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The Structure Development of Yogurt with Vegetable Ingredients

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Abstract*: The development of functional foods with specified beneficial properties is one of the main directions in the problem of maintaining and preserving the health of the population. To solve this problem in the diet include products produced using dietary supplements made from natural ingredients. In addition, the problem of creating food with a certain texture made it possible to isolate thickeners and gelling agents food additives that regulate the consistency and form the structure of the product. The department of technology of meat, dairy products and chemistry of the Bashkir State Agrarian University developed two functional combined products, dairy and vegetable yogurt: 1 using dried horse milk and 2 using dried horse milk and vegetable component, pumpkin seed flour, identified their physicochemical properties and the proposed method for studying structure formation in fermented milk products. The study on the example of yoghurt 1 proved that when lactic acid fermentation proceeds, a decrease in the carbohydrate content of the product leads to an increase in its acidity and this causes a change in the consistency of the product. As a result, a ferment with lactic acid bacteria, producing exopolycarbohydrates, dried mare's milk and a vegetable component from pumpkin flour containing pectins, turned out to be good thickeners and gelling agents. All of the above components of yogurt are natural raw materials, so they can be used as safe food additives in the production technology of fermented milk products. In addition, the introduction of dried mare's milk contributes to the correction of the protein composition of the milk mixture, and the use of pumpkin seed flour in the production of yogurt enhances the beneficial properties of yogurt. This research is very relevant from the point of view of developing useful functional products.

***Index Terms*: functional foods, yoghurt, food additives, dried mare's milk, pumpkin seed flour, lactic fermentation, milk coagulation, structure formation**

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I. INTRODUCTION

Nutrition is one of the main factors affecting the normal growth and development of the body, performance and human health. In the early 80s, the concept of a healthy diet was formulated, and in this connection, so-called functional foods became very popular. The main task of functional products is not to heal and heal, but to prevent diseases and aging of the body. Functional products are diverse, so they are classified according to their effects on the body:

1) fortifying action: due to the essential nutrients that make up the product, provide optimal livelihoods;

2)preventive actions: provide prevention of exacerbations of chronic diseases and the emergence of new due to the correction of negative effects: dietary, antimutagenic, ballast, immunostimulating;

3)adaptogenic action: under conditions of increased loads, they ensure optimal functioning of the body, have an energetic, calming, tonic effect;

4)special purposes: increase resistance to extreme effects, for example, in sports, are used in the complex treatment of individual pathologies as part of a therapeutic diet [1-4].

From the point of view of the above classification of functional products, dairy products have all the listed types of physiological effects on the human body. As fermented milk products contain vitamins (especially B group), calcium and high-grade protein, characterized by high biological value, they have a therapeutic and prophylactic effect, as they stimulate appetite, increase the bioavailability of micronutrients, improve the secretory and motor function of the gastrointestinal tract, favorably affect intestinal microbiocenosis [5, 6].Fermented milk products have a tonic, prophylactic, adaptogenic effect and are used in the complex treatment of certain pathologies.

Fermented milk products, yoghurts, are well proven in baby food, for the manufacture of which they use mixtures of starter microorganisms (thermophilic lactic acid streptococci and Bulgarian lactic acid bacillus), which have a high content of dry non-fat milk substances [7] and are characterized by pronounced functional properties:

1) due to the partial breakdown of milk protein to peptides and free amino acids, its allergenic properties are reduced, its digestion and assimilation is facilitated,

which makes it possible for children with food allergies and hypotrophy to use this product,2) when lactic acid fermentation proceeds as a result of the lactic acid bacteria vital activity, lactose content decreases, free fatty acids accumulate during partial hydrolysis of fats, biologically active substances are synthesized, therefore children with partial lactase deficiency can consume yogurts while reducing pancreatic lipase and hypotrophy;

3) yogurt consumption is a good prevention of rickets, and later osteoporosis, since calcium, which is part of milk, is involved in the formation of bone tissue;

4) lactic acid resulting from lactic fermentation has good bactericidal properties. It prevents the growth of pathogenic microflora and promotes the growth of normal intestinal microflora, in addition, in the presence of lactic acid increases the bioavailability of calcium, phosphorus and iron, therefore yogurts are recommended to be used in the diet of children suffering rickets, anemia [5-9].

Creating an environmentally friendly balanced diet that ensures the normal functioning of all organs and tissues of the body is very important today. To solve this problem in the diet include products produced using dietary supplements (BAA). Supplements - supplements made from natural biologically active components, they are used for the introduction into the diet together with basic foods to enrich the body with the necessary micronutrients [11].In addition, the problem of creating food with a certain texture allowed us to distinguish a group of substances – food additives that regulate the consistency and form the structure of the product – thickeners and gelling agents [12-16].

The purpose of this work is the development of two functional combined products, dairy and vegetable yogurts: **1** – using dried mare's milk and **2** – using dried mare's milk and a vegetable component, studying structure formation and determining their physicochemical properties. For this it was necessary to solve the following tasks: to get yoghurt **1** and **2**, to determine the content of lactose, the acidity of the product and the conditional viscosity.

II. MATERIALS AND METHODS

The objects of the study were prototypes of yogurt **1** and **2**. The raw materials used for making yogurt, in line with current standards. Dry mare's milk was produced in the kumys shop of the sanatorium "Yumatovo" by spray drying.

It's no secret that the quality of yogurt is influenced by the chemical composition of milk, depending on many factors [17].Normalization of milk can reduce the influence of these factors on the composition of milk. In the production process, indicators such as acidity and sugar or sweetener content in yogurt can be adjusted, and the viscosity and consistency of the product is determined by the protein content of the raw materials, so milk enrichment is important (increase in SOMO). According to existing standards, the content of SOMO in yogurt without fillers should be at least 9.5% [18]. For the enrichment of natural milk in the industry is widely used whole or skimmed milk powder [19]. The use of dry mare's milk allows you to improve the consumer properties of yogurt, as milk mares has a high biological value. In [20], to characterize the biological value of yogurt proteins from mare's milk, an amino acid rate was determined, indicating the content of amino acids in this product compared to the content of the same amino acids in an ideal protein. It has been established that yogurt from Mare's milk contains all the essential amino acids, among which methionine and phenylalanine were limiting. Dry mare's milk is no less valuable than whole milk. In work [21], the most optimal amount of mare's milk powder for the production of yogurt was determined; it makes up 2% of the mass of the normalized mixture.

Pumpkin seed flour, rich in both essential and non-essential amino acids, is famous for its high content of natural, easily digestible zinc, a complex of vitamins of group B, vitamin C, and dietary fiber. The use of flour brings to normalization of metabolism, stimulate immunity, improve the functioning of the main organs and systems of the human body, increase mental and physical performance [22, 23].

As a starter, a series of YoFlex® cultures was used, consisting of the single strain lactic acid bacteria Lactobacillusdelbrueckii of the subspecies bulgaricus and Streptococcusthermophilus. This starter has a number of advantages, without the addition of appropriate food additives, you can get dairy products with a very mild flavor and with a given texture [12, 24, 25].

Figure 1 shows the production scheme of fermented milk products **1** and **2**.

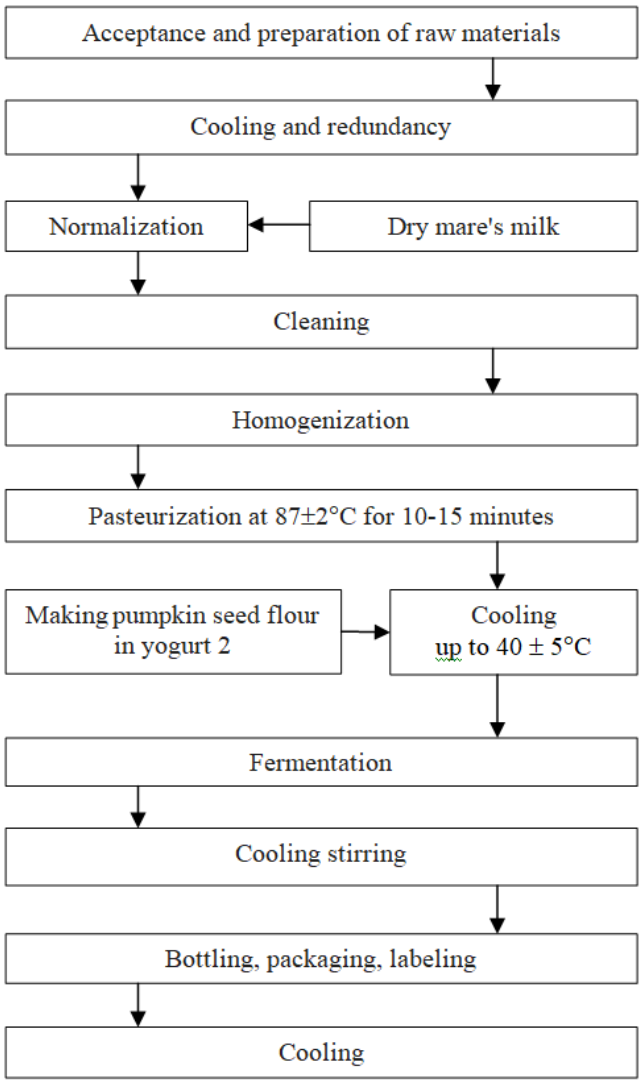

Figure 1 – Flowchart of production of yogurt

In cooked yoghurts, the carbohydrate content, titratable acidity and conditional viscosity of products,

the content of vitamin C and calcium were determined. The carbohydrate content was determined according to GOST R 54667 - 2011, titrated acidity according to GOST 3624-92, determination of conditional viscosity using a viscosity meter B3 - 246, determination of vitamin C according to GOST 30627.2-98, determination of calcium ions according to GOST ISO 12081-2013 [26-30]. The results obtained were statistically processed by known methods of mathematical analysis.

III. RESULTS AND DISCUSSION

The basis of the manufacture of a number of dairy products are the processes of the deep breakdown of milk sugar under the action of microorganisms. To determine the lactose content of the product, it was necessary to choose a suitable analysis method (table 1).

Table 1 - Determination of lactose in milk by various methods

Method	ώ±Δώ-confidence interval	ε- relative error of the average results
Iodometric method	(4,66±0,31)% 4,7%*	6,65%
Bertrand method	(4,61±0,27)% 4,7%*	5,85%
Refractometry method	(5,40±0,95)% 4,7%*	17,6%

*- manufacturer data

All of the considered methods give reproducible results, but the further experiment is based on a more accessible iodometric method.

Milk is a complex polydisperse system. The dispersed phases of milk are presented in the form of colloidal particles of protein and fat. The remaining components of milk, such as lactose, dissolved salts, vitamins, are in the ion - molecular state. During the synthesis of milk, a single equilibrium system of milk is formed. Any external changes, such as temperature, acidity of the medium, etc., can lead to disruption of the dynamic equilibrium between the components of milk.

It is known [31, 32] that the surface of milk micelles is covered with κ - caseins, which include fairly long hydrophilic macropeptide chains that form a “hairy” protective layer that does not allow micelles to stick together in an aqueous solution. Native casein micelles in an aqueous solution have a negative electrical charge arising from the dissociation of various functional groups of caseins, which ensures the stability of the micellar solution in milk.

The repulsion of milk micelles from each other is due to the presence on the surface of an elastic layer consisting of macropeptide k-casein residues, and the appearance of an electric charge of the same name during the dissociation of micellar calcium caseinate (CaCAS)

$$CaCAS \leftrightarrow Ca^{2+} + CAS^{2-} \quad (1)$$

When the fermentation reaction proceeds, the milk sugar is first broken down into glucose and galactose under the influence of the enzyme lactase, and then glucose is subjected to fermentation. Since for Streptococcusthermophilus and Lactobacillusdelbrueckiissp. Bulgaricus is characterized by homofermentative lactic fermentation [33 - 35], then the reaction product is lactic acid, which leads to an increase in the titrated acidity of the fermented milk product (Fig.2).

Using the basic principles of the theory of aggregative stability of dispersed systems — the DLVO theory — the authors of work [31] described the process of acid coagulation of milk.

During acid coagulation, the ionic equilibrium in the surface layer of the micelle shifts, the negative charge decreases, and the protective layer of the micelle is destroyed.The charge reduction on the surface of the micelle occurs as a result of the hydrogenation of the macropeptide residues k-casein (CMP)

$$CMP^{-} + H^{+} \leftrightarrow CMP \quad (2)$$

In addition, the decrease in the additional charge of micelles due to the equilibrium shift (1) to the left is caused by the hydrogenation of colloidal calcium phosphate (CCP), as a result of which calcium ions are formed

$$CCP + 2H^{+} \leftrightarrow CCP^{*} + Ca^{2+} \quad (3)$$

CCP * - hydrogenated form of colloidal calcium - phosphate complex

Each of these factors is characterized by energy, the value of which is determined by the kinetics of changes in the respective charges: the negative electric charge of q-casein q_{CMP}, depending on the concentration of dissociated macropeptide residues, and the additional negative electrical charge of q_{CAS} micelles proportional to the concentration of dissociated calcium caseinate molecules.

The authors of [31] proved the significant role of calcium ions in milk coagulation, formed as a result of the interaction of colloidal calcium phosphate with hydrogen ions.

Thus, as a result of the formation of lactic acid, the negatively charged groups of casein are neutralized and the colloidal calcium phosphate is removed from the casein micelles. At the same time, the dzeta potential of casein micelles decreases, leading to the complete destruction of the intestinal structure of casein, a decrease in its hydration and the aggregation of hydrophobic particles due to the distemper particles Van der Waals forces.

Violation of the colloidal stability of micelles leads to their sticking together and the formation of a grid of milk gel into which the fat globules are captured. The result is a clot that serves as the basis for the production of various dairy products.

In [35], the thermodynamic method proved the formation of an ordered structure in fermented milk products. Musina O.N. and Lisin P.A. studied the water activity, the yield stress and the thermodynamic parameters of yogurt - free energy (75.46 J / mol), enthalpy of destruction of the structure of yogurt (13.38 J / mol), entropy (– 0.2008 J / mol K) of the fermented milk products.

The negative value of entropy indicates the occurrence of an ordered structure when receiving yogurt, and low energy values indicate the prevalence of hydrophobic interactions. According to Puradier's formula, the concentration of intermolecular bonds, associated with the gel stiffness, was calculated, with a limiting stress of 207 Pagel stiffness was 41.8 mol / m^3.

In addition, Streptococcusthermophilus and Lactobacillusdelbrueckiissp. Bulgaricus belong to the starter cultures producing exopolicarbohydrate capable of increasing the viscosity and moisture–holding capacity of acid clots, i.e. are natural thickeners – stabilizers.

In Figures 2, 3, the carbohydrate content in the product, the titratable acidity and the conditional viscosity of fermented milk products, are determined within 10 days. From the data obtained it can be seen that a decrease in the carbohydrate content in the product (Fig. 2) leads to an increase in its acidity (Fig. 2), and this is the cause of the change in the viscosity of the product (Fig. 3). The viscosity of the product varies ambiguously. By P.A Rebinder viscosity change for 6 days corresponds to the induction period, the stage of flocculation and metastable equilibrium, during which aggregates consisting of casein macromolecules related to coagulation - condensation structures arise.Thus, a change in both the carbohydrate content in yoghurt**1**, and the acidity of the product, and its consistency, testifies to the interrelation of the ongoing processes (Fig. 2-5).Both intermolecular Van – der – Waalsforces and interactions based on the formation of chemical bonds are manifested in them [37].

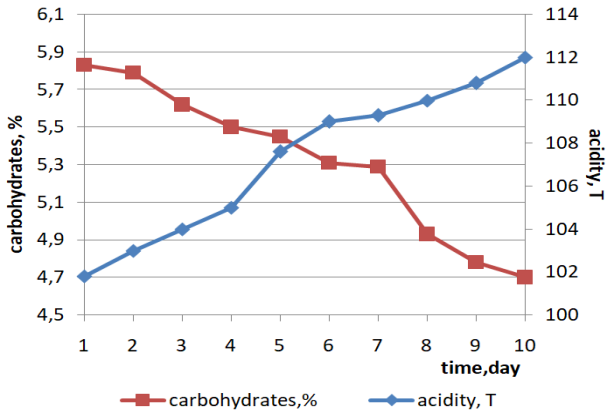

Figure 2 - Changes in carbohydrate content and acidity of yogurt 1 during 10 days

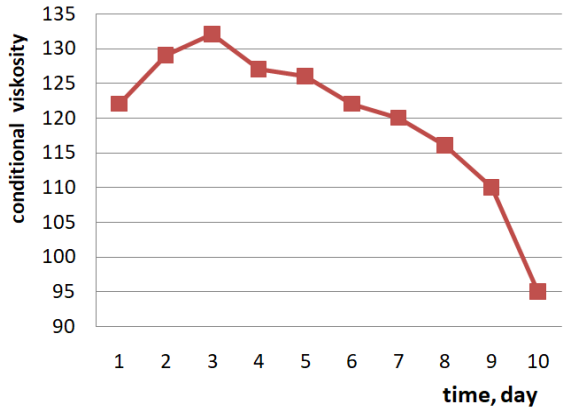

Figure 3 – Changes of yogurt 1 consistency within 10 days

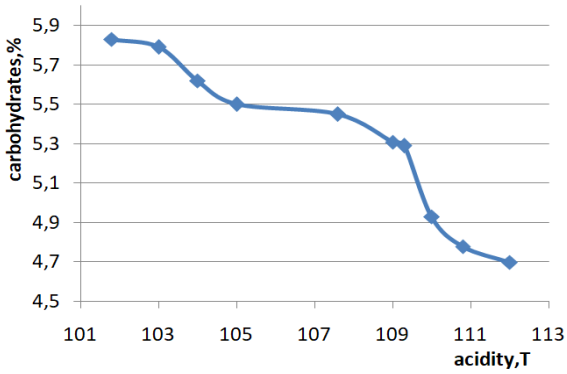

Figure 4 - The relationship between the amount of carbohydrates in the product 1 and the acidity

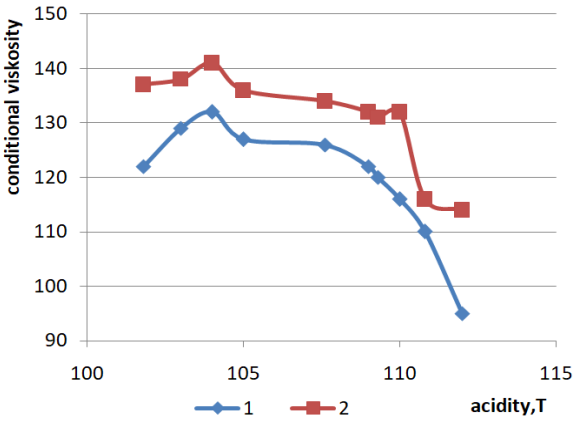

Figure 5 - Effect of the acidity on the viscosity of yogurt: 1 - without vegetable additives, 2 - with the addition of pumpkin flour

In this regard, when studying the structure formation in yogurt **2** with a herbal additive for 10 days, only the change in the acidity of the product and the conditional viscosity was monitored. But first they picked up the required amount of pumpkin flour. For this, we conducted a series of organoleptic studies aimed at determining the most optimal amount of herbal supplements in yogurt, as a result of which it was found that adding 1% pumpkin seed to the product gives the product a pleasant taste and color.

The herbal component leads to an increase in the calcium content (in yogurt **1**–91.01 ± 0.19%, in yogurt **2**– 95.06 ± 0.15%) and vitamin C (in yogurt **1**–0.90 ±0.03 mg / kg, in yogurt **2**–1.08 ± 0.09 mg / kg) in a fermented milk product and has a positive effect on its consistency, which varied according to a complex law for 10 days. First, the viscosity increased slightly, and then began to decrease, and the patterns of change in the conditional viscosity of yogurt with herbal supplements are repeated as in control yogurt, with only one difference, all the changes are not as obvious as in yogurt without additives (Fig. 5).

It is known that pumpkin flour contains pectins - polycarbohydrate belonging to the group of polygalacturonans, which are structural components of plants. Pectins are widely used in food as a good gelling agent, in addition, pectins are able to bind toxic elements and radionuclides, therefore pectins are a valuable additive in the production of food for medical and prophylactic purposes.

In an acidic environment, the dissociation of free carboxyl groups is suppressed, which prevents electrostatic repulsion of the chains. In the presence of Ca^{2+} ions, calcium bridges are formed, connecting pectin molecules [12, 23].

Apparently, therefore, the viscosity in the product with the plant component has become higher compared to the control sample and the structure formation proceeded first, due to the destruction of the micellar structure of casein, and secondly, due to the formation of spatial structure when pectin chains come together.

A similar phenomenon, an increase in the viscosity of the solution, was observed by A.V. Krupin in studying the effect of agar on shear stress in whey. In his work, he showed that an increase in serum agar concentration leads to an increase in gel strength, i.e. whey proteins are an additional structure-forming agent [37].

And the authors N.A. Bugayets and others showed that the joint presence of pectin and gelatin in the solution, which, like casein, is of protein nature, leads to an increase in the viscosity of the solution in the pH = 3.8 - 6.2 range. This is due to the fact that macromolecules of gelatin and pectin in an acidic environment have opposite charges, which contributes to the formation of complexes of gelatin and pectin [38].

The decrease in viscosity of solutions in both products with an increase in the acidity of the medium is associated with the destruction of the yogurt structure caused by the appearance of positive charges in the casein molecule due to the protonation of free amino group lysine, guanidine groups of arginine and imidazole groups of histidine, and a decrease in the production of exopolysaccharides by starter cultures (the most optimal pH value for this equals 6).

IV. CONCLUSION

So, as a result of the studies conducted on the example of dairy - vegetable yogurts, the interrelation of the processes of lactic acid fermentation with the formation of its structure has been experimentally proved. The data obtained as a demonstration material can be used in the educational process in the preparation of milk production technologists. As thickeners and gelling agents, ferment was used with lactic acid bacteria producing exopolycarbohydrate, dried mare's milk and vegetable component from pumpkin flour containing pectins, that is, natural raw materials, which solves the problem of using safe food additives in the production technology of dairy products.

Conflict of interest

The authors declare no conflict of interest.

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