Development Technology of Functional Food of Cholesterol Lowering Yogurt

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Abstract - **The aim of the research is to study the development technology of functional food of cholesterol lowering yogurt. Two strains of lactobacillus plantarum with cholesterol lowering ability were used as the starter cultures. In single factor experiment, optimal rate, inoculum, optimal fermentation period and temperature was determined. Box-Behnken Design was performed. Chemical composition analysis, sensory evaluation and storage was determined.**

Key words - Cholesterol-lowering yogurt; Lactobacillus plantarum; Fermented camel milk "Khoormog"; Functional food

I. INTRODUCTION

In modern nutrition concept, functional foods are defined as the food and beverage products that derived from naturally occurring substances or those similar in appearance to potentially helpful products. It includes any modified food or food ingredient that provide specific benefits and/or reduce the risk of disease beyond nutritional functions[1]. Current foods are rich in lipids, such as cholesterol which is associated with risks of heart disease[2, 3, 4]. Many researches have been studied to solve the problem, including the use of probiotics which defined by FAO/WHO as the living microorganism can provide the host health benefits when administered in a sufficient amount.

According to FAO/WHO standards, yogurt is defined as the coagulated milk product which produced through the fermentation of *Lactobacillus delbrueckii ssp. bulgaricusand* and *Streptococcus thermophilus*[5]. However, in the recent years, more and more yogurt containing probiotics have been developed using more species of *Lactobacillus* and *Bifidobacterium* at the level up 10^{-6} cfu/g[6].

The development technology of cholesterol lowering yogurt is produced under the fermentation process of stirred yogurt production with some modification using two strains of *lactobacillus plantarum* with cholesterol lowering ability which isolated from traditional fermented camel milk "*Khoormog*" collected in Inner Mongolia, China as the starter cultures of functional yogurt.

II. MATERIALS AND METHODS

1.Starter Cultures

Lactobacillus plantarum strains AM2-6 (isolation from Alxa right banner, Inner Mongolia, China) and BM2-5 (isolation from Bayannur, Inner Mongolia, China) were used as the starter cultures of development technology of cholesterol lowering yogurt in comparison with the standard strain *Lb. platarum* NBRC 15891^{T} (Inner Mongolian Agricultural University).

2.Milk Preparation

The hydrated whole milk powder was prepared for cholesterol lowering yogurt production. Whole milk powder pretreated and standardized according to GB 19644-2010 and GB 19301-2010. The quality inspection of hydrated milk was determined according to GB 19644-2010.

3.Optimal Rate of Starter Cultures

The production process of cholesterol lowering yogurt was based on the traditional production process of stirred yogurt with some modification using the cholesterol lowering strains together with freeze dried commercial yogurt starter power according to the yogurt production process of GB 19302-2010. The starter cultures were inoculated at different rate according to the method of Li H. et al, 2008[7]. After finish the fermentation, the pH value, titratable acidity and viable count of each yogurt product was determined.

4.Inoculum of Starter Cultures

The starter cultures were inoculated at different inoculum. After finish the fermentation, the pH value, titratable acidity and viable count of each yogurt product was determined.

5.Optimal Fermentation Period and Temperature

The starter cultures were fermented under different temperature. After finish the fermentation, the pH value, titratable acidity and viable count of each yogurt product was determined.

6.Box-Behnken Design

The optimal production process of development technology of cholesterol lowering yogurt was obtained through response surface experiment design. Box Behnken Design was performed using the Design-Expert 8.0.6 software.

7.Chemical Composition Analysis

The production process under the optimal fermentation conditions of cholesterol lowering yogurt was obtained and new yogurt product was produced under the optimal conditions. The chemical composition analysis of protein, fat and ash was determined according to GB 5009. 6-2016, GB 5009. 5-2016, and GB 5009. 4-2016, respectively. The total solid content was determined according to GB 5413. 39-2010. And the non-fat solid was calculated.

8.Sensory Evaluation

Sensory evaluation was performed by seven trained researchers after milk fermentation. Sample preparation, laboratory and personnel requirements, evaluation items, standards and methods, data processing was analyzed according to sensory regulation of yogurt RHB 103-2004.

9.Storage and Shelf Life

The cholesterol lowering yogurt was refrigerated and stored under 4℃ for 21 days according to GB 19302-2010. The pH, titratable acidity and viable count was determined in every 7 days.

III. RESULTS AND DISCUSSION

1.Optimal Rate of Starter Cultures

The cholesterol lowering yogurt was produced using *Lactobacillus plantarum* strains AM2-6 and BM2-5 according to the yogurt production process of GB 19302-2010. Briefly, the whole milk powder was 12.5% (w/v) hydrated and heated under 73℃ for 15 s (pH 6.5, GB 19644-2010). Then rapidly cooled to 43℃, the production starter cultures were inoculated together with the commercial starter powder at the rate of 1:1, 2:1, 1:2, respectively[7]. The inoculum was 3% of raw milk. Fermentation under 43℃. The pH value, titratable acidity, viable count and sensory evaluation score of each yogurt product was shown in Table 1.

According to the sensory evaluation score, the strain rate AM2-6:BM5-2 of 2:1, showed a stronger fermentation ability than that in other rate. So that it can be considered as the optimal rate of the selected starter cultures.

TABLE 1. EFFECT OF DIFFERENT RATE OF STARTER CULTURES ON THE YOGURT.

Strain Rate *AM2-6:BM5-2*	**Time**	**pH**	**Titradable Acidity (°*T*)**	**Viable Count** *(Log 10 CFU/g)*	**Refrigerated Yogurt State**	**Sensory Score**
1:1	2 h	6.25	21		The curd texture is slightly soft and little whey is separated. Milky white color.	
	4 h	5.54	38			
	6 h	4.65	77			
	8 h	4.37	94	7.56		75
2:1	2 h	6.02	25		The curd texture is moderate and little whey is separated. Milky white color.	
	4 h	5.37	44			
	6 h	4.51	85			
	8 h	4.34	96	7.83		78
1:2	2 h	6.34	19		The curd texture is slightly soft and little whey is separated. Milky white color.	
	4 h	5.49	40			
	6 h	4.69	75			
	8 h	4.45	89	7.43		76

All data was repeated twice and recorded as mean±SD.

2. Inoculum of Starter Cultures

Whole milk powder was 12.5% (w/v) hydrated, standardized and heated under 73℃ for 15 s as mentioned above. After rapidly cooled to 43℃, the production starter cultures were inoculated together with the commercial starter powder at the rate 1:1, The inoculum was 1%, 2%, 3%, 4% and 5% of raw milk respectively. Fermentation under 43℃. The pH value, titratable acidity, viable count and sensory evaluation score of each yogurt product was determined as mentioned above (Table 2).

According to the sensory evaluation score, the strain rate inoculum of 4% of raw milk, showed a stronger fermentation ability than that in other inoculum. So that it can be considered as the optimal inoculum of the selected starter cultures.

3.Optimal Fermentation Period and Temperature

Whole milk powder was hydrated, standardized and heated as mentioned above. After rapidly cooled to 43℃, the production starter cultures were inoculated together with the commercial starter powder at the rate of 1:1, the inoculum was 3% of raw milk. Fermentation temperature was under 39℃, 41℃, 43℃ and 45℃ for fermentation. The pH value, titratable acidity and viable count of each yogurt product was determined as mentioned above to obtain the optimal fermentation period and temperature (Table 3).

According to the sensory evaluation score, the fermentation temperature under 43℃, showed a stronger fermentation ability than that in other fermentation temperature. So that it can be considered as the optimal fermentation temperature of the selected starter cultures.

TABLE 2. EFFECT OF DIFFERENT INOCULUM OF STARTER CULTURES ON THE YOGURT.

Inuculum	Time	pH	Titradable Acidity (°*T*)	Viable Count *(Log 10 CFU/g)*	Refrigerated Yogurt State	Sensory Score
1%	2 h	6.33	20		The curd texture is slightly soft and some whey is separated. Milky white color.	
	4 h	5.93	26			
	6 h	5.02	58			
	8 h	4.67	75	7.26		72
2%	2 h	6.34	19		The curd texture is slightly soft and some whey is separated. Milky white color.	
	4 h	5.74	32			
	6 h	4.85	67			
	8 h	4.57	82	7.34		73
3%	2 h	6.05	24		The curd texture is slightly soft and little whey is separated. Milky white color.	
	4 h	5.34	45			
	6 h	4.56	82			
	8 h	4.38	93	7.45		75
4%	2 h	5.98	26		The curd texture is moderate and little whey is separated. Milky white color.	
	4 h	5.24	49			
	6 h	4.47	88			
	8 h	4.23	103	7.49		78
5%	2 h	6.02	23		The curd texture is moderate and some whey is separated. Milky white color.	
	4 h	5.37	43			
	6 h	4.52	84			
	8 h	4.36	94	7.43		74

All data was repeated twice and recorded as mean±SD.

TABLE 3. EFFECT OF DIFFERENT FERMENTATION TEMPERATURE ON THE YOGURT.

Fermentation Temperature *℃*	Time	pH	Titradable Acidity (°*T*)	Viable Count *(Log 10 CFU/g)*	Refrigerated Yogurt State	Sensory Score
39℃	2 h	6.41	19		The curd texture is slightly soft and some whey is separated. Milky white color.	
	4 h	5.87	27			
	6 h	4.93	62			
	8 h	4.56	82	7.03		72
41℃	2 h	6.43	18		The curd texture is slightly soft and some whey is separated. Milky white color.	
	4 h	5.78	31			
	6 h	4.83	68			
	8 h	4.46	88	7.12		75
43℃	2 h	6.02	25		The curd texture is slightly soft and little whey is separated. Milky white color.	
	4 h	5.43	42			
	6 h	4.46	88			
	8 h	4.39	92	7.28		79
45℃	2 h	6.24	21		The curd texture is moderate and little whey is separated. Milky white color.	
	4 h	5.66	34			
	6 h	4.69	75			
	8 h	4.49	86	7.06		73

All data was repeated twice and recorded as mean±SD.

4.Box-Behnken Design

In Box-Behnken Design of the optimal production process of cholesterol lowering yogurt, 3 numeric factors and 3 responses were set. The 3 numeric factors were inoculation rate of AM2-6 and BM5-2 strains, the inoculum and fermentation temperature (Table 4). And 3 responses were pH, titradable acidity and the viable count of each produced yogurt (Table 5).

TABLE 4. THE NUMERIC FACTORS OF BOX-BEHNKEN DESIGN.

Numeric factors	Code	Level	
		Low	High
Rate	X_1	1:2	2:1
Inoculum (%)	X_2	1	5
Temperature (℃)	X_3	39	45

TABLE 5. THE RESPONSES OF BOX-BEHNKEN DESIGN.

Responses	R_1	R_2	R_3
Code	pH	Titradable acidity (°T)	Viable count Log 10 CFU/g

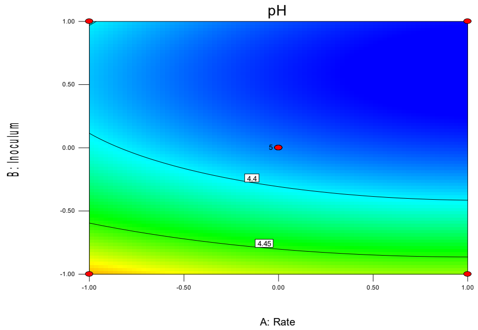
According to the Box-Behnken Design, the final equation in terms of coded factors was performed as the following formula:

$$R_1 = +4.38 - 0.017 * X_1 - 0.051 * X_2 - 0.011 * X_3 - 2.500E\text{-}003 * X_1 * X_2 - 2.500E\text{-}003 * X_1 * X_3 + 0.000 * X_2 * X_3 + 7.500E\text{-}003 * X_1^2 + 0.045 * X_2^2 + 0.045 * X_3^2.$$

$$R_2 = +93.00 + 1.17 * X_1 + 3.17 * X_2 + 0.67 * X_3 - 2.500E\text{-}003 * X_1 * X_2 + 2.500E\text{-}003 * X_1 * X_3 + 0.000 * X_2 * X_3 - 0.50 * X_1^2 - 2.83 * X_2^2 - 2.67 * X_3^2.$$

$R_3 = +7.43 + 0.069 * X_1 + 0.029 * X_2 + 5.000E\text{-}003 * X_3 - 2.500E\text{-}003 * X_1 * X_2 + 0.000 * X_1 * X_3 + 0.000 * X_2 * X_3 + 0.024 * X_1^2 - 0.036 * X_2^2 - 0.079 * X_3^2$.

The effect of inoculation rate, inoculum and fermentation temperature on the pH, titradable acidity and viable count which designed by Box-Behnken Design of the optimal production process of cholesterol lowering yogurt was shown in Fig 42 A-I.



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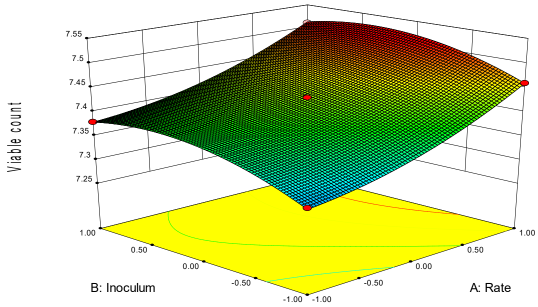

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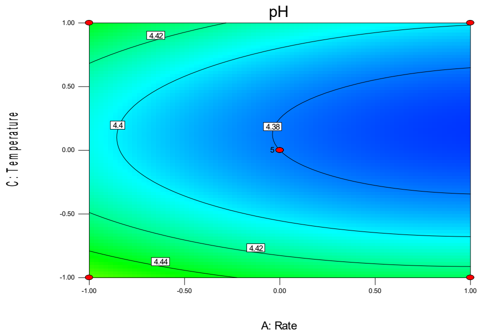

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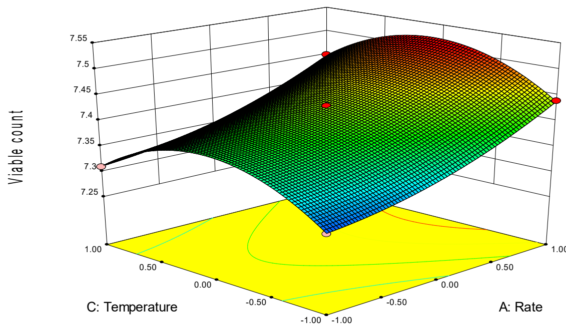

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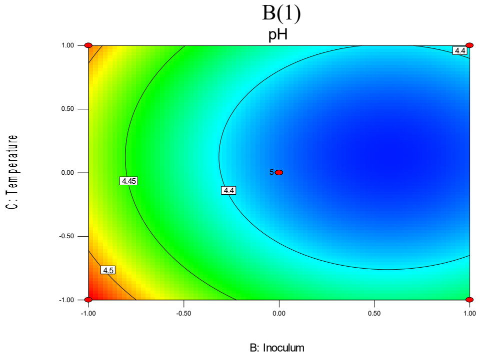
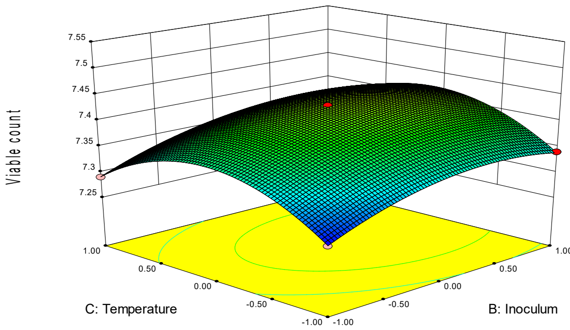


C(1)



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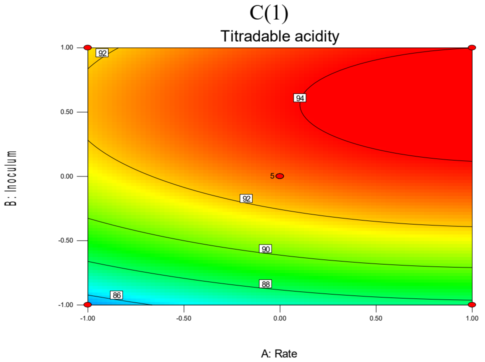
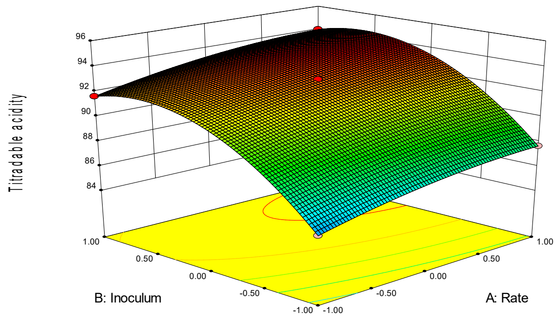


D(1)



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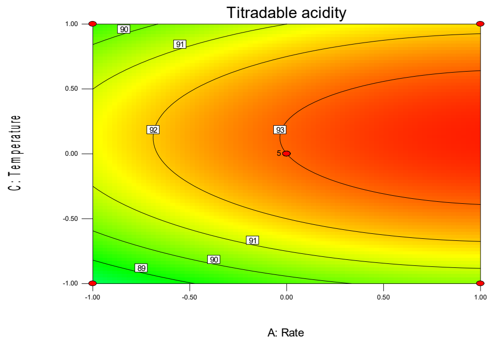
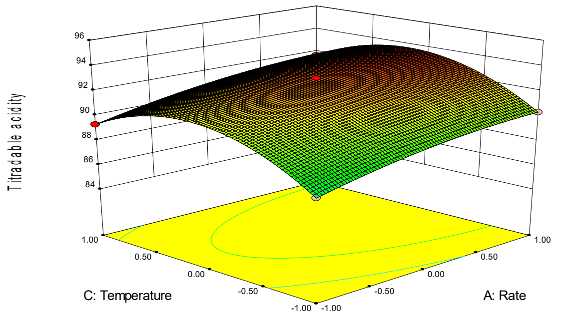


E(1)



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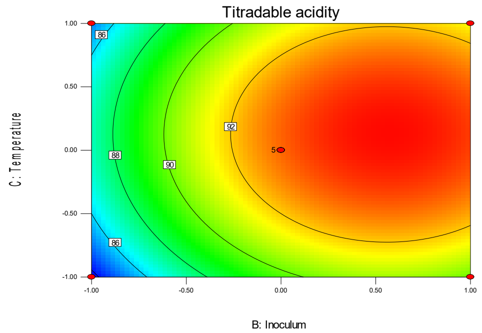
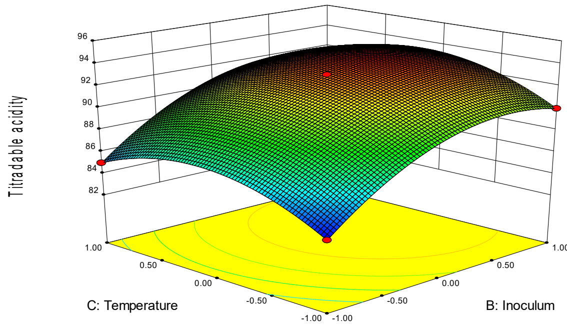


F(1)



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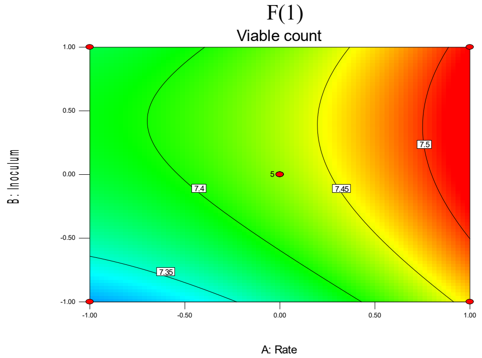
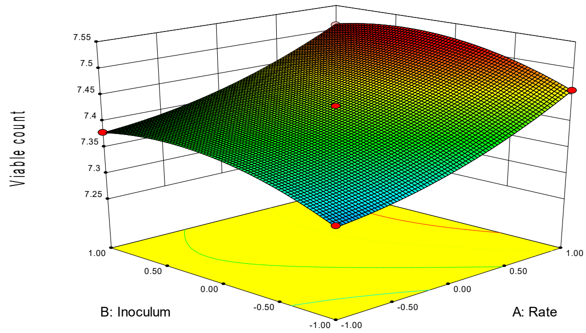


G(1)



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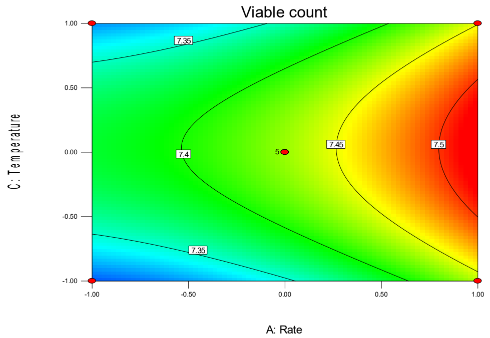
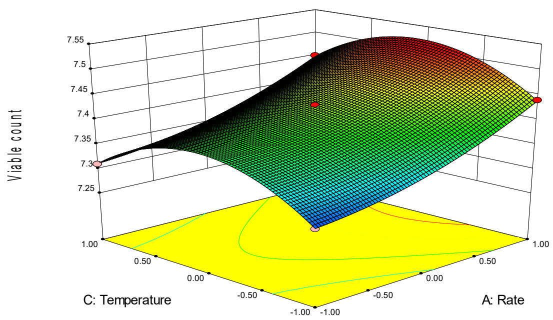
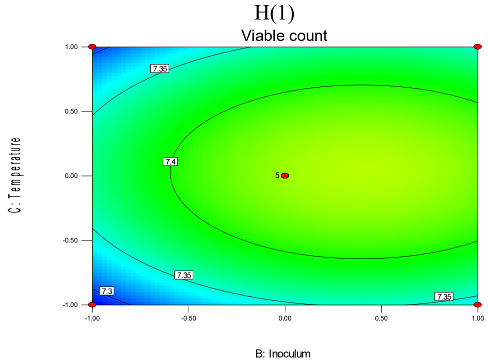


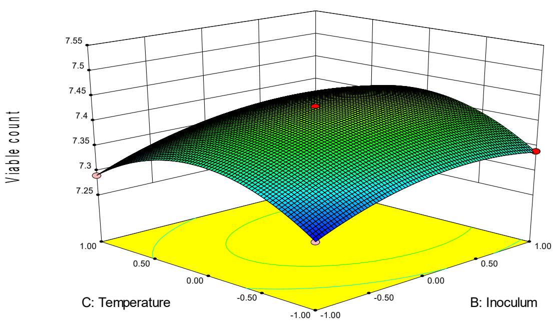
H(1)



H(2)



I(1)



I(2)

Fig 1. The Box-Behnken Design of the optimal production process of cholesterol lowering yogurt. A: the effect of inoculum and rate on pH value. B: the effect of temperature and rate on pH value. C: the effect of inoculum and temperature on pH value. D: the effect of inoculum and rate on titradable acidity. E: the effect of temperature and rate on titradable acidity. F: the effect of inoculum and temperature on titradable acidity. G: the effect of inoculum and rate on viable count. H: the effect of temperature and rate on viable count. I: the effect of inoculum and temperature on viable count. (1): Contour, (2): 3D surface.

The most desirable conditions combined the numeric factors on the responses according to the Box-Behnken Design was 2:1 of inoculation rate, 4.02% of inoculum, under 42.26℃ of fermentation temperature. The results of the orthogonal test by Box-Behnken Design were consistent with the optimal condition results of single factor experiment in the actual operation which the inoculation rate of 2:1, 4% of inoculum and fermentation under 43℃. The most desirable conditions of development technology of cholesterol lowering yogurt by Box-Behnken Design was shown as Fig 2 A - C, at the prediction of 85.9%.

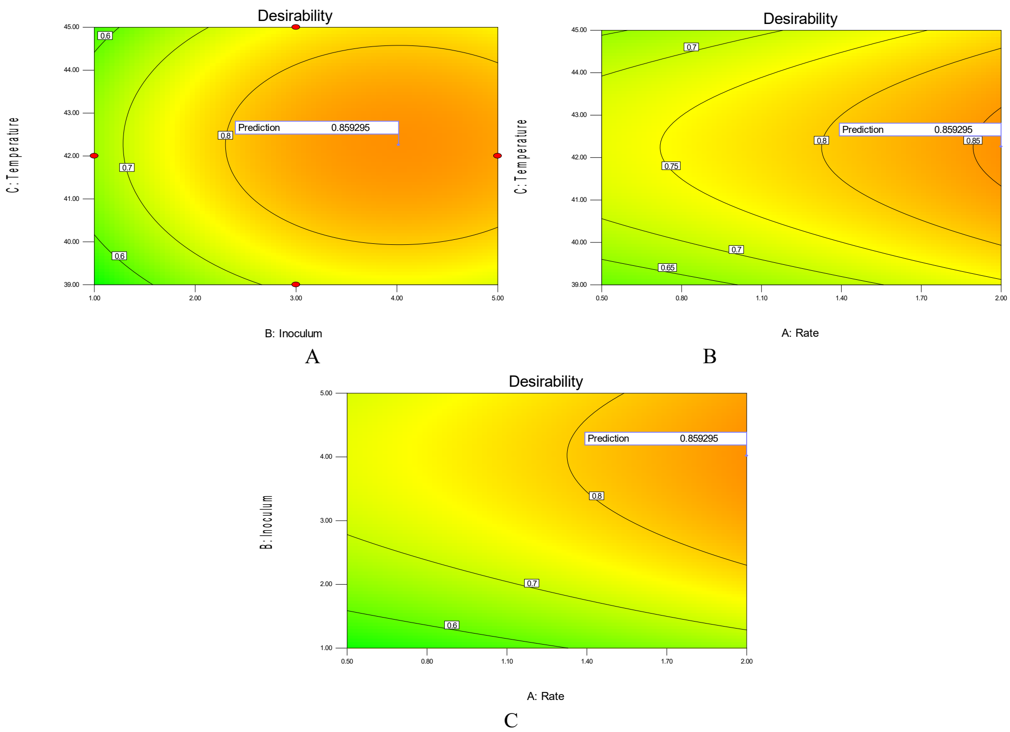


Fig 2. The most desirable conditions of development technology of cholesterol lowering yogurt, at the prediction of 85.9%. A:the effect of the inoculum and temperature on optimal inoculation rate of 2:1. B: the effect of temperature and rate on 4% of inoculum. C: the effect of inoculum and rate under 43℃ fermentation.

5.Chemical Composition Analysis

The production process under the optimal fermentation conditions of cholesterol lowering yogurt was obtained and new yogurt product was produced under the optimal conditions, 2:1 of inoculation rate, 4% of inoculum and under 43℃ of fermentation temperature. The results of chemical composition analysis were shown as Table 6.

TABLE 6. THE CHEMICAL COMPOSITION OF CHOLESTEROL LOWERING YOGURT.

Cholesterol Lowering Yogurt	**Fat** ***(g/100g)***	**Protein** ***(g/100g)***	**Ash** ***(%)***	**Non-Fat Solid** ***(g/100g)***	**pH**	**Titradable Acidity** ***(°T)***
	3.15±0.08	2.97±0.13	0.87±0.01	10.31±1.23	4.45±0.09	89±1.83

All data was repeated twice and recorded as mean±SD.

6.Storage and Shelf Life

The cholesterol lowering yogurt was refrigerated and stored under 4℃ for 21 days. The pH and titratable acidity of cholesterol lowering yogurt product was determined in every 7 days (Table 7).

The results of chemical composition analysis and storage were closed to the past researches on development technology of yogurt[8, 9]. Therefore, the development technology of cholesterol lowering yogurt could be considered to apply in the factories.

TABLE 7. PH AND TITRATABLE ACIDITY OF CHOLESTEROL LOWERING YOGURT DURING STORAGE.

Cholesterol Lowering Yogurt	**1 Day**	**7 Day**	**14 Day**	**21 Day**
pH	4.45±0.09	4.37±0.12	4.29±0.08	4.23±0.07
Titradable Acidity (°T)	88±1.8	93±2.3	98±1.7	102±2.1

All data was repeated twice and recorded as mean±SD.

IV. ACKNOWLEDGMENT

Especially grateful to the professor Menghebilige provided the relevant experimental conditions in the course of the completion of the present study.

V. CONCLUSION

The cholesterol lowering yogurt was produced using 2 strains of *Lactobacillus plantarum* strains AM2-6 and BM2-5 with cholesterol lowering ability which isolated from Inner Mongolian fermented camel milk "*Khoormog*" as the starter cultures. The development technology of cholesterol lowering yogurt was obtained by both single-factor experiment and Box-Behnken Design. The optimal conditions of development technology of cholesterol lowering yogurt was as follows: 2:1 of inoculation rate, 4% of inoculum, 43°C of fermentation temperature.

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