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DETERMINING PATTERNS OF LACTOSE HYDROLYSIS IN LIQUID CONCENTRATES OF DEMINERALIZED WHEY

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The object of this study was the process of milk sugar hydrolysis in liquid whey concentrates. The problem solved was the intensification of the lactose hydrolysis reaction in liquid whey concentrates through the use of a combination of leavening and enzyme preparations. Patterns in the lactose hydrolysis process in liquid concentrates of demineralized whey were studied.

It was established that the use of an enzyme preparation for liquid whey concentrates does not make it possible to achieve a degree of lactose hydrolysis higher than 75–77 % within 10 h. The simultaneous use of enzyme and leavening preparations for 6 hours ensures the conversion of more than 95 % of lactose for concentrates with a mass fraction of dry substances of 10–30 % and more than 90 % for a 40 % concentrate. The rational duration of lactose hydrolysis in demineralized whey concentrates for the combination of preparations is 6 hours for 10 and 20 % concentrates, and 8 h for 30 and 40 % concentrates, which enables lactose hydrolysis at the level of 96.8–100 %. The rheological properties of concentrates with a solids content of 30 and 40 % indicate that these systems have a high ability to restore the structure.

The dynamics of monosaccharide formation during hydrolysis are similar for 10 and 20 % concentrates, in which galactose slightly predominates the systems. Data on the increase in glucose content in fermented 30 and 40 % concentrates contradict the known data regarding the consumption of glucose by the acidophilic bacillus and its conversion to galactose. This may indicate suppression of the activity of the acidophilic bacillus under conditions of increased osmotic pressure in the concentrates.

The results of the work could be used in the technology of whey ice cream, as well as dairy products that require adjustment of the chemical composition, primarily in terms of protein and lactose content

Keywords: lactose hydrolysis, enzyme, acidophilic bacillus, whey concentrate, galactose, glucose

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1. Introduction

The market of protein ingredients obtained from whey is one of the most dynamic and profitable in the field of production of technical milk protein concentrates. Significant volumes of whey accumulated at milk processing enterprises and the rapid expansion of the range of dietary dairy products encourage manufacturers to look for new approaches to the use of secondary dairy resources. Thus, there is a development of the technology of liquid concentrates from whey of various origins, which have been tested in the composition of yogurts [1], sour milk gels and drinks [2, 3], sour cream [4], and cream [5].

However, the use of liquid whey concentrates has not been explored in detail in whey-based ice cream technology. The protein content in ice cream depends on its type, component composition, and may be regulated at the state level. For whey ice cream, there are no legislative and international requirements regarding its chemical composition, in particular, protein content. With a low content of protein in whey, as the main raw material, it becomes impossible to ensure its standard content (2.5–4.0 %) in ice cream, which reduces the nutritional value of the product and leads to defects in taste, consistency, and texture.

To bring the indicators of whey ice cream to the generally accepted level in terms of protein content, it is advisable to choose a milk base that would ensure the performance of certain technological functions in the composition of the product. The cheapest source of whey proteins in ice cream is condensed and dry whey. However, whey contains up to 70–75 % of lactose from the total dry matter content, the excess of which in ice cream causes crystallization [6]. Decreasing the lactose content or excluding it from the composition of ice cream by enzymatic hydrolysis could prevent the occurrence of defects in consistency during its storage. Lactose hydrolysis products are also characterized by an increased degree of sweetness, which makes it possible to reduce the sugar content in ice cream [7]. Partial hydrolysis of lactose can also be achieved by fermenting ice cream mixtures with lactic acid bacteria. However, the combination of both methods of lactose hydrolysis in whey concentrates with the simultaneous use of enzyme and leavening preparations has not been studied, which confirms the need for additional research into this area. That is why the scientific substantiation of the modes of complex hydrolysis of lactose in demineralized whey concentrates is a relevant field of applied research.

2. Literature review and problem statement

The most common in the food industry are physical methods of removing lactose from whey by filtration through membranes and dialysis. In the scientific literature, there is information on the use of physical methods in the production of liquid whey concentrates for further use as a basis for dairy products. For example, paper [8] reported obtaining liquid whey concentrates with a mass fraction of dry substances of 15.08–19.08 % by the ultrafiltration method. In addition, diafiltration was applied, the main purpose of which was to reduce the mass fraction of lactose and salts to obtain a concentrate with a reduced content of lactose and mineral salts at the level of 2.37 % (for concentrates without diafiltration – 4.45–6.06 %). The same technology was applied in [9], in which the combination of ultrafiltration and continuous diafiltration using NF permeate gives the desired result for the effective removal of lactose from buttermilk concentrate. The established rational parameters of this process are a pressure of 1.5 MPa and a temperature of 50 °C.

Physical methods of lactose removal are implemented at large milk processing complexes that have the financial opportunity to purchase, install, and maintain specialized equipment. At the same time, the use of biological methods of lactose hydrolysis, which are much more affordable, is more promising for small dairy enterprises.

A likely option to overcome related difficulties is to use microorganisms with pronounced β-galactosidase activity or enzyme preparations of β-galactosidase.

It is known that restored whey is not inferior to milk as a nutrient medium for lactobacilli, and the speed of the enzymatic reaction depends only on the initial lactose content in the dairy system [10]. Lactic acid bacteria *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus acidophilus* are distinguished by high β-galactosidase activity among known microorganisms [11].

This is the approach used in work [12], which was focused on the selection of microorganisms for the purpose of developing fermentation cultures for the production of fermented milk products. The highest activity was observed for the composition of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbruecki* subsp. *bulgaricus*, which fermented about 26 % lactose in buttermilk and 30 % in buttermilk-retentate mixture.

The results of another study [13] indicate that the fermentation of the yogurt base for ice cream with the combination of DVS: FD YF-903+LA-5 fermentation cultures takes place in 5 hours (up to pH 4.68), and with VIVO Yogurt sourdough – in 7 hours (up to pH 4.62). At the same time, the mass fraction of lactose in the fermentation process decreases by 28–33 %.

But in the case of production of acidophilic ice cream based on liquid concentrates of demineralized whey, it will be impossible to achieve a significant reduction of lactose only if probiotic cultures are used. Therefore, the additional use of lactase enzyme preparations may be appropriate; however, there are no data on the joint use of leavening and enzyme preparations in the indicated food systems. This is probably due to the fact that the patterns of lactose hydrolysis in whey concentrates may differ from the known data established by predecessors and require detailed study for each system.

There are two main types of lactase enzymes commercially available in the market of technological preparations – neutral and acidic. The enzyme preparation β-galactosidase is produced from the yeast *Kluyveromyces lactis, Kluyveromyces fragilis, Saccharomyces lactis* or *Kluyveromyces marxianus* (Singh & Sambyal, 2023). Currently, its production is carried out by the largest players in the market of food additives: DSM Food Specialties (TM "Maxilact", Heerlen, the Netherlands), Godo, Nagase and Amano (Japan) for manufacturers of Halactase (TM "Chr. Hansen", Öresund, Denmark), Lactozyme Pure (TM "Novozymes", Bagsvard, Denmark) and Dupont (TM "Godo YNL2", Wilmington, Delaware, USA).

In [14], it was established that all enzyme preparations produced from *Kluyveromyces lactis* yeast have the same mechanism of action, but there is a difference in the degree of their purification. That is why, in order to form the proper conditions for the enzymatic action of β-galactosidase, it is necessary to strictly follow the manufacturer's instructions regarding the recommended concentration of the enzyme, the acidity of the medium, as well as the temperature and duration of the lactose hydrolysis process in food systems.

Other commercial enzyme preparations are also available, including those produced from *Aspergillus oryzae*. However, they are not common for use in food technology due to the higher price and limited conditions of use (different optimal ranges of active acidity, temperature, and duration of the process) [15]. In addition, there is information about their negative impact on quality indicators of finished products. In work [16] it was established that the analysis of the content of non-protein nitrogenous compounds in whey hydrolyzed by an enzyme preparation of mushroom origin indicates an increase in their content with an increase in the concentration of the enzyme preparation. The preparation of acid lactase in its composition may contain impurities of proteolytic enzymes, which with the accumulation of non-protein nitrogenous compounds was accompanied by the deterioration of the organoleptic characteristics of the product.

The most researched preparation for the hydrolysis of lactose in dairy products is neutral lactase TM "GODO-YNL2", which has a predictable effect on the course of the process. One of these approaches is shown in work [17], the authors of which investigated the process of lactose hydrolysis in cheese whey with apple pectin and found that the enzyme "GODO-YNL2" provides a degree of hydrolysis of 55–60 % at an active acidity of 6.0–6 .5 pH units, temperature 30 °C, and treatment duration 5–6 h.

This confirms that the simultaneous use of enzyme and leavening preparations can intensify this process, which is especially appropriate, considering the high content of lactose in dry demineralized whey and restored concentrates that will be made from it. The regularities of lactose hydrolysis in such food systems may differ from the known data established by predecessors and require detailed study.

In addition to the technological effect of lactose hydrolysis, which will prevent its crystallization and the appearance of defects in the quality of ice cream, there are other advantages of using this process. During hydrolysis, lactose is split into monosaccharides – glucose and galactose. If glucose is found in vegetables and fruits, galactose in its pure form is not found in food products [18]. It is generally accepted that as a result of the hydrolysis of milk sugar by β-galactosidase, the formation and accumulation of glucose and galactose occurs in equal quantities; however, when applying enzymeolysis, their mass fraction will change due to the action of the acidophilic rod.

According to [16], under optimal temperature conditions, the degree of hydrolysis was maximal and led to the predominant formation of glucose, and in the case of an increase in the fermentation temperature, the degree of lactose hydrolysis decreased, and galactose prevailed in the medium. The ratio between the mass fractions of glucose and galactose ranged from 1.08 % to 1.6 %, depending on the type of enzyme preparation and fermentation temperature.

Lactose occupies one of the last places on the relative scale of sweetness. Compared to sucrose, it is 5–6 times less sweet and has a sweetness index of 16 conditional units [19]. At the same time, the products of its hydrolysis have a much higher degree of sweetness, which is 73 units for glucose and 32 for galactose [19]. Due to the accumulation of galactose and glucose, the product based on hydrolyzed whey will have a more pronounced sweet taste, which will make it possible to reduce the need for sugar.

That is why the use of liquid hydrolyzed concentrate of demineralized whey in the composition of whey ice cream will solve the following tasks:

– to partially replace sugar due to the increased sweetness of monosaccharides in the composition of the concentrate with a simultaneous increase in the content of dry substances to the level of a full-fat analog (30–40 %);

– to prevent excessive crystallization of lactose during ice cream storage, which leads to the formation of consistency defects (sandiness, flouriness);

– to increase the content of biologically complete whey proteins in the product.

All this gives reason to assert that it is expedient to conduct a study aimed at substantiating lactose hydrolysis regimes in liquid concentrates of demineralized whey.

3. The aim and objectives of the study

The purpose of our work is to determine patterns in the lactose hydrolysis process in liquid concentrates of demineralized whey. This will make it possible to substantiate rational modes of lactose hydrolysis for the further use of liquid whey concentrates in dairy products technologies.

To fulfill the set goal, the following tasks of scientific research were stated:

– to investigate the course of lactose hydrolysis when using enzyme and leavening preparations;

– to determine the patterns of accumulation of monosaccharides in food systems depending on the preparation used;

– to determine the main physical-chemical parameters of liquid hydrolyzed whey concentrates.

4. The study materials and methods

4. 1. The object and hypothesis of the study

The object of our research is the technology of liquid concentrates of demineralized whey.

The main hypothesis of the study assumes that the joint use of leavening and enzyme preparations could intensify the process of lactose hydrolysis in demineralized whey concentrates, which will allow their further use in dairy products as functional and technological ingredients.

At the stage of conducting research, it was assumed that the combination of enzyme and leavening preparations could synergistically affect the process of lactose hydrolysis, increasing its speed and efficiency. It was predicted that changes in temperature, pH, and reaction time would have a controlled effect on the hydrolysis process and on the properties of the final product. It was also believed that partial hydrolysis of lactose could improve the organoleptic properties of the product, in particular reducing sweetness and improving its texture.

To simplify the study, a specific leavening agent was chosen, which limits the universality of the results, as it does not take into account all possible types of leavening agents for whey. In addition, the optimized temperature and pH may also not cover all the variations that occur during scaling. The limited selection of enzymes may limit conclusions about the effectiveness of other enzymes or their combinations. These simplifications made it possible to create controlled conditions, but at the same time narrowed the completeness of the evaluation of the technology for the production of liquid concentrates of demineralized whey.

4. 2. Researched materials and equipment used in the experiment

Dry demineralized whey with a demineralization level of 90 % (AT "Milk Alliance", Ukraine) was chosen as an input raw material for the production of concentrates, which contains, in terms of dry matter: ash – no more than 2.5 %, lactose – no less than 79 %, protein – not less than 10.7 %. The solubility index of dry demineralized whey is 0.5 cm^3 of raw sediment.

For the fermentation of whey concentrates, a liquid preparation of β-D-galactosidase-hydrolase (lactase) with the commercial name GODO-YNL2 ("Danisko", Denmark) was used, which is a producer of breeding strains of *Kluyveromyces lactis*. Under standard conditions of milk hydrolysis for 24 hours at a temperature of 4.4–7.2 °C, the recommended amount of the preparation GODO-YNL2 (containing 10 % β-galactosidase) is 100 g per 100 liters of milk.

For fermentation of fermented samples with residual lactose content, the fermentation preparation "*L. acidophilus LYO 50 DCU-S*" ("Danisko, Denmark) was used – a single-strain lyophilized probiotic culture at the recommended dose of 5 g per 100 liters of milk.

Dry demineralized whey was restored in drinking water at a temperature of 40–45 °C to obtain concentrates with a mass fraction of dry substances from 10 to 40 %. The concentrates were filtered, pasteurized at a temperature of 80–82 °C for 3–5 min, cooled to a temperature of 40–43 °C, and fermented with the preparation GODO-YNL2 and leaven based on the leavening preparation "*L. acidophilus LYO 50 DCU-S*" in various combinations.

4. 3. Methods for investigating mixtures and ice cream

The mass fraction of dry substances in concentrates was determined by the arbitration method, and the protein content was determined by the Kjeldahl method. The fat content was measured according to the modified Gerber method, the acidity was measured by inserting the electrodes of a potentiometric analyzer into the concentrates at a temperature of 20 °C.

Viscosity characteristics were determined on a rotary viscometer "Rheotest II" with a "cylinder-cylinder" measuring system by taking deformation kinetics curves. The research was carried out at a temperature of 20 °C.

Shear stress τ (Pa) was measured at twelve values of the shear rate gradient D in the range from 3 to 1312.2 s^{-1} during forward and reverse motion. The maximum effective viscosity of the practically undamaged structure (γ=3 s^{-1}), the minimum effective viscosity of the marginally destroyed structure (γ=1312.2 s^{-1}) and the effective viscosity of the restored structure (γ=3 s^{-1}) were recorded). The degree of restoration of the structure of the concentrates (thixotropic ability) was determined as a percentage by the difference in the values of the effective viscosity of the practically intact structure at the beginning and at the end of the measurement at a shear rate gradient (γ=3 s^{-1}) [20].

Water activity in whey concentrates and ice cream mixes was determined on a water activity analyzer "HygroLab 2" (Rotronic, Switzerland) at a temperature of 20 °C in the measurement range of 0–1 Aw (0–100 % rh).

The mass fraction of carbohydrates (lactose, glucose, and galactose) in whey concentrates was determined by the method of high-performance liquid chromatography on a chromatograph of model LC-6A (Shimadzu, Japan) with a refractometric detector, column SCR-101-N (250×4.7 mm). Deionized degassed water was used as an eluent, the flow rate was 0.5 ml/min.

The degree of hydrolysis was expressed as a percentage, according to the lactose content in the fermented samples relative to its initial content.

Statistical analysis was performed using the Statistika 10 software. Data were expressed as the mean value with standard deviation of triplicate measurements. Differences were considered reliable at validity α=0.95. Construction of flow rheograms of ice cream mixtures was carried out in the Microsoft Excel 2016 environment (Taiwan). To ensure the reliability of the results, the study was conducted three times.

5. Research results based on liquid concentrates of demineralized whey

5. 1. Patterns of lactose hydrolysis

At the first stage of the study, liquid whey concentrates were produced (Table 1). Their mass fraction was brought to the level of 10–40 % on the basis that subsequently their inclusion in the composition of ice cream would significantly increase the level of dry substances in the finished product.

Table 1

Chemical composition and physical-chemical parameters of non-fermented liquid whey concentrates ($p \le 0.05$, n=3)

Concentrate with mass fraction of solids, %	Active acidity, pH units	Water activity, units	Mass fraction of lactose, %
10	6.6±0.1	0.985±0.006	7.8±0.1
20	6.5±0.1	0.980±0.002	15.5±0.1
30	6.2±0.1	0.974±0.001	23.3±0.4
40	6.1±0.1	0.966±0.001	30.8±0.3

From the given data (Table 1), it can be seen that the level of lactose is quite high, especially for concentrates with a mass fraction of dry substances of 30–40 %, which requires measures to adjust the carbohydrate composition of these food systems.

In order to carry out the hydrolysis of lactose in liquid whey concentrates, the following schemes were adopted:

– scheme 1. Fermentation of liquid concentrates of demineralized whey with the enzyme preparation GODO-YNL2;

– scheme 2. Fermentation of liquid concentrates of demineralized whey with the simultaneous use of the enzyme preparation GODO-YNL2 and the leavening preparation "*L. acidophilus LYO 50 DCU-S*".

With the simultaneous application of GODO-YNL2 and a leavening agent, it is assumed that during the lag phase of *L. Acidophilus* development (2–4 h), the enzyme should have time to detect hydrolytic activity at an active acidity of pH≥5.7.

According to scheme 1, the effectiveness of the use of the GODO-YNL2 enzyme preparation for the hydrolysis of lactose in liquid whey concentrates under variable process parameters was tested (Fig. 1).

According to Fig. 1, the enzyme preparation shows the highest activity during the first 4 hours at the lowest concentration (0.1 %). An increase in the mass fraction of the enzyme somewhat intensifies the hydrolysis process, especially for systems with the highest lactose content. The active phase of the enzyme preparation is prolonged up to 6 hours.

The dynamics of lactose hydrolysis in liquid concentrates are similar for samples with 10 and 20 % dry substances, for which a sharp increase in the degree of hydrolysis is observed during the first 2–4 h, after which the reaction slows down.

A further increase in the mass fraction of dry substances in concentrates to 30–40 % reduces the speed of the process even at the initial stages, only by increasing the concentration of the enzyme to 0.3–0.4 %, the dynamics of the process become similar to concentrates of 10 and 20 %.

In general, the use of only the enzyme preparation at the specified concentrations does not make it possible to achieve a degree of lactose hydrolysis higher than 75–77 %, which indicates the need to find ways to intensify this process.

That is why, at the next stage, the effectiveness of the combination of enzyme and leavening preparation to speed up the process of hydrolysis of milk sugar was investigated.

For this study, the mass fraction of the enzyme preparation in liquid concentrates was taken as follows:

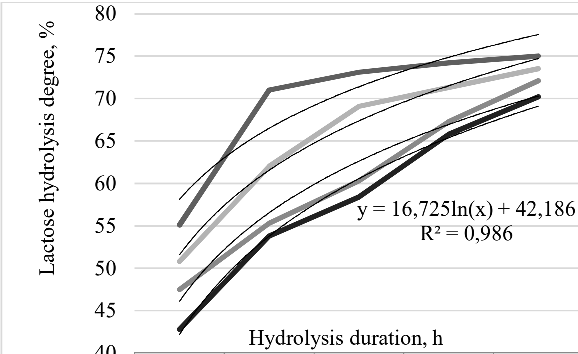
– concentrate 10 % – 0,1 % enzyme;
– concentrate 20 % – 0,2 % enzyme;
– concentrate 30 % – 0,3 % enzyme;
– concentrate 40 % – 0,4 % enzyme.

The mass fraction of leavening preparation "*L. acidophilus LYO 50 DCU-S*" was unchanged for all samples at the rate of 5 g of starter per 100 l of sample.

The results of the dynamics of lactose hydrolysis under the joint action of various drugs are shown in Fig. 2.

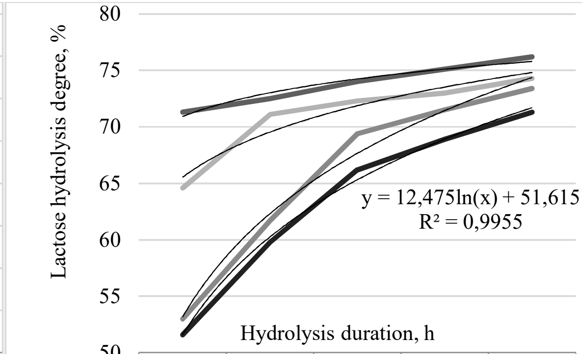
According to Fig. 2, fermentation culture prolongs the active phase of lactose hydrolysis up to 6 hours, which ensures the splitting of more than 95 % of milk sugar for concentrates with a mass fraction of dry substances of 10–30 % and more than 90 % for a 40 % concentrate.

Thus, the rational duration of lactose hydrolysis in demineralized whey concentrates is 6 hours for 10 and 20 % concentrates, and 8 hours for 30 and 40 % concentrates, which ensures lactose hydrolysis at the level of 96.8–100 %.



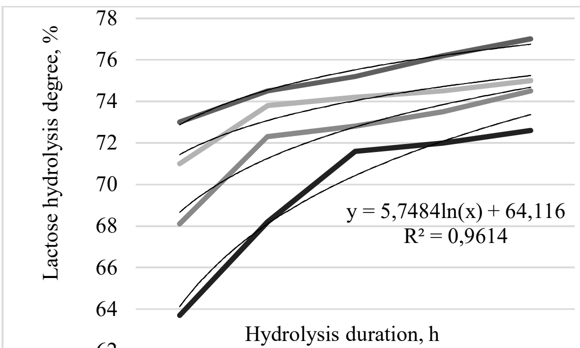
	2	4	6	8	10
10%	55,1	71,0	73,1	74,2	75,0
20%	50,8	62,0	69,1	71,3	73,5
30%	47,5	55,3	60,3	67,3	72,1
40%	42,8	53,8	58,4	65,8	70,2

a



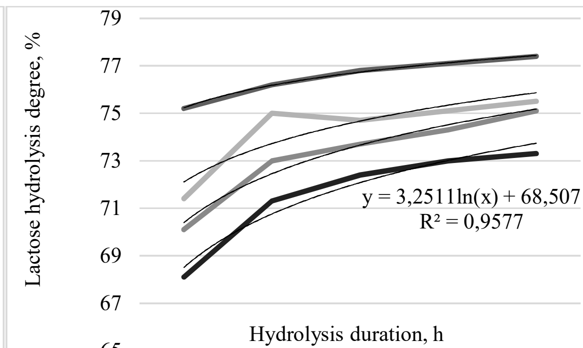
	2	4	6	8	10
10%	71,3	72,5	74	75,1	76,2
20%	64,6	71,1	72,3	73	74,3
30%	53	61,7	69,4	71,5	73,4
40%	51,6	59,8	66,2	68,9	71,3

b



	2	4	6	8	10
10%	73	74,5	75,2	76,2	77
20%	71	73,8	74,2	74,5	75
30%	68,1	72,3	72,8	73,5	74,5
40%	63,7	68,2	71,6	72	72,6

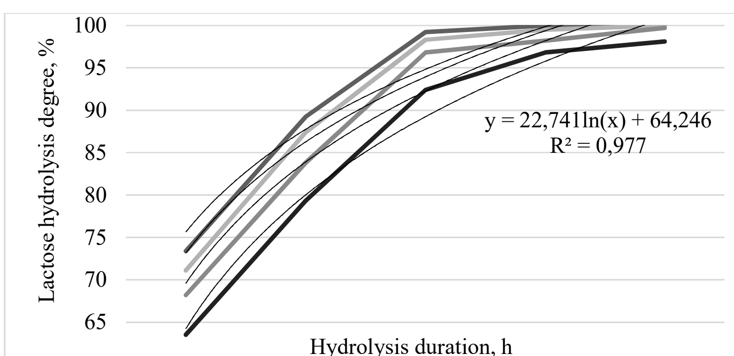
c



	2	4	6	8	10
10%	75,2	76,2	76,8	77,1	77,4
20%	71,4	75	74,7	75,1	75,5
30%	70,1	73	73,7	74,3	75,1
40%	68,1	71,3	72,4	73	73,3

d

Fig. 1. Hydrolysis of lactose in liquid whey concentrates at different concentrations of the enzyme preparation: *a* – 0.1 %; *b* – 0.2 %; *c* – 0.3 %; *d* – 0.4 %



	2	4	6	8	10
10%	73,4	89,2	99,2	100,0	100,0
20%	71,1	87,4	98,3	99,5	100,0
30%	68,2	83,8	96,8	98,2	99,7
40%	63,5	79,3	92,4	96,8	98,1

Fig. 2. Dynamics of lactose hydrolysis in liquid whey concentrates under the joint action of enzyme and leavening preparations

5. 2. Results of investigating carbohydrate composition in liquid hydrolyzed whey concentrates

For a deeper understanding of the peculiarities of the lactose hydrolysis process in liquid whey concentrates, the carbohydrate composition of sugars in fermented samples was investigated (Table 2).

Table 2

Carbohydrate composition of liquid hydrolyzed whey concentrates ($p \leq 0.05$, $n=3$)

Concentrate with m.s.dr. mat., %	Mass fraction of lactose, %	Mass fraction of glucose, %	Mass fraction of galactose, %	Glucose to galactose ratio
10	0.06±0.00	3.71±0.05	4.03±0.09	1:1.09
20	0.26±0.01	7.23±0.03	8.01±0.01	1:1.10
30	0.41±0.01	11.51±0.01	11.38±0.02	1:0.98
40	0.97±0.01	14.38±0.04	13.49±0.01	1:0.94

The remaining amount of milk sugar after hydrolysis does not exceed 1 % of the initial concentration, which opens up opportunities for the use of such concentrates in the production of products with a reduced lactose content or low lactose.

The dynamics of monosaccharide formation due to lactose hydrolysis are similar for 10 and 20 % concentrates, in which galactose slightly predominates the systems. However, for concentrates of 30 and 40 %, there is an equilibration of the ratio between monosaccharides with a slight predominance of glucose. Such changes can be caused both by the conditions of the hydrolysis process (duration, temperature, etc.), and by the presence of an acidophilic bacillus that can more actively consume glucose and produce it into galactose (10 and 20 % concentrates). With an increase in the mass fraction of dry substances in the studied samples to 30 and 40 %, the activity of the acidophilic bacillus may not be so pronounced due to potential changes in the osmotic pressure of the medium, which may to some extent inhibit the consumption of one monosaccharide and its conversion to another.

That is why it is important to pay attention to the activity of water in fermented concentrates, which will give an indirect idea of the relationship between this indicator and the osmotic pressure in the water environment of the studied food systems and the possible influence on the activity of the acidophilic bacillus in relation to monosaccharides.

5. 3. Results of investigating physical and chemical indicators of liquid hydrolyzed whey concentrates

In order to understand possible changes occurring in whey concentrates after hydrolysis carried out with the help of a combination of drugs, their viscosity-speed characteristics before and after fermentation were investigated (Table 3).

The effective viscosity of concentrates increases with the increase in the content of dry substances in the system, which undoubtedly affects the ability of the structure to recover. Hydrolysis of lactose slightly reduces the effective viscosity, which may be related to the decrease in the molecular weight of carbohydrates. However, the thixotropy of the systems increases after fermentation, indicating that the strength of the bonds formed by hydrolysis of lactose is likely to be higher than in non-hydrolyzed concentrates. The rheological properties of 30 and 40 % concentrates indicate that these systems have a high ability to restore destroyed samples. This allows us to make an assumption that their use in ice cream in further studies is appropriate for adjusting quality indicators, in particular during long-term storage.

Analysis of the physical and chemical parameters of liquid hydrolyzed concentrates provides a clear understanding of the state of water in the systems, as well as the main nutritional characteristics (Table 4).

Table 3

Rheological characteristics of liquid whey concentrates

M.s.dr.mat. in concentrate, %	Effective viscosity (mPa·s) at variable shear rate gradient			Time of ultimate structure destruction (γ=1312.2 s^{-1}), min	Degree of structure recovery, %
	γ=3 s^{-1} (forward)	γ=1312.2 s^{-1}	γ=3 s^{-1} (reverse)		
Unfermented					
10	135.8±1.25	10.1±0.3	70.7±1.00	2.9±0.1	52.06
20	220.4±2.54	11.2±0.2	120.4±2.05	2.4±0.2	54.62
30	295.3±3.18	14.5±0.2	188.2±1.45	2.2±0.2	63.73
40	340.1±1.87	16.6±0.4	240.5±3.84	3.1±0.1	70.71
Fermented					
10	119.8±3.56	15.4±0.1	78.5±2.14	2.7±0.2	65.52
20	195.6±2.01	17.8±0.1	130.4±1.66	2.8±0.2	66.67
30	280.5±2.24	18.0±0.3	190.9±2.45	2.6±0.1	68.05
40	328.2±4.81	18.4±0.2	255.1±2.92	3.0±0.1	77.72

Table 4

Physicochemical parameters of liquid whey concentrates

Indicator	Concentrate with m.s.dr.mat., %			
	10	20	30	40
Mass fraction of protein, %	1.09±0.01	2.21±0.03	3.30±0.01	4.44±0.01
Mass fraction of fat, %	0.10±0.00	0.21±0.00	0.31±0.01	0.42±0.01
Total mass fraction of carbohydrates, %	7.98±0.11	15.55±0.19	23.90±0.08	31.73±0.20
Active acidity, units pH	5.49±0.01	5.30±0.02	5.18±0.02	5.12±0.01
Water activity, unit	0.958±0.02	0.950±0.03	0.940±0.02	0.933±0.04

According to Table 4, the activity of water in hydrolyzed concentrates decreases with an increase in the mass fraction of dry substances. These values are lower than the activity of water in non-hydrolyzed concentrates, which may indicate an increase in osmotic pressure in the presence of monosaccharides with the corresponding formation of a stronger network that better retains moisture. This is especially noticeable in concentrates of 30 and 40 **%**, the high content of protein and carbohydrates in which significantly affect the formation of the main characteristics in these systems.

6. Discussion of the results related to lactose hydrolysis and physicochemical parameters of liquid whey concentrates

It was reported [21] that the maximum efficiency of lactose hydrolysis after 2 h of reaction was obtained using β-galactosidase *K. lactis* at 37 °C. In milk, the highest degree of hydrolysis was 73.84 %. For whey, the maximum efficiency of hydrolysis was at the level of 74.98 %, and for permeate – 69.42 %. For *K. lactis* β-galactosidase, 100 % hydrolysis efficiency was not achieved under any of the conditions evaluated, which correlates with the data obtained in this study using only the enzyme preparation.

However, in paper [21] it was determined that the enzyme preparation from *K. lactis* yeast provides the same conditions for the process in both milk and whey, regardless of the concentration of the enzyme under study. In this study, increasing the concentration of the enzyme had a direct effect on enhancing the conversion process of milk sugar to monosaccharides (Fig. 1). This dynamic was also reported in other studies [22, 23].

A fourfold increase in enzyme concentration doubles the concentration of hydrolyzed lactose in milk after 12 h of the process, which was tested on the example of raw and pasteurized milk using four commercial *Kluyveromyces* β-galactosidases at 2 °C for 72 h [23].

There are examples of studies [22] reporting significantly higher rates of lactose hydrolysis using the *Kluyveromyces* enzyme. The use of β-galactosidase *Kluyveromyces* at 2 °C for 48 h of reaction ensures 100 % efficiency of lactose hydrolysis for pasteurized milk. Also known is study [24], which evaluated the physicochemical, microbiological, and organoleptic characteristics of lactose-free milk using β-galactosidase *K. lactis* at 10 °C. Under these conditions, the rate of hydrolysis was recorded at the level of 90 % after 21 h of reaction. The differences in the above data [24, 24] compared to the reported results in this experiment consist in conducting the hydrolysis reaction for a much longer time. In addition, the activity of enzymes is affected by numerous environmental factors, among which temperature is one of the most important parameters. Achieving a high degree of hydrolysis in this study is also possible due to the reaction temperature at the level of 40–42 °C, which is higher than that used in work [24]. However, the obtained results can be considered satisfactory since, according to [25], reducing the concentration of lactose to 70–80 % in dairy products is sufficient for most people with lactose intolerance.

Regarding increasing the efficiency of the lactose hydrolysis process with the simultaneous use of enzyme and leavening preparations (Fig. 2), it can be noted that the information in the scientific literature is extremely limited. Based on the data on the use of probiotic cultures in dairy products to reduce the lactose content, it should be noted that some of them really have a high ability to convert milk sugar. This approach was used in study [26], in which it was established that probiotic cultures *L. delbrueckii subsp. bulgaricus* B-5b and *L. helveticus* LH-17 make it possible to achieve a degree of lactose hydrolysis of about 75 %. In this study, due to the joint action of the drugs, lactose hydrolysis increased by 21.8 % compared to the single action of the lactase enzyme. The reason may be the use by the authors of study [27] of special ultrasonic treatment of systems before the start of hydrolysis.

The hydrolysis of lactose with the formation of the corresponding monosaccharides with the simultaneous predominance of galactose in the system (Table 2) is a consequence of the fact that the acidophilic bacillus consumes glucose and converts it to galactose. It was reported [28] that the content of lactose, glucose and galactose in various fermented milk products was investigated by enzymatic methods. Lactose was reduced in all fermented products. After 11 days of storage of yogurt, the lactose content decreased to about 2.3 g/100 g compared to 4.8 g/100 g in unfermented milk. In the same period, the galactose content increased from traces in milk to 1.3 g/100 g in yogurt. The results were similar with acidophilus and bifidum milk. As is known, galactose prevents the occurrence and simplifies the course of various diseases associated with brain function disorders, and it can also be an additional source of energy for the body and affect the metabolism, in particular of carbohydrates [29]. However, for the 30 and 40 % concentrates, there was a slight predominance in the glucose system, which may indicate a decrease in the activity of the acidophilic bacillus under conditions of increased osmotic pressure.

Despite the lower effective viscosity of liquid concentrates due to the presence of lactose hydrolysis products, their thixotropy increases for all samples (Table 3), which is especially noticeable in systems with a high content of dry substances, in particular protein. The reason for this may be the formation of complexes of whey proteins with monosaccharides, which can improve the rheological characteristics of mixtures. Work [30] compared the influence of whey proteins with various monosaccharides on the rheological properties of mixtures. It is shown that the presence of d-glucose, d-fructose, d-alose, and d-psicose additionally increases the resistance to foaming and foam resistance of the mixtures, and this value only increases during the mixing interval from 15 to 30 min. This shows that the presence of a large amount of monosaccharides in systems based on whey proteins can have a certain synergistic effect for the formation of “carbohydrate-protein” complexes.

The study of water activity in non-fermented and fermented concentrates (Tables 1, 4) demonstrates the influence of monosaccharides on the state of water in the studied samples. A slight decrease in the activity of water in fermented whey concentrates due to the hydrolysis of lactose will to some extent affect the processes of forming the physicochemical parameters of ice cream as a polydisperse food system. Therefore, in further work, it will be necessary to investigate the cryoscopic temperature of ice cream samples based on hydrolyzed concentrates of demineralized whey.

The chemical composition of liquid concentrates of demineralized whey (Tables 1, 4) is slightly different from the composition of liquid whey concentrates given in the literature, which usually have a higher protein content (5.09–11.93 %), but a lower content of dry matter (14 .09–26.45 %), while the fat content ranges from 0.43–0.78 % for low-fat concentrates to 6.43–7.82 % for full-fat concentrates [2, 8]. The use of highly demineralized whey to obtain concentrates in this study significantly increases the lactose content to 30.8 %, which can significantly affect the quality of ice cream in the future. However, the hydrolysis of lactose in whey concentrates allows reducing its content to 0.98 %, which is lower than the value (4.95 %) reported in another study [2]. At the same time, a high proportion of solids (39.92–40.01 %) in liquid concentrates could provide the recommended level of solids in ice cream in the range of 25–35 %, which is especially important for low concentrates. The difference in the obtained values can be explained by the use of whey of different origins, as well as the use of special technologies for the production of concentrates.

Whey ice cream is a product of the amateur group, which causes the absence of clear requirements for its chemical composition, as well as defects in organoleptic indicators. For this reason, the prospect of further work is to investigate the potential technological functions of liquid hydrolyzed whey concentrates in the composition of ice cream, in particular during long-term storage.

The limitation of our study is that the intensification of the lactose hydrolysis process in liquid whey concentrates was achieved by combining a combination of drugs. However, the possibility of using other sourdough cultures, including multi-strain ones, was not investigated.

Among the potential shortcomings of the research, it can be noted that the hydrolysis process with the joint use of enzyme and leavening preparations is more time-consuming than in the case of using specialized equipment for removing milk sugar using a physical method.

7. Conclusions

1. The use of the GODO-YNL2 enzyme preparation in liquid whey concentrates does not allow the degree of lactose hydrolysis to be reached higher than 75–77 %. Simultaneous use of enzyme and leavening preparations "*L. acidophilus LYO 50 DCU-S*" within 6 hours provides decomposition of more than 95 % of milk sugar for concentrates with a mass fraction of dry substances of 10–30 % and more than 90 % for a 40 % concentrate. The rational duration of lactose hydrolysis in demineralized whey concentrates for the combination of drugs is 6 hours for 10 and 20 % concentrates, and 8 hours for 30 and 40 % concentrates, which ensures lactose hydrolysis at the level of 96.8–100 %.

2. The dynamics of monosaccharide production due to lactose hydrolysis are similar for 10 and 20 % concentrates, where galactose slightly predominates the system. However, for concentrates of 30 and 40 **%**, the ratio of monosaccharides is balanced with a slight predominance of glucose, which is explained by the suppression of the activity of the acidophilic rod under conditions of increased osmotic pressure in the concentrates.

3. The rheological properties of concentrates with a mass fraction of dry substances of 30 and 40 % indicate that these systems have a high capacity for recovery. Their water activity is lower than non-fermented concentrates, which affects the increase in osmotic pressure.

Conflicts of interest

The authors declare that they have no conflicts of interest in relation to the current study, including financial, personal, authorship, or any other, that could affect the study, as well as the results reported in this paper.

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Data availability

All data are available, either in numerical or graphical form, in the main text of the manuscript.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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