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ORIGINAL ARTICLE

The Effect of Lactose, Protein and Zinc on Biotechnological Characteristics of Cheese Whey Using Kluyveromyces Yeasts

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ABSTRACT

Background: In the last decade, the volume of cheese and cheese-based production has been growing steadily. This study aimed to determine the effect of lactose, protein and zinc on biotechnological characteristics of cheese whey using Kluyveromyces yeast.

Methods: The yeast strains *Kluyveromyces lactis* Y-2037, Y-2035 and *Kluyveromyces marxianus* Y-2042 were cultivated using a laboratory fermenter and orbital shaker thermostat for 72 hours at 34°C. To assess the differences related to whey composition, we changed the concentration of lactose, protein or zinc ions. Main characteristics of the investigations were yeast growth rate, lactose processing, ethanol production and fermentation efficiency.

Results: Yeast strains converted lactose from 82.7% to 89.9% and produced ethanol from 17.1 to 20.9 g^{-1} L^{-1} with a fermentation efficiency of 74.9-91.6%. The substrate feeding increased the lactose processing and ethanol production by 1.58-1.66 and 1.44-1.54 times, respectively. With a high initial lactose concentration in whey, an improvement in bioconversion rates, and ethanol production were observed with an increase by 1.87-2.02 times.

Conclusion: Cultivation in a low-protein environment as shown to worsen the biotechnological characteristics of fermentation. High concentrations of zinc ions contributed to an increase in the efficiency of the lactose conversion to ethanol too; while the percentage of processed lactose increased by 3.1-5.1%, and fermentation efficiency increased to 14.5%.

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Introduction

In the last decade, the volume of cheese and cheese-based production has been growing steadily and intensively all over the world, especially in middle- and low-income countries. Global cheese production in 2023 was about 22.4 million tons and it has an upward trend in the future too (1, 2). The cheese-making process belongs to high-waste industries, and up to 90% of dairy raw materials eventually turn into a by-product, named cheese

whey (named whey in short). The volume of global whey production is estimated at 200 million tons per year, and it increases by 1-2% annually. While no more than 25% of this amount can be processed into other food products, the rest requires recycling, and this puts a burden on producers (3). Experts note that manufacturers of small and medium-sized businesses tend to receive more waste during the cheese making and they are unable to ensure their full utilization due to the relatively high cost of purification technologies. As a result, more than half of lactose-containing waste is sent to surface water bodies without any processing, with inevitable damage to the environment (4-6).

Cheese whey has an acidic reaction with pH from 4.3 to 5.6 and it contains from 40 to 50 g^{-1} L^{-1} of lactose, up to 6 g^{-1} L^{-1} of protein, up to 4 g^{-1} L^{-1} of lipids, vitamins and mineral salts, which makes it an excellent raw material for the food industry, including biotechnology. At the same time, these substances consider the whey to be a dangerous pollutant that can cause the growth of natural microflora, excessive oxygen consumption, and ecosystem disruption if it is released into water reservoir (3, 7). Based on the current state of the problem, all plants where lactose-containing waste is generated in should process and/or neutralize this waste. Fermentation of carbohydrates using microorganisms, primarily yeast, is the technology of choice in this case. As in other environmental biotechnologies, one of the factors ensuring economic feasibility and practical success is the combination of the processing with the biosynthesis of compounds that are useful products or raw materials for the synthesis of other useful compounds (8, 9). Ethanol is an ideal product for this conversion in case of lactose-containing waste utilization, and its production is a promising trend for the development of new energies at the scale of specific industries (7, 10, 11).

Kluyveromyces yeast has proven to be an attractive microbial system for industrial biosynthesis and environmental biotechnologies (12, 13). They have a unique ability to lead to the lactose cleavage, which makes it valuable in dairy industry for food production and processing of lactose-containing waste. This property of *Kluyveromyces* yeast is due to the presence of two genes including *LAC12*, which encodes the enzyme lactose permease with the function of active transport of lactose into the cell, and *LAC4*, which is responsible for the synthesis of β-galactosidase, catalyzing the hydrolysis of lactose to glucose and galactose (6, 14). Among the representatives of this genus, *K. lactis* is one of most actively used one in the dairy industry for lactose cleavage, as well as a highly effective microbial system for biosynthetic processes and environmental biotechnologies (15, 16).

All the above dictate the need to develop and implement new effective biotechnologies for processing cheese production waste by converting lactose into ethanol, including use of modern highly productive strains of microorganisms. To test the lactose fermentation technology, we selected two representatives of *Kluyveromyces* species. The current study evaluated the effect of lactose, protein and zinc on biotechnological characteristics of cheese whey using Kluyveromyces yeast.

Materials and Methods

The whey for this work was obtained at a cheese-making factory located in Elan village of Volgograd region, Russia. To remove yeast cells and coarse component, we passed the whey through a microfilter and stored at a temperature of +4°C until use. Before work, the lactose and protein content in the cheese whey was normalized to 42.5 g^{-1} L^{-1} and 6.4 g^{-1} L^{-1} , respectively. For fermentation, we used two strains of *K. lactis*, labeled according to the VKPM catalog of the Russian National Bioresource Center as Y-2035 and Y-2037, and one strain of *K. marxianus* Y-2042. To ensure the fastest possible growth of the yeast culture, we grew the inoculum on a specially prepared nutrient medium. To do this, 10% sucrose solution at a concentration of 60 g^{-1} L^{-1} , 10% suspension of 1% soy flour and 5% *Saccharomyces cerevisae* Y-187 hydrolysate were added to 75% of the whey by volume.

Inoculation was carried out in glass vessels containing 150 mL of the nutrient medium and 5 mL of activated yeast strains, covered with cotton-gauze stoppers and sterilized in an autoclave. After cooling at 30°C, 10 mL of activated culture of the corresponding yeast strain was added to each vessel under sterile conditions. Then, we removed 10 mL from each vessel to measure the initial values and placed all vessels in an ES-20 orbital thermostat shaker (BioSan, Latvia) for 36 hours at a controlled temperature of 34°C and a table speed of 100 per minute. Three experiences were repetited for each yeast strain. Resulting from normalization of each inoculum in optical density, its yeast content was 10^8 cells/mL, and the pH of the medium was 4.35.

The basic nutrient medium for yeast cultivation in the main part of the study was native cheese whey containing 42.5 g^{-1} L^{-1} of lactose. Table 1 shows how the whey composition was modeled in six experimental series with variations in concentration of lactose, protein, and zinc ions. For lactose, one of the options was provided for maintaining constant lactose concentration throughout the entire time of yeast cultivation.

Table 1: The content of model series with differ concentrations of main substances.

Variable	Series	Lactose, g-1 L-1	Protein, g-1 L-1	Zn^{2+}, mM/L
Without changes	N	42.5	6.4	<0.7
Lactose concentration	Lc1	42.5	6.4	<0.7
	Lc2	85.0		
Protein белка	Pt1	42.5	3.2	<0.7
	Pt2		<0.5	
Zinc ion concentration	Zn1	42.5	6.4	4.0
	Zn2			8.0

In the Ps2 series, the yeasts grew in a medium with deproteinized whey, and in the Pt1 series, it was mixed with the initial whey, so that the protein concentration became 3.2 g^{-1} L^{-1} .

The main biotechnological process took place in a laboratory fermenter FA-02 (Prointech, Russia) with a 2.0l working chamber that included two fermentations of whey from each series (14 samples in total). Before starting work, all removable fermenter units were sterilized by autoclaving, while the working chamber underwent sterilization along with 1.55 liters of nutrient medium, as the non-removable part of the device and the metal surface of the housing that were additionally treated with 70% ethanol. The inoculum was directly loaded into the fermenter from cultivation vessel using a peristaltic pump in a volume of 50 mL. Cultivation took 72 hours with controlled values of following parameters including (i) atmospheric pressure, (ii) air humidity of at least 80%, (iii) free aeration and venting, (iv) speed of fermenter stirrer of 100 revolutions per minute), and (v) temperature of 34.0°C. pH was maintained by periodic adding medium through a peristaltic pump of 20% sodium hydroxide solution. The readings of the built-in pH sensor and sampling for laboratory analysis were recorded immediately before the start of incubation and after 12 hours, 24 hours, 48 hours and 72 hours. Before the analysis, these samples were purified from microorganisms and dispersed components of the medium using a filter with a pore diameter of 0.2 microns. Four more fermentations of each series (28 samples), we observed the same conditions in vessels containing 93 mL of the corresponding whey and 3 mL of inoculum on an orbital shaker thermostat.

To construct growth curves, we incubated samples on 96-well plates, while each well completely repeated the composition of one of the "large" samples. The data on the optical density of the medium obtained every 3 hours, and every 6 hours after 12 hours of fermentation were used for calculations. The study was performed on an iMark microplate analyzer (Bio-Rad Laboratories Inc., USA), while the initial value of optical density at a wavelength of 600 nm (OD600) was standardized to 0.1 (17). The specific growth rate [(μ as 10^8 cells/(L×h)] was determined by the

following formula: $\mu=d\ OD_{600}/(dT\times OD_{600})$, where dN was the maximum difference in optical density per unit time during the exponential growth phase and dT was the time between these measurements.

The concentration of lactose and ethanol in culture medium was determined enzymatically on a SmartSpectPlus spectrophotometer (Bio-Rad Laboratories Inc., USA) using ready-made kits manufactured by R-Biopharm AG (Germany) and Stereoglass S.r.l. (Italy), respectively. The results were expressed in g^{-1} L^{-1} . The final fermentation parameters were calculated based on the quantitative data for each strain that were percentage of processed lactose (LP, %); average rate of lactose processing in 72 hours (RL_{72}, g^{-1} L^{-1} h^{-1}); the average rate of ethanol formation in 72 hours RE72, g^{-1} L^{-1} h^{-1}) and fermentation efficiency, which was calculated using the following formula (18): $E_f=185.9\times E_{72}/L_0$, where coefficient of 185.9 summarized expression of theoretical maximum output during the formation of ethanol from lactose; E_{72} was the ethanol concentration after 72 hours of fermentation, and L_0 was the lactose concentration in the initial medium. In the Lc1 series, additional lactose portions were considered to maintain the consistency of its concentration in cultural medium, which ranged from 20.4 g to 22.8 g of lactose per 1 liter during incubation.

The quantitative results were processed and graphically visualized using the Statistica 12.0 software package (StatSoft Inc., USA). The analysis of variance was carried out for all quantitative samples obtained in the work. Since the Kolmogorov criterion established a nonparametric distribution in all cases, the statistical description was presented in the format of the median and the interquartile range as Me, Q1÷Q3. Mann-Whitney criterion was used to determine the statistical significance of the differences. Intra-group comparisons were performed using the Kraskel-Wallis criterion, and comparisons between groups using the Mann-Whitney criterion. The differences were considered statistically significant at $p<0.05$.

Results

Figure 1 shows the dynamics of the number of cells during cultivation under controlled conditions on a medium containing whey of the initial composition. It reflects the phase character of yeast growth in culture; while the lag phase lasted 8-12 hours, the exponential growth phase was followed by 16-24 hours of fermentation. By 36 hours of cultivation, all strains showed signs of steady growth, and by 60 hours, *K. marxianus* culture had entered the stationary growth phase. The total increase in cell mass over 72 hours ranged from 3.4×10^8 cells/L in *K. lactis* Y-2037 to 4.9×10^8 cells/L in *K. marxianus* Y-2042. The calculated specific growth rate of *K. lactis* Y-2035, Y-2037, and *K. marxianus* Y-2042 yeasts was 0.78×10^8, 0.10×10^8, and 0.14×10^8 cells/(L×h), respectively.

Table 2 presents quantitative data on changes in lactose and ethanol concentrations under these cultivation conditions. It is clearly seen that all the yeast strains were able to process lactose to one degree or another, and its concentration decreased markedly starting from the 12th hour of fermentation. Within 72 hours, *K. lactis* Y-2035 strain converted 87.5% lactose in unchanged whey, *K. lactis* Y-2037 strain converted up to 89.9%, and *K. marxianus* Y-2042 converted up to 82.7%. Fermentation of lactose in whey in all cases was accompanied by the formation of bioethanol, the concentration of which in the medium by 72 hours of the experiment was near 20.0 g^{-1} L^{-1} .

Table 3 shows the results of variation in lactose concentration during cultivation. In the Lc1 series, constant feeding of the culture medium with a whey concentrate containing 120 g^{-1} L^{-1} of lactose led to its concentration remaining close to the initial one. During 72 hours of fermentation, lactose was additionally fractionated into the whey with *K. lactis* Y-2035, Y-2037 and *K. marxianus* Y-2042 in a total amount of 22.8 g, 25.3 g and 20.4 g per 1 liter of medium, respectively. As a result of applying this technology, the total volume of lactose processing increased by 1.58-1.66 times. These changes led to the share of lactose processing, which increased by just over 4% for all studied strains. Under conditions of maintaining the lactose concentration near 42.5 g^{-1} L^{-1} , the ethanol production increased in all yeast cultures, exceeding similar values in the N series by 1.44-1.54 times. The fermentation efficiency in *K. lactis* Y-2035 and Y-2037 cultures remained virtually unchanged using this technology, while *K. marxianus* Y-2042 yeast showed a 16.2% increase in fermentation efficiency.

The usage of whey with 85.0 g^{-1} L^{-1} of lactose led to a proportional increase in the total amount of its processing, but it had virtually no effect on other fermentation parameters. The final lactose concentrations decreased in the same proportion after 72 hours of fermentation as in the N series, and

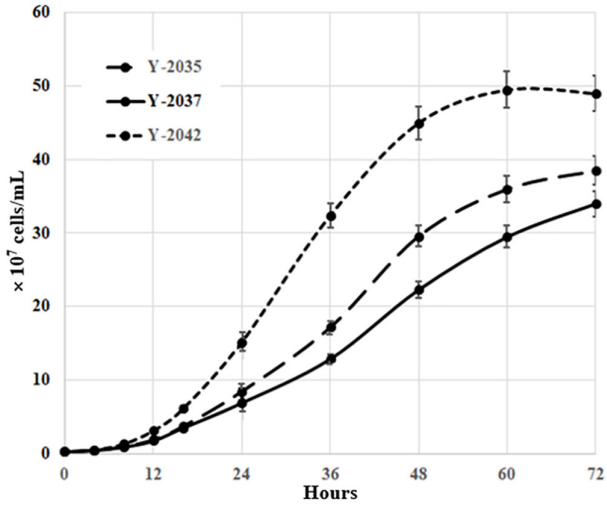

Figure 1: Growth curves of *K. lactis* Y-2035, Y-2037 and *K. marxianus* Y-2042 during cultivation in a whey-based medium on 96-well microplates.

Table 2: Changes in lactose and ethanol concentration during cultivation in unchanged whey-based medium.

Time, h	Strain		
	***K. lactis* Y-2035**	***K. lactis* Y-2037**	***K. marxianus* Y-2042**
Lactose, g-1 L-1			
0	42.5 (40.7÷44.9)		
12	29.4 (27.7÷30.8)*	25.5 (24.2÷26.3)*	32.8 (29.0÷35.3)*
24	13.0 (11.7÷14.4)*	11.2 (10.0÷12.5)*	15.7 (14.1÷17.5)*
48	9.3 (8.4÷10.4)*	7.1 (6.7÷7.9)*	10.9 (9.6÷11.4)*
72	5.3 (4.7÷5.8)*	4.3 (3.9 ÷ 4.8)*	7.3 (6.6÷8.1)*
Ethanol, g-1 L-1			
0	0.4 (0.2÷0.5)		
12	4.7 (4.3÷5.2)*	6.5 (6.0÷7.2)*	4.9 (4.3÷5.4)*
24	12.3 (10.9÷13.8)*	13.7 (12.1÷15.4)*	10.2 (9.2÷11.4)*
48	17.6 (15.6÷19.8)*	19.0 (16.9÷21.3)*	14.5 (13.1÷15.9)*
72	19.8 (17.4÷21.2)*#	20.9 (19.3÷22.6)*	17.1 (15.7÷18.3)*

* and # indicate statistically significant differences with the initial values before fermentation.

Table 3: Characteristics of lactose processing and ethanol production in a whey-based medium with varying lactose concentration.

Parameter	Strain		
	***K. lactis* Y-2035**	***K. lactis* Y-2037**	***K. marxianus* Y-2042**
Maintaining lactose concentration near 42.5 g-1 L-1 (Lc1 series)			
Lactose, g-1 L-1	40.8 (38.5÷42.1)#	39.7 (37.9÷41.6)#	41.2 (39.4÷42.2)#
Processed lactose, %	91.3 (85.8÷94.9)	93.7 (88.5÷96.2)	88.2 (83.0÷91.1)#
Ethanol, g-1 L-1	28.6 (25.3÷31.4)#	32.1 (29.7 ÷ 34.6)#	25.0 (22.8÷27.3)#
Fermentation efficiency, %	88.6 (82.6÷91.2)	94.0 (88.5÷97.0)	85.4 (80.4÷87.1)#
Whey with lactose concentration of 85.0 g-1 L-1 (Lc2 series)			
Lactose, g-1 L-1	8.7 (8.1÷9.2)#	6.9 (6.5÷7.4)#	13.1 (12.3÷13.9)#
Processed lactose, %	89.8 (84.7÷93.8)	91.9 (86.2÷92.6)	84.6 (79.8÷88.9)
Ethanol, g-1 L-1	37.1 (33.5÷39.8)#	39.5 (35.8÷42.6)#	34.5 (31.2÷37.1)#
Fermentation efficiency, %	90.6 (84.5÷93.3)	94.2 (88.6÷97.1)	90.7 (84.2÷93.9) #

# indicates statistically significant differences.

the percentage of processed lactose did not change for all studied strains. In this series, an increase in the final ethanol concentration was recorded by 1.87-2.02 times. The fermentation efficiency increased slightly in *K. lactis* Y-2035 and Y-2037 cultures and increased 1.21-fold in *K. marxianus* Y-2042; while its activity was maximally dependent on the concentration of lactose.

Table 4 presents data about the value of protein content on whey processing. A decrease in whey protein concentration to 3.2 g^{-1} L^{-1} was accompanied by an alteration of fermentation parameters due to all yeast strains usage, but the differences were no more than 3% and they were considered insignificant. In comparison with the series, the protein whey concentration was 6.4 g^{-1} L^{-1}. A less intensive ethanol production was observed. By 72 hours, its concentration was lower in the culture of *K. lactis* Y-2035 by 3.6 g^{-1} L^{-1} , in the culture of *K. lactis* Y-2037 by 3.1 g^{-1} L^{-1} , and in the culture of *K. marxianus* Y-2042 by 4.5 g^{-1} L^{-1} . These changes led to a natural decrease in the percentage of processed lactose by 4.1-4.5%.

Cultivation in deproteinized whey resulted in 1.9-2.8 g^{-1} L^{-1} more lactose remaining in the medium than at the initial protein content of 6.4 g^{-1} L^{-1} (N series). Accordingly, the percentage of processed lactose decreased by 4.0% in the culture of *K. lactis* Y-2035, by 4.7% in the culture of *K. lactis* Y-2037, and by 6.6% in the culture of *K. marxianus* Y-2042. Ethanol production decreased in these cultures strongly by 2.2-3.6 g^{-1} L^{-1} , fermentation efficiency was lower by 15.9%, 13.7% and 9.7%, respectively.

Table 5 reveals the dependence biotechnological parameters on the zinc ion concentration. When we added zinc ions to the whey at 4.0 mM/L, there was slight raising lactose utilization, and it was somewhat more noticeable in the culture of the *K. marxianus* Y-2042. An increase in the concentration of zinc ions by 8.0 mM/L caused large changes in amplitude. The amount of converted lactose increased by 1.6-2.1 g^{-1} L^{-1} , depending on the cultivated yeast strain, the percentage of processed lactose increased by 4.5% in *K. lactis* Y-2035, by 3.7% in *K. lactis* Y-2037 and by 5.1% in *K. marxianus* Y-2042.

Table 4: Characteristics of lactose processing and ethanol production in a whey-based medium with varying protein concentration.

Parameter	Strain		
	***K. lactis* Y-2035**	***K. lactis* Y-2037**	***K. marxianus* Y-2042**
Whey with protein concentration of 3,2 g-1 L-1 (Pt1 series)			
Lactose, g-1 L-1	5.9 (5.3÷6.4)	4.7 (4.2÷5.0)	8.4 (7.8÷8.9)
Processed lactose, %	86.1 (81.1÷91.4)	88.9 (83.6÷92.7)	80.2 (75.9÷85.5)
Ethanol, g-1 L-1	19.7 (17.9÷21.0)	20.8 (18.7÷22.2)	16.3 (15.0÷17.7)
Fermentation efficiency, %	86.4 (82.2÷91.5)	91.1 (89.0÷97.4)	71.3 (65.8÷73.1)
Deproteinized whey (Pt2 series)			
Lactose, g-1 L-1	7.6 (7.0÷8.3)#	6.2 (5.7÷6.8)#	10.1 (9.0÷10.9)#
Processed lactose, %	82.1 (78.0÷86.9)	85.4 (80.2÷89.7)	76.2 (72.7÷80.3)#
Ethanol, g-1 L-1	16.2 (14.8÷17.5)#	17.8 (16.3÷19.2)	14.9 (13.7÷16.1)#
Fermentation efficiency, %	70.9 (64.5÷75.8)#	77.9 (82.3÷93.1)#	65.2 (61.8÷70.3)#

# indicates statistically significant differences.

Table 5: Characteristics of lactose processing and ethanol production in a whey-based medium with varying zinc ion concentration.

Parameter	Strain		
	***K. lactis* Y-2035**	***K. lactis* Y-2037**	***K. marxianus* Y-2042**
Maintaining lactose concentration near 42.5 g-1 L-1 (Lc1 series)			
Lactose, g-1 L-1	4.5 (4.2÷4.9)	3.7 (3.3÷4.0)	6.5 (5.9÷7.0)
Processed lactose, %	89.4 (83.1÷92.0)	91.3 (84.8÷94.2)	84.7 (78.5÷89.9)
Ethanol, g-1 L-1	20.2 (18.3÷21.9)	21.1 (19.1÷23.2)	18.3 (16.5÷19.8)
Fermentation efficiency, %	88.5 (82.1÷93.6)	92.1 (86.4÷96.0)	80.2 (72.3÷86.5)#
Whey with lactose concentration of 85.0 g-1 L-1 (Lc2 series)			
Lactose, g-1 L-1	3.4 (3.1÷3.6)#	2.7 (2.5÷3.0)#	5.2 (4.8÷5.5)#
Processed lactose, %	92.0 (85.3÷95.1)	93.6 (87.6÷95.9)	87.8 (81.4÷90.2)
Ethanol, g-1 L-1	20.9 (19.1÷22.4)	21.4 (19.2÷23.3)	20.4 (18.5÷22.1)#
Fermentation efficiency, %	91.6 (85.9÷95.5)	94.8 (87.3÷97.6)	89.4 (83.2÷94.8)#

# indicates statistically significant differences.

The ethanol concentration after three days of whey fermentation with the addition of 4.0 mM/L zinc ions did not differ statistically from one in the unchanged whey for all strains of studied yeast, although the fermentation efficiency of the *K. marxianus* Y-2042 strain increased by 5.3%. In the series with the concentration of zinc ions by 8.0 mM/L, the raising of ethanol production became more noticeable, fermentation efficiency increased in *K. lactis* Y-2035, Y-2037 and *K. marxianus* Y-2042 cultures by 4.8%, 3.2% and 14.5%, respectively.

Discussion

Dairy production has a global spread and its ingredients and biotechnological characteristics are of great importance in health status of the consumers (19-21). Even though yeast *Saccharomyces spp.* is by far the leaders in modern biotechnology (1), in case of working with dairy products and processing dairy waste, we are increasingly turning to representatives of the genus *Kluyveromyces*, which have valuable sets of characteristics and properties for this (22-24). The results obtained by testing selected *K. lactis* strains showed their high ability to utilize lactose and convert part of this product into ethanol. According to the values of key indicators of the biotechnological process, these data turned out to be like the results of previous studies with other yeast strains (25-27).

To assess the effectiveness of fermentation, we calculated the utilization rate of the main substrate and the formation rate of final product. These results were demonstrated in Figure 2. The calculation of the rate of lactose utilization and rate of ethanol formation showed that they were determined for *K. lactis* strains around 0.7 and 0.4 g^{-1} L^{-1}, respectively. The fermentation efficiency of these strains was 86.8% for the *K. lactis* Y-2035 and 91.6% even more for *K. lactis* Y-2037. The strain of *K. marxianus* Y-2042 with fermentation efficiency of 74.9%, turned out to be less productive.

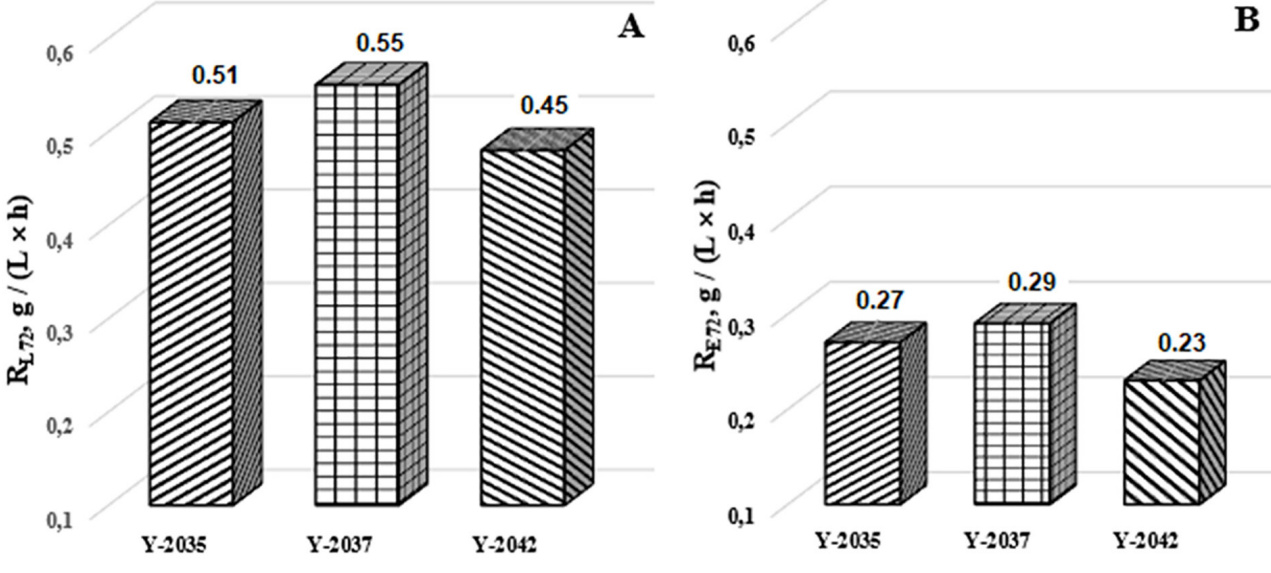

Figure 2: The rate of lactose utilization (A, R_{L48}) and ethanol synthesis (B, R_{E48}) during cultivation of *K. lactis* and *K. marxianus* strains for 72 hours in a whey-based medium.

Evaluation of the fermentation efficiency, as expected, showed for lactose to be incompletely converted into ethanol during processing. As is known, almost half of lactose must be oxidized for energy purposes, and the maximum theoretical ethanol output by weight in this process is only 0.538 (18). Since the task of ecological biotechnology in our case was lactose oxidation;, while the question of the amount of ethanol produced in this case is to some extent secondary. The fermentation process is subject to many factors that can significantly affect the conversion of carbohydrates to bioethanol by a particular yeast strain. The most important of these factors are cultivation conditions, primarily the composition of whey, the presence of ecotoxicants, growth stimulants, temperature, pH, and aeration. Optimization of these parameters can significantly increase ethanol output (24, 28).

The concentration of lactose in the medium is an important factor for its conversion. The used concentration of slightly more than 40 g-1 L-1 proved to be quite sufficient for the utilization of 82.7% lactose (*K. marxianus* Y-2042) to 89.9% (*K. lactis* Y-2040), although concentrations up to 100 g-1 L-1 are currently used to increase the efficiency of bioconversion and ethanol output (27). Since the present study we have positioned ourselves as ecological biotechnology for the purification of whey from lactose, out of economic reasons, we decided to try to abandon the expensive procedures for protein isolation and whey concentration, leaving the native lactose content in it.

The presence of proteins in the whey is important for the vital potential and enzymatic activity of yeast. This process may become particularly important if the goal of whey processing is not only to utilize lactose, but also to give the whey the properties of potential raw materials for manufacturing functional beverages with a high content of amino acids and bioactive peptides (29). In frequent cases when initially deproteinized whey is used as a raw material, certain changes must be made to the cultivation protocol (30).

Naturally, the dynamics of protein utilization, changes in the peptide and amino acid content of whey and yeast enzymatic systems because of proteolysis deserve a separate detailed study and discussion, which, unfortunately, is beyond the scope of this article. Zinc is known to be an important element for the normal growth, metabolism, and physiology of yeast. In addition to acting as a cofactor for many enzymes, zinc is also essential for the structural stability of zinc finger proteins, many of which have important effects on cellular metabolic processes (31, 32). Recent results demonstrated the involvement of zinc finger proteins in cellular stress responses, with an emphasis on increasing yeast stress tolerance in bioethanol production (10).

During cultivation, in addition to biological threats of contamination, yeast faces several types of stresses, including nutrient intake with an increase in the amount of ethanol and other fermentation products (33-35). On the one hand, extreme values of temperature, osmotic pressure, and unusual concentrations of trace elements can have a negative effect on the entire fermentation process, and at the same time, they can be used as stimulators of reproduction, growth, and targeted synthetic activity of yeast cultures (36). One of the ways to solve this biotechnological problem is to use strains with initially high resistance to extreme influences (37).

Most certain and nearest approaches provided methods of classical and molecular selection, genetic engineering in the form of transgenesis or genomic editing to improve the converting ability of yeast strains with respect to lactose (38, 39). The main tasks that will have to be solved in this case are effective protection against contamination, increasing the activity of key lactose delivery enzymes into cells and bioconversions, as well as increasing resistance to inhibitory substances, including the main product of ethanol. In general, this study highlighted the importance of studying *Kluyveromyces* yeast as one of the most suitable biological objects for the disposal of lactose-containing waste from the dairy industry and, to a certain extent, for bioethanol production. Environmental friendliness, energy security and economic factors will support the interest of dairy producers in this biotechnology in the future.

Conclusion

The conducted study provided evidences that modern strains of *Kluyveromyces spp.* can dispose 82.7% to 89.9% of lactose from cheese whey for 72 hours at a controlled temperature of 34°C, stirring and aeration in order to form 17.1-20.9 g^{-1} L^{-1} of ethanol. The fermentation efficiency in this process ranged from 74.9% to 91.6%. The lactose feeding regime as a two fold increase in its concentration in whey, or additional administration of zinc ions could increase the efficiency of lactose conversion to ethanol, while cultivated in a low-protein environment or it was completely removed from whey that worsened the biotechnological characteristics of fermentation. Yeast culture *K. lactis* Y-2037 was the best studied strain, and its use was recommended for processing lactose-containing dairy waste to obtain valuable raw materials and/or reduce environmental pollution, as well as for the internal production of bioethanol.

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Authors' Contribution

K.ais S.A- original draft Writing - review and V.V.N editing, Writing -original draft, Methodology, and Kasim S.A - review and proofreading.

Conflict of Interest

The authors declare that there is no conflict of interest associated with this study.

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