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# Optimization of physicochemical, textural and sensory properties of low-fat yogurt by adding fenugreek and basil seeds gum and investigating its effect on *Bifidobacterium bifidum* viability

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**ABSTRACT**

The use of fat substitutes with prebiotic properties, such as seeds gum, can be a suitable solution to improve the quality characteristics of low-fat products. In this research, response surface method with central composite design used to investigate the effect of basil seeds (0-0.2%) and fenugreek seeds gum (0-0.2%) on the physicochemical, textural and organoleptic properties of low-fat yogurt. The results showed that with the increase in the amount of gums in the low-fat yogurt formulation, the pH decreased. Addition of basil and fenugreek seeds gum reduced the syneresis of low-fat yogurt, while the water holding capacity and viscosity increased. Based on the results of sensory evaluation, the highest overall acceptance score was obtained in the treatments containing intermediate amounts of gums. The results of optimization with the central composite design showed that the best sample with optimum physicochemical and sensory properties was obtained when 0.14% of basil seed gum and 0.13% of fenugreek seed gum were used in the yogurt formulation. In the second part of the research, the viability of *Bifidobacterium bifidum* in the optimal yogurt formulation and different characteristics of low-fat yogurt was evaluated during the storage period. The results showed that by adding probiotic bacteria and increasing the storage time, the pH of the samples decreased while the acidity increased. The findings indicated that during the storage time, the amount of syneresis and viscosity of all samples increased, while the addition of probiotic bacteria did not have a significant effect on these parameters. Based on the results of counting probiotic bacteria, the use of optimal amounts of basil and fenugreek seeds gum had a positive effect on the viability of probiotic bacteria. In general, the results of this research showed that the use of basil and fenugreek seeds gum as a fat substitute in probiotic low-fat yogurt formulation can improve the quality properties of low-fat yogurt and increase the viability of probiotic bacteria.

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## 1-Introduction

Dairy products are major components of consumers' diet. However, the high fat content of these products has reduced their popularity among health-conscious consumers. There is considerable scientific evidence that high and/or prolonged consumption of high-fat products increases the risk of diseases such as obesity, type 2 diabetes, high blood pressure, and cardiovascular disease [1]. Therefore, the production of low-fat dairy products has been a major priority for food technologists over the past few decades. However, due to the key role of milk fat in texture and flavor of dairy products, the production of low-fat dairy products, including yogurt, faces serious challenges [2]. Yogurt is one of the most popular dairy products, which is formed by lactic fermentation of milk by thermophilic lactic acid bacteria. This dairy product has several health benefits such as anticancer effects, lowering blood cholesterol, anti-allergy, improving the bioavailability of calcium and other nutrients, controlling gastrointestinal infections, stimulating the immune system, and prolonging life. By reducing fat in yogurt, the texture and taste of low-fat yogurts are affected, which is not welcomed by consumers [3]. In recent years, the use of fat substitutes has been proposed to improve the quality characteristics of yogurt [4]. Hydrocolloids are among the fat substitutes that have been widely used in recent years. Some of these compounds, in addition to their role as fat substitutes, are known as prebiotic compounds. Prebiotics are non-digestible carbohydrate dietary fibers that stimulate the growth and proliferation of bacteria such as Bifidobacterium and Lactobacillus in the colon, thereby improving host health [5]. Among the indigenous Iranian hydrocolloids, basil seed gum and fenugreek seed gum have received attention in recent years. Fenugreek is one of the oldest medicinal plants with exceptional medicinal and nutritional profile. Fenugreek is grown in many parts of the world, including India, Central Asia, and North Africa. Fenugreek seed galactomannan is similar to that of locust bean, guar, and tara beans, and consists primarily of galactose and mannose [6]. Galactomannans have various applications in food, pharmaceutical and cosmetic industries such as fillers and matrix materials in tablet production, coating materials in microencapsulation processes, packaging film production, consistency enhancers and stabilizers in emulsion and suspension systems, and emulsifying, texture modifying and gelling agents. They can also be used as dietary fiber and fat substitutes [7]. Basil (*Ocimum basilicum* L.) is one of the endemic plants in Iran and is mainly used as a pharmaceutical plant [8]. Besides its use as a traditional medicine, basil seeds are commonly incorporated into food products, such as desserts and beverages, and are used as a source of dietary fiber in Iran and some regions of Asia. These seeds, when soaked in water, swell into a gelatinous mass, which has a reasonable amount of gum. It has been reported that the polysaccharides extracted from basil seed comprise two major fractions, glucomannan, xylan and glucan [9-10]. Basil seed gum (BSG) has shown good functional properties comparable to some commercial food hydrocolloid [8]. Basil and fenugreek seed gum have high potential for replacing fat in low-fat yogurt. The aim of this study was to optimize the formulation of low-fat yogurt using basil and fenugreek seeds gum as a substitute for milk fat, and also to investigate the effect of adding the aforementioned gums on the viability of the probiotic bacterium

*Bifidobacterium bifidum* and various characteristics of low-fat yogurt during storage.

## 2-Materials and methods

### 2.1. Materials

Basil and fenugreek seeds (reputable herbal stores in Tehran), milk (Pegah, Tehran), yogurt starter (Denisco, Denmark), and probiotic bacteria (Christian Hansen, Denmark) were purchased. All chemicals were purchased from Merck, Germany.

### 2.2. Methods

#### 2.2.1. Extraction of basil seed gum

Basil seeds were mixed with deionized water in a ratio of 1:50 at 50°C and pH 7. After 20 min, the resulting mixture was centrifuged for 20 min at 5000 rpm. The collected gum dried by vacuum oven at 50°C. Finally, the dried extracted gums were ground and packed in the plastic bags and stored under dry and cool conditions [11].

#### 2.2.2. Extraction of fenugreek seed gum

After cleaning the fenugreek seed, it was ground by a mill and passed through a 30-mesh sieve. In order to extract the seed gum, water was added to the seed in a ratio of 45 to 1. The water and seeds were mixed using a stirrer at a constant speed of 1500 rpm and temperature of 60°C. After the required time (3 hours), the seeds were separated from the hydrocolloid solution. The resulting solution was precipitated by addition of acetone for 24 hours. The collected gum was dried by vacuum oven at 35°C [12].

#### 2.2.3. Production of low-fat yogurt

Low-fat milk (1.5% fat) was placed on a steam bath and after reaching a temperature of 45°C, different amounts of fenugreek and basil seeds gum (according to the experimental design) were added to the milk and stirred until completely hemogenized (it should be noted that the dry matter was standardized to 12% by adding dry milk). All treatments were pasteurized at 90°C for 10 min and after cooling to 42°C, commercial starter (at this stage, probiotic bacteria were added at a rate of 0.2 g/L in probiotic treatments) was added to the samples. Then, the samples were transferred to a 42°C incubator and after reaching pH=4.6, the samples were transferred to a 5°C refrigerator [13]. In the second stage of the research, a low-fat yogurt sample containing optimum amounts of gums was produced and its various characteristics were compared during storage (1, 7, 14, and 21 days after production) with a low-fat sample containing probiotics, low-fat yogurt (without gums), and low-fat probiotic yogurt (without gums).

#### 2.2.4. Physicochemical analysis

##### 2.2.4.1. pH and acidity

The pH measurement was performed using a digital pH meter (Metrohm model 827, Switzerland). Also, the titratable acidity of the samples was determined after mixing 10 g of the samples with 20 mL of distilled water and titrating using 0.1 N sodium hydroxide and 0.5 mL of phenolphthalein reagent until a pink color appeared [14].

##### 2.2.4.2. Syneresis

About 25 g of yogurt sample was poured onto filter paper (Whatman No. 43) and placed on glass funnels. The amount of serum removed after 3 hours was calculated by gravity as the percentage of water separated [15].

##### 2.2.4.3. Water holding capacity

About 20 g of yogurt samples were poured into a falcon and centrifuged at 1250 × g for 10 min. The amount of serum removed was weighed after 10 min and then the water holding capacity was calculated [15].

##### 2.2.4.4. Viscosity

The viscosity of yogurt samples was measured using a viscometer at 4°C. Spindle No. 5 with a shear rate of 60 rpm was used and the viscosity value was recorded after 30 seconds [16].

#### 2.2.5. *Bifidobacterium bifidum* count

Yogurt samples were cultured using the purplate method after preparing serial dilutions using 0.1% peptone water in RCA medium that only allows the growth of *Bifidobacterium*, and incubated under anaerobic conditions (anaerobic jar containing gas pack) at 37°C for 72 hours [17].

#### 2.2.6. Sensory evaluation





[ DOI: 10.22034/FSCT.22.162.73 ]

Different yogurt samples were coded as three-digit numbers and were presented to the panelists under the same conditions. The sensory properties of low-fat yogurt, including color, flavor, texture, and overall acceptability, were evaluated by 15 people using a 5-point hedonic test [18].

#### 2.2.7. Experimental design and statistical analysis

RSM was employed to optimize the formulation ingredients of low-fat yogurt according to a 5-level-2-factor central composite design (CCD). The experimental design with coded and actual values is presented in Table 1. The design consisted of 13 experiments with 5 center points to calculate the repeatability of the method. According to experimental design, yogurt samples were prepared with varied concentration of fenugreek and basil seeds gum and evaluated for physicochemical and organoleptic characteristics. A second-order polynomial equation (Eqn 1) was fitted to the obtained experimental data for responses:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j + \varepsilon_{ij} \quad (1)$$

where Y is the response (physicochemical and rheological characteristics), β0, βi, βii and βij are regression coefficients for intercept, linear, quadratic and interaction coefficients, respectively and Xi and Xj are the independent variables. Response surface analysis, mapping of plots and response optimization were performed using statistical package Design-Expert, version 8.0.0 (Stat-Ease Inc., Minneapolis, USA).

**Table 1.** Uncoded and coded levels of independent variables used in the RSM design

| Independent variable | Symbols | Range and levels | | | | |
|---|---|---|---|---|---|---|
| | | -1 | -0.5 | 0 | +0.5 | +1 |
| Basil seed gum (%) | $X_1$ | 0 | 0.05 | 0.10 | 0.15 | 0.20 |
| Fenugreek seed gum (%) | $X_2$ | 0 | 0.05 | 0.10 | 0.15 | 0.20 |

## 3-Results and discussion

### 3.1. pH and acidity

A statistical analysis of the effects of fenugreek and basil seeds gum on the pH and acidity of low-fat yogurt samples showed that both variables had a significant linear effect ($p<0.05$) on the pH and acidity of low-fat yogurt (Table 2). In interpreting of the analysis of variance table, a positive value of the regression coefficient indicates an increasing effect of the independent variable on the response, while a negative coefficient has the opposite effect. Accordingly, with an increase in the amount of basil and fenugreek seeds gum in the low-fat yogurt formulation, the acidity increased, while the pH decreased (Table 2, Figure 1). These findings can be attributed to the prebiotic effect of these gums, which causes more growth of yogurt starters, resulting in an increase in the amount of acid produced, followed by an increase in acidity and a decrease in pH [19]. Similarly, Danker et al. (2007) and Hassan et al. (2015) reported that with increasing in guar gum and watercress seed mucilage the pH of yogurt decreased while the acidity increased [20-21]. Also, the results of Milani et al. (2011) regarding the addition of guar gum to frozen yogurt and Almasi et al. (1400) regarding the addition of basil gum to low-fat yogurt were similar to the results of this study [22-23]. According to the data analysis, the final models obtained for pH





[ DOI: 10.22034/FSCT.22.162.73 ]

and acidity in low-fat yogurt were determined as follows (Equations 2 and 3, respectivly).

$$Y_1 = 4.27 - 0.09X_1 - 0.06X_2 \quad (2)$$

$$Y_2 = 0.92 + 0.08X_1 + 0.07X_2 \quad (3)$$

$X_1$ and $X_2$ are basil seeds gum and fenugreek seeds gum, respectively.

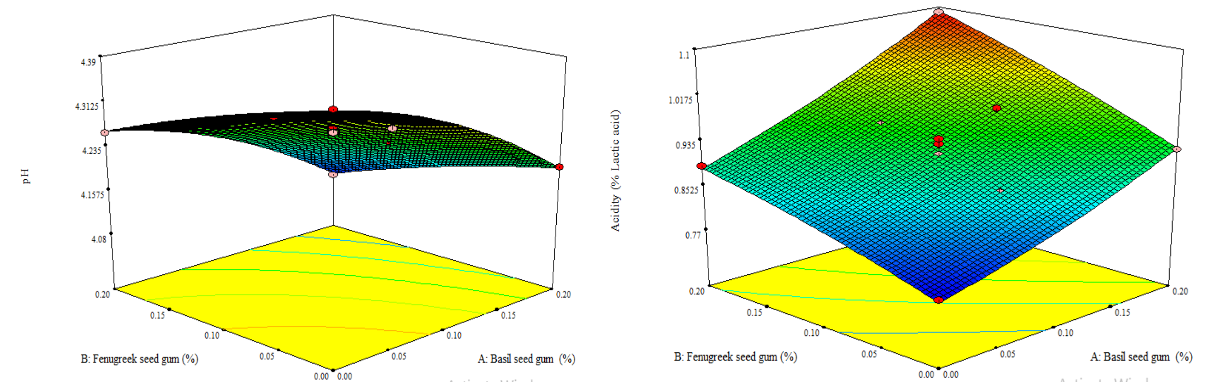


**Fig 1.** Response surface plots for effects of gums on pH and acidity of low-fat yogurt

**Table 2.** Regression coefficients and analysis of variance (ANOVA) for predicted models for different responses

| Source | Ph | | Acidity (%) | | Syneresis (%) | | WHC (%) | | Viscosity (Cp) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Coefficient | P value | Coefficient | P value | Coefficient | P value | Coefficient | P value | Coefficient | P value |
| Constant | 4.27 | <0.0001 | 0.92 | <0.0001 | 14.27 | 0.0020 | 59.45 | <0.0001 | 3691 | <0.0001 |
| Linear | | | | | | | | | | |
| $X_1$ | -0.09 | <0.0001 | 0.08 | <0.0001 | -5.23 | 0.0016 | 9.30 | <0.0001 | 1165 | <0.0001 |
| $X_2$ | -0.06 | <0.0001 | 0.07 | <0.0001 | -4.64 | 0.0031 | 7.71 | <0.0001 | 968.8 | <0.0001 |
| Interaction | | | | | | | | | | |
| $X_{12}$ | 0.002 | 0.588 | 0.015 | 0.128 | -2.65 | 0.0489 | 2.26* | 0.0014 | 142.5 | 0.0318 |
| Quadratic | | | | | | | | | | |
| $X_{11}$ | -0.028 | 0.155 | 0.012 | 0.753 | 3.02 | 0.5230 | -6.13 | 0.0113 | -211 | 0.3604 |
| $X_{22}$ | -0.008 | 0.651 | -0.008 | 0.816 | 2.32 | 0.6213 | -3.53 | 0.0904 | -205 | 0.3734 |
| $R^2$ | 0.9907 | - | 0.9650 | - | 0.9024 | - | 0.9940 | - | 0.9827 | - |
| $R^2$-adjust | 0.9840 | - | 0.9400 | - | 0.8327 | - | 0.9897 | - | 0.9875 | - |
| Lack of fit | - | 0.398 | - | 0.392 | - | 0.272 | - | 0.073 | - | 0.207 |
| CV | 0.21 | - | 1.90 | - | 13.79 | - | 1.58 | - | 3.00 | - |

$X_1$& $X_2$ are Basil seed gum and Fenugreek seed gum, respectively.

### 3.2. Syneresis

Syneresis is the separation of serum or whey from yogurt, which is one of the important disadvantages of yogurt. Syneresis occurs due to the shrinkage of the three-dimensional structure of the protein network of yogurt, which causes the release of whey from the product [24]. The findings showed that the linear effects of the variables and their interaction on water loss were significant ($p<0.05$) (Table 2). According to Figure 2 with the increase in basil and fenugreek seeds gum, the syneresis decreased, which was due to the formation of a denser gel network by hydrocolloids in the yogurt formulation and the water binding properties of these hydrocolloids. Similarly, Karim et al. (2018) reported that the addition of inulin reduced the syneresis in yogurt. They also explained that the addition of inulin increased the dry matter content and consistency of yogurt, and on the other hand, inulin caused the formation of a gel network, and rapid water absorption occurred by this gel network, so syneresis decreased [25]. Similar results were reported by Ghaibi and Ashrafi (2019) and Almasi et al. (2010) with the addition of inulin and gum arabic and basil gum on the amount of syneresis in low-fat yogurt, respectively [23, 26].. Finally, the following equation was obtained for the syneresis in yogurt.

$$Y = 14.27 - 5.23X_1 - 4.64X_2 - 2.65X_1X_2 \quad (4)$$

$X_1$ and $X_2$ are basil seeds gum and fenugreek seeds gum, respectively.

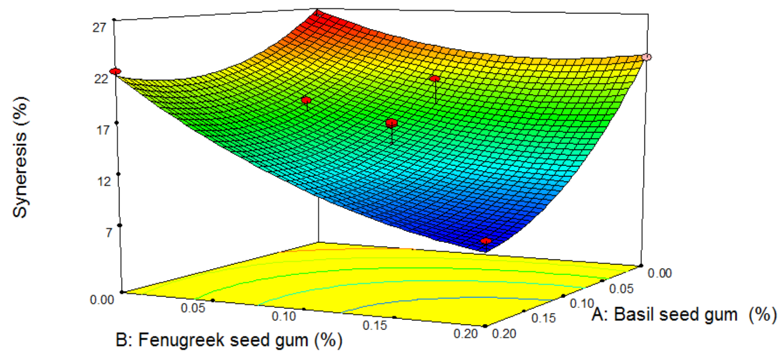


**Fig 2.** Response surface plots of interaction effects of gums on syneresis of low-fat yogurt.

### 3.3. Water holding capacity

Water holding capacity is the ability of yogurt texture to prevent water from leaving the product and migrating to its surface. The three-dimensional protein network structure is responsible for retaining and trapping water molecules in the product [27]. Statistical findings indicated that both independent variables had a significant linear effect ($p<0.05$) on water holding capacity, and their interaction and the quadratic effect of basil seed gum were also statistically significant ($p<0.05$) (Table 2). By increasing the amount of basil and fenugreek seeds gum in the low-fat yogurt formulation, the water-holding capacity increased (Figure 3). The increase in the water-holding capacity of yogurt as a result of the addition of gums can be attributed to the increase in the amount of dry matter in yogurt and the creation of a denser structure, a more stable gel network, and lower porosity, which increased the water-holding capacity of yogurt [28]. Similar to the results of present study, Lajevardi et al. (2010) reported that adding locust bean gum and xanthan gum, increased the water-holding capacity of yogurt [29]. In another study, Gantomore et al. (2024) also reported that adding modified whey protein increased the water-holding capacity of low-fat yogurt [30]. Equation 5 shows the final model obtained to explain the changes in the water-holding capacity of low-fat yogurt based on the formulation components.

$$Y = 59.45 + 9.30X_1 + 7.71X_2 + 2.26X_1X_2 - 6.13X_1^2 \quad (5)$$

$X_1$ and $X_2$ are basil seed gum and fenugreek seed gum, respectively.




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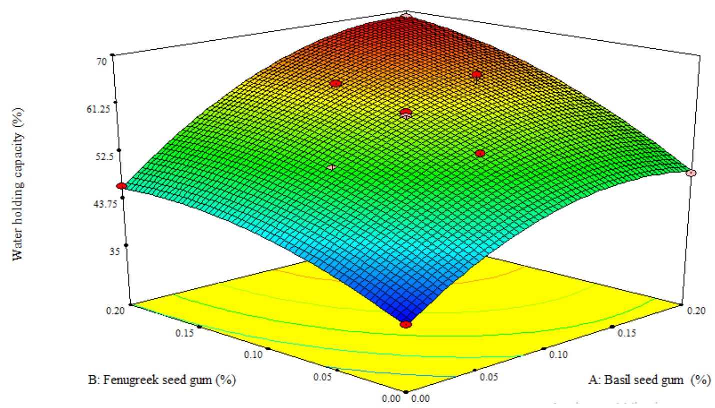



**Fig 3**. Response surface plots for interaction effects of gums on WHC of low-fat yogurt.

### 3.4. Viscosity

The results of viscosity analysis of low-fat yogurt samples showed that basil and fenugreek seeds gum had a significant linear effect ($p<0.05$) on the viscosity of low-fat yogurt (Table 2). Viscosity is influenced by several factors such as type, quality and concentration of the ingredients, especially fat and stabilizers, and temperature [31]. The results (Figure 4) showed that the increase in basil and fenugreek seeds gum increased the viscosity of low-fat yogurt. Hydrocolloids increased viscosity by binding free water in the samples. Saha and Bhattacharya (2010) reported that the increase in viscosity is caused by the interaction between the polymer chains of the thickening compounds and in high-viscosity systems, the molecules in the gel network formed come into more contact with each other and the mobility of the dispersed phase particles decreases. Hydrocolloids create coherent and dense networks and cause immobility and involvement of the dispersed phase in the suspension phase [32]. In previous studies it has been reported that addition of basil seeds gum increased the viscosity of low-fat yogurt [23, 33]. The final model obtained for viscosity is shown in Equation 6.

$$Y = 3691.29 + 1165.922X_1 + 968.89X_2 + 142.5X_1X_2 \quad (6)$$

$X_1$ and $X_2$ are Basil seed gum and Fenugreek seed gum, respectively.

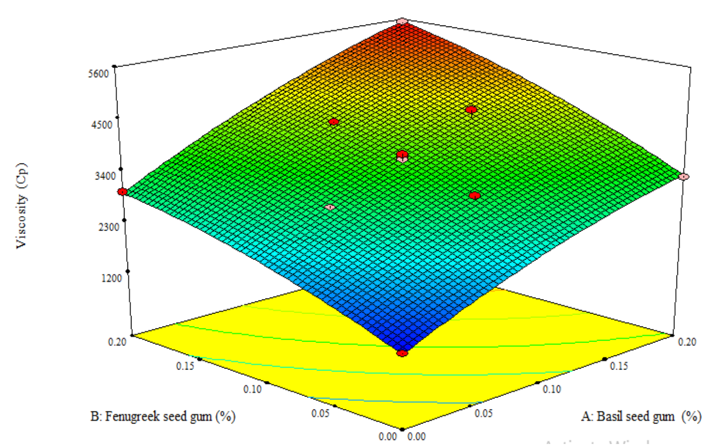



**Fig 4.** Response surface plots for interaction effects of gums on viscosity of low-fat yogurt.

### 3.5. Organoleptic characteristics

Statistical findings (Table 3) from the evaluation of the sensory characteristics of low-fat yogurt samples containing basil and fenugreek seeds gum showed that the addition of basil and fenugreek gum to yogurt formulation had a significant linear effect on the acceptability of the texture, flavor and overall acceptance of the products ($p<0.05$), while the effect of the mentioned variables on color acceptability was not significant ($p>0.05$).

**3.5.1. Flavor**

A review of the statistical findings (Table 3) indicated that changing the concentration of basil and fenugreek seeds gum in the low-fat yogurt formulation had a significant linear effect on flavor ($p<0.05$). The results (Figure 4) showed that with increasing the amount of basil and fenugreek seeds gum, the flavor score of low-fat yogurts increased. Almasi et al. (2021) reported that the addition of basil seeds gum improved sensory properties, which could be due to high water absorption and the jelly-like state created in the texture [23]. Tavakolipour et al. (2014) also stated that the taste score of low-fat yogurts increased with the addition of modified corn starch [34]. Equation 7 shows the final model obtained for flavor acceptability:

$$Y_1 = 3.90 + 0.26X_1 + 0.14X_2 \quad (7)$$

$X_1$ and $X_2$ are basil seeds gum and fenugreek seeds gum, respectively.

**Table 3** Regression coefficients and analysis of variance for predicted models for different responses

| Source | Flavor | | Texture | | Overall acceptability | |
|---|---|---|---|---|---|---|
| | Coefficient | P value | Coefficient | P value | Coefficient | P value |
| Constant | 3.90 | 0.0067 | 3.97 | <0.0001 | 4.01 | <0.0001 |
| Linear | | | | | | |
| $X_1$ | 0.26 | 0.0023 | -0.17 | 0.0122 | -0.29 | 0.0125 |
| $X_2$ | 0.14 | 0.0452 | -0.12 | 0.0435 | -0.22 | 0.0371 |
| Interaction | | | | | | |
| $X_{12}$ | -0.04 | 0.5233 | -0.12 | 0.0495 | -0.15 | 0.1463 |
| Quadratic | | | | | | |
| $X_{11}$ | -0.19 | 0.4656 | -1.87 | <0.0001 | -1.05 | 0.0252 |
| $X_{22}$ | -0.11 | 0.6733 | -1.07 | 0.0015 | -1.85 | 0.0016 |
| $R^2$ | 0.8603 | - | 0.9965 | - | 0.9893 | - |
| $R^2$-adjust | 0.7605 | - | 0.9940 | - | 0.9817 | - |
| Lack of fit | - | 0.1191 | - | 0.7643 | - | 0.5583 |
| CV | 3.14 | - | 3.58 | - | 6.10 | - |

$X_1$& $X_2$ are Basil seed gum and Fenugreek seed gum, respectively.

**3.5.2. Texture**

Statistical results (Table 3) showed that basil and fenugreek seeds gum had a significant effect ($p<0.05$) on texture acceptability. Also, the interaction between variables was statistically significant ($p<0.05$). Examination of the graph (Figure 4) of the texture acceptability of low-fat yogurt from the panelists' perspective showed that increasing the concentration of basil and fenugreek seeds gum had a dual effect on the texture acceptability, and the highest texture acceptability score was related to treatments containing the middle range of gums. Due to water holding capacity of hydrocolloids, the water absorption and consequently consistency of





[ DOI: 10.22034/FSCT.22.162.73 ]

yogurt was improved. Rady et al. (2009) reported that the use of modified wheat starch in low-fat yogurt formulation improved the product's texture acceptance among consumers [35]. Kim et al. (2020) also reported the positive effect of adding basil seed gum on the texture score of low-fat yogurts [33]. At high gum concentrations, the texture acceptability among panelists decreased significantly, which is likely due to the high viscosity of samples containing high amounts of gums, which negatively affects the texture of yogurt. Equation 8 shows the final model obtained for texture acceptability:

$$Y_2 = 3.97 - 0.17X_1 - 0.12X_2 - 0.12X_1X_2 - 1.87X_1^2 - 1.07X_2^2 \quad (8)$$

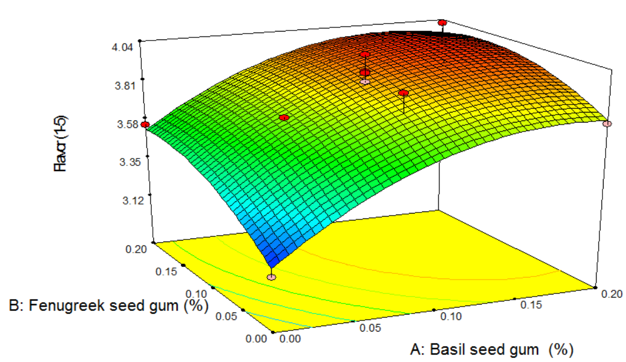
$X_1$ and $X_2$ are basil seeds gum and fenugreek seeds gum, respectively.

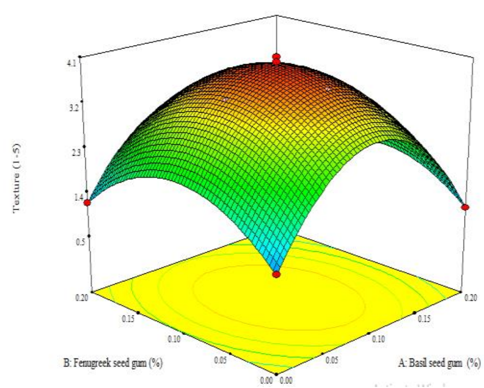
### 3.5.3. Overall acceptance

The evaluation of overall acceptance of low-fat yogurt (Table 3) showed that both independent variables had a significant effect on overall acceptance. Their quadratic effects were also significant ($p \leq 0.05$). The results (Figure 4) indicated that increasing the amount of gums had a dual effect on overall acceptance of low-fat yogurt, and the highest overall acceptance score was obtained in treatments containing intermediate levels of gums. In gel-like foods, the most important parameter that influences their acceptance is the texture of the food. The texture of yogurt has a direct impact on mouth feel, and mouth feel in gel-like foods is an important characteristic that influences other factors. The hydrocolloids, due to their water absorption, strengthen the texture of yogurt and create a desirable, fat-like mouth feel [36]. The decrease in overall acceptance of samples at high gum concentrations is probably due to the negative effect of gums on the texture of yogurt, which is not appreciated by the panelists. These results were similar to the findings of Norouzi et al. (2021) in investigating the effect of different amounts of balango seed gum on the overall acceptance of low-fat yogurt among consumers [37]. Dalili et al. (2017) also reported the positive effect of okra mucilage and guar gum on the sensory properties of low-fat yogurt [19]. Equation 9 shows the final model obtained for overall acceptance:

$$Y_3 = 4.01 - 0.29X_1 - 0.22X_2 - 1.05X_1^2 - 1.85X_2^2 \quad (9)$$

$X_1$ and $X_2$ are basil seed gum and fenugreek seed gum, respectively.





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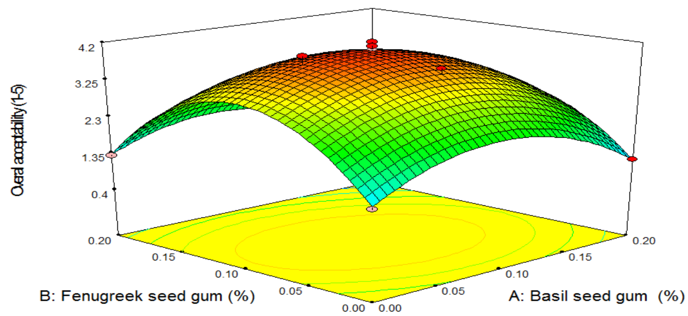


**Fig 4.** Response surface plots for effects of formulation ingredients on organoleptic characteristics of low-fat yogurt.

### 3.6. Formulation optimization

The results of the optimization showed that by using 0.14% basil seedss gum and 0.13% fenugreek seeds gum in the yogurt formulation, a low-fat product with characteristics close to full-fat yogurt can be produced. To confirm the accuracy of RSM models in predicting the optimum formulation, the low-fat yogurt sample with the proposed formulation was prepared and its physicochemical and organoleptic attributes were compared with those predicted by the models. The result of one-sample t-test verified that the experimental and predicted values of all responses were not statistically different ($p>0.05$)(Table 4).

**Table 4.** Predicted and experimental values for responses at the optimum point ingredients

| | pH | Acidity | Syneresis | WHC | Viscosity | Flavor | Texture | Total point |
|---|---|---|---|---|---|---|---|---|
| Predicted | 4.21 | 0.974 | 11.12 | 64.50 | 4410.8 | 3.99 | 3.47 | 3.46 |
| Experimental | 4.25±0.23 | 0.982±0.08 | 11.67±0.85 | 63.96±1.04 | 4350±82.1 | 3.89±0.11 | 3.52±0.12 | 3.55±0.13 |

### 3.7. Evaluation of yogurt characteristics during storage

#### 3.7.1. pH and Acidity

Based on the statistical findings (Table 5), during the storage period, the pH of all yogurt treatments, unlike acidity, suffered a significant decrease ($p<0.05$). During the storage period, the pH of the control sample (low-fat yogurt without added gum) and the control containing probiotic bacteria was significantly ($p<0.05$) higher than the treatments containing optimal amounts of gums (probiotic or non-probiotic). The results also indicated that the highest and lowest acidity levels at different stages of storage were related to the probiotic treatment containing optimal amounts of gums and the control sample, respectively. The decrease in pH of the samples during storage are due to the activity of lactic acid bacteria. Also, as mentioned in the previous sections, the reason for the lower pH and higher acidity in the samples containing gum was probably due to the stimulation of the metabolic activity of acid-producing starter bacteria. The addition of probiotic bacteria increased acidity and decreased pH, which could be due to the activity of *Bifidobacterium bifidum* at low pH and the consumption of lactose, followed by an increase in acidity. The present results were similar to the findings of Faraji et al. (2016) in investigating the effect of gum arabic and tragacanth on the pH and acidity of low-fat yogurt containing the probiotic bacterium *Lactobacillus casei* [38].. Ghasempour et al. (2010) reported that yogurt samples containing the probiotic bacteria *Lactobacillus acidophilus* and *Bifidobacterium bifidum* had lower pH and higher acidity than the control sample [39].

**Table 5.** Means±SD of pH and acidity of different low-fat yogurt samples during storage

| Treatment | pH |
|---|---|





[ DOI: 10.22034/FSCT.22.162.73 ]

| | Day 1 | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|
| Control low-fat | 4.39±0.04[aA] | 4.33±0.02[bA] | 4.25±0.01[cA] | 4.09±0.02[dA] |
| Optimized low-fat | 4.25±0.03[aB] | 4.18±0.04[bC] | 4.11±0.01[cC] | 4.01±0.02[dB] |
| Control low-fat+ Probiotic bacteria | 4.37±0.03[aA] | 4.28±0.04[bB] | 4.20±0.04[cB] | 4.06±0.03[dA] |
| Optimized low-fat+ Probiotic bacteria | 4.23±0.03[aB] | 4.13±0.05[bD] | 4.07±0.05[cD] | 3.98±0.05[dB] |

| Treatment | Acidity | | | |
|---|---|---|---|---|
| | Day 1 | Day 7 | Day 14 | Day 21 |
| Control low-fat | 0.780±0.006[dD] | 0.795±0.003[cD] | 0.812±0.001[bD] | 0.850±0.001[aD] |
| Optimized low-fat | 0.982±0.005[dB] | 0.994±0.002[cB] | 1.09±0.001[bB] | 1.15±0.005[aB] |
| Control low-fat+ Probiotic bacteria | 0.789±0.001[dC] | 0.810±0.005[cC] | 0.832±0.007[bC] | 0.869±0.005[aC] |
| Optimized low-fat+ Probiotic bacteria | 0.994±0.009[dA] | 1.09±0.005[cA] | 1.18±0.006[bA] | 1.29±0.001[aA] |

[a-d] Means within the same row with different superscripts are significantly different at p<0.05.
[A-D] Means within the same column with different superscripts are significantly different at p<0.05.

### 3.7.2. Syneresis

The results of statistical analysis (Table 6) showed that the syneresis of all samples increased with increasing storage time ($p<0.05$). Also, during the storage period, the syneresis of low-fat samples containing optimal amounts of gums was lower than that of samples without gums ($p<0.05$), but the addition of probiotic bacteria had no significant effect on this parameter ($p>0.05$). Less syneresis with the addition of gums can be attributed to the denser gel network in the presence of hydrocolloids and their water absorption properties. Regarding the increase in water loss during storage, it can also be said that the decrease in pH during the storage period caused a change in the natural form of the protein and, due to protein denaturation, the water binding capacity decreased and syneresis increased [40]. Similar to the results of this study, Razmkhah et al. (2019) reported an increase in syneresis of samples containing pectin and marjoram and basil seed gums during storage [41]. Almasi et al. (2014) and Behnia et al. (2014) also reported a negative effect of storage time on syneresis of low-fat yogurt [23, 42].

**Table 6.** Means±SD of syneresis of different low-fat yogurt samples during storage

| Treatment | Syneresis | | | |
|---|---|---|---|---|
| | Day 1 | Day 7 | Day 14 | Day 21 |
| Control low-fat | 25.87±0.19[cA] | 25.90±0.22[cA] | 26.73±0.12[bA] | 27.25±0.20[aA] |
| Optimized low-fat | 11.67±0.22[cB] | 11.75±0.17[bcB] | 11.95±0.30[bB] | 12.43±0.31[aB] |
| Control low-fat+ Probiotic bacteria | 25.15±0.25[cA] | 25.39±0.25[cA] | 26.43±0.32[bA] | 26.90±0.25[aA] |
| Optimized low-fat+ Probiotic bacteria | 11.43±0.29[cB] | 11.44±0.315[cB] | 11.83±0.29[bB] | 12.33±0.21[aB] |

[a-d] Means within the same row with different superscripts are significantly different at p<0.05.
[A-D] Means within the same column with different superscripts are significantly different at p<0.05.

### 3.7.3. Viscosity

The findings (Table 7) indicated that the viscosity of all samples increased significantly ($p<0.05$) during the storage period, and at the same storage intervals, the viscosity of the treatments containing basil and fenugreek seeds gum was higher than the low-fat control sample (probiotic or non-probiotic) ($p<0.05$). It should also be noted that according to the findings, the addition of probiotic bacteria had no significant effect ($p<0.05$) on the viscosity. In general, the increase in viscosity during the storage period is probably due to changes in protein-protein bonds and an increase in the water-binding capacity of the gums, which increases the sample's resistance to flow, or apparent viscosity [23]. Nateghi (2019) achieved similar results while investigating the possibility of producing low-fat stirred yogurt using gum zedo [36]. Almasi et al. (2019) also reported similar findings [23].

**Table 7.** Means±SD of viscosity of different low-fat yogurt samples during storage

| Treatment | Viscosity | | | |
|---|---|---|---|---|
| | Day 1 | Day 7 | Day 14 | Day 21 |




[ DOI: 10.22034/FSCT.22.162.73 ]

| | | | | |
|---|---|---|---|---|
| Control low-fat | 1299±53$^{dB}$ | 1690±61$^{cB}$ | 2046±70$^{bB}$ | 2473±73$^{Ab}$ |
| Optimized low-fat | 4350±82$^{dA}$ | 4832±95$^{cA}$ | 5232±92$^{bA}$ | 5621±87$^{aA}$ |
| Control low-fat+ Probiotic bacteria | 1310±56$^{dB}$ | 1578±91$^{cB}$ | 2001±71$^{bB}$ | 2560±82$^{aB}$ |
| Optimized low-fat+ Probiotic bacteria | 4439±90$^{dA}$ | 4841±96$^{cA}$ | 5168±87$^{bA}$ | 5770±98$^{aA}$ |

$^{a-d}$ Means within the same row with different superscripts are significantly different at p<0.05.
$^{A-D}$ Means within the same column with different superscripts are significantly different at p<0.05.

#### 3.7.4. *Bifidobacterium bifidum* count

According to the Iranian National Standard No. 11325, yogurt is considered probiotic if the microbial count of each probiotic species used is not less than $10^6$ cfu/mL until the end of the storage date [43]. The results of the *Bifidobacterium bifidum* counting (Table 8) in different yogurt samples during the storage period showed that the changes in the number of *Bifidobacterium bifidum* in both samples followed the same pattern and decreased significantly with increasing storage time (p<0.05). The statistical results also showed that at all storage stages, the number of *Bifidobacterium bifidum* in the sample containing optimal amounts of fenugreek and basil seeds gum was significantly higher than that in the probiotic control sample (p<0.05). The survival of probiotic bacteria in yogurt depends on factors such as the species used, the reaction between the species presents in the culture medium, the chemical composition of the fermentation medium, the final acidity, milk solids, temperature, and inoculation levels [40]. In the sample containing optimal amounts of gums, the viability of probiotic bacteria was higher than in the sample without gums. Adding prebiotics can increase the nutrients of the product [20]. Also, with increasing storage time and bacterial activity, the acidity of the samples increases and the amount of available nutrients decreases, which will probably cause a decrease in the number of bacteria with increasing storage time. Danker et al. (2006) reported that enriching yogurt with prebiotics, especially inulin, leads to improved viability of probiotic bacteria in yogurt during storage [20]. Similar to the present results, Dalili et al. (2017) reported that adding guar gum and okra to low-fat yogurt formulation had a positive effect on the viability of *Bifidobacterium bifidum* during storage [19]. Hosseini et al. (2016) also reported that the survival of the probiotic bacterium *Lactobacillus paracasei* in non-fat stirred yogurt samples containing maltodextrin and xanthan was higher than the control sample [40].

**Table 8.** Means±SD of *bifidobacterium bifidum* count (log10 cfu/ml) of different low-fat yogurt samples

| Treatment | *Bifidobacterium bifidum* | | | |
|---|---|---|---|---|
| | Day 1 | Day 7 | Day 14 | Day 21 |
| Control low-fat+ Probiotic bacteria | 7.35±0.11$^{aB}$ | 7.05±0.08$^{bB}$ | 6.53±0.08$^{cB}$ | 6.03±0.15$^{dB}$ |
| Optimized low-fat+ Probiotic bacteria | 7.95±0.15$^{aA}$ | 7.64±0.06$^{bA}$ | 7.32±0.16$^{cA}$ | 6.94±0.15$^{dA}$ |

$^{a-d}$ Means within the same row with different superscripts are significantly different at p<0.05.
$^{A-D}$ Means within the same column with different superscripts are significantly different at p<0.05.

## 4-Conclusion

In the first part of this study, the effect of fenugreek and basil seeds gum on the physicochemical and sensory properties of low-fat yogurt was investigated using the response surface methodology. In general, the results of this part of the study showed that by using optimal amounts of basil seed gum (0.14%) and fenugreek (0.13%) in the formulation of low-fat yogurt, a product with desirable physicochemical and sensory properties can be produced. In the second part of this study, the low-fat yogurt sample containing optimal amounts of gums was compared with the low-fat probiotic sample containing optimal amounts of gums, the low-fat sample (without gum), and the low-fat probiotic sample (without gum) during the storage period. The results showed that with increasing storage time, the pH decreased, unlike the acidity. Also, based on the findings, the addition of probiotic bacteria caused a decrease in pH and an increase in acidity. The syneresis and viscosity of all samples increased with increasing storage time, and the addition of probiotic bacteria had no significant effect on these parameters ($p<0.05$). The results of the probiotic bacteria count (*Bifidobacterium bifidum*) also indicated that the use of basil and fenugreek seeds gums in low-fat yogurt formulations had a positive effect on increasing the survival of probiotic bacteria.

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# بهینه‌سازی خصوصیات فیزیکوشیمیایی، بافتی و حسی ماست کم‌چرب با افزودن صمغ دانه شنبلیله و ریحان و بررسی تاثیر آن بر زنده‌مانی بیفیدوباکتریوم بیفیدوم

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**چکیده**

**استفاده از جایگزینهای چربی با خواص پری‌بیوتیکی از جمله صمغ دانه‌ها می‌تواند راهکار مناسبی برای بهبود ویژگی‌های کیفی محصولات کم‌چرب باشد. در این پژوهش از روش سطح پاسخ با طرح مرکب مرکزی به منظور مطالعه اثر صمغ دانه ریحان در پنج سطح (۰-۰/۲ درصد) و شنبلیله (۰-۰/۲ درصد) بر خواص فیزیکوشیمیایی، بافتی و ارگانولپتیکی ماست کم‌چرب استفاده گردید. نتایج نشان داد که با افزایش میزان صمغها در فرمولاسیون ماست کم‌چرب میزان pH کاهش یافت. میزان آباندازی ماست کم‌چرب با افزودن صمغ دانه ریحان و شنبلیه کاهش یافت در حالی که ظرفیت نگهداری آب و ویسکوزیته افزایش پیدا کرد. بر پایه نتایج ارزیابی حسی نیز بیشترین امتیاز پذیرش کلی در تیمارهای حاوی مقادیر میانی صمغها کسب گردید. نتایج بهینه‌سازی با طرح مرکب مرکزی نشان داد که بهترین نمونه با خواص فیزیکوشیمیایی و حسی مطلوب زمانی حاصل میشود که فرمولاسیون ماست شامل ۰/۱۴ درصد صمغ دانه ریحان و ۰/۱۳ درصد صمغ دانه شنبلیله باشد. در قسمت دوم پژوهش اثر افزودن مقادیر بهینه صمغ‌های ذکر شده بر زنده‌مانی باکتری پروبیوتیک بیفیدوباکتریوم بیفیدوم و ویژگیهای مختلف ماست کم‌چرب طی دوره نگهداری مورد بررسی قرار گرفت. نتایج نشان داد که با افزودن باکتری پروبیوتیک و افزایش زمان نگهداری میزان pH نمونه‌ها کاهش یافت در حالی که اسیدیته افزایش پیدا کرد. یافته‌ها حکایت از آن داشت که در طی دوره نگهداری میزان آب‌اندازی و ویسکوزیته تمامی نمونه‌ها افزایش پیدا کرد در حالی که افزودن باکتری پروبیوتیک اثر قابل توجهی بر این پارامترها نداشت. استفاده از مقادیر بهینه صمغ دانه ریحان و شنبلیه اثر مثبتی بر زندهمانی باکتریهای پروبیوتیک داشت. به طور کلی نتایج این پژوهش نشان داد که استفاده از صمغ دانه ریحان و شنبلیله به عنوان جایگزین چربی در فرمولاسیون ماست کم‌چرب پروبیوتیک می‌تواند راهکاری جهت بهبود خواص کیفی ماست و افزایش زنده‌مانی باکتری پروبیوتیک باشد.**



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