pectin in the development of functional products of normalized composition.

Objects and methods of research

Objects of research: cow's raw milk that meets the requirements of regulatory documents of the Russian Federation; pectin (esterification degree 75%), produced in Belgorod region (TC 9199-012-01014470-04 "Apple pectin, biologically active food additive"), whey- polysaccharide fraction (WPF); protein-lipid fraction (PLF).

Methods of research. At physical and chemical research and study of the properties of objects standard and common methods were used:

- determination of the mass fraction of moisture and solids with infrared thermogravimetric methods on the moisture analyzer Evlas-2M and drying apparatus APS-1 (Analit-Service), as well as arbitration method – dry the sample to constant weight in a drying cabinet at a temperature of 102-105°C;
- determination of raw and pasteurized milk composition at US-analyzer "Laktan 1-4" (Sibagro-pribor, Russia);
- determination of solids content in WPF on refractometer IRF-454 B2M (JSC "Komz");
- determination of the mass fraction of fat by the Gerber acidic method;
- determination of the protein mass fraction by Kjeldal;
- determination of calcium by complexometric method;
- determination of titratable acidity by titrimetric method;
- determination of lactose by iodometric method;
- determination of chloride ions by titration with silver nitrate;

The content of protein, vitamins, macro - and microelements was determined in the testing laboratory of Belgorod State Agrarian University, registered in the State Register of Russia, accredited in the accreditation system of analytical laboratories.

In the studies, a 5% aqueous solution of apple pectin, technological in physical properties, was used. Pectin powder was dissolved in hot water with a temperature of 70±2°C, the solution was filtered through a strainer to ensure homogeneity and cooled to a temperature of 22±2°C. Thesolution had organoleptic characteristics typical for apples. The active acidity (pH) of the obtained solution corresponded to 2.5 ±0.1 units.

Milk raw material was pasteurized at a temperature of 85-87°C without aging for the purpose of maximum flocculation of the protein fraction, was cooled to a temperature of 22±2°C and mixed with a 5% aqueous solution of pectin with a temperature of 22±2°C, which ensures equal distribution of the components in the mixture.

In the study of the effect of pectin concentration and temperature of the mixture on the state of the polydisperse milk system, a laboratory method was used. A wide range of pectin concentration and two temperature variants of mixture fractionation were studied: 6-8 and 39-40°C. From each sample, 30 cm^3 of the mixture was measured in two rows of test tubes (three times repeatability), the initial height of the mixture layer was measured and one row of test tubes was placed in the refrigerator (temperature 6-8°C), the other row – in a thermostat (temperature 39-40°C). Samples were visually evaluated at 30-minute intervals. In the case of whey separation, the height of its layer was measured; the efficiency of the process was estimated in %to the initial height of the mixture.

To study the mechanism of pectin effect on milk, a comprehensive study using analytical and instrumental methods was conducted. Fractionationtemperaturewas 6-8°C, duration– 2 hours.

The experimental data were processed by standard methods of dispersion, regression and correlation analysis. Processing of experimental data, plotting were carried out on the PC using the package of applications Statistica 10.0 and Microsoft Office Excel 2010.

RESULTS

The state of a polydisperse milk system at changes in the pectin concentration and the temperature of the mixture

The pectin content from 0.2 to 1.0 with a step of 0.2 g per 100 g on polydisperse state of whole pasteurized milk was studied. Milk with the following parameters was used: fat content 3.6 ± 0.2%., protein 2.86 ± 0.05%, DSMR (dry skimmed milk residue) 8.25 ± 0.05%, titratable acidity 16-17°T, pH 6.67-6.72.

It should be noted: when the pectin content of 0.2 g per 100 g of milk, no visible changes occur, the destabilization of the polydisperse milk system begins with the pectin content of 0.4%. The process was considered complete when the indicators at the previous level were stabilized. The ageing time of the mixtures is two hours.

The effect of pectin concentration and temperature of the mixture on the efficiency of fractionation of whole pasteurized milk is shown in figure 1.

The dynamics of the process in cold and warm milk is almost the same, but the fractionation efficiency is slightly higher at a mixture temperature of 39-40°C, probably due to a decrease in the viscosity of the environment. From data analysis (Fig. 1) the conclusion is obvious – in industrial conditions, fractionation is advisable to carry out at a temperature no higher than 8°C and the pectin content within 6-8 g per 1 kg of milk.

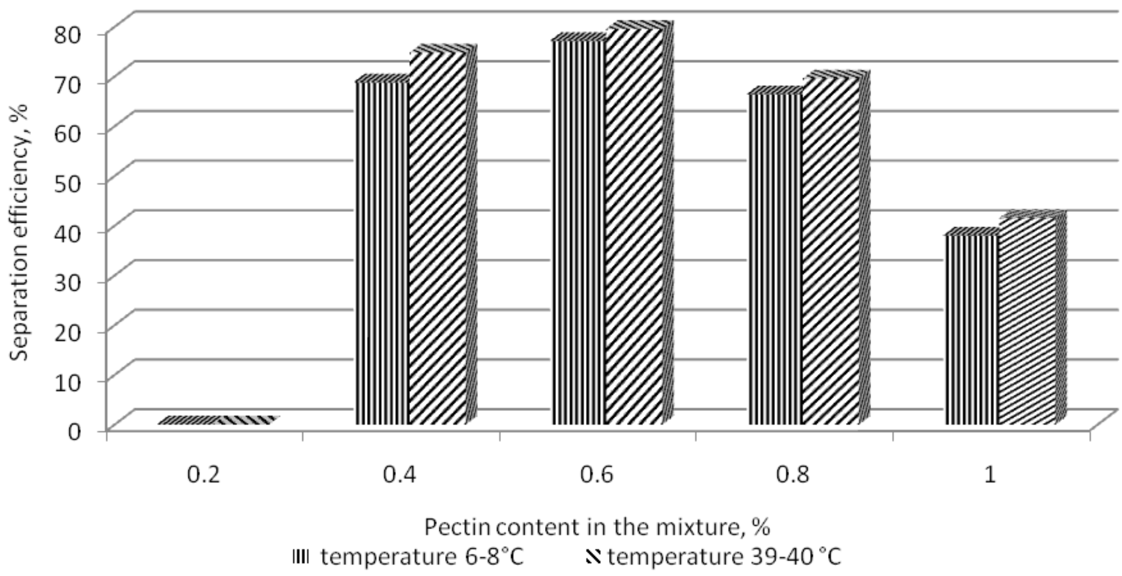

Figure 1: Effect of pectin concentration and temperature of the mixture on the efficiency of fractionation of whole pasteurized milk.

The fact of stabilization of the process at a certain constant for each pectin concentration level is repeatedly confirmed. Figure 2 shows the trend of milk fractionation with 4 hoursduration. The stability of the fractions separation is maintained after 24 hours.

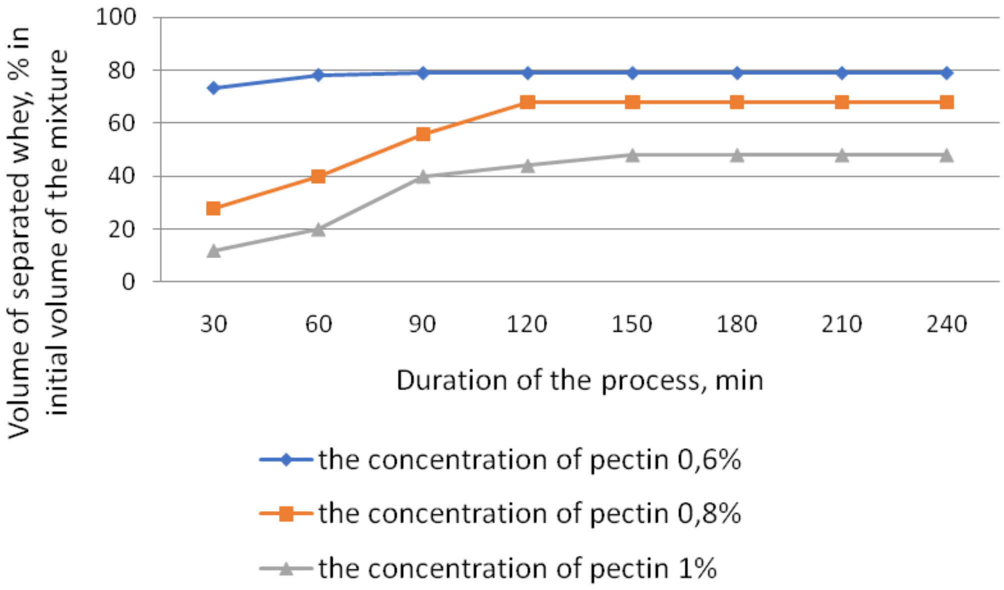

Figure 2: Dynamics of skim milk fractionation with pectin

The obtained results (Fig.2) confirm the fact of the maximum division of milk into fractions at pectin concentration (0.6-0.8%) to the mass of milk and, having reached a certain level, the process is stabilized. Inaddition, certainconditionsmustbecomplied:

- pectin is implemented in the form of an aqueous solution for the purpose of equal distribution in the mass of milk at stirring;
- fractionation occurs only in stationary mode. In the case of continuous mixing, it is not observed. The process is reversible: after stopping the mixing, the fractionation begins again.

Theoretical aspects of the effects of pectin on milkraw material

For the theoretical explanation of the observed effect of milk raw material fractionation with pectin in a narrow concentration zone, there are a number of hypotheses presented in the review article of A.G. Khramtsov, V.V. Molochnikov, R.I. Ramanauskas. According to the authors, the mechanism of fractionation is not fully studied and the different behavior of pectin in milk is not explained, at a concentration beyond this zone [11].

In this article, we propose to discuss the ion-osmotic theory of milk fractionation with pectin, which, understandably, explains the mechanism of milk fractionation at pectin content of 0.6-0.8% (recount to dry). According to the proposed theory, the mechanism of pectin action on milk occurs in the form of successive physical and chemical processes:

1. Electrostatic repulsion of equally (negatively) charged particles: pectin, casein micelles, and fat globules in fatty raw materials.

2. The manifestation of the particle mass gradient.

Lighter in molecular weight pectin is exfoliated into the upper part of the mixture; heavier particles (casein micelles and fat globules) remain in the lower part of the mixture. This conclusion follows from comparison of the measured molecular weight of apple pectin in the range (2.5-3.5) × 10^4 Da (daltons) and only in some cases can reach 2×10^5 Da, the mass of the hydrated casein micelles is equal to 1.3×10^9 Da, the fat globules are much greater than the mass of the casein micelles.

3. Electrostatic attraction of milk plasma ions to acidic groups of pectin molecules.

Apple pectin solution exhibits acidic properties; pH is 2.5 ± 2 units.The pectin molecules should attract cations of plasma and milk, primarily potassium ions, as predominant by weight over the rest of the milk cations, with the formation of water-soluble salt with the ionic type of chemical bond. Calcium, in our opinion, does not participate in the osmotic process, as the concentration of the ionized form of calcium is low (no more than 8-10% of the total calcium content of milk). Calcium in milk is in a bound form in the composition of the casein micelles and mineral colloid (Sol) – $[CaHPO_4]_x$ and therefore the main part of the milk calcium remains in the concentrated fraction.

4. The concentration of the active ions of the milk plasma linked by ionic bonds in the pectin layer.

Ion bond, unlike covalent bonds, does not have directivity and saturation. It occurs due to electrostatic attraction of differently charged ions to each other regardless of the direction. Herewith attracted oppositely charged ions retain the ability to electrostatically attract other oppositely charged ions. Such mutually attractive ions in milk are potassium cations (K^+) and to a lesser extent sodium (Na^+) because of their low content in milk, anions – chlorine ions (Cl^-) as prevailing by weight in milk and having chemical affinity to alkali metal ions.

5. Increasing the osmotic pressure in the pectin layer, by moving potassium, sodium, chlorine ions to the pectin layer.

Osmotic pressure depends on the number of low molecular molecules and ions under other standard conditions. High molecular compounds (HMC) affect slightly on the value of osmotic pressure. In milk, the osmotic pressure (0.65-0.66 MPa) is createdwith lactose and salt ions. According to literature sources and our calculations, the osmotic pressure created with lactose at a milk temperature of 20°C is equal to 0.33 MPa [12].

**6. Equalization and stabilization of osmotic pressure between the fractions of milk having been divided under the influ
ence of pectin.**

The gradient of osmotic pressure, which arose between the layers in the system“milk-pectin”, causes the movement of solvent molecules (water) in the pectin layer to reduce the resulting shift of osmotic pressure to osmotic equality in the general system. Processes of this type are quite fast and, having reached equilibrium, remain stable for a long time.

For effective destabilization of the polydisperse milk system with pectin of used brand 0.6-0.7 g (recount to dry) in 100 g of milk is required. Fractionation of pasteurized skim milk ends in 30-60 minutes, of pasteurized whole and normalized milk (with or without homogenization) the process ends in 2 hours. The separation effect is stable, does not change for a long time.

At the content below 0.6% to the mass of milk, the ability of pectin to concentrate active ions around itself decreases, at the same time the osmotic pressure in the WPF drops and the efficiency of the mixture separation declines.

With a content of more than 0.8 g per 100 g of milk the ability to self-association of pectin moleculesincreases. In the protein-lipid fraction drops of WPFappear, there is an effect that looks like peptization of Sol. At a pectin content of 2% or more, a homogeneous mixture of increased viscosity is formed.

For the argument of the proposed mechanism of pectin action on milk indicators that play, in our opinion, the main role in the regulation of osmotic pressure – pectin, lactose and monovalent ions were investigated. The concentration of monovalent ions was judged by the content of chloride ions.

The argument of the theoretical aspects of the effects of pectin on milk

In the experiment pasteurized whole milk with initial parameters was used: fat –3.8±0.2%, protein – 2.8±0.05%; DSMR– 8.2±0.05%; dry substance (fat + DSMR) – 12.0±0.2 %. Titratable milk acidityis 17±1°T.

For calculations on the distribution of milk components between the obtained fractions, information on the practical yield of fractions is needed (table 1).

Table 1: Practical yield of fractions (n=3)

Initial milk		Whey- polysaccharide fraction		Protein-lipid fraction	
mass,%	dry substance, %	yield from milk mass,%	dry substance, %	yield from milk mass,%	dry substance, %
100	12.0±0.2	78.2±0.5	6.3-6.4	21.8±0.5	30.8±0.5

It should be especially noted that the content of dry substances in WPF practically does not depend on the fat content in milk, varies within 6.3-6.4% and does not change with the increase in the process duration. The dry substances content in WPF is proposed to use to control the end of the fractionation process in industrial conditions; it is easy to measure it with a refractometer.

To assess the distribution of lactose and chloride ions between WPF and PLF, the content of natural moisture in the raw material was determined and, taking into account their solubility in water, the concentration was recalculated for the water fraction.

The content of lactose and chloride ions in whey-polysaccharide and protein-lipid fractions is given in table 2.

Table 2: Content of lactose and chloride ions in whey-polysaccharide and protein-lipid fractions (n=3)

Fraction	Water content, gper 100 gof raw material	Lactose content, g		Chloride ions content, mg	
		per 100 g of natural moisture fraction	per 100 g of aquatic environment faction	per 100 g of natural moisture fraction	per 100 g of aquatic environment faction
WPF	93.7±0.5	4.43 ± 0.05	4.72 ± 0.05	114.0 ± 0.2	121.7 ± 0.2
PLF	69.2±0.5	3.27 ± 0.05	4.73 ± 0.05	4.0 ± 0.2	5.7 ± 0.2

With the visible difference in the lactose content in WPF and PLF (table 2), the calculation of its concentration in the aquatic environment faction showed that pectin does not affect the distribution of lactose having a neutral reaction of environment in aquatic solution. The concentration of lactose in the aquatic environment faction is the same.

As previous studies showed whey proteins and the molecular form of casein fractions are not involved in the fractionation process. A slight gradient in the mass of pectin molecules and called proteins can be considered as the reason.

Chloride ions are distributed differently. As one would expect (table 2), chloride ions are almost completely transferred to the WPF layer, therefore, chemically there should be K^+ and Na^+ milk cations. The results having been obtained confirm reliably the role of pectin and monovalent milk plasma ions in the ion-osmotic mechanism of fractionation of raw milkwith pectin. Monovalent milk plasma ions ensure the movement of water into the pectin layer to equalize the osmotic pressure in the system to form a well-formed diluted layer of WPF.

One of the fractionation hypotheses [11] indicates a positive role of calcium in the process of milk fractionation with pectin. Our study refutes this proposition. The study was conducted on skim milk containing 0.65% pectin (recount to dry). In the test samples, the calcium content was increased by implementing a 4%

solution of calcium chloride into the mixture. The mixture was kept at a temperature of 20±2°C for 2 hours. The effect of the ionized calcium content on the efficiency of milk fractionation with pectin is given in table 3.

Table 3: The influence of the ionized calcium content in milk on efficiency of the milk fractionation with pectin (n=3)

Studied mixture	Calcium content, mg/%	Systemcharacteristic	Dry substance in WPF,%
Milk+pectin (control)	136 ± 5	The protein concentrate is well separated. Wheyistransparent	6.2 ± 0.1
Milk+pectin+15% $CaCl_2$	156 ± 5	The protein concentrate is well separated. Wheyiscloudy	6.2 ± 0.1
Milk+pectin+25% $CaCl_2$	170 ± 5	The protein does not concentrate; it takes half the volume of the mixture. Wheyiscloudy	6.3 ± 0.1
Milk+pectin+50% $CaCl_2$	204 ± 5	The protein does not concentrate; it takes more than half of the volume of the mixture.Whey is cloudy	6.3 ± 0.1

The additional implementation of calcium chloride into milk reduces the effect of fractionation (table 3), and it is quite logical. The natural content of the ionized form of calcium in milk is low. Increasing the content of ionized calcium reduces the charge of pectin and casein micelles. As a result, the forces of particles repulsion decrease; the degree of dispersion of casein micelles increases and simultaneously calcium is bound by carboxyl groups of pectin with the formation of salt of chelated structure incapable of dissociation. Lowering the ionization of pectin reduces the polarity of pectin and its ability to attract monovalent milk ions, which reduces the ability of pectin to fractionate milk.

Practical implementation of the proposed theory of milk fractionation with pectin in the development of functional products of normalized composition

For the industrial application of the process of milk fractionation with pectin, it is necessary to normalize the fat in the product; this requires the rules of normalizing the fat in the original milk. The basis for the normalization of milk fat for fractionation is the principle used in the production of canned food.

We offer to use the degree of concentration (n) of fat in dry matter PLF on the contents of fat in milk source dry substance:n = F_{fr}^{PLF} / F_{fr}^{milk}. Itisexperimentallyestablished: thepectincontentof0.65% tomilkweightofn = 2 ± 0.1. **Example of calculation**: for product development with humidity not more than 80% (78% accepted) milk fat for fractionation should be equal to 1.5%, with a dry substance content in milk of at least 11%. When milk is fractionated with pectin, each of the obtained fractions (WPF and PLF) is selectively enriched with certain functional ingredients [13, 14].

When predicting the composition of products based on PLF and WPF the relative constancy of protein content in fractions should be taken into account. The total protein content in WPF is 0.61-0.62% with predominance of whey proteins in it. In the protein-lipid fraction – 12-13%, the fraction consists mainly of casein, the proportion of whey proteins does not exceed 0.3%.

When assessing the degree of raw materials enrichment and further product with functional ingredients the redistribution of milk minerals and vitamins between PLF and WPF must be taken into account (table4).

Technologically and physiologically, calcium and phosphorus (more precisely phosphates) are of particular importance from macronutrients. It was found that the content of calcium and phosphorus in PLF increases by more than 1.7 times relative to milk. Both elements structurally enter the casein micelles and the colloidal form of calcium phosphate. In WPF, calcium is in the form of soluble forms of salts (phosphates and citrates) and in the bound pectin form.

Microelements are usually bound with proteins, but they can be boundwith pectin, in both cases, complex salts of chelated structure are formed. Zinc and iron are also concentrated in the protein shell of fat globules [12]. According to the results (table 4), in PLF relative to milk zinc content increases by 44.2%, iron – by 31.6%.

Using the mechanism of milk fractionation with pectin proposed above, it is possible to predict the content of vitamins in fractions. Vitamins soluble in fat, as non-polar compounds, should and remain in PLF. Water-soluble vitamins do not react to the presence of pectin. The content of water-soluble vitamins, at recounting to an aqueous environment, in WPF and PLF is almost the same, but the absolute content in the total mass of fractions of water-soluble vitamins in PLF is less than in WPF. Evaluation of the raw materials enrichment with mineral elements and vitamins is given in table 4.

Table 4: Evaluation of raw material enrichment with mineral elements and vitamins (n=3, V<5%)

Indicator	Daily consumption, mg	Content in 100g			
		milk: fat content 1.5%, dry substance 11± 0.5%		PLF: fat content 6.1± 0.5%, dry substance22 ± 0.5%	
		component mass, mg	% of daily consumption	component mass, mg	% of daily consumption
Mineral elements					
Ca	1000	136.00	13.6	250.0	**25.00**
P	800	93.30	11.7	163.0	**20.38**
Mg	400	14.20	3.5	19.4	4.85
Zn	12	1.39	11.5	2.0	**16.67**
Fe	13	0.57	4.4	0.8	6.15
Vitamins					
A	1.0	0.033	3.3	0.198	**19.8**
E	1.5	0.075	5.0	0.262	**17.7**
C	90	17.690	19.7	1.490	1.65

Thus, at the stage of obtaining raw materials, it is possible to provide the future product with such functional ingredients as Ca, P, Zn, vitamins A and E.

The authors of the work on the basis of fraction in natural form developed more than 10 technologies and functional product formulations [14].

CONCLUSION

Fractionation of milk raw material with pectin is a promising process for use in the technology of functional products. The process is simple, environmentally friendly,there are no denaturing changes in the system. The obtained fractions are characterized by high food and biological value. The presented argument of the ion-osmotic mechanism working in milk under the influence of pectin will allow controlling this process.

This approach provides the possibility of rationing the composition of fractions for the production of functional products, to predict the content of fat and protein, vitamins and minerals in the final product.

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