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The Impact of Barley Bran Powder on the Physicochemical, Rheological, and Sensory Properties of Probiotic Low-Fat Cheese: A Response Surface Methodology Approach

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Background: This study was conducted to produce probiotic cheese containing barley (*Hordeum vulgare*) bran, optimizing its physicochemical, rheological, and sensory characteristics while maximizing the viability of *Lactobacillus acidophilus* over a nine-day storage period at 4 °C.

Methods: Using the Response Surface Method (RSM), 13 treatment combinations were designed, including barely bran concentrations ranging from 0% to 4% and storage durations extending up to 42 days. Two types of cheese were evaluated: probiotic cheese and probiotic cheese containing barley bran. Comprehensive assessments included microbial viability, physicochemical variables (such as pH and acidity), rheological properties, and sensory evaluation.

Results: The highest viability of probiotics, recorded at 8.7 log CFU/g, was observed in the probiotic low-fat cheese on day 1 with a barley bran concentration of 4%. The physicochemical analysis indicated suitable pH and acidity levels. Rheological characteristics, such as viscosity and hardness, were enhanced in barley bran samples, whereas adhesiveness showed no significant change. The color index (L*) decreased in the samples with added barley bran.

Conclusion: Incorporating 2.37% barley bran into the cheese formulation resulted in a product with optimal physicochemical, rheological, and sensory properties. In addition, the population of *L. acidophilus* bacteria increased to 8.21 log CFU/g, and these characteristics were maintained for approximately nine days during refrigerated storage (4 °C).

1. Introduction

Dairy products, particularly cheese, play a vital role in human nutrition (da Cruz et al., 2009). Cheese is one of the good sources of protein, vitamins, and minerals; however, it is notably low in dietary fiber (Gahruie et al., 2015). Given this deficiency, there is a growing interest in developing dairy products containing fiber, thereby enhancing their

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nutritional value. The addition of plant-based ingredients rich in fiber, such as barley bran, wheat fiber, and inulin, has gained popularity in this context (Hasani et al., 2016; Karaca et al., 2019). Barley bran contains polysaccharide and hemicellulose compounds and has both soluble (beta-glucan) and insoluble (cellulose) dietary fibers. These components serve as low-energy and reasonably-priced ingredients in food production (Ghaemi et al., 2010). Furthermore, barley bran is recognized as a significant source of dietary fiber (Abdul-Hamid & Luan, 2000). The fiber in barley is indigestible and plays a crucial role in controlling cardiovascular diseases, type 2 diabetes, glycemia control, blood lipid levels, increasing intestinal motility, preventing or eliminating constipation, regulating appetite, preventing cancer risk, and enhancing the immune system, thereby contributing significantly to overall health (Lampe, 1999; Slavin, 2013). Probiotics are defined as live microorganisms that, when consumed in enough quantities, offer health benefits to the host. A critical quality indicator for probiotic products is the viability of probiotic bacteria 10^7 CFU/mL of bacteria until consumption is needed to be beneficial in providing health (Asadzadeh et al., 2021). Their health benefits include increasing immune response, reducing serum cholesterol, vitamin synthesis, anticancer activity, and antibacterial activity (Karimi et al., 2012). *L. acidophilus* bacteria are microaerophilic and their growth on solid culture medium is mainly intensified in anaerobic conditions or reduced oxygen pressure and the presence of CO_2. These bacteria are the natural flora of the small intestine of humans and animals. They grow and multiply well in the small intestine due to the low surface tension created by bile salts (Lamoureux et al., 2002). Unfortunately, these microorganisms in dairy products cannot survive and maintain themselves in the production due to unfavorable conditions such as low pH and increased organic acids during cold storage), or they may even change the flavor of the product (Hasani et al., 2016). Prebiotics, particularly oligosaccharide compounds such as soluble fibers, can increase the growth or activity of lactic acid bacteria. Consuming prebiotic compounds in food increases the activity of probiotics in the intestine, and as a result, they have useful effects on human health. Fibers may be used as a food (prebiotic) by probiotic bacteria and increase their shelf life (De Vrese & Offick, 2010; Malago et al., 2011). Consequently, using prebiotics can be a method to increase the viability of probiotic bacteria in food products with low pH. Adding barley bran in high amounts to probiotic low-fat cheese is no longer within the beyond research with the aid of the usage of the RSM software. In the study by Hasani et al. (2016), they added barley bran to yogurt, which was different from our product, which is cheese. In addition, optimization with RSM was not done in their study. Also, in the study of Mousavi et al. (2019a), flaxseed was added to probiotic yogurt. Which was different from our product, which is cheese and barley bran. The purpose of this study is to investigate the prebiotic effect of barely bran on the probiotic *L. acidophilus* bacteria in probiotic cheese and to find the best conditions and physicochemical, rheological, and sensory characteristics of two low-fat kinds of cheese (0% barely bran probiotic cheese and 2% and 4% probiotic cheese) by using RSM software.

2. Materials and Methods

The milk used in this research was low-fat pasteurized milk (1.5%), purchased from Pegah Company (Hamadan, Iran). Barley bran was sourced from OAB Company (Tehran, Iran). Further, cheese curd (starter), microbial rennet, and the probiotic strain *Lactobacillus acidophilus* (PTCC 1643) were obtained from the Collection Center of Industrial Fungi and Bacteria (Tehran, Iran).

2.1 Preparation of probiotic low-fat Cheese (control sample)

Milk samples (100 mL) were heated for 30 min at a temperature of 63-65 °C. After cooling to 35°C, a starter culture containing *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*, obtained from Chr. Hansen Co. (Copenhagen, Denmark), was inoculated at a rate of 0.5% (w/w). Probiotic bacteria (*L. acidophilus*) were then inoculated to the milk samples at the rate of 10^8-10^9 CFU/mL. Subsequently, 0.02% (W/V) calcium chloride was added. When the pH reached 5.6, 0.001% (W/V) microbial rennet, acquired from the Collection Center of Industrial Fungi and Bacteria (Tehran, Iran), was added to the milk. To enhance rennet efficiency, the milk was maintained at around 35 °C during the coagulation period. After 1 h, the formed clot was placed under the pressure using a sterile weight for 6 h to extract water. The dehydrated clot was then immersed in 20% (W/V) sterile salt water for 8 h. Following this, the cheese samples were transferred to sterile 8% salt water and kept for 15 days at a temperature of 12-14 °C. After this initial ripening period, the samples were kept for 42 days at 4 °C for the final ripening period (Ehsani et al., 2011).

2.2 Preparation of probiotic low-fat Cheese containing barely bran

To prepare this type of cheese, barley bran powder (2% and 4%) was added to 100 mL of milk, followed by heating. The next steps were similar to the approach in section 2.1. Finally, 13 treatments of probiotic low-fat cheese were prepared (Table 1).

2.3 Measurement of the viability of probiotic bacteria

10 g of probiotic low-fat cheese samples (0%, 2%, and 4 %) were added to five flasks, to which 90 mL of 0.1% peptone water was added. The mixtures were thoroughly mixed, and 7 dilutions (from 10^{-1} to 10^{-7}) were prepared from each sample. Then, 0.1 mL of the dilutions at 10^{-5}, 10^{-6}, and 10^{-7} were plated, creating surface cultures. This plating process was performed in duplicate. The plates were then transferred to an incubator at 37°C for 24-48 h. After 24-48 h. Following the incubation period, colony counts were conducted using the following formula (Hasani et al., 2016).

$$\text{CFU/ml: } \frac{\text{No.of colonies} \times \text{Total dilution factor}}{\text{volume of culture plated in ml}}$$

2.4 Measurement of pH

First, the pH meter (Denver Instruments, TX, USA) was calibrated with buffers pH values of 4 (acidic), 7 (neutral), and 9 (alkaline). Then, 10 g of the cheese sample was transferred to a 100 mL beaker, which was placed under the electrode pH meter. The pH value was then recorded as indicated by the device (Hasani et al., 2016; Nematollahi et al., 2016).

2.5 Measurement of acidity

The cheese samples were completely homogenized with a homogenizer (Krones, Germany). Then, 10 g of the homogenized cheese was transferred to a flask, to which 20 mL of distilled water and 0.5 mL of phenolphthalein reagent (Merck, Germany) were added. The mixture was then mixed thoroughly and slowly. The resulting solution was titrated with 0.1 N sodium hydroxide (Merck, Germany) until a pale pink color appeared (Mousavi et al., 2019a).

2.6 Measurement of rheological properties

The viscosity, hardness, and adhesiveness of the cheese samples were assessed with a Zwick Texture Analyzer (Roller Company, Ulm, Germany) at the Institute of Nutritional Research and Food Industry, Shahid Beheshti University of Medical Sciences. The probe used in this test was a cylindrical type with a diameter of 38 mm. The penetration speed of the probe into the sample was 1 mm per second and the penetration depth was 30 mm (Azari-Anpar et al., 2017a).

2.7 Measurement of sensory properties

The sensory high-quality of the cheese samples was evaluated by 30 panelists using the hedonic scale (5-point hedonic method). The prepared cheese samples were analyzed for sensory parameters such as color, odor, taste, and texture. A score of 5 indicated excellent quality, while a score of 1 represented very poor quality (Azari-Anpar et al., 2017a).

2.8 Measurement of color indicators

Color indicators, including L*, a*, and b* were measured using a HunterLab (Colorflex EZ, Virginia 20190, USA). The L* index indicated lightness, the a* index indicated the green-red color, and the b* index indicated the blue-yellow color (Mousavi et al., 2019a).

2.9 Optimization

Using the RSM software program, the optimal conditions for producing probiotic low-fat cheese containing barley bran were determined. The goal contained maximizing both independent variables (storage time and barley bran) and the response variables (viability of *L. acidophilus*, viscosity, hardness, color, smell factor, taste, and texture) while minimizing adhesiveness. Furthermore, the variables of pH and acidity were maintained within specified ranges (Table 2).

Table 1. Thirteen treatments were designated for two types of low-fat cheese by RSM (central composite design)

RUN	(X1)	(X2)
1	1	0
2	42	0
3	21	2
4	21	2
5	42	4
6	21	2
7	21	2
8	21	0
9	21	2
10	42	2
11	21	4
12	1	2
13	1	4

Table 2. Goals of optimal level for the production of probiotic low-fat cheese containing barley bran

Goals of independent variables		Goals of Responses									
Storage time	Barley bran	Viability of *L. acidophilus*	pH	Acidity	Viscosity	Adhesiveness	Hardness	L*	a*	b*	Sensory evaluation
Max	Max	Max	In range	In range	Max	Min	Max	Max	In range	In range	Max

2.10 Statistical analysis

Using Design Expert 7.0.0 statistical software, thirteen treatments were developed for two types of low-fat cheese: probiotic cheese and probiotic cheese containing barley bran. The RSM method was used to optimize probiotic cheese containing barley bran. In this approach, the usage of the central composite design (CCD) with 3 levels and 5 repetitions for the central point, storage time (X1), and barley bran concentration (X2) were considered as independent variables (Table 2). Storage times of 0, 21, and 42 days were evaluated, while barley bran concentrations of 0%, 2%, and 4% were assessed. The response variables were pH, acidity, viability of *L. acidophilus*, viscosity, adhesiveness, hardness, color index, and sensory assessment. Significant variations among treatments were calculated through One-way ANOVA in the RSM software. The significance level for all investigated variables was less than 0.01 ($p < 0.01$). The characteristics and testing methods of the cheese were compared and analyzed against the Iranian national standard quantity 13418.

3. Results and Discussion

3.1 Evaluation of the viability of L. acidophilus

The survival method of *L. acidophilus* is reported in Figure 1a. The viability of *L. acidophilus* increased from 7.1 log CFU/g to 8.7 log CFU/g. If the number of bacteria obtained is

10^6 CFU/mL, it can be concluded that barley bran functions as a prebiotic, enhancing the viability of probiotic bacteria (Hasani et al., 2016). The highest concentration of viable *L. acidophilus* cells in samples containing 4% was recorded at 8.7 log CFU/g on the first day of storage, while the lowest concentration in control samples, devoid of barley bran, was observed on the 42nd day (7.1 log CFU/g). Comparative assessments between cheese samples containing 2% barley bran and control samples revealed that the former demonstrated greater, viability of *L. acidophilus* during storage at days 1, 21, and 42. Furthermore, the viability of *L. acidophilus* in cheese samples with both 2% and 4% barley bran decreased with a lower slope during days 1, 21, and 42 (Figure 1a), whereas samples without barley bran decreased more steeply ($p < 0.01$). This research indicates that the fiber in barley bran acted as a prebiotic and caused the growth and strengthening of *L. acidophilus* as a probiotic. Our results were similar to the results of Heshmati et al. (2016), who reported that adding barley bran (1.2%) and rice bran (1.2%) to 100 g of probiotic yogurt containing *L. acidophilus* accelerated the viability of *L. acidophilus* for 28 days compared to control samples. Additionally, our results were similar to the results of Hasani et al. (2016), which demonstrated that adding rice bran (1.2%) to 100 g of probiotic-stirred yogurt containing *L. acidophilus* significantly increases bacterial growth over 28 days. While the study by Azari-Anpar et al. (2017b) was against our results, indicating that the addition of 5% aloe vera gel to probiotic yogurt decreased the quantity of *Bifidobacterium lactis* bacteria on the 28th day of storage from 8.74 log CFU/g to 7.79 log CFU/g and decreased the number of *L. acidophilus* bacteria from 7.94 log CFU/g to 7.80 log CFU/g by the 28th day of storage.

3.2 Evaluation of pH

The pH levels in samples containing *L. acidophilus* and barley bran during storage on days 1, 21, and 42 were significantly lower than those in control samples containing only *L. acidophilus* (Figure 1b) ($p < 0.01$). The pH values of samples containing *L. acidophilus* and barley bran, as well as the control cheese samples ranged from 4.1 to 5.2. The highest pH value was recorded on the first day of storage, in the probiotic cheese samples without barley bran (pH = 5.2). Conversely, the lowest pH level was observed on day 42 and the concentration of 4% barley bran (pH = 4.1). This comparison shows that the fiber in barley bran caused more growth and activity of *L. acidophilus* and pH decreased. Our results were consistent with the results of Vahedi et al. (2008). They investigated the optimization of fruit yogurt and assessed its quality assessment during storage and showed that the pH value decreased by 0.2 units over time during storage due to microbial. Motamedzadegan et al. (2015) examined the effect of gelatin type on the functional characteristics of non-fat yogurt and reported a decrease in pH following incubation. This reduction in pH is attributed to the metabolic activity of yogurt bacteria, which produces and the result of acid and enhances the protein network of milk.

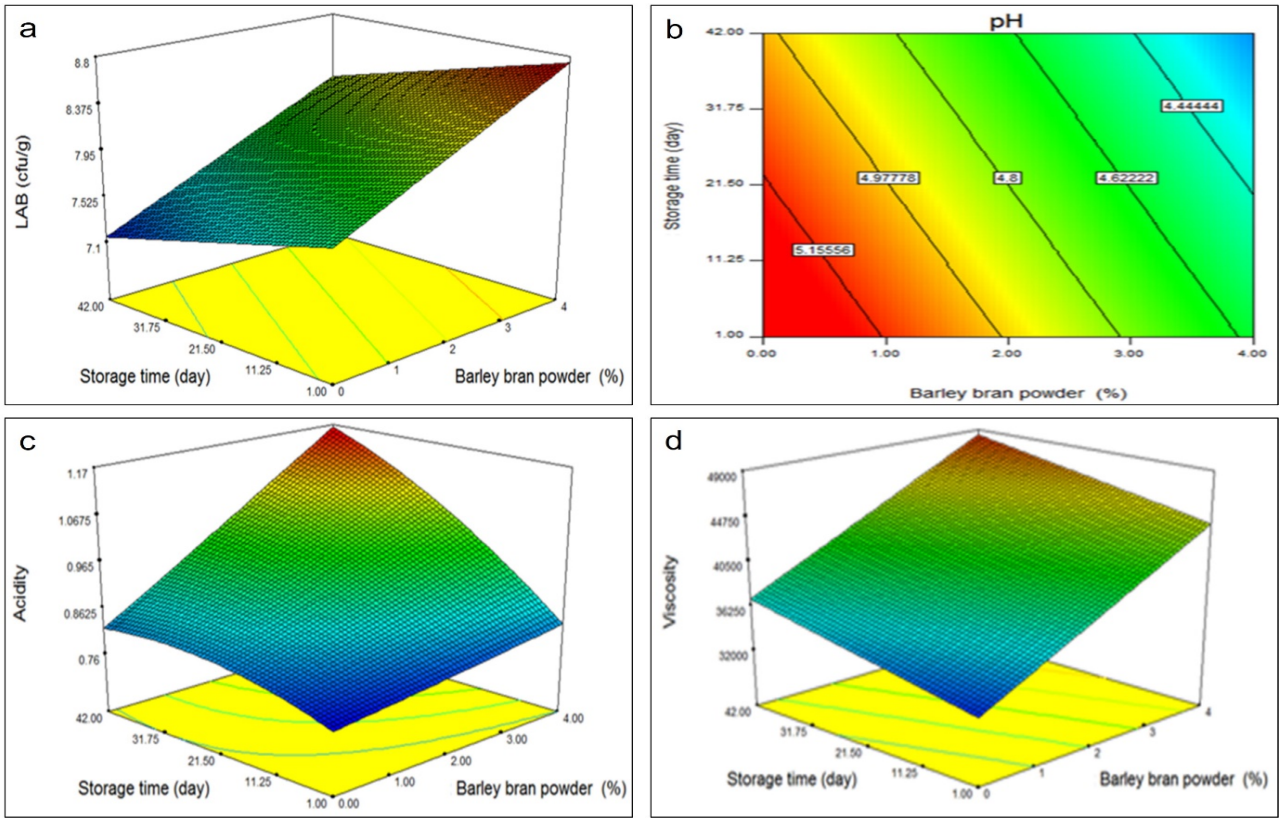

Figure 1. Viability of *L. acidophilus* (a), PH (b), acidity (c), and viscosity (centipoise) (d) of probiotic low-fat cheese samples during days 1, 21, and 42

3.3 Evaluation of acidity

The acidity levels in the samples containing *L. acidophilus* and barley bran during storage were significantly higher than those in the control samples, which contained only *L. acidophilus* (Figure 1c) ($p < 0.01$). The acidity range of the samples with *L. acidophilus* and barley bran, as well as the control cheese samples, was from 0.76 to 1.15 (%W/W). The highest acidity was seen on day 42 in probiotic cheese with a concentration of 4% barley bran (1.15% W/W), the lowest acidity was on day 1 and in probiotic cheese without barley bran (just containing *L. acidophilus*) (0.76% W/W). These changes can be due to more growth and activity of *L. acidophilus* bacteria in the presence of barley bran and more lactic acid production. The production of high lactic acid causes the death of bacteria, and the high acidity in whole grain samples is probably due to the nutritional value of whole grain cheese. Our results were similar to the results of Hasani et al. (2016). They reported the acidity of different yogurt samples after 1, 7, 14, 14, and 28 days of storage in the refrigerator, yogurt samples containing rice bran and probiotics (92 % W/W) had significantly higher acidity than those without bran (0.84% W/W). In general, an increase in bran levels correlates with elevated acidity, which can be explained by the greater fermentation of sugars into lactic acid due to the increased proliferation of these bacteria.

3.4 Evaluation of viscosity

The viscosity of probiotic cheese samples containing barley bran during storage on days 1, 21, and 42 was lower than that of probiotic cheese samples without barley bran. The range of viscosity changes of probiotic samples containing barley bran and probiotic cheese samples without barley bran was from 31743 to 49321 centipoise (Figure 1d). The highest viscosity recorded was for probiotic cheese samples with 4% barley bran (49321 centipoise) and the lowest viscosity was observed in probiotic cheese samples without barley bran (31743 centipoise). Rezaei et al. (2013) showed that adding guar gum to frozen yogurt significantly increased viscosity from 1522 mPa.s to 3305 mPa.s compared to the control sample without gum. In this research, the incorporation of barley bran increased the viscosity of 29926 centipoises in probiotic yogurt samples and 25968 centipoises in standard yogurt samples.

3.5 Evaluation of adhesiveness

Adhesiveness indicates the force required to overcome the bond between the surface of the coagulum and the surface of the remaining material, serving as an indicator of the textual desirability of the product for consumers (Fadela et al., 2009). The adhesiveness of probiotic cheese samples containing barley bran during storage on days 1, 21, and 42 was lower than that of probiotic cheese samples without barley bran (Figure 2a). The range of changes in the adhesion properties of probiotic samples containing barley bran and probiotic cheese samples without barley bran was observed from 0.21 to 0.58 N. The results of BahramParvar et al. (2013) were similar to our results. They reported that the addition of capacarrageenan at the rate of 0.02% reduces the adhesiveness (-16.2 g) of ice cream compared to the formulation without this hydrocolloid (-9.2 g).

3.6 Evaluation of hardness

The amount of hardness in probiotic cheese samples containing barley bran during storage on days 1, 21, and 42 was higher than probiotic cheese samples without barley bran (Figure 2b) ($p < 0.01$). The range of hardness changes of probiotic samples containing barley bran and probiotic cheese samples without barley bran was 18.53 to 33.8 N. The highest level of hardness on days 1 to 42 was observed in probiotic cheese samples containing 4% barley bran (33.8 N) and the lowest hardness level on days 1 to 42 samples was observed in probiotic cheese without barley bran (18.53 N). Our results were not similar to those of Azari-Anpar et al. (2017b). They reported that adding 5% of aloe vera gel to yogurt reduces the hardness of yogurt from 76.62 to 66.89 g due to the presence of salicylic acid in aloe vera gel. This can probably be due to the jelly and soft texture of aloe vera, and the water is retained inside the product, causing the level of hardness to decrease.

3.7 Evaluation of color index

A three-dimensional diagram illustrating the changes in the color index L* (lightness) of different cheese samples in response to different concentrations of barley bran is presented in Figure 2c. In probiotic cheese with a concentration of 4% barley bran, the L* index of the samples was 87.36. With the increase in the concentration of barley bran, the brightness L* of the samples decreases ($p < 0.01$). This can probably be due to the darker color of barley bran compared to that of the cheese, which adversely affected the brightness parameter, L*. The changes in the color index a* (Figure 2d) in probiotic cheese with 4% barley bran were -2.8 and in samples of probiotic cheese without barley bran were -7.9. Also, the color index b* (Figure 2e) in probiotic cheese with 4% barley bran was 44.2, compared to 25.12 for the samples without barley bran. Our results were consistent with the results of Zomorrodi (2013). They reported that adding wheat fiber (1.2%) to fruit yogurt decreases the amount of brightness (L*) from 73.94 to 72.04. In the study of García-Pérez et al. (2005), the amount of a* and b* increased in yogurt samples containing orange fiber, which was consistent with our results. In our study, the changes of color index a* in probiotic cheese with 4% barley bran was -2.8, and in probiotic cheese samples without barley bran was -7.9. Similarly, the color index b* for probiotic cheese with 4% barley bran was 44.2, while it was 25.12 for the samples without barley bran.

3.8 Evaluation of sensory properties

The cheese samples were evaluated for color, odor, taste, and texture on storage days 1, 21, and 42. The results showed that probiotic cheese samples had higher scores compared to

those containing barley bran. In terms of color, probiotic cheese samples with and without barley bran were rated between 3.9 and 5 points. The highest color score was recorded for the probiotic cheese sample without barley bran on day 1 (5 points) and the lowest was for the sample containing 4% barley bran on day 42 (3.9 points) (Figure 3a).

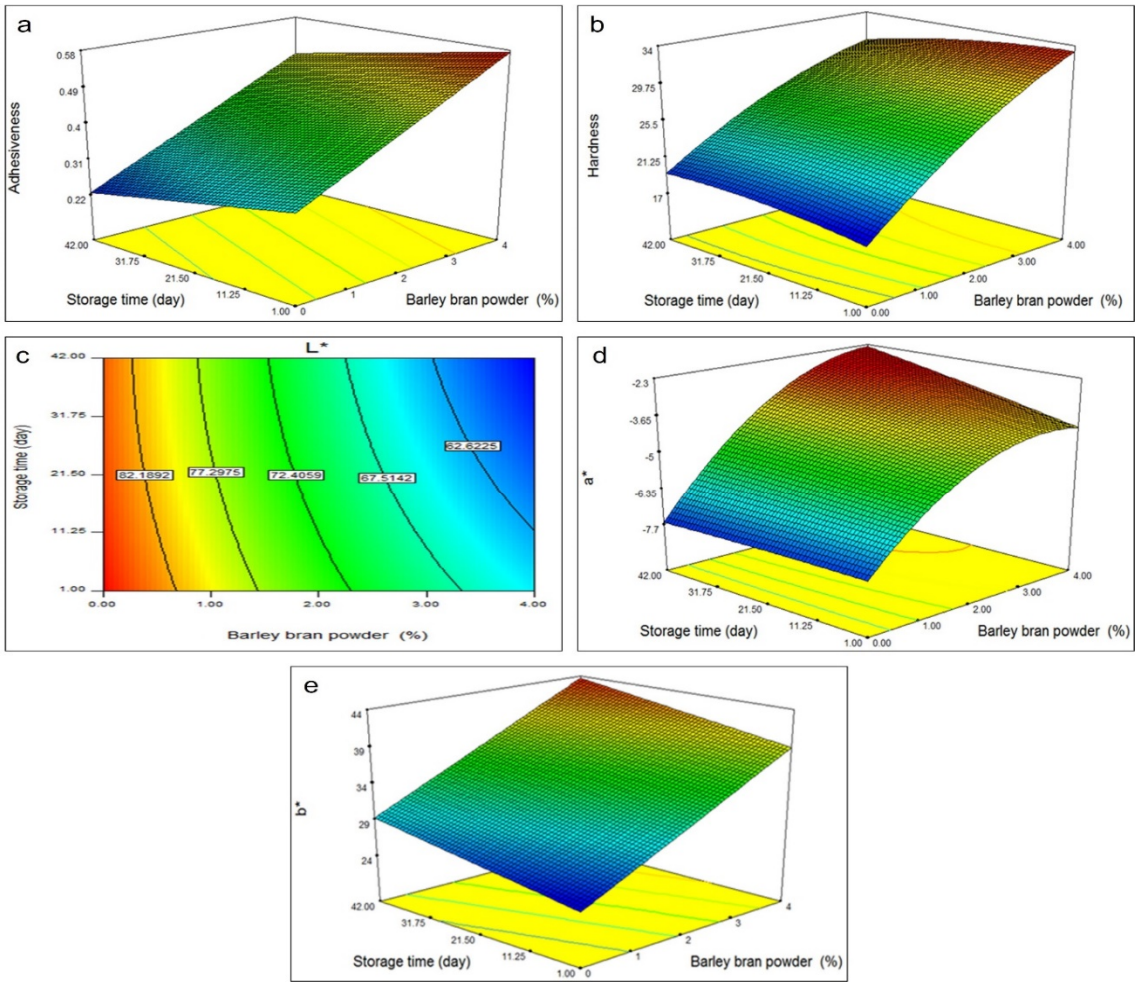

Figure 2. Adhesiveness (a), hardness (b), L* (c), a* (d), and b* (e) of probiotic low-fat cheese samples during days 1, 21, and 42

Regarding odor evaluation, the scores for probiotic cheese samples containing barley bran and probiotic cheese samples received scores from 2.1 to 4.9. The highest odor score was again for the probiotic cheese sample without barley bran on day 1 (4.9 points), whereas the lowest odor score was for the sample containing 4% barley bran on day 42 (2.1 points) (Figure 3b). In terms of taste, scores for probiotic cheese samples varied from 1.9 to 4.9. The highest taste score was the probiotic cheese sample on day 1 (4.9 points) and the lowest taste score was the probiotic cheese sample containing 4% barley bran on day 42 (1.9 points) (Figure 3c). For texture characteristics, probiotic cheese samples containing barley bran and probiotic cheese samples were scored from 2.9 to 5 points. The highest texture score was the probiotic cheese sample on day 1 (5 points) and the lowest texture score was the probiotic cheese sample containing 0% barley bran (without barley bran) on day 42 (2.9 points) (Figure 3d). Overall, with the addition of barley bran and the increasing storage time, the color score decreased. Overall, adding barley bran decreased the color score and generally increasing the storage time decreased the color score. These findings are similar to those of Mousavi et al. (2019a), who reported that the addition of flaxseed powder to probiotic yogurt decreased sensory evaluation scores.

3.9 Optimization

To find the optimal conditions for the production of probiotic cheese containing barley bran, optimization was done using the RSM software program. Barley bran concentration and storage time were considered independent variables, and dependent variables including probiotic viability, pH, acidity, viscosity, adhesiveness, hardness, color index, and sensory characteristics were considered as responses. Thirteen designed treatments

(Table 2) using the RSM software program were analyzed. Obtained optimum levels were 2.37% barley bran and 8.88 days storage time, 8.21 log CFU/g probiotic viability, pH: 4.83, acidity: 0.86% W/W, viscosity: 40277cp, adhesiveness: 0.4532 N, hardness: 28.62 N, color index, and sensory characteristics are shown in Figure 4. A study by Mousavi et al. (2019b) surveyed the optimization of probiotic yogurt containing flaxseed, and their result was similar to our result. They reported that addition of 4% flaxseed concentration can maintain microbial, physicochemical, and rheological properties and sensory evaluation for 13 days of storage time.

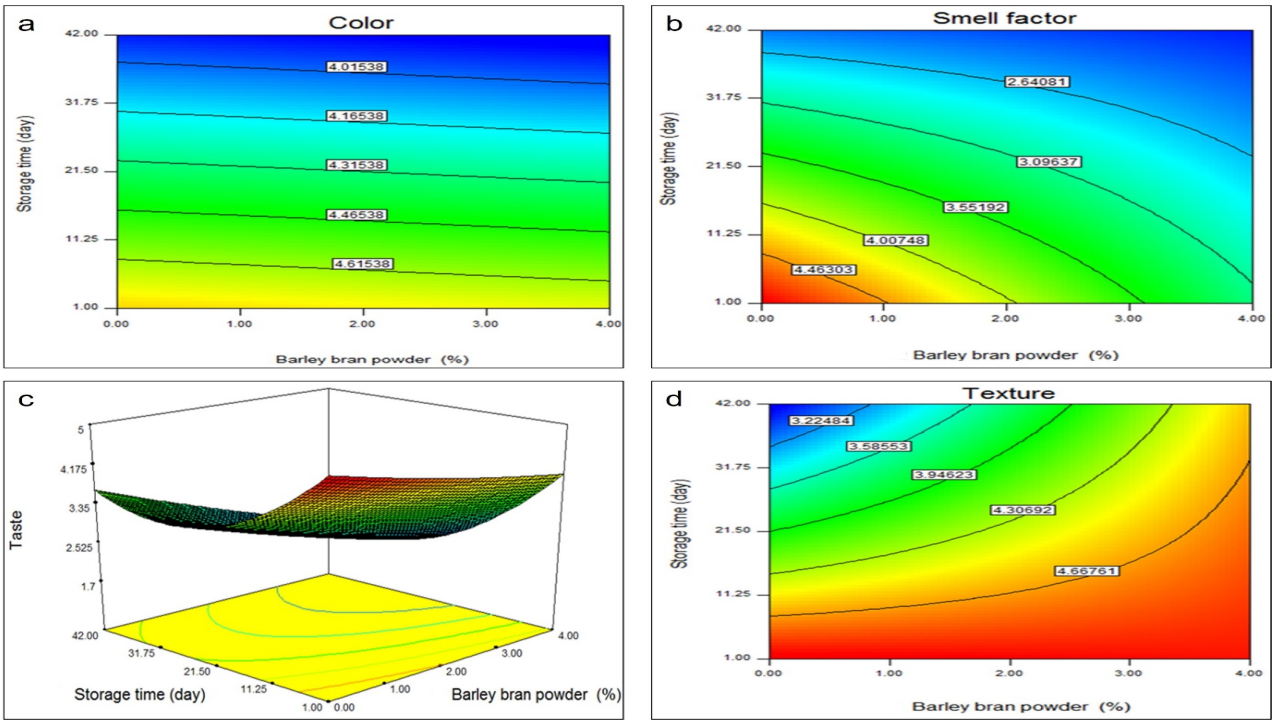

Figure 3. Sensory evaluation of probiotic low-fat cheese samples contains color(a), smell factor(b), taste(c) and texture(d) during days 1, 21 and 42

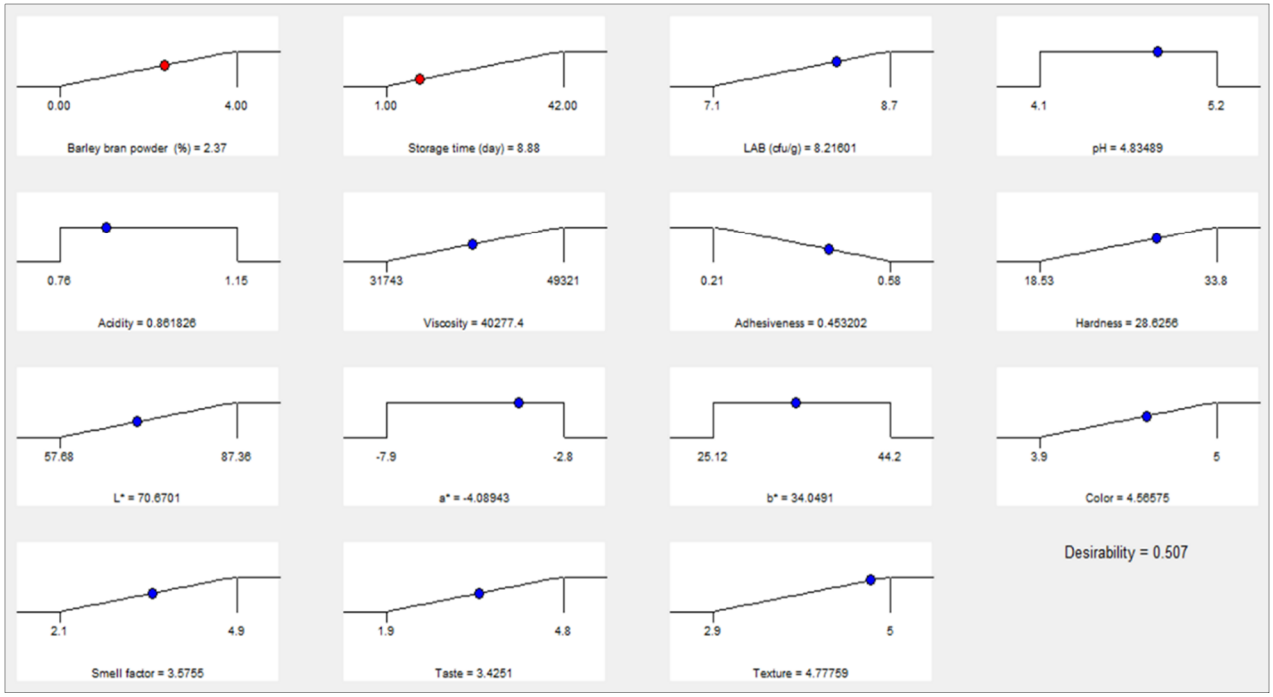

Figure 4. The optimum value of different variables for the production of probiotic cheese containing barley bran

4. Conclusion

This study was conducted to develop a product that contains probiotic bacteria and barley bran powder with the highest viability of probiotic bacteria alongside the best physicochemical, rheological, and sensory characteristics. By using RSM software and considering optimal parameters for two types of cheese, it was concluded that the addition of 2.37% barley bran resulted in a functional low-fat probiotic cheese with a probiotic viability of 8.21 log CFU/g, a pH of 4.83, acidity of 0.86% W/W, viscosity of 40277cp, adhesiveness of 0.4532 N, and hardness of 28.62 N. Furthermore, this cheese exhibited favorable color and sensory characteristics, which were maintained for approximately 9 days during refrigerated storage.

Authors' Contributions

Mohammad Reza Shadan, Malihe Mousavi, Zahra Poureshagh: Data curtain; Methodology. Maryam Bigomi, Adel Mirza Alizadeh, Ali Heshmati: Methodology; Writing-review & editing. Fahimeh Khoushabi, Anahita Shadan: Methodology.

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Conflicts of Interest

The authors declare no conflict of interest.

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Ethical considerations

In the article by Zahra Poureshagh, derived from the doctoral dissertation, it was noted that compliance with ethical considerations was not regarded as a requisite (Ethics no. IR.ZAUMS.REC.1402.488).

Using artificial intelligence

This research does not utilize any artificial intelligence (AI) techniques.

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