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EFFECTS OF DIFFERENT LACTASES AND RATIOS ON THE PREPARATION OF LOW-LACTOSE PREBIOTIC LIQUID GOAT MILK

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ABSTRACT
Consumption of dairy products provides consumers with nutrients and is beneficial to human health, but lactose intolerance prevents some consumers from consuming dairy products. Using lactase to hydrolyze lactose and generate galactooligosaccharides (GOS) can not only avoid lactose intolerance but also increase the prebiotics of dairy products and improve the functionality of the product. This study investigated the effects of 14 lactases and their ratios on the generation of GOS and the hydrolysis of lactose using goat milk. It was found that there were significant differences in the effects of lactase on the synthesis of GOS and lactose hydrolysis. The rate of lactose hydrolysis (LHR) and GOS concentration are 4.70%-80.62%, and 0.82 g/L-12.60 g/L respectively. The best effect is achieved when lactase E10 and E5 are in a ratio of 4:1, The GOS concentration was 14.587±0.20 g/L and the LHR was 91.880±0.01%, respectively.

1. Introduction

When it comes to dairy products, goat milk is the most like breast milk. with the most complete nutrition and the most easily absorbed by the human body. The fat particles of goat milk are one-third of the volume of milk, which is more conducive to human absorption, and long-term moderate drinking of goat milk will not cause weight gain. In addition, the vitamins and trace elements in goat milk are significantly higher than in cow milk. However, a large proportion of people suffer from “lactose intolerance” and cannot effectively break down lactose in the body, they cannot drink goat milk. In Africa and South America, more than half the population is lactose intolerant, and the impact is more pronounced in some Asian countries, where lactose intolerance affects almost 100% of the population. (Garcia et al., 2011).

At present, there are four main methods to reduce lactose content in dairy products, namely, physical removal, chemical acid hydrolysis, genetic engineering technology, and enzymatic hydrolysis. The physical removal method and chemical acid hydrolysis method use membrane technology and high-temperature hydrolysis of strong acid and alkali respectively, which are lower in cost, but will lose nutrients in milk, so these two methods are used less. Transgenic technology has developed a new idea for the research of low-lactose dairy products. By connecting the cDNA encoding the precursor of intestinal lactase to the egg cell, the mammals that produce low-lactose liquid milk can be cultured without affecting the protein level in the milk. At present, this method is still in the process of exploration. (Liang, 2013). Enzymatic hydrolysis is to decompose lactose into glucose and galactose by adding exogenous lactase. This method can prevent the loss of other nutrients in the milk, not only that, due to the increase of glucose content in the milk to increase the sweetness, glucose, and galactose are more easily absorbed by the human body, so the enzymatic hydrolysis method is currently the main processing technology for processing low-lactose dairy products.

The products of lactose hydrolysis are glucose and galactose on the one hand, glucose is an energy source that can be absorbed directly by the body, so more easily converted into energy for the human body (Wang, 2009); Galactose, is the other hand, it is an essential monosaccharide for the body's brain and mucous membranes (Sun et al., 2004). Moreover, glucose and galactose can be combined into GOS by transglycosidation using lactase (Huang et al., 2020). GOS can reduce the secretion of inflammatory factors and relieve inflammation.

GOS is the most common prebiotic. The use of prebiotics can control the balance of intestinal flora and produce short-chain fatty acids that facilitate intestinal transport (MDSL S A et al., 2014). Butyric acid, propionic acid, and short-chain fatty acids have anti-cancer properties, these three compounds are the primary byproducts of the GOS process in the body (Cummings et al., 1981).

With the vigorous development of the dairy industry in recent years, many hydrolyzed lactose products have been developed at home and abroad, such as yogurt, buttermilk, cheese, milk, fermented milk, and so on (Jelen et al., 2003). Some of the lactose-rich whey produced in the production of cheese is discarded, causing environmental problems (Pesta et al., 2007). Some studies have shown that GOS can be produced by enzymolysis of whey by lactase, which can turn waste into treasure (Nedim et al., 2002). So now more and more research are to synthesize GOS by using milk or by-product whey and whey permeate. However, there are relatively few studies on the synthesis of GOS from goat milk products.

At present, most of the experiments at home and abroad use a single lactase to decompose lactose to synthesize GOS. The LHR varies greatly under different experimental conditions, but the change in GOS production is not much. So, in this study, different complex lactase enzymes were selected to explore their combined effects on lactose hydrolysis and GOS production in goat milk (Wang et al., 2006; Zhang et al., 2008)

In our previous study, the effects of 14 commercially available lactose enzymes on the preparation of enzymatic hydrolyzed goat milk were studied and the optimal enzyme ratio was improved (Xu et al., 2021). In this study, we used the same enzyme preparation to investigate the effects of its type and ratio on the LHR in goat milk and the production of GOS, providing a guide for the preparation of prebiotic goat milk.

2. Materials and methods

2.1.Experimental raw materials and handling The raw material used in the experiment is full-fat sheep milk powder from the laboratory. Then the raw materials are treated and rehydrated at the ratio of 1:8, after sterilization, a certain amount of lactase is added for enzymatic hydrolysis, and then the enzyme is removed and cooled, and finally, the

Enzymatic hydrolyzed goat milk required for the experiment is obtained.

2.2.Lactase

14 commercially available lactose enzymes from previous experiments were selected for this study (Xu et al., 2021).

2.3.Screening of different types of lactases

Considering the cost of the experiment, after the milk was sterilized 65 ℃ for 20 minutes,14 lactase enzymes were added to goat milk at 0.1% each, enzymolysis at 40℃for 3h, and then enzymolysis at 100℃ for about ten minutes. After cooling, the content of glucose, galactose, and lactose in enzymatically hydrolyzed goat milk could be determined, then the LHR and the galactose oligosaccharide could be calculated. It was selected as a lactase that hydrolyzed lactose readily and contained a high level of GOS.

2.4.Matching Optimization of enzymatic hydrolyzed goat milk

Based on the results of lactase screening, the influence of complex enzymes on the content of GOS and the LHR was studied, and the ratio of complex enzymes was determined by mixing experiment design.

2.4.1.Measurement of galactose and lactose content

The galactose and lactose content after the addition of lactase to goat milk is usually based on a lactose/galactose test kit. It is based on the principle that in a weak acid environment (pH of about 6.6), Glucose and D-galactose are produced when lactase hydrolyzes lactose. Then, in a weak base environment (pH of about 8.6), D-galactose reacts with galactose dehydrogenase (Gal-DH) and coenzyme I to give galacturonic acid and coenzyme II. Therefore, the amount of coenzyme II corresponds to the amount of lactose and galactose to a certain extent, and its absorbance can be determined by a spectrophotometer at 340 nm.

2.4.2.Measurement of glucose content

While glucose in goat milk by biosensor analyzer. The measurement process is as follows: firstly, a water filter membrane of 0.2 µm is used to filter the samples which are the pure goat milk and goat milk added with lactase, and then a glucose standard solution of 1 g/L is used to calibrate the biosensor. Finally, Glucose concentration was calculated by adding 25 g/L of the filtered filtrate to the biosensor. The samples were verified by repeated experiments.

2.4.3.Measurement of LHR and galactose oligosaccharide content

The LHR can be computed based on the change in lactose quantity before and after the comparison of lactase enzymolysis through the determination methods of lactose, galactose, and glucose mentioned above. In line with the conservation of mass concept, the quantity of glucose, galactose, and galactose oligosaccharides generated is equal to the amount of lactose ingested, in other words, the quantity of galactose oligosaccharide is comparable to the amount of lactose consumption subtract the amount of growth of galactose and glucose. The LHR and galactose oligosaccharide were calculated according to previous experiments (Xu et al., 2021).

2.5.Color Measurement

The color difference of goat milk is usually determined using CIE color analysis (International Commission on Illumination) which utilizes the spectrophotometer Minolta CM-5. Before analyzing the sample, a whiteboard must be used to zero and calibrate the sample. During the measuring process, the average value is determined three times. The distinction in color between the experiment and the blank group is denoted by ΔE. ΔE can be calculated according to previous experiments (Xu et al., 2021).

2.6.Organoleptic evaluation of liquid goat milk

According to Jing and Liu's (2020) method, the color, tissue state, odor, and taste of the enzymatically hydrolyzed goat milk were organoleptically evaluated.

3. Results and discussions

3.1.Analysis of GOS and LHR from lactose hydrolysis by different lactase

As shown in Figure 1, the lactase type has a noteworthy impact on the GOS content and LHR in goat milk, and the outcomes differ according to the enzyme. In the figure, the LHR ranged from 4.696% to 80.617%, and GOS content ranged from 0.823 g/L-12.599 g/L. Among them, 5 LHRs were higher than 50%, which were E11 (54.834%), E13 (56.630%), E14 (69.635%), E5 (71.755%), E10 (80.617%). The LHR of the remaining 9 lactase enzymes were all lower than 50%, and the lowest one was E2 (only 4.696%). There were 4 kinds of galactooligosaccharides with content higher than 6 g/L, namely E11 (6.146 g/L), E9 (8.356 g/L), E10 (10.421 g/L), and E14 (12.599 g/L). The other ten enzymes had GOS contents less than 6 g/L, and the lowest was E2 (0.823 g/L). The GOS content and LHR are not always positively correlated, as can be seen from the comparison. For example, the GOS concentration of E10 is only 10.421 g/L, despite the LHR being 80.61%, suggesting a low glycoside transfer efficiency and a high enzymatic hydrolysis efficiency. On the contrary, even though E14's LHR is just 69.635%, enzymatically hydrolyzed goat milk has a 12.599 g/L GOS concentration, suggesting a poor level of enzymatic hydrolysis efficiency, Transglycoside efficiency is high.

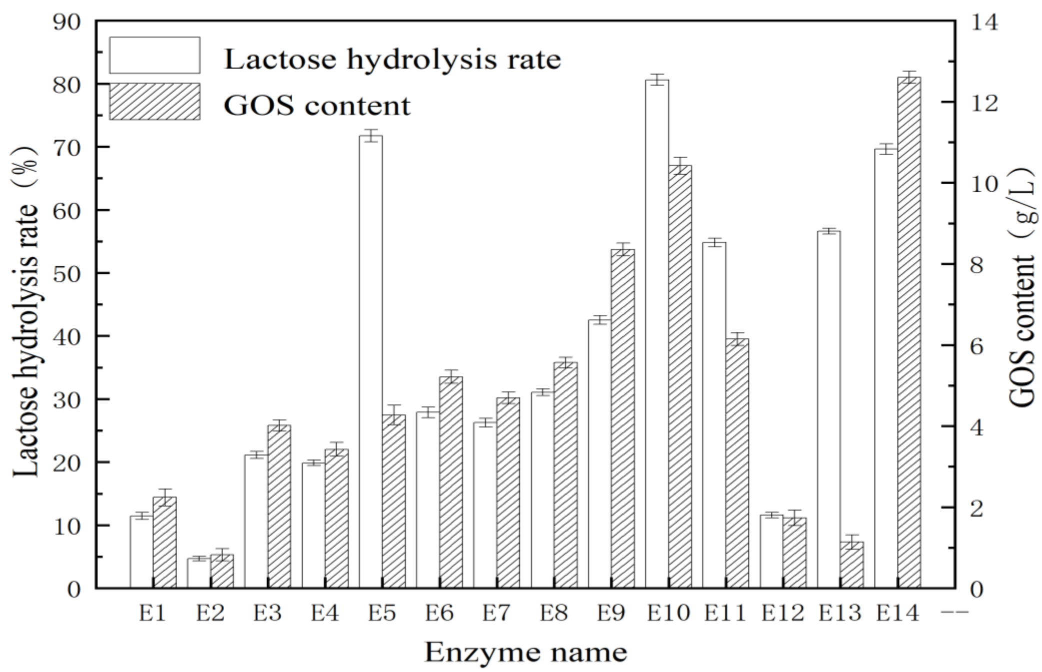

Figure 1. The impact of various lactase types on the LHR and the amount of GOS in goat milk

As can be seen from Table 1, contrasted with the goat milk's control sample color value (L=93.39±0.02, a=-2.10±0.02, b=7.1±0.03), the variation in color of the enzymatically hydrolyzed goat milk had significant changes: the milk color became darker, and most of the milk was reddish and yellow. Among them, the E8 enzymolysis milk's color became dark and yellowish green, and the E12 enzymolysis milk's color became dark and bluish green, indicating that different types of lactases possess varying impacts on the color value of goat milk. The amount that the color number is off-center from low to high is L* (92.96~90.07), a*(-1.19~-2.43), b* (8.00~5.18). The appearance of color difference is due to the different types of lactases leading to changes in the color parameters of goat milk, and then leading to changes in the color of goat milk. The overall color difference (ΔE), can be computed, as indicated by formula 3. At ΔE =1, the minimum color variation that is discernible to the human eye is present., which is five times the threshold for visual identification. Identify it as 1NBS. The difference between the two colors is more noticeable the larger the ΔE; on the other hand, the difference is less noticeable. If it is in the range of 0 to 0.5 NBS, it means that there is little to no color variation between the two colors; If it is between 0.5 and 1.5 NBS, it indicates a small difference. If it is between 1.5-3NBS, the color distinction between the two colors can be clearly distinguished. When the color difference is greater than 3.0NBS, The most noticeable distinction between the two colors (Chen et al., 2013).

Table 1. The impact of various lactases regarding the hue of goat milk that has been hydrolyzed enzymatically.

Enzyme	L*	a*	b*	ΔE
E1	92.77±0.01	-1.80±0.01	7.3±0.03	0.72
E2	90.23±0.18	-2.43±0.01	7.44±0.10	3.19
E3	91.66±0.02	-1.19±0.01	7.98±0.03	2.15
E4	90.31±0.04	-2.47±0.01	8.00±0.03	3.23
E5	92.65±0.02	-1.71±0.07	7.37±0.034	0.87
E6	92.38±0.01	-1.53±0.01	7.65±0.02	1.29
E7	92.87±0.01	-1.83±0.01	6.99±0.01	0.59
E8	91.48±0.80	-2.12±0.13	7.54±0.02	1.96
E9	90.07±0.01	-2.25±0.02	6.73±0.39	3.34
E10	92.96±0.01	-1.98±0.01	7.20±0.01	0.46
E11	92.60±0.01	-2.10±0.01	7.51±0.04	0.89
E12	91.26±0.07	-2.38±0.01	5.18±6.38	2.88
E13	92.48±0.11	-2.12±0.03	6.77±0.16	0.97
E14	92.87±0.01	-1.97±0.01	6.85±0.14	0.59

Figure 2 shows that the color variation of goat milk is significantly influenced by the kinds of lactase. The variation in milk color caused by lactase enzymolysis ranges from 0.46NBS to 3.34NBS, among which E12 (2.88NBS), E3 (2.15NBS), and E8 (1.96NBS) have entire color disparity between 1.5NBS and 3.0NBS, respectively. The total chromatic difference of E9 (3.34NBS), E4 (3.23NBS), and E2 (3.19NBS) exceeded 3.0NBS, respectively, and the remaining eight lactases had chromatic differences of less than 1.5NBS.

From Figure 3, it is evident that the organoleptic assessment of goat milk is significantly influenced by the kind of lactase, and the sensory scores of enzymolysis goat milk range from 38.5-69.5, in which the organoleptic evaluation score with the greatest value is E10 (69.5±2.12), while the lowest score is E2 (38.5±3.54).

Among them, E2, E4, E8, E9, and E12 enzymatic hydrolyzed goat milk tastes bitter, this could be connected to the way that proteins in liquid goat milk are hydrolyzed by enzymes. (Wang et al., 2020). Pure goat milk is less sweet than goat milk made with additional lactase enzymes, which should be due to the increased sweetness of lactose after hydrolysis to produce glucose.

In summary, the enzymolysis effect of lactase on goat milk was summarized, the suitable lactases are E10, E5, E14, and E13 in order, these enzymatic hydrolysis of goat milk had higher GOS content and LHR, had less impact on organoleptic evaluation and color.

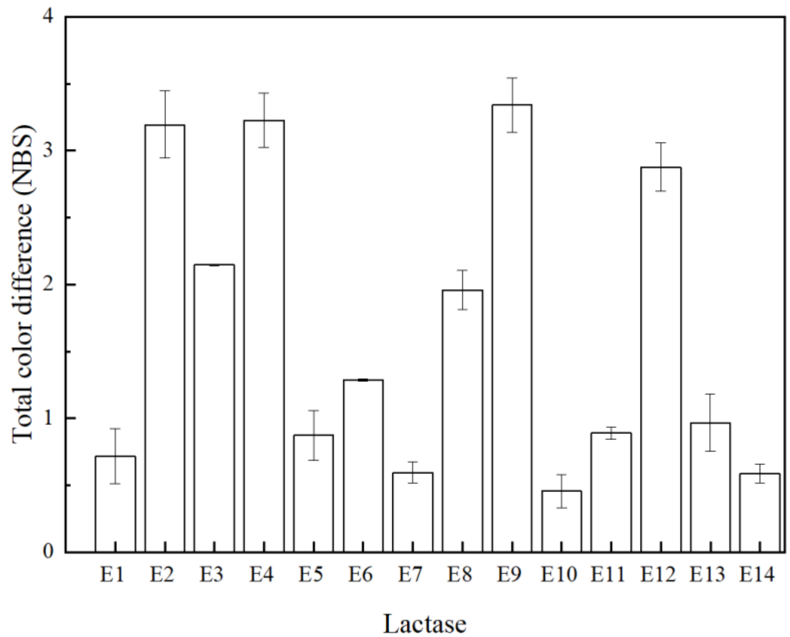

Figure 2. The impact of various lactase types on color ΔE of goat milk

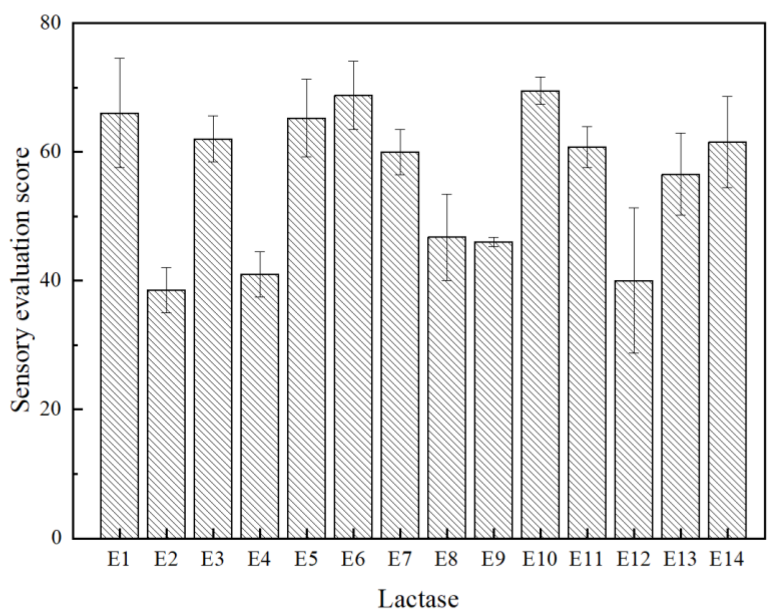

Figure 3. The organoleptic evaluation of goat milk after enzymolysis by different lactase types

3.2.Effects of different lactase combinations on GOS production from hydrolyzed lactose

Based on the test findings mentioned above, lactase E10, E5, E14, and E13 with good enzymolysis ability were selected, and the additive quantity of mixed lactase was established at 0.06%. The test findings are shown in Table 2.

As can be seen from Table 2, different lactase combinations have a noteworthy impact on the GOS content and LHR in goat milk. The GOS content varies from 12.237g /L-14.667 g/L and the LHR of goat milk varies from 69.583%-85.000%. The LHR from low to high were E10+E14 (69.583%), E10 (74.738%), E10+E5+E13+E14 (78.750%), E10+E13 (81.250%), and E10+E5 (85.000%). Production of GOS from low to high were E10 (12.237g/L), E10+E14(12.298 g/L), E10+E5+E11+E14(12.697 g/L), E10+E5 (14.476 g/L), and E10+E13(14.667 g/L). It showed that the E10+E5 had greater hydrolysis and glycoside transfer efficiency than the others, while the hydrolysis efficiency of E10+E13 was lower than that of E10+E5, and its GOS content was similar to that of E10+E5. Therefore, E10 and E5 were selected as test compound enzyme preparations.

Table 2 The impact of combining lactases on degree of hydrolysis and GOS in goat milk

Enzymes	LHR（%）	GOS（g/L）
E100.06%）	74.738±0.001	12.237±0.020
E100.03%）+E5（0.03%）	85.000±0.007	14.476±0.014
E100.03%）+E13（0.03%）	81.250±0.001	14.667±0.010
E100.03%）+E140.03%）	69.583±0.002	12.298±0.013
E100.03%）+E5（0.01%）+ E13（0.01%）+ E14（0.01%）	78.750±0.001	12.697±0.018

Different compound experimental designs were used to optimize the lactase ratio

Table 3. The setup and outcomes of the goat milk hydrolysis experiment using lactases E5 and E10

NO	A(E10)	B(E5)	LHR(%)	GOS(g/L)
1	0.667	0.333	88.817	13.911
2	1	0	87.603	13.610
3	0.5	0.5	84.521	13.115
4	0.5	0.5	84.177	12.982
5	0.25	0.75	73.831	11.168
6	1	0	87.982	13.723
7	0.75	0.25	92.166	14.509
8	0	1	44.352	4.751
9	0.333	0.667	84.751	13.177
10	0	1	39.982	3.625

A mixture test was created using DesignExpertV8.0.6 to determine the ideal complex lactase ratio. The design and outcomes of the mixed experiment are displayed in Table 3 to Table 5.

Based on the test findings shown in Table 3, the quadratic regression equations of complex lactase (E10 and E5), LHR (Y1), and GOS content (Y2) were obtained by Design-Expert8.0.6. The quadratic regression equation is (1) and (2):

$$Y_1=86.81A+43.71B+87.61AB \quad (1)$$

$$Y_2=13.41A+4.57B+18.68AB \quad (2)$$

Table 4 demonstrates the significance of the regression equation and the no-significance of the missing fitting component, which suggests that the regression equation in place is well-fitted. The P in the table for the linear combination of influencing factors and AB is less than 0.0001, meaning that the influence on the rate of lactose hydrolysis of enzymolized goat milk is greater when E10 and E5 are raised in a linear proportion. The quadratic regression equation determination coefficient, or R2, is 96.82%, making it possible to use the model to forecast the rate at which complex lactase (E10+E5) Enzymatic hydrolyzed goat milk.

Table 5 demonstrates the significance of the regression equation and the no-significance of the missing fitting component, which suggests that the regression equation in place is well-fitted. The P in the table for the linear combination of influencing factors and AB is less than 0.01, meaning that the influence on the rate of lactose hydrolysis of enzymolized goat milk is greater when E10 and E5 are raised in a linear proportion. The quadratic regression equation determination coefficient, or R2, is 96.12%, making it possible to use the model to forecast the GOS content of goat milk hydrolyzed by complex lactase (E10+E5).

Finally, according to the examination of the regression equation, E10:E5=0.8:0.2 is the ideal complex lactase ratio. The GOS was 14.633 g/L, and the LHR was 92.208% under these circumstances. The confirmed GOS content and LHR was 14.587±0.20 g/L and 91.880±0.01%, and they were in close agreement with the estimates. The ideal lactase E10+E5 ratio found by the composite design is achievable, according to the data.

Dairy products are an important source of nutrients for the human body, providing more than 10 essential nutrients for maintaining blood health, nervous, immune systems, and promoting the growth and repair of various parts of the body. People with "lactose intolerance" are unable to efficiently break down lactose, which is the main sugar in dairy products, thus affecting the dairy industry. Therefore, reducing the amount of lactose in dairy products by hydrolyzing lactose with combined lactose enzyme.

Table 4 The LHR of goat milk by （E5+E10）was analyzed using variance

source	Degree of freedom	sum of square	Mean square	F	P	Significance
model	2	3114.53	1557.27	106.55	＜0.0001	***
Linear mixing	1	2193.5	2193.5	150.08	＜0.0001	***
AB	1	921.03	921.03	63.02	＜0.0001	***
error	7	102.31	14.62			
Lack of Fit	4	92.63	23.16	7.18		
Pure error	3	9.68	3.23			
sum	9	3216.84				

Table 5 The GOS content of goat milk by complex lactases （E5+E10）was analyzed using variance

source	Degree of freedom	sum of square	Mean square	F	P	Significance
model	2	134.11	67.06	86.68	＜0.0001	***
Linear mixing	1	92.25	92.25	119.25	＜0.0001	***
AB	1	41.86	41.86	54.12	0.0002	**
error	7	5.42	0.77			
Lack of Fit	4	4.77	1.19	5.51	0.0963	
Pure terror	3	0.65	0.22			
sum	9	139.53				

Lactase can also use glucose and galactose to synthesize GOS through glycoside transfer, which can reduce the secretion of inflammatory factors and relieve inflammation and can be digested and utilized by colon microorganisms. It is an important nutrient and immune regulatory substance and is intimately linked to the early growth and development of newborns and young children. The primary determinant of GOS content, according to Ruiz-Matutte et al (2012), was lactase's hydrolysis rate of lactose breakdown. When lactose has hydrolyzed 75–90% of it, the amount of GOS is gradually increased, and when the LHR exceeds 99%, the content of GOS begins to gradually decrease. The GOS content was the highest when the LHR 99.3%, and the lowest when the LHR was 99.9%.

At present, there are few studies on the decomposition of lactose in goat milk at home and abroad. Most studies are on the decomposition of lactose in milk by lactase, and the lactase used is single enzyme: It has been found that when the enzyme amount is 2500 U/L, the hydrolysis temperature is 40°C, and the hydrolysis temperature is 2h, the hydrolysis effect of lactose in milk is the best, and the hydrolysis rate is 74.50% (Wang et al., 2013); When the lactase dosage is 0.16%, the temperature is 45.3°C, and the pH4.95, the predicted hydrolysis rate of lactose in milk powder is 76.87% (Rong et al., 2019). These studies are based on single enzyme that changes the amount of addition, temperature, and other conditions. Therefore, this study first studied the effects of 14 lactase enzymes and their ratios on the production of LHR and GOS in goat milk, and screened out the more suitable enzymes: E10, E5, E14 and E13. Then, the compound lactase combination was applied to goat milk, and the compound experimental design was adopted to optimize the ratio and obtain the optimal ratio (E5: E10=1: 4), the GOS content was 14.587±0.20g/L, LHR was 91.880±0.01%, and the transglycosylation and hydrolysis rates were higher than those of single enzymes.

4. Conclusions

Using goat milk as raw material, the effects of 14 lactose enzymes and their ratio on LHR and GOS production were studied. A considerable difference was seen between the impact of various lactose enzymes on the GOS and LHR, with GOS ranging from 0.82 g/L to 12.60 g/L and LHR ranging from 4.70% to 80.62%. The selected E10, E5, E14, and E13 were more suitable than other enzymes. The best effect is achieved when lactase E10 and E5 are in a ratio of 4:1, the GOS content was 14.587±0.20 g/L and the LHR was 91.880±0.01%, respectively.

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