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Effects of Lycium Barbarum Polysaccharides on Properties of Fermented Milk Gel and Optimization of Lycium Barbarum Polysaccharides Yogurt Process

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Abstract: Lycium barbarum polysaccharide was considered as a new raw material in the making of functional yogurt. The present study was conducted to study the gel properties of acidity, water holding capacity, viscosity, hardness, consistency, cohesion, viscosity and microbiological characteristics in yoghurt samples produced with different concentrations of lycium barbarum polysaccharide. Based on the sensory score of yogurt, the orthogonal experiment was used to optimize the technology of lycium barbarum polysaccharide yogurt on the basis of single factor experiment. The results showed that lycium barbarum polysaccharide had certain influence on the microbiological characteristics of the finished yogurt. Adding lycium barbarum polysaccharide could improve the acidity, water holding capacity, texture characteristics of the fermented milk. When the amount of lycium barbarum polysaccharide added was 0.8%, the water holding capacity, hardness, consistency, cohesion and viscosity were maximized. The optimum conditions for lycium barbarum polysaccharide yoghurt were fermentation time 7h, fermentation temperature 42°C, strain inoculum 0.1%, lycium barbarum polysaccharide 0.8%, and sensory evaluation under this condition was 97.6 points. Adding lycium barbarum polysaccharide could effectively improve the gel properties and related physical indicators of fermented milk, and improve the quality of fermented milk. The results laid a theoretical foundation for the development of new functional yogurt.

Keywords: Lycium Barbarum Polysaccharide, Yogurt, Lactobacillus, Texture

1. Introduction

Lycium barbarum, also known as wolfberry and white thorn, is a traditional and popular medicinal and edible homologous plant in China because of its remarkable health care effect. Polysaccharide is the main active component of Lycium barbarum. It belongs to water-soluble protein polysaccharide. It is composed of six monosaccharides making up with arabinose, glucose, galactose, mannose, xylose and rhamnose. The molecular weight is as high as 10-2 250kDa [1]. At the same time, lycium barbarum polysaccharide (LBP) also contains galacturonic acid, glucuronic acid and amino acids [2]. The content of LBP in Ningxia is the highest due to it is correlated with altitude, up to more than 8%. LBP has low sweetness and has the function of reducing blood sugar, tonifying kidney and liver, enhancing immunity and so on. LBP has no toxic side effects and no adverse effects on human health [3]. It has great potential in the development of functional health food.

With the continuous improvement of people's requirements for healthy diet, functional yogurt is more and more favored by people. In developed countries, there are more than 3000 kinds of functional yogurt, and the sales volume is second only to fresh milk [4]. Some scholars applied arctium lappa polysaccharide to yogurt processing, which significantly improved the taste and tissue state, and significantly improved the number of live bacteria [5]. Gunenc have made seabuckthorn polysaccharide into yogurt processing, the water holding rate of yogurt is as high as 100% [6]. Some scholars have added seaweed polysaccharide to yogurt, which has a certain effect in increasing the number of lactic acid bacteria, improving the fermentation process, the taste and flavor of yogurt [7]. Functional polysaccharides enter the human body with yogurt as the carrier, which is very beneficial in promoting human intestinal health and preventing cardiovascular diseases. Although some scholars developed solidified lycium barbarum yogurt, only the process formula was optimized, and the research on the effect of LBP on the quality of yogurt was rare [8]. This paper studied the effects of LBP with different amounts on the quality of yogurt and the proliferation and quantity of lactic acid bacteria in yogurt during storage, which laid a theoretical foundation for the development of new functional yogurt.

2. Materials and Methods

2.1. Materials

Raw milk was obtained from the experimental farm of Yangzhou University.

Lycium barbarum polysaccharide (LBP) was bought in the market from Xi'an Lisen Biotechnology Co., Ltd.

Yoghurt starter (Yo-mix 300, containing Lactobacillus bulgaricus, Streptococcus thermophilus and Bifidobacterium) was bought from Danisco (China) Co., Ltd.

2.2. Manufacture of LBP Yogurt

Raw milk was heated to 60°C, then 6% sugar and 0.6% -1.4% LBP was added, and stir continuously to dissolve the additives and homogenize. The mixtures were pasteurized at 95°C for 5 minute, and then cooled to 42°C, the milk was inoculated with starter that 1 DCU corresponds to 5 kg of milk. The cups were incubated at 42°C until coagulation and stored at 4°C. Samples analyzed as fresh after 24h and periodically during storage period for 28d. All experiments were carried out in triplicate.

2.3. Detection and Analysis

2.3.1. Lactic Acid Bacteria Count

Lactic acid bacteria count were determined by an assay modified from Waldemar [9].

2.3.2. Acidity Determination

Acidity determination were determined by an assay modified from Dorota [10].

2.3.3. Water-Holding Capacity

Water-holding capacity were determined by the method described by HUANG [11].

2.3.4. Texture Analysis

The texture analysis of yogurt were determined by the method described by Mousavi [12].

2.3.5. Experimental Design

According to the results of single factor experiment, four factors including fermentation time (A), fermentation temperature (B), strain inoculation amount (C) and LBP addition amount (D) were studied by a single factor experiments. Each factor was set with three levels, and L_9 (3^4) orthogonal experimental was designed. The factor levels are shown in Table 1.

Table 1. *Factors and levels of orthogonal experiment.*

	fermentation time /h (A)	fermentation temperature /°C (B)	strain inoculation amount /% (C)	LBP addition amount /% (D)
1	5	38	0.06	0.6
2	6	40	0.10	0.8
3	7	42	0.14	1.0

2.3.6. Sensory Evaluation

The sensory evaluation of yogurt samples was performed by a method of adjustment by Staffolo [13].

2.4. Statistical Analyses

The experiment was repeated three times. The average, and the standard deviation was calculated. All of the analyses were carried out using the SPSS vesion 11.5.06 (sep, 2002. SPSS Inc., 1999-2002).

3. Results and Discussion

3.1. Effect of LBP Amounts on the Content of Lactic Acid Bacteria in Yogurt During Storage

The changes of the number of viable bacteria of LBP yoghurt during the 28 day storage period were shown in figure 1, figure 2 and figure 3. It could be seen that the number of viable bacteria of the three lactic acid bacteria reached the peak in about a week with the extension of storage time, and then showed a gradually decreasing trend. The number of viable lactic acid bacteria in the blank control group was also similar to that of LBP yogurt. However, the number of viable lactic acid bacteria in yogurt containing LBP was significantly higher than that in the control group after 14 days of storage ($p<0.05$). It showed that LBP could maintain the number of viable lactic acid bacteria, but there was a difference in the addition concentration [14]. It was speculated that it may be related to the quality of LBP.

Streptococcus thermophilus and *Lactobacillus bulgaricus* have a high number of live bacteria, and the total number of bacteria after 28 days of storage was more than 10^8 CFU/ml. As a combination of two common fermentation strains for yoghurt fermentation, they grew well in yoghurt due to their good interaction. It could be seen from figures 1 and 2 that after the fermentation of yogurt added with LBP, the number of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* was significantly better than that of the control group. It was always higher than that of the control group during storage, indicating that LBP had an obvious effect on maintaining the number of live *Streptococcus thermophilus* and *Lactobacillus bulgaricus* during storage.

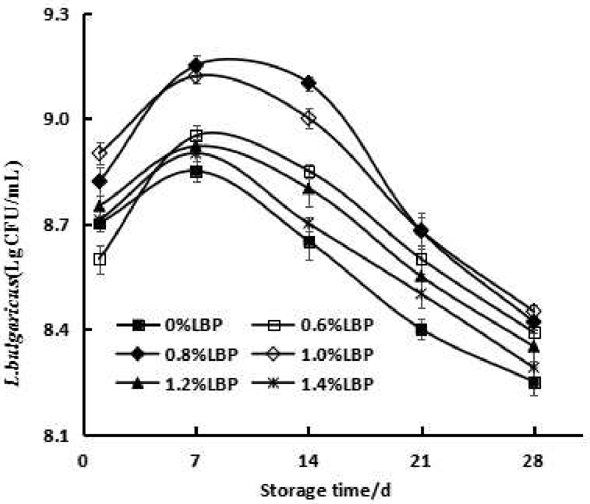

Figure 1. *Effects of LBP content on the growth of L. bulgaricus in yogurt.*

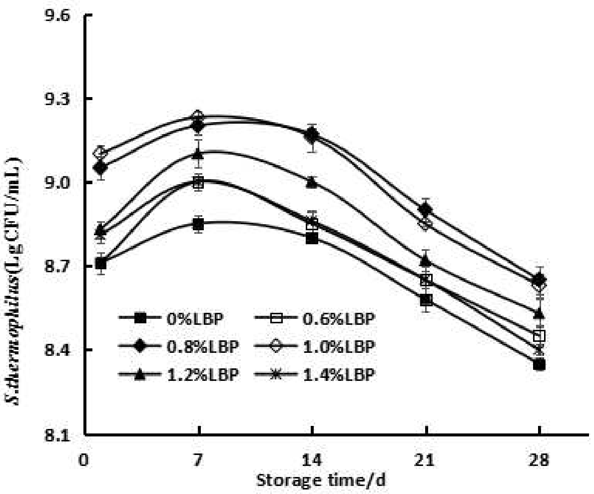

Figure 2. *Effects of LBP content on the growth of S. thermmophilus in yogurt.*

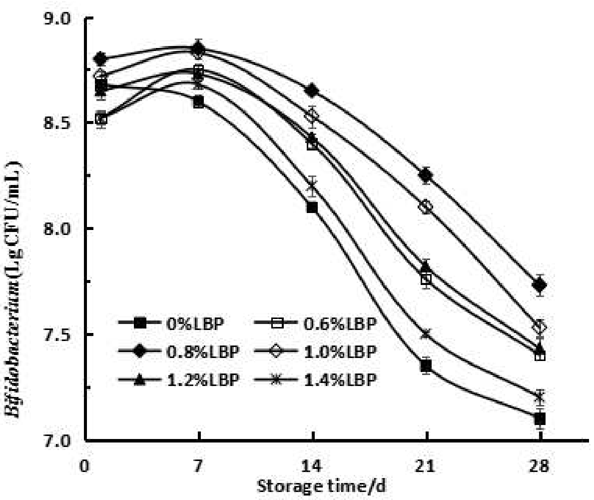

Figure 3. *Effects of LBP content on the growth of Bifidobacterium in yogurt.*

Figure 3 showed that the total number of *Bifidobacteria* after 28 days of storage was relatively small, from 8.7 to 7. The number of bacteria was about 10^7CFU/ml. The analysis might be due to its poor tolerance to oxygen. The number of viable *Bifidobacteria* in LBP yoghurt was higher than the control group, which showed a trend that the number of viable *Bifidobacteria* in LBP group with a mass fraction of 0.8% was the highest. When the addition amount was greater than 0.8%, the number of viable *Bifidobacteria* decreased with the increase of the addition amount.

3.2. Effect of LBP on Acidity of Yogurt

Figure 4 showed the effect of LBP with different amounts on the acidity of yogurt. After the completion of yogurt fermentation, the acidity of LBP yogurt was significantly higher than that of the control group. When LBP was added with a mass fraction of 0.8%, the maximum acidity of yogurt was 78° T. After adding LBP ≥ 1.0%, the acidity decreased, but it was still higher than that of the control group. The lack of acidity can weaken the gel structure of yogurt. In the yogurt added with LBP, lactic acid bacteria decompose lactose and LBP into glucose and galactose, and further enhance the ability to further decompose lactic acid. When adding 0.8% LBP, lactic acid will reach the maximum.

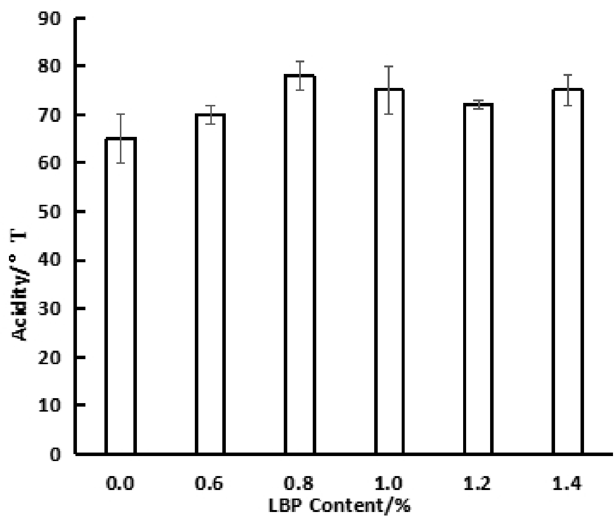

Figure 4. *Effects of LBP content on the acidity of yogurt.*

3.3. Effect of LBP on Water Holding Capacity of Yogurt

Water holding capacity was one of the important standards to measure the quality of yogurt. Strong water holding capacity indicateed that yogurt had good stability [15]. It could be seen from Figure 5 that the water holding capacity of yogurt added with LBP was quite different from that of the control group. After fermentation, the highest water holding capacity of fermented yogurt with 1.0%LBP, indicating that the addition of LBP increased the gel network of yoghurt, which might be more stable by hydrogen bonds between the hydroxyl groups and the amino groups in the milk protein [16]. Some scholars had also shown that the addition of polysaccharides could reduce the spontaneous separation of whey, which might be related to the fat in yogurt [17]. However, with the increase of LBP content, the water holding capacity decreased obviously and the surface of yogurt was uneven. The main reason was that the addition of LBP destroyed the three-dimensional network of casein gel which existed in yogurt, which is consistent with the rheological and texture results.

3.4. Effect of LBP on Texture of Yogurt

Table 2 showed the texture data of yogurt added with different amounts of LBP after fermentation and stored at 4°C for 24 hours. Overall, the four indexes of yogurt after adding LBP were significantly higher than those in the control group without LBP ($p < 0.05$), indicating that the addition of LBP significantly improved the texture of yogurt.

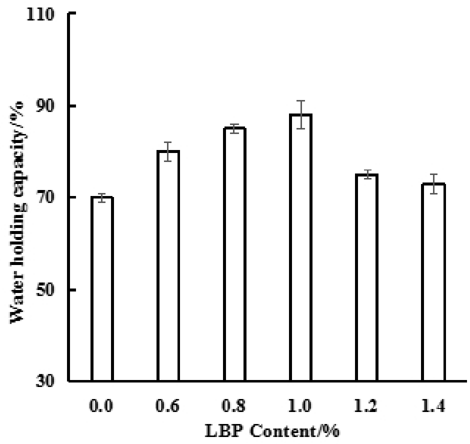

Figure 5. *Effects of LBP content on the water holding capacity of yogurt.*

Table 2. *Effects of LBP content on texture of yogurt.*

LBP content/%	Hardness/g	Consistency/g.s	Cohesion/g	Viscosity/g.s
0.0	43.06±1.30a	345.70±14.24a	42.44±3.28a	58.18±4.38a
0.6	56.96±2.46b	507.12±30.02d	63.11±4.98c	78.80±3.66c
0.8	63.00±1.59c	480.16±14.27c	83.31±2.99d	83.79±1.79d
1.0	66.23±1.27d	482.20±18.35c	82.08±3.20d	75.41±4.71c
1.2	60.87±2.40c	454.54±12.67b	66.08±3.11c	70.40±1.54b
1.4	60.30±2.28c	427.41±20.03b	53.30±0.24b	65.29±3.93b

Note: different lowercase letters indicate significant differences between groups ($p < 0.05$).

The effect of LBP addition on the texture of yogurt was as follows: in terms of hardness index, when 1.0% LBP was added, the hardness value of yogurt reaches the maximum, and when the LBP addition was less than 1.0%, the hardness increased with the increase of concentration. When LBP was added more than 1.0%, the hardness of yogurt decreased significantly, but there was no significant difference between 1.2% and 1.4% ($p>0.05$). Consistency, adding LBP to yogurt, gel strength was increased and thicker. When 0.6%LBP dosage increased, the gel strength reached the maximum. However, with the addition of LBP increased, the gel strength decreased significantly. There was no significant difference between 0.8% and 1% groups ($p>0.05$), and significantly higher than that in 1.2% and 1.4% groups ($p<0.05$). It was known that the gel strength of milk coagulant was mainly related to the protein content and total solids content in milk [18]. The addition of LBP played a certain stabilizer role. The gel strength of LBP yogurt was better than that of the control group ($p<0.05$). With the addition of more LBP, the strength of casein gel network was destroyed to some extent. The consistency of yogurt decreased with the addition of higher LBP. The consistency of yogurt in the groups with LBP 0.8%, 1.0%, 1.2% and 1.4% was significantly lower than that in the group with LBP 0.6% ($p < 0.05$). The trend of cohesion and viscosity was similar to that of hardness, which reached the maximum at 0.8% and 1.0%. The addition of 1.2% and 1.4% significantly decreased the cohesion and viscosity of yogurt, but there was no significant difference between 1.2% and 1.4% ($p>0.05$). The results showed that proper addition of LBP (0.8% ~ 1.0%) could improve the texture of yogurt. The overall texture quality of yogurt in 0.8% and 1.0% groups was better than that in other groups.

3.5. Optimization of LBP Yogurt Process

3.5.1. Single Factor Test Results

It could be seen from Figure 6(a) that with the extension of fermentation time, the sensory score of yogurt increases first and then decreases. When the fermentation time was 7 h, the sensory score of yogurt was the highest. When the fermentation time was less than 7 h, the fermentation of yogurt was incomplete. Otherwise, the fermentation time was higher than 7 h, the quality of yogurt was not ideal because of excessive fermentation. It could be seen from Figure 6(b) that temperature had a significant impact on the sensory score of yogurt. 42°C was the most suitable temperature for yogurt fermentation. Too low or too high temperature would affect the growth rate of lactic acid bacteria in yogurt. As showed in Figure 6(c), when the inoculation amount was 0.10%, the quality of yogurt was the best. When the inoculation amount was too low, the quality of yogurt was very poor. When the inoculation amount was higher than 0.14%, the sensory score of yogurt also decreases significantly. It could be seen from Figure 6(d) that the sensory effect of LBP on yogurt was mainly in viscosity and wire drawing. When the addition amount of LBP was 0.8%, the sensory score was also the highest. With the increase of the addition amount of LBP, the sensory score of yogurt decreased slightly.

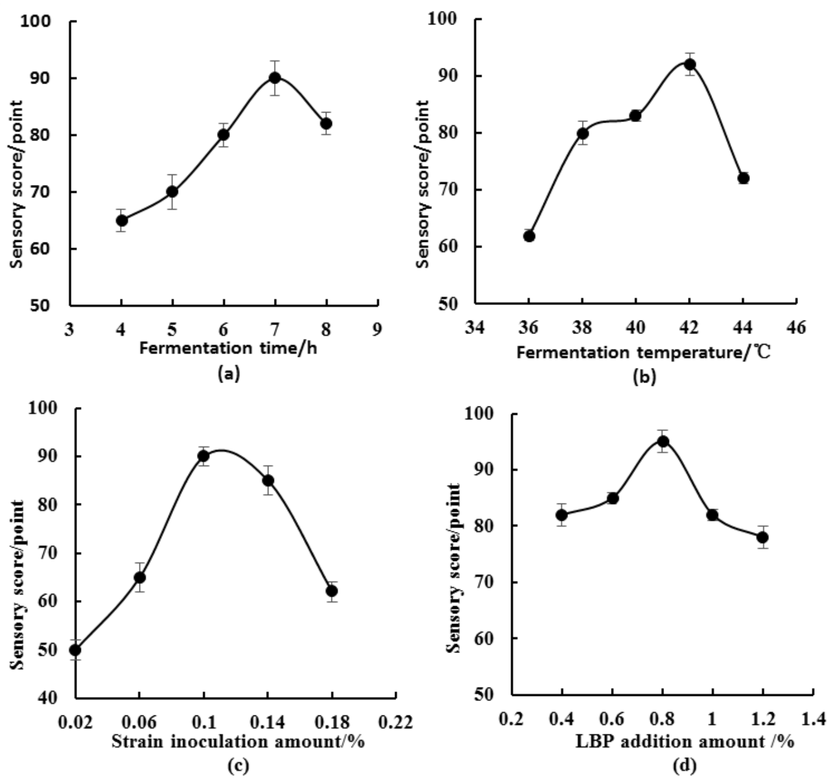

Figure 6. *Effects of different factors on sensory score of the LBP yogurt.*

Table 3. *The results of orthogonal test.*

	fermentation time /h (A)	fermentation temperature /°C (B)	strain inoculation amount /% (C)	LBP addition amount /% (D)	Sensory score / point
1	1	1	1	1	54.2
2	1	2	2	2	84.6
3	1	3	3	3	80.5
4	2	1	2	3	78.4
5	2	2	3	1	82.8
6	2	3	1	2	73.0
7	3	1	3	2	81.9
8	3	2	1	3	77.3
9	3	3	2	1	83.2
K1	73.100	71.500	68.167	73.400	
K2	78.067	81.567	82.067	79.833	
K3	80.800	78.900	81.733	78.733	
k1	24.367	23.833	22.722	24.467	
k1	26.022	27.189	27.356	26.611	
k3	26.933	26.300	27.244	26.244	
R	7.700	10.067	13.900	6.433	

3.5.2. Orthogonal Experiment

It could be seen from Table 3 that the optimal combination of orthogonal test results was No. 2 (A1B2C2D2), and the sensory score was 84.6. The primary and secondary order of the influence of various factors on the sensory score of LBP yogurt was C (inoculation amount) > B (fermentation temperature) > A (fermentation time) > D (addition amount of LBP). The horizontal combination of the optimal process was A3B2C2D2. The sensory score was 97.6, which was better than orthogonal test No. 6. Therefore, A3B2C2D2 was selected as the best process of LBP yoghurt, that was, fermentation time 7 h, fermentation temperature 42°C, inoculation amount 0.1%, LBP addition amount 0.8%.

4. Conclusion

Lycium barbarum polysaccharide will affect the lactic acid bacteria content, acidity, water holding capacity, rheological properties and texture indexes of yogurt. The yogurt with 0.8% Lycium barbarum polysaccharide has the best quality. Adding Lycium barbarum polysaccharides can effectively improve the gel properties and other physical and chemical indexes of yogurt, and improve the quality of yogurt. On the basis of single factor experiment, the process of seabuckthorn polysaccharide yoghurt was optimized by orthogonal experimental design. The results showed that the optimum process conditions of Lycium barbarum polysaccharide yoghurt were fermentation time 7 h, fermentation temperature 42°C, strain inoculation amount 0.1% and Lycium barbarum polysaccharide addition amount 0.8%.

In the future, we will further explore the mechanism of Lycium barbarum polysaccharides affecting the fermentation characteristics of yogurt, including the analysis of the effects of Lycium barbarum polysaccharides on yogurt gel structure by means of cryo electron microscopy, which is conducive to the application of Lycium barbarum polysaccharides in other functional foods.

Conflicts of Interest

The authors declare that all the authors do not have any possible conflicts of interest.

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