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SCREENING OF LACTASES SUITABLE FOR THE PREPARATION OF LOW-LACTOSE PREBIOTIC LIQUID MILK AND OPTIMISATION OF THEIR COMBINATION

– Research paper –

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ABSTRACT: Lactose intolerance is an important factor restricting the consumption of dairy products. Lactase is used to hydrolyze lactose in milk while generating galactooligosaccharides (GOS), thereby reducing the incidence of lactose intolerance. We used cow milk as raw materials, and selected enzyme preparations with high lactose hydrolysis rate and strong GOS generation ability from 14 commercially available lactase enzymes. The lactose hydrolysis rate is 5.85%-81.38%, and the GOS content is 0.03 g/L- 13.10 g/L. The mixing experiment design determined the two lactase enzymes (E10 and E11) ratio and the optimal enzymatic hydrolysis process of low-lactose prebiotic milk: compound lactases (E10:E11=0.756:0.244) addition 0.11%, 55℃ for 5h, lactose hydrolysis rate and GOS content were 98.02% and 19.69g/L, respectively, and the remaining lactose content was about 0.97 g/L.

Keywords: Lactase, cow milk, enzymatic hydrolysis, galacto-oligosaccharide, prebiotics

INTRODUCTION

Cow milk is the raw material for the main variety of dairy. Most of the dairy products available on the market are made from cow milk. The main components in milk are water, protein, fat, lactose, minerals, vitamins, enzymes, nucleosides and nucleotides, hormones and growth factors, etc. It contains all the nutrients known so far, and is the closest food to perfection. However, because a large part of the population suffers from "lactose intolerance", they cannot drink pure milk. Lactose intolerance worldwide affects more than 50% of the population in South America, Africa and Asia, and it also affects almost 100% of the population in some Asian countries. (Garcia H S et al., 2011). This makes these people unable to enjoy this natural nutrient, and to a certain extent also affects the consumption of dairy products.

In order to solve this problem, currently, lactase is mainly used to hydrolyze lactose to reduce the content of lactose in milk, which can achieve the purpose of preventing lactose intolerance, thereby increasing the number of consumers. Lactose is enzymatically decomposed into glucose and galactose. Glucose, as an energy source directly absorbed by the human body, is easier to supply energy. Galactose is a monosaccharide necessary for the human brain and mucosal tissues and is also closely related to infant brain development (Zhang et al., 2004). In addition, lactase can also synthesize galacto-oligosaccharide (GOS) by using glucose and galactose after enzymatic hydrolysis of lactase by transglycosidation (Huang et al., 2020), GOS could also be used as a value-added factor of human intestinal flora to prevent diarrhea and constipation to a certain extent. It can also improve the quality of some foods. Manufacturers of lactose, yogurt and frozen desserts often use lactase to enhance the texture, sweetness, solubility (Gro Z, 2008), palatability and digestibility of cream (Abada E A et al., 2019). At the same time, lactose hydrolysis makes the yogurt viscosity higher, relatively prolongs the acidity and milk shelf life.

Some studies have found when the enzyme addition was 1500 NLU/L, pH=6.8, and 38°C, lactose enzymatic hydrolysis for 3 h, the lactose hydrolysis

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rate is 75.2% (Zhang et al., 2008). At 40°C and the lactose hydrolysis rate is 52%, only 15% of GOS can be obtained (Barbara R C et al., 2016). When the enzyme addition is 0.15%, the enzymatic hydrolysis temperature is 38.15℃, and the enzymatic hydrolysis time is 3 h, the lactose hydrolysis rate is 92.67%, and the GOS content is 14.02% (Wu et al., 2018).

Prebiotics are mostly non-digestible oligosaccharides, which are ingested by humans and used as proliferation factors by the intestinal probiotic flora (Onishi et al., 2010). It usually has anti-caries, anti-carcinogenesis (Crittenden R G et al., 1996), promotes the absorption of mineral elements, regulates the balance of intestinal flora, stimulates the growth of beneficial bacteria (Rabiu B A et al., 2001), and enhances the body's resistance (Jing Shan, 2019). As a common prebiotic, GOS is also very effective. Short-chain fatty acids (SCFA) are the key product of GOS fermentation in the colon, and the propionic acid and butyric acid among them have been proved to be of positive significance for the prevention of colon cancer (Cummings et al., 1981). Consumption of prebiotics can ensure a healthy intestinal microbiota and is easy to produce short-chain fatty acids that have a positive effect on intestinal transport. Mice fed with 90% of GOS were used to study the intestinal microbiota, and it was found that GOS enrich the intestinal flora of mice (Mônica de Souza Lima Sant'Anna et al., 2014; Monteagudo-Mera A et al.,2016). The addition of prebiotics also has a certain impact on product quality. Studies have found that adding GOS to goat milk ice cream can reduce calories and increase sweetness. Compared with full-fat ice cream, it has larger bubbles, smaller ice crystals, and lower melting temperature. The melting rate is higher (Balthazar C F et al.,2017).

In this study, cow milk was used as the raw material, and the enzyme preparations with high lactose hydrolysis rate and high galactooligosaccharides content were selected from 14 kinds of commercially available lactases, the effects of different combinations of selected lactase enzymes on the lactose hydrolysis rate and GOS content were compared, and then the lactases ratio was optimized through the design of mixing experiments, which provides reference for the development of low-lactose prebiotic liquid milk.

MATERIALS AND METHODS

Lacatses and preparation of low-lactose prebiotic liquid milk

The lactases used in the experiment are shown in Table 1.

The Arla whole milk (Arla Foods Dairy Company, Denmark) used in the research was purchased from Jingdong Mall. The liquid milk was divided into 200 mL yogurt cups with a sample amount of 150 mL, sterilized at 65℃ for 20 minutes, a certain amount of lactase was added to the cooled milk for enzymatic hydrolysis for a period of time, and then the enzyme was inactivated at 95℃ to 100℃ for 10 minutes. After it was cooled, low-lactose prebiotic liquid milk can be obtained.

Table 1. Beta galactosidase

Lactase name	specification	Vendor
Lactase 1（E1）	Food grade	Guangzhou Xinzhiwei Biological Technology Co., Ltd.
Lactase 2（E2）	Food grade	Henan Wanbang Industrial Co., Ltd.
Lactase 3（E3）	Food grade	Xinxiangyuan Food Ingredients Supermarket
Lactase 4（E4）	Food grade	Mr. Liu Additives
Lactase 5（E5）	Food grade	Zhejiang Bodanheng Food Additives
Lactase 6（E6）	Food grade	Yixin Biological Technology Co., Ltd.
Lactase 7（E7）	Food grade	Shiweixian Ingredients Mall
Lactase 8（E8）	Food grade	Unparalleled Hu Gaochao
Lactase 9（E9）	Food grade	Henan Huayue Chemical Products Co., Ltd.
Lactase 10（E10）	Food grade	Novozymes Biotechnology Co., Ltd.
Lactase 11（E11）	Food grade	Ubaojia Food Flagship Store
Lactase 12（E12）	Food grade	Shanghai Youlezi Food Ingredients
Lactase 13（E13）	Food grade	Zhejiang Yinuo Biological Technology Co., Ltd.
Lactase 14（E14）	Food grade	Pangbo Biological Technology Co., Ltd.

Lactase Screening

After pure milk is sterilized at 65°C for 20 minutes and cooled to 40°C, 14 kinds of lactases were added at a 0.1% amount and then enzymatically hydrolyzed at a constant temperature of 40°C for 3 hours, and then the enzymes were inactivated at 95°C~100°C for 10 minutes and cooled,The content of lactose, galactose, and glucose was determinee and the lactose hydrolysis rate and galactooligosaccharide (GOS) content were calculated, and the lactase enzymes with high lactose hydrolysis rate and GOS content were screened out.

Preparation of low-lactose prebiotic liquid milk

Some lactase enzymes with high lactose hydrolysis rate and GOS content were screened, and they were used to study the effects of single and combined enzymes on the lactose hydrolysis rate and GOS content of low lactose prebiotic liquid cow milk, respectively, and then the compound enzyme ratios were optimized using a mixed-feed experimental design.

Determination of lactose and galactose

The content of lactose and galactose in milk before and after enzymatic hydrolysis was determined using a lactose/galactose kit (R-BIOPHARM AG). The principle is that β-galactosidase can enzymatically hydrolyze lactose to produce D-glucose and D-galactose at pH 6.6. At pH 8.6, D-galactose can react with coenzyme I and D-galactose dehydrogenase (Gal-DH) to obtain D-galactonic acid and coenzyme II. The amount of NADPH reflects the amount of lactose and D-galactose, and its absorbance value can be measured at 340 nm.

Sample pretreatment: 1g of enzymatic hydrolysate and 60mL of distilled water were placed in a 100mL volumetric flask, and then placed in a water bath at 70°C for 15 minutes at constant temperature and shaking, 5 mL of Carrez-I solution, 5mL of Carrez-II solution and 10 mL of sodium hydroxide (0.1 M) solution was added to clarify the solution, and then water was added to the mark, Finally the sample was filtered and dilute if necessary.

Glucose determination

The glucose in the low-lactose prebiotic liquid milk is directly measured by the biosensor analyzer (S-10, Siemantec Technology Co., Ltd, Shenzhen, China). The enzymatically hydrolyzed milk (the blank is pure milk) is filtered with a 0.2 μm water filter membrane, and the biosensor is calibrated with a 1 g/L standard glucose solution. Draw 25 μL of filtrate and add it to the biosensor to get the concentration of glucose (g/L). Each sample was tested three times, and the average value was taken as the result.

Lactose hydrolysis rate and determination of galactooligosaccharide

The lactose hydrolysis rate can be calculated according to the changes in the amount of lactose before and after enzymatic hydrolysis. Galactooligosaccharides are counted as disaccharides. According to the principle of conservation of mass, the amount of lactose consumed is equal to the increase in glucose, galactose and galactooligosaccharides, that is, the amount of galactooligosaccharide is equal to the consumption of lactose minus the increase in galactose and glucose.

The calculation formula of lactose hydrolysis rate is given in eq. (1). The formula for calculating the content of galactooligosaccharide (GOS) is eq. (2).

Color determination

CIE (International Commission on Illumination) color analysis was performed using Minolta CM-5 spectrophotometer. The sample needs to be zeroed and calibrated with a whiteboard before the sample is analyzed. The average value is measured three times during the sample measurement. The color difference between the sample and the control group can be expressed by △E (eq. (3)).

Sensory evaluation

The sensory evaluation including colour, organization status, odor, and taste for low-lactose prebiotic liquid milk was measured by referring to the methods of Jing and Liu (2020).

$$\text{Lactose hydrolysis rate (\%)} = (C_{\text{The amount of lactose in unhydrolyzed milk}} - C_{\text{The amount of lactose in enzymatically hydrolyzed milk}}) / C_{\text{The amount of lactose in unhydrolyzed milk}} \times 100 \quad (1)$$

$$\text{GOS content (g/L)} = C_{\text{Lactose consumption}} - C_{\text{Increased glucose}} - C_{\text{Increased galactose}} \quad (2)$$

$$\triangle E = [(\triangle L)^2 + (\triangle a)^2 + (\triangle b)^2]^{1/2} \quad (3)$$

RESULTS AND DISCUSSION

Effect of different lactase to produce GOS

As Figure 1 shows, the type of lactase has a significant effect on the lactose hydrolysis rate and GOS content in milk. The results vary with enzymes. The lactose hydrolysis rate in the experiments ranges from 5.848% to 81.380%, and the GOS content is 0.030 g/L- 13.101 g/L. There are 6 types with lactose hydrolysis rate higher than 50%, namely E10 (81.380%), E11 (78.288%), E14 (72.238%), E5 (70.647%), E13 (62.357%), E12 (55.770%), The lactose hydrolysis rates of the other eight lactases are all lower than 50%, the lowest is E1, only 5.848%; there are 5 samples with GOS higher than 6 g/L, E10 (13.101 g/L), E14 (11.498) g/L), E12 (10.640 g/L), E9 (7.445 g/L) and E5 (6.684 g/L). The GOS content of the remaining 9 enzymes hydrolyzed liquid milk is lower than 6g/L, the lowest is only It is 0.030g/L (E1). Comparing the lactose hydrolysis rate and GOS content shows that the two are not necessarily positively correlated. The lactose hydrolysis rate of E11 is 78.288%, but the GOS content is only 5.362 g/L, indicating that the enzyme has high hydrolysis efficiency but low transglycosidation efficiency. E9 is the opposite of E11, and its lactose hydrolysis rate is only 40.175%, but enzymatic hydrolysis The GOS content of liquid milk is 7.445 g/L, indicating that the enzymatic hydrolysis efficiency is low, but the transglycosidation efficiency is high.

Table 2 shows that, compared with the chromaticity value of the control milk (L=93.27±0.01, a=-0.97±0.03, b=9.29±0.02), the chromaticity value of the milk hydrolyzed by lactase has a significant change. It may be related to the changes in milk fat, protein composition and lactose content after lactase hydrolysis (Figueroa A et al., 2020). Lactase enzymatic hydrolysis darkens the color of liquid milk and most of it is reddish-yellow. Among them, the color of E9 enzymatically hydrolyzed milk turns darker to yellowish green, while the color of E7 enzymatically hydrolyzed milk turns darker to reddish blue, indicating that the type of lactase has different effects on the chromaticity of milk, and the degree of color deviation from the center is higher. The descending order is L* (90.91~93.05), b* (8.99~11.11), a* (-1.24~-0.32).

Due to the type of lactase, the color parameters of milk will change, resulting in color difference. According to formula 2-8, the total color difference △E (NBS) can be calculated. When ΔE=1, it is 1 NBS, which is equivalent to 5 times the visual recognition threshold, which is the smallest color difference that the human body can distinguish. When the △E is smaller, the difference between the two colors is less obvious, otherwise, the difference is more obvious. If it is between 0 and 0.5 NBS, the color difference between the two colors is difficult to recognize with the naked eye; if it is between 0.5 and 1.5 NBS, it means that there is a slight difference; if it is between 1.5 and 3 NBS, the substance can be clearly noticed The chromatic aberration between. When the color difference exceeds 3.0 NBS, the difference between the two colors is very obvious (Chen et al., 2013).

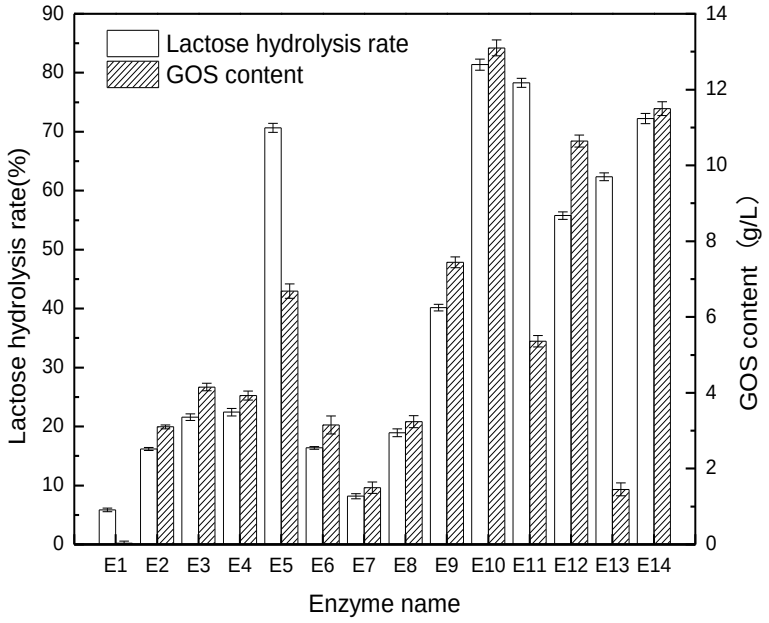

Figure 1. The effects of different lactases on the lactose hydrolysis rate and GOS content of cow milk

Table 2. The effect of different lactases on the color value of enzymatically hydrolyzed cow milk

Sample number	Enzyme name	L*	a*	b*	△E
1	E1	93.02±0.14	-0.95±0.01	9.62±0.02	0.41
2	E2	92.11±0.06	-1.17±0.01	10.48±0.21	1.67
3	E3	91.46±0.15	-0.32±0.01	10.39±0.07	2.22
4	E4	91.63±0.21	-0.97±0.03	11.02±0.01	2.39
5	E5	92.87±0.12	-0.87±0.01	9.75±0.07	0.62
6	E6	92.81±0.02	-0.82±0.01	9.59±0.01	0.57
7	E7	92.87±0.30	-0.88±0.10	8.99±0.01	0.51
8	E8	92.84±0.36	-0.64±0.10	9.71±0.02	0.68
9	E9	91.02±0.01	-1.20±0.01	11.11±0.03	2.90
10	E10	92.92±0.12	-0.95±0.01	9.52±0.01	0.42
11	E11	92.95±0.01	-0.87±0.01	9.71±0.03	0.53
12	E12	90.91±0.01	-1.24±0.01	11.10±0.08	2.99
13	E13	93.05±0.03	-0.94±0.01	9.47±0.01	0.28
14	E14	92.80±0.02	-0.89±0.01	9.73±0.034	0.65

It can be observed from Figure 2 that the type of lactase has a significant effect on the color difference of enzymatically hydrolyzed milk. The color difference of lactase enzymatically hydrolyzed milk ranges from 0.28-2.99 NBS. Among them, the total color difference is between 1.5 and 3.0 NBS, respectively, with E12 (2.99 NBS), E9 (2.90 NBS), E4 (2.39 NBS), E3 (2.22 NBS) and E2 (1.67 NBS). The color differences of the other nine lactase enzymes are all lower than 1.5 NBS.

It can be seen from Figure 3 that the type of lactase has a significant impact on the sensory evaluation of milk. The sensory score of enzymatic milk is 33.75-71.94, of which E10 (71.94±0.80) has the highest sensory evaluation score, and E4 (33.75±13.44) sensory evaluation. The evaluation score is the lowest.

Among them, the enzymatic hydrolysis of E2, E4, E8, E9 and E12 tastes bitter in liquid milk, which may be related to the enzymatic hydrolysis of protein in liquid milk (Wang et al., 2020). Milk prepared by enzymatic hydrolysis of other lactase enzymes is sweeter than pure milk. This is because lactose is hydrolyzed to produce glucose and increase the sweetness. The enzymolysis ability of lactase is different, and the sweetness of the enzymatically hydrolyzed liquid milk is slightly different. Among them, the E10 and E14 enzymatically hydrolyzed milk is sweeter than the rest of the enzymatically hydrolyzed milk.

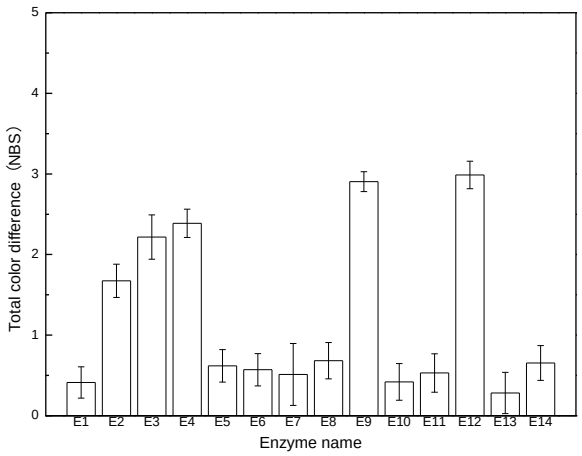

Figure 2. The effects of different lactases on the color △E of cow milk

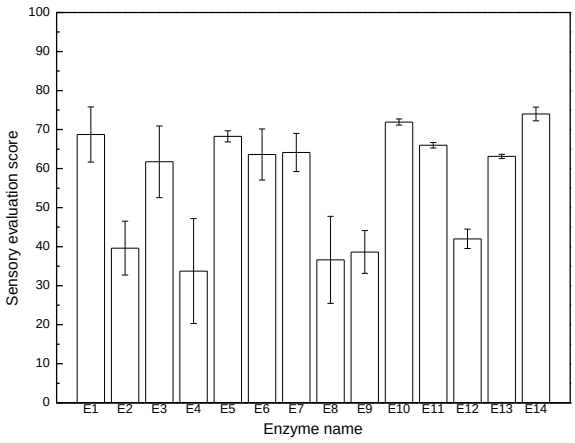

Figure 3. The sensory evaluation of enzymatic hydrolysis of cow milk by different lactases

After considering the comprehensive results of lactase enzymatic hydrolysis of cow's milk, the lactase enzymes suitable for enzymatic hydrolysis of cow's milk are: E10, E11, E14, E5. Among them, considering the high lactose hydrolysis rate and GOS content of E10 enzymatically hydrolyzed liquid milk, its small effect on color and sensory evaluation, and the cost is lower than E14, so E10 was selected as the main test enzyme preparation.

Effect of Lactase Combination on Hydrolyzed Lactose to Produce GOS

According to the above test results, the lactase enzymes with better enzymolysis ability were screened out: E10, E11, E14, E5, considering the cost, the total added amount of combined lactase was set to 0.06%, the combination of lactase, the ratio and the test results See Table 3.

As indicated in Table 3, the lactase combination has a significant effect on the lactose hydrolysis rate and GOS content in milk. The hydrolysis rate of milk lactose ranges from 77.061% to 84.770%, and the GOS content is 12.644 g/L-14.397 g/L. The lactose hydrolysis rate from high to low is E10+E11 (84.770%), E10+E5+E11+E14 (82.248%), E10+E14 (80.859%), E10+E5 (80.009%) and E10 (77.061%) ; The content of GOS produced in descending order is E10+E11 (14.397 g/L), E10+E5+E11+E14 (13.791 g/L), E10+E5 (13.540 g/L), E10+E14 (13.397 g) /L) and E10 (12.644 g/L), indicating that the hydrolysis efficiency and transglycosidation efficiency of E10+E11 are higher than others, and the enzymatic hydrolysis effect of combined enzymes is better than that of single enzyme, so E10 and E11 are selected for milk.

Mixing experiment design to optimize the ratio of lactases

The lactase formula is designed by using Design Expert V8.0.6 to design a mixing test to find the best ratio of compound lactase. The mixing experiment design and the results are show in Table 4. The analysis of variance for Lactose hydrolysis rate and GOS content are given in Table 5 and Table 6.

Table 3. The effect of lactase combination on hydrolysis degree and GOS content of of cow milk

Enzyme combination	Lactose hydrolysis rate（%）	GOS content（g/L）
E10（0.06%）	77.061±0.013	12.644±0.010
E10（0.03%）+E5（0.03%）	80.009±0.001	13.540±0.020
E10（0.03%）+E11（0.03%）	84.770±0.008	14.397±0.061
E10（0.03%）+E14（0.03%）	80.859±0.012	13.442±0.013
E10（0.03%）+ E5（0.01%）+ E11（0.01%）+ E14（0.01%）	82.248±0.005	13.791±0.012

Table 4. The experiment design and results of enzymatic hydrolysis of cow milk by lactases E10 and E11

Serial number	Design order	A-E10	B-E11	Lactose hydrolysis rate（%）	GOS content（g/L）
1	4	0.667	0.333	91.645	16.451
2	2	1	0	87.251	15.158
3	5	0.5	0.5	84.462	14.769
4	6	0.5	0.5	87.685	16.211
5	8	0.25	0.75	73.129	13.497
6	1	1	0	87.649	16.142
7	3	0.75	0.25	91.235	17.560
8	9	0	1	65.339	12.536
9	7	0.333	0.667	80.230	13.944
10	10	0	1	33.865	5.907

Table 5. The variance analysis of the hydrolysis rate of cow milk by lactases E10 and E11

source	sum of square	Degree of freedom	Mean square	F value	p value	Significance
model	2306.70	2	1153.35	15.98	0.0025	**
Linear mixing	1660.66	1	1660.66	23.01	0.0020	**
AB	646.04	1	646.04	8.95	0.0202	*
error	505.22	7	72.17			
Lack of Fit	4.63	4	1.16	0.006943	0.9998	not significance
Pure error	500.58	3	166.86			
sum	2811.92	9				

Table 6. Analysis of variance of GOS content in enzymatic hydrolysis of cow milk by E10 and E11

source	sum of square	Degree of freedom	Mean square	F value	p value	Significance
model	72.73	2	36.37	10.27	0.0083	**
Linear mixing	52.36	1	52.36	14.79	0.0063	**
AB	20.37	1	20.37	5.76	0.0475	*
error	24.78	7	3.54			
Lack of Fit	1.29	4	0.32	0.041	0.9950	not significance
Pure error	23.49	3	7.83			
sum	97.51	9				

According to the test results in Table 4, the second regression fitting was performed on the test results by Design-Expert 8.0.6, and the hydrolysis rate (Y1) and GOS content of compound lactase (E10 and E11) enzymatically hydrolyzed liquid milk lactose (Y1) and GOS content (Y2) Quadratic regression equation. The quadratic regression equations are (5) and (6):

$Y_1=87.32A+49.81B+73.38AB$ (5)

$Y_2=15.83A+9.17B+13.03AB$ (6)

Table 5 indicates that the regression equation model is significant, and the lack of fit term is not significant, indicating that the established regression equation fits well. Among them, the p value of linear mixing of influence factors is less than 0.01, and the p value of AB is less than 0.05, indicating that when E10 and E11 increase in a linear ratio, the effect on the hydrolysis rate of enzymatic milk lactase is more significant. The coefficient of determination R^2 of the quadratic regression equation is 82.03%, indicating that 82.03% of the response value change can be explained by this model. The correction coefficient of determination R^2_{adj}=76.9%, which is relatively close to R^2, further illustrates the significance of the model. Therefore, this model can be used to predict the lactose hydrolysis rate of the compound lactase (E10+E11) enzymatic hydrolysis of milk.

It can be seen from Table 6 that the regression equation model is significant, and the lack of fit term is not significant, indicating that the established regression equation fits well. The p value of the linear mixture of influence factors is <0.01, and the p value of AB is <0.05, indicating that the increase of E10 and E11 in a linear ratio has a significant effect on the GOS content of enzymatically hydrolyzed milk. The coefficient of determination R^2 of the quadratic regression equation is 74.59%, indicating that 74.59% of the response value change can be explained by this model. The correction coefficient of determination R^2_{adj}=67.33%, which is relatively close to R^2, further illustrates the significance of the model.

Therefore, this model can be used to predict the GOS content of compound lactase (E10+E11) enzymatically hydrolyzed milk.

By analyzing the regression equation, the optimal ratio of compound lactase is E10:E11=0.756:0.244. Under these conditions, the lactose hydrolysis rate was 91.704%, and the GOS content was 16.607 g/L. The verified lactose hydrolysis rate and GOS are 90.110±0.01% and 16.356±0.20 g/L, respectively, which are close to the predicted values, indicating that it is feasible to optimize the compound lactases ratio by using mixture experiment design.

"Lactose intolerance" hinders the development of the dairy industry. The use of lactase to hydrolyze lactose can achieve the purpose of preventing lactose intolerance, and the GOS produced by the hydrolysis of lactose by lactase can prevent diarrhea as a value-added factor of human intestinal flora and also improve the quality of some foods. Many studies used a single lactase enzyme was used to hydrolyze lactose, which leads to a high hydrolysis rate of lactose, but low GOS content, indicating that the lactase used in it has high hydrolytic activity but poor transglycosidic activity. Ruiz-Matutte et al. (2012) found that the lactose hydrolysis rate is the key factor for the GOS content. When 75-90% of the lactose is hydrolyzed, the GOS formed reaches the maximum value of about 10,000mg/L, and then when it exceeds 99% When the lactose is hydrolyzed, it gradually drops to a value well below 5g/L. In this study, the two selected lactase enzymes (E10 and E11) were added and acted on cow milk at the same time. The composition was optimized through the design of the mixing experiment, so that the efficiency of hydrolysis and transglycosidation was high, the lactose hydrolysis rate and GOS content were 98.02% and 19.69g/L, respectively, and the above problems were overcome.

There is also a lot of research using whey or lactose as raw materials to prepare galactooligosaccharides, and then GOS is separated and sold as food functional factors, but the separation and purification of GOS increases the cost**.** In this study, milk was used as a raw material to directly add two lactases, which reduced the content of lactose and increased the content of GOS. It can be directly used as a functional dairy product, which not only avoids lactose intolerance, but also increases the prebiotic properties of milk. It also reduces the cost of separation and purification of GOS, which is conducive to industrial applications.

CONCLUSION

There were significant differences in the hydrolysis rate (5.848% to 81.380%) and GOS content (0.030 g/L to 13.101 g/L) generated by the 14 lactase enzymes, The suitable lactase enzymes for cow milk were E10, E11, E14 and E5 in order.The mixture experiment design determined the E10 and E11 enzymes ratio and the optimal enzymatic hydrolysis process of low-lactose prebiotic milk were as follows: compound lactases (E10:E11=0.756:0.244) addition 0.11%, 55℃ for 5h, lactose hydrolysis rate and GOS content were 98.02% and 19.69g/L, respectively, and the remaining lactose content was about 0.97 g/L, which provides a reference for the production of low-lactose prebiotic liquid milk.

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