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БИОТЕХНОЛОГИЧЕСКИЕ ОСНОВЫ ФУНКЦИОНАЛЬНОГО НАПИТКА НА ОСНОВЕ МОЛОЧНОЙ СЫВОРОТКИ

BIOTECHNOLOGICAL BASES OF A FUNCTIONAL DRINK BASED ON WHEY

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Аннотация

Обоснована актуальность ферментативного гидролиза молочной сыворотки как основы для получения функциональных напитков. Проведен сравнительный анализ ферментативной активности различных ферментов и разработана композиция функционального напитка.

Ключевые слова: гидролизованная молочная сыворотка, функциональные напитки, креатин моногидрат, глюкозо-фруктозный сироп.

Abstract

The relevance of obtaining whey after enzymatic hydrolysis for various types of functional drinks is substantiated. A comparative analysis of the enzymatic activity of different enzymes was carried out, and a composition of a functional drink was developed.

Key words: hydrolyzed whey, functional drinks, creatine monohydrate, glucose-fructose syrup.

Introduction

The problem of the full and rational use of whey is relevant, regardless of the volumes received, methods of organization and forms of ownership of production. Whey, despite its unique composition, is not fully processed in most dairy processing plants [1]. Currently, about 21% of whey is used in Russia for food purposes [2], and the rest is used to feed farm animals, irrigate fields, or drain into wastewater.

Milk whey, into which about 50% of milk solids pass [3], is considered one of the most promising sources of raw materials for obtaining food, clinical, therapeutic and dietary ingredients. Milk whey does not have a negative effect on the human body, has practically no contraindications for use. It is noted that it has an active stimulating effect on the secretory function of the digestive organs and can be used for medicinal purposes [4].

The chemical composition of milk whey is quite rich, diverse and includes more than 200 essential components. A number of studies have confirmed that it is a biologically valuable raw material, since it contains all the essential amino acids, which gives the products prepared using whey functional properties [5,6]. In the dry matter of whey, the main components are distributed as follows: lactose – 70%, nitrogenous substances – 14.5%, fat – 7.5% and mineral salts – 8%. The high biological value of whey is due to protein substances, as well as vitamins, hormones, organic acids, immune bodies and microelements [7].

During the production of cheese and cottage cheese, the main whey proteins, β-lactoglobulin and α- lactalbumin, pass into whey. The share of β-lactoglobulin accounts for 7-

12% of the total amount of milk proteins, and the share of α- lactalbumin - 2-5%. Whey proteins are rich in essential amino acids (lysine, tryptophan, methionine, threonine) and cystine, which makes them one of the most biologically valuable part of milk proteins [7]. Whey proteins are used by the body for structural metabolism, mainly for the regeneration of liver proteins, the formation of hemoglobin and blood plasma [8]. Also, whey proteins, β-lactoglobulin and α- lactalbumin, have important functional and biologically active properties, which are significantly increased upon hydrolysis by various enzymes [9]. Due to the high nutritional and biological value of whey, it is often used as a natural raw material for various drinks [10]. The prospects for the use of whey as the main ingredient for obtaining functional drinks are due to the following factors:

- composition and properties;
- relatively low cost and availability;
- a radical solution to the environmental problem through non-waste processing;
- seasonal coincidence of the maximum consumption of beverages by the population and the production of whey at most enterprises;
- the expediency of using natural liquid whey in dietary and clinical nutrition [11].

The technology for the production of drinks from whey can vary significantly depending on the type of its pre-treatment: the use of natural whey; removal of whey proteins (serum clarification); hydrolysis of nitrogenous or carbohydrate components; dry matter concentration; fermentation. Thus, whey drinks belong to one of the most significant product groups.

Knowing the features of the technology of each group of beverages makes it possible to evaluate the effectiveness of developing innovative products for a particular enterprise, taking into account its features and technical capabilities [12].

One of the modern trends in solving this problem is enzymatic hydrolysis, especially the incomplete hydrolysis of proteins. Research in this direction is an important link in solving the fundamental problem of providing the human body with a complete protein diet. Innovative products, which include milk protein hydrolysates, can significantly increase their nutritional and biological value, which is especially important when organizing sports nutrition [13].

It is known that hydrolysis is actively used to cleave proteins by proteolytic enzymes to peptides and amino acids. During hydrolysis, peptide bonds are broken, and after the addition of a water molecule, peptides and amino acids are released (Figure 1).

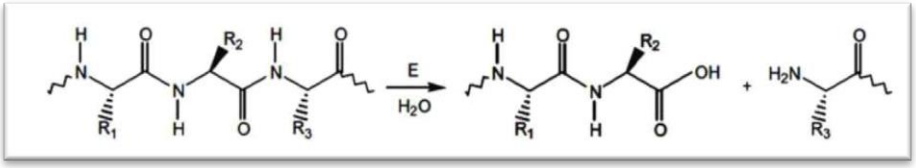


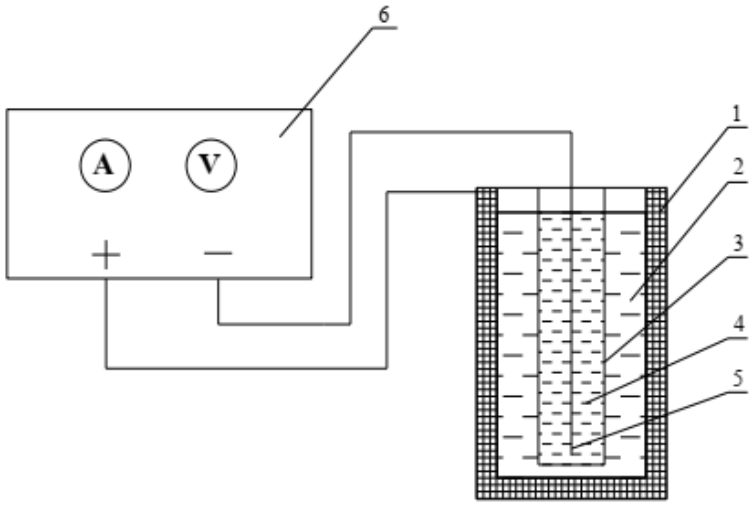
Figure 1 – Scheme of cleavage of the peptide bond: R1, R2, R3 - amino acid radicals; E - enzyme

The newly formed peptides act as new substrates for the enzyme [7]. Enzymatic hydrolysis proceeds under normal, in contrast to extreme, parameters commonly used in chemical and physical treatment [14]. It has been proven that enzymatic hydrolysis makes it possible to obtain hydrolysates with desired properties. The advantage of enzymatic hydrolysis of whey proteins is a high speed under relatively mild conditions – atmospheric pressure and a temperature not exceeding 50°C. As a result of hydrolysis, there is practically no destruction of amino acids and a decrease in the biological value of the final product. A feature of proteolytic enzymes is their specificity with respect to the type of peptide bond, which makes it possible to obtain products with varying degrees of protein hydrolysis, which are used in bakery, dairy, and other food industries [15].

The ultrafiltration concentrate (retentate) of cheese whey by the enzyme preparations

Promod 439L and Flavorpro 766 MDP is known, due to which peptides with high antioxidant properties were obtained that have a positive effect on metabolic processes in the human body [16]. In addition, protein hydrolysates have functional and technological properties: better solubility; stability during high-temperature processing; high resistance to precipitation under the influence of a number of factors (pH, the presence of metal ions) [7].

To evaluate the enzymatic hydrolysis of whey with enzymes of various proteolytic activity, studies were carried out on the effect of electrochemical activation on the efficiency of the process. Electrochemical activation is a combination of reactions in the space charge region, near the surface of the electrodes, and electrochemical effects on the medium containing ions and molecules of dissolved substances in non-equilibrium charge transfer through the electrode-electrolyte interface under conditions of minimal heat release. The main structural transformations of water molecules occur near the electrode surface, where the electrical voltage is several orders of magnitude higher [17]. Milk whey was subjected to electrochemical treatment in a laboratory electroactivation installation (Figure 2).



The designs of electroactivation installations (electrolyzers) are different, but the essence of the process is the same. The simplest electrolyzer consists of a container, two electrodes (1.5), a semi-permeable ion-exchange membrane (3) made in the form of a partition or a bag into which one of the electrodes (5) is immersed. The process of obtaining anode (acidic) and cathode (alkaline) water is as follows: when the rectifier (6) is connected to the AC network, the latter supplies a positive DC charge to the anode (1), and negative to the cathode (5). In the prepared solution, under the influence of direct current, an electrolytic process occurs, as a result of which an acidic medium is formed near the anode, anolyte (2) with pH 2.0 - 2.5), and near the cathode, an alkaline, catholyte (4) with pH 10, 0 - 12.0.

We have studied the effect of electrochemical activation on serum in the presence of a pancreatic enzyme with different proteolytic activity (enzyme 1 – 250 IU Ph. Eur "; enzyme 2 – 370 IU Ph. Eur; enzyme 3 – 900 IU Ph. Eur; enzyme 4 – 1000 U Ph. Eur). For research, reconstituted whey was used, with a mass fraction of solids of 6.0%, which was pasteurized at a temperature of 66 ± 2 ° C, which was then cooled to a temperature of 47 ± 2 ° C, at which electrochemical activation was performed. The hydrolysis process was carried out at a temperature of 38 ± 2 °C by adding pancreatin enzyme and holding for 14-16 hours. To inactivate the enzyme, hydrolyzed whey was heated to a temperature of 66±2°C and kept for 15 minutes.

Figures 3 and 4 show graphical dependencies characterizing the change in pH and the efficiency of acid formation depending on the proteolytic activity of the introduced enzyme preparation, respectively, with and without electrochemical activation.

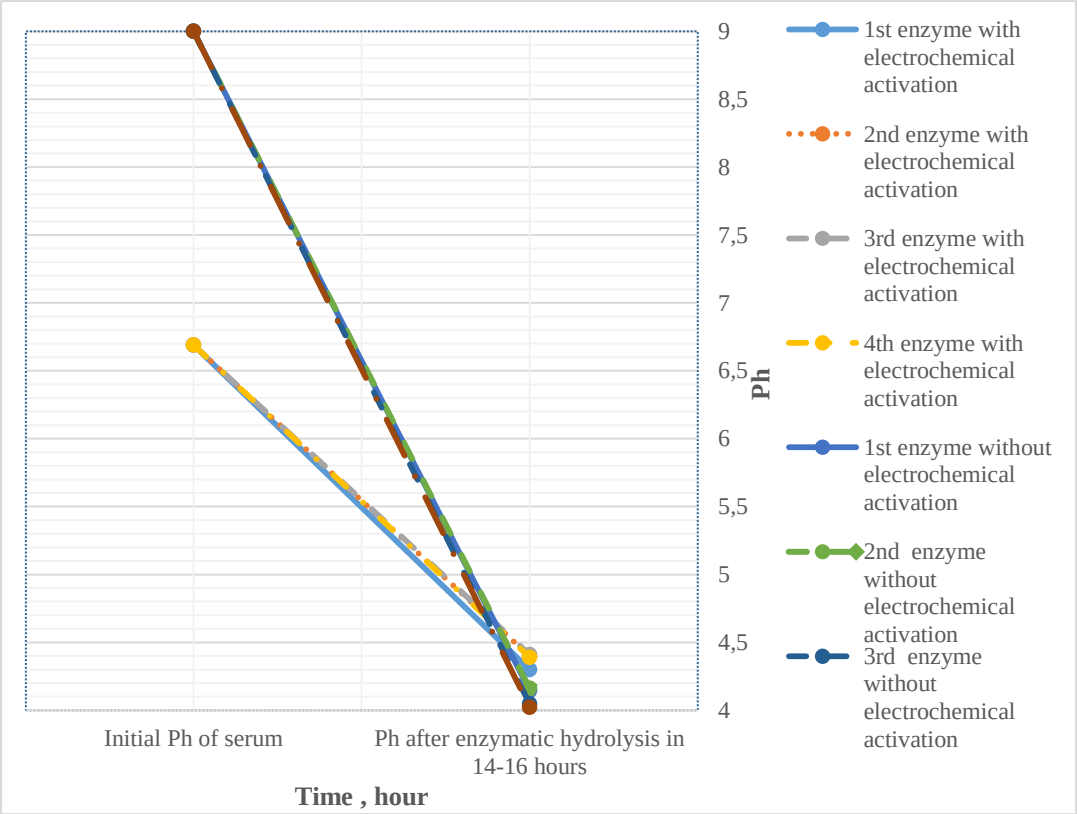


Figure 3 – Dynamics of pH change depending on the proteolytic activity of the introduced enzyme preparation and electrochemical activation.

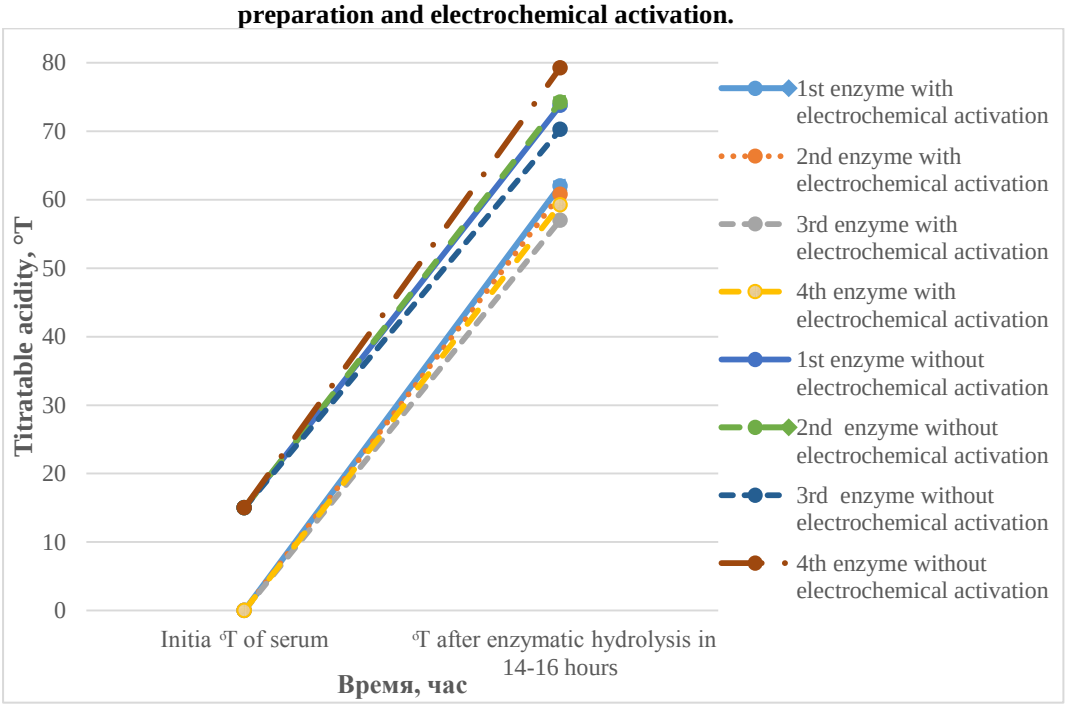


Figure 4 – Efficiency of acid formation depending on the proteolytic activity of the introduced enzyme preparation and electrochemical activation.

As can be seen from the graphs, the initial value of the titratable and active acidity of the samples after electrochemical activation was, respectively, 4.0 ± 0.5 °T and pH 6.9 ± 0.04, and the final value was 60.0 ± 3.0 ° T and pH 4.37 ± 0.04, and for samples without electrochemical activation, respectively, the initial value is 9.0 ± 1.0 °T and pH 4.09 ± 0.06, the final value is 60.0 ± 3.0 ° T and pH 4.37±0.04.

It was found that the effect of electroactivation on titratable acidity was not manifested, in contrast to the active pH acidity, which was taken into account when creating a sports drink. In further studies, a proteolytic enzyme 1–250 IU Ph was used. Eur, with a lower cost, since no significant difference in the effect of enzyme activity was observed.

It is known that creatine in the human body is synthesized from arginine, S-adenosylmethionine and glycine, primarily in the pancreas, liver [18], as well as in the brain, testicles [19, 20].

Creatine as a dietary supplement is available in various forms, such as creatine anhydrous, creatine monohydrate, creatine ethyl ester, creatine malate, creatine pyruvate, and others [21]. The main data on creatine monohydrate relate to the area of its influence on human performance and health. It was noted that with the regular use of this supplement, strength indicators increase, without changing the fat mass and muscle morphology [22]. Although people respond differently to creatine supplementation, it is generally accepted that creatine supplementation increases body stores, promotes faster post-exercise recovery, and improves exercise performance [23].

It should be noted that creatine enters the body mainly with meat, fish and other animal products [24]. Its daily intake in humans is about 2 g per day [25]. Thus, to maintain creatine stores in the body, it is necessary to synthesize or take about 2 g of creatine daily, however, during sports or heavy physical activity, the daily intake of creatine increases [23]. With constant sports, taking into account the increase in the consumption of creatine by the body, it becomes necessary to include it in food, in particular, in the composition of a functional drink based on whey.

We have studied the effect of creatine monohydrate on the development of lactic acid microflora containing *Lactococcus lactis, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus helveticus, Propionibacterium freudenreichii ssp. Shermanii*, while developing a functional drink based on whey.

During the study, reconstituted whey was used, with a mass fraction of solids of 6.0%, pasteurized at a temperature of 66±2°C. Pasteurized whey was subjected to electrochemical activation and enzymatic hydrolysis with a proteolytic enzyme 1–250 IU Ph. EUR. Further, after enzyme inactivation, creatine monohydrate was added to the samples in the amount of 0.5%, 1% and 1.5% of the total serum volume. The starter culture of lactic acid microorganisms was used in an amount of 5% of the serum volume. During the experiment, fermentation continued for 6 hours at the optimum temperature for fermentation of 38 ± 2 °C. During the fermentation, the pH and titratable acidity of the samples were controlled. Figures 5 and 6 show graphical dependencies characterizing the efficiency of acid formation and pH for a starter culture containing *Lactococcus lactis, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus helveticus, Propionibacterium freudenreichii ssp. shermani* with different doses of creatine monohydrate.

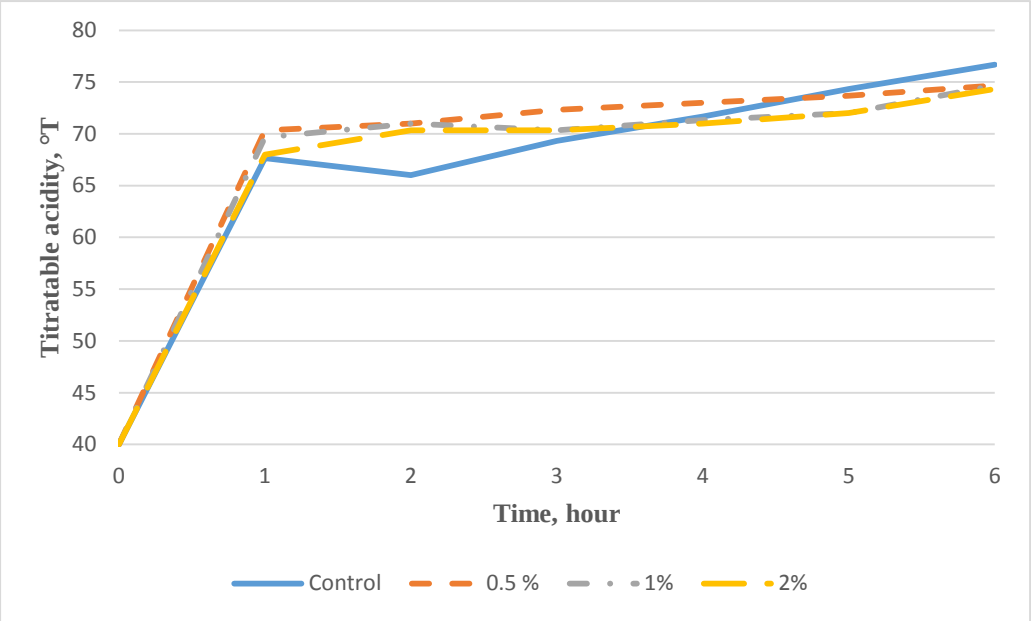


Figure 5 – Efficiency of acid formation of starter containing *Lactococcus lactis, Streptococcus thermophilus , Lactobacillus acidophilus , Lactobacillus helveticus , Propionibacterium freudenreichii ssp . Shermanii,* with various doses of creatine monohydrate

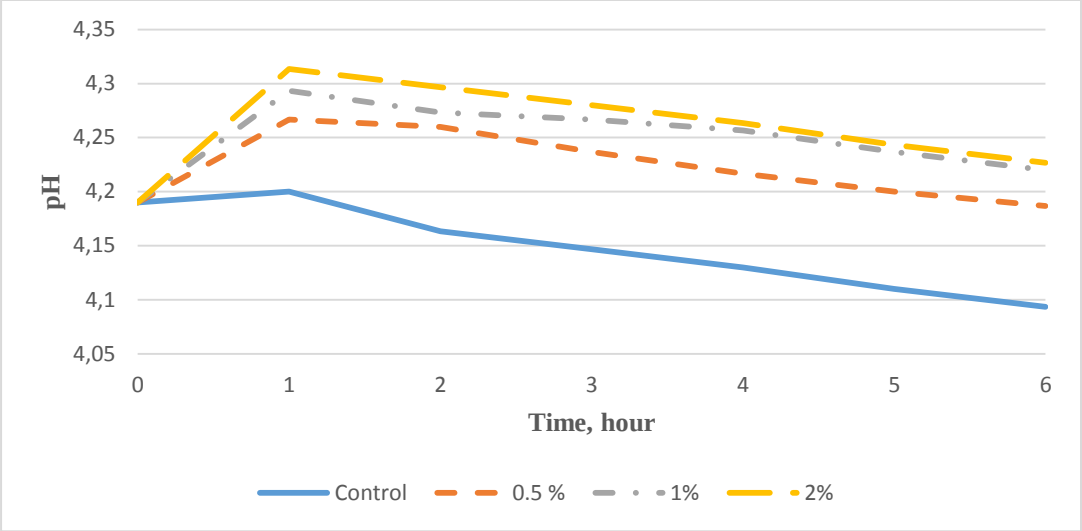


Figure 6 – Dynamics of pH of the starter containing *Lactococcus lactis, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus helveticus, Propionibacterium freudenreichii ssp. Shermanii,* with various doses of creatine monohydrate

The conducted studies show that creatine monohydrate practically does not affect the acid formation efficiency of the starter, however, there is a change in the pH value within acceptable limits, so it was decided to use a dose of creatine monohydrate in the amount of 1% of the total whey volume. This value of creatine monohydrate is of fundamental importance, since in terms of 200 ml the dose of creatine monohydrate is 2 g, which corresponds to the daily intake of the drug [25].

To give pleasant organoleptic properties to a functional drink, we chose glucose - fructose syrup (GFS) as a sweetener. Firstly, this is due to the fact that GFS is a natural sweetener obtained from corn by successive enzymatic liquefaction and saccharification of starch to a high-glucose syrup. Secondly, in terms of sweetness level and taste profile, GFS is comparable to sucrose and provides a taste enhancement, since its sweetness is quickly and early determined by taste buds, but is not delayed, which leads to better perception [26]. GFS also provides stability, freshness, texture, color, fluidity, and consistency in foods compared to sucrose [27]. The GFS used by us, obtained by isomerization of a part of D-glucose into D-fructose, contained 20–50% of solids.

Whey was reconstituted to a solids content of 6.0% and subjected to enzymatic hydrolysis. After enzymatic hydrolysis, GFS was added in the amount of 5%, 7%, 9% of the serum mass. The samples were then fermented with a starter containing *Lactococcus lactis, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus helveticus, Propionibacterium freudenreichii ssp. shermani.* After ripening, an organoleptic evaluation of the obtained samples was carried out, recorded for each criterion, summarized in the form of a final assessment using a discrete ten-point scale. At the same time, the indicator "excellent" was considered at 10 points, "good" – at 8 points, "unsatisfactory" and "poor", respectively, at 2 and 1 points. Each expert additionally described the nature of sensations during organoleptic evaluation [28]. The results are shown in table 2.

Table 2 – Organoleptic indicators of a functional drink with different doses of GFS

GFS doses	Organoleptic indicators	Grade
Control	Sour smell, sour taste, yellow color	2.3
5 %	Sweet and sour smell, sour taste, yellow color	5
7%	Sweet smell, pleasant sweet taste, yellow color	8.5
9 %	Sweet smell, sickly sweet taste, yellow color	6.5

Analysis of the data obtained showed that the use of GFS significantly improves the organoleptic characteristics of the drink, while its optimal amount is 7% of the total whey mass.

Based on the results of the experiments, a technology for a functional drink for sports nutrition was developed, adapted to industrial conditions (Figure 7).

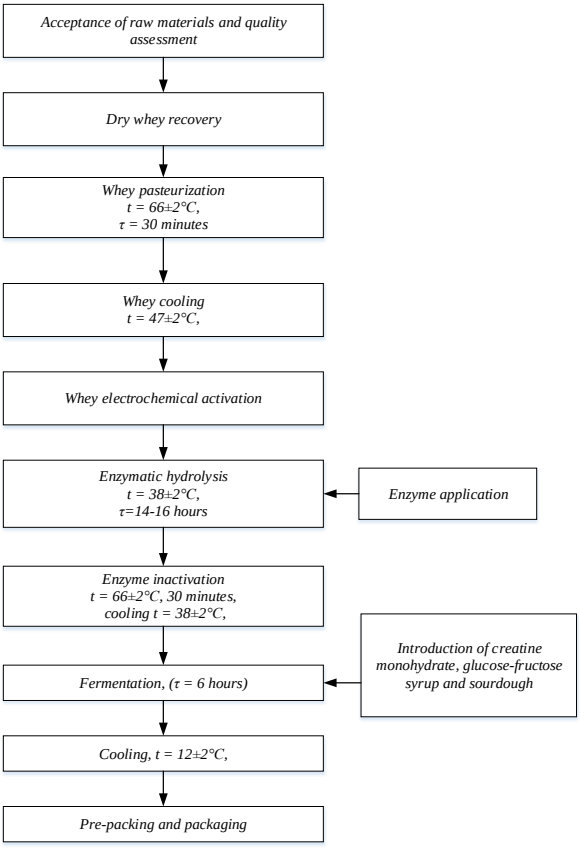


Figure 7 – Schematic diagram of the production of a functional drink using whey.

In order to ensure the quality of sports nutrition drinks, the incoming raw materials are controlled – whey powder, then it is restored to a solids content of 6.0%.

Whey is pasteurized at 66 ± 2 °C with a holding time of 30 minutes. The mixture is cooled to a temperature of 47 ± 2 °C and electrochemical activation is carried out, ensuring a constant pH for the enzyme preparation equal to 4.37.

After electrochemical activation, the serum is cooled to a temperature of 38 ± 2 °C and a hydrolytic enzyme is added. Hydrolysis of whey is carried out within 14 - 16 hours.

At the end of hydrolysis, the whey is pasteurized for 30 minutes at a temperature of 66 ± 2 °C, and cooled to the starter addition temperature of 38 ± 2 °C.

A sourdough containing *Lactococcus lactis, Streptococcus therm o philus, Lactobacillus acidophilus, Lactobacillus helveticus, Propionibacterium freudenreichii ssp. Shermanii,* contribute in the amount of 5%. Creatine monohydrate is added in the amount of 1%, glucose-fructose syrup in the amount of 7%. The mixture of components is stirred. The fermentation of the drink is carried out until the acidity of the product reaches 75 - 80 °T, the sports drink is cooled to a temperature of 12 ± 2 °C. Before bottling, the product is stirred.

The research results confirmed the expediency of using whey after enzymatic hydrolysis to obtain functional drinks. The developed technology for the production of a sports drink takes into account the creation of a fermented whey base, the optimal amount of creatine monohydrate and GFS in order to give the drink functional properties, a pleasant aroma, with hints of sourness and a sweet taste.

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