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Optimization of composition of probiotic stirred yogurt containing *lactobacillus acidophilus LA5* by response surface methodology

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ABSTRACT

Response surface methodology was applied to investigate the effect of three independent variables, including fat (8, 6.68, 4.75, 2.82, 1.5%), salt (1, 0.82, 0.55, 0.28, 0.10%) and solids-non-fat (SNF) (15, 13.68, 11.75, 9.82, 8.50%) on the probiotic stirred yogurt. The results of organoleptic evaluation showed that samples with composition of 4.75% fat, 1% salt, 11.75% SNF and 6.68% fat, 0.82% salt, 13.68% SNF obtained the highest score at the first day and samples with composition of 8 fat, 0.55 salt, 11.75% SNF and 4.75 fat, 0.55 salt, 15% SNF had the lowest score on the 21st day. According to the results of sensory evaluation, probiotic yogurt with composition of 5.45 fat, 0.28 salt, 13.68% SNF was selected as optimum sample and compared to commercial stirred yogurt (1.4 fat, 0 salt, 10% SNF) as control sample with respect to microbial characteristics. Statistical analysis showed that the percentage of fat, SNF and salt had no significant effect on pH of samples, but the SNF and salt and their interaction had a significant ($p<0.05$) effect on acidity. The results of microbial analysis showed that composition, storage time and interaction of them had a significant ($p<0.01$) effect on bacterial and probiotic count. Decreasing in the number of starter and probiotic bacteria in the control sample was due to an increase in the acidity. Finally, 5.45 fat, 0.28 salt and 13.68% SNF is introduced as the best probiotic stirred yogurt formulation.

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1-Introduction

Emerging functional foods have created diversity in food product development. These foods not only provide suitable nutritional sources, but also promote the health of consumers. Today, the most significant functional foods include probiotics, prebiotics, and synbiotics. Yogurt is the most popular fermented dairy product and is produced and marketed as the most important commercial probiotic product in the world. Dairy products, including yogurt, can play a crucial role as carriers of probiotic bacteria, serving as a means of delivering them to consumers [1]. According to researchers, yogurt is a fermented dairy product made from fresh milk by two microorganisms: Streptococcus thermophilus and Lactobacillus bulgaricus [2]. The popularity and high consumption of this dairy product is attributed to its nutritional value (rich in carbohydrates, proteins, fats, minerals, and vitamins) and the beneficial effects of its starter bacteria (maintaining the balance of the microbial flora in the gastrointestinal tract) as well as its therapeutic properties, including skin health, anti-cancer effects, weight control, and more [3,4]. Today, various types of yogurt are produced based on their physical, chemical, and flavor characteristics. The most common commercial types include set yogurt, stirred yogurt, drinkable yogurt, and frozen yogurt. Stirred yogurt is produced by fermenting milk and stirring the curd to break the firm gel structure, resulting in a viscous liquid [5]. The physical characteristics and structure of stirred fermented products, such as yogurt, are essential and important criteria for consumer acceptance. However, due to the fact that commercial starter bacteria in yogurt do not survive well in the gastrointestinal tract and exhibit beneficial properties, there has been an increasing interest in consuming probiotic yogurt [6]. Probiotics are live microorganisms that, when administered in adequate amounts, confer beneficial effects on the host. The existing belief regarding the beneficial effects of probiotics is based on the fact that the gut microbiota plays a protective role against various diseases; the primary effect of probiotics is manifested through the stabilization of gut microbial flora [7]. It has been observed that the regular consumption of probiotics is effective in reducing the incidence of various diseases, particularly in high-risk populations (such as hospitalized children, those who do not consume breast milk, or those living in deprived conditions). The viability and metabolic activity of probiotic products must be maintained throughout all stages of food processing, from production to digestion by the consumer. The production of probiotic yogurt requires the correct selection of microbial strains and an appropriate carrier food matrix, along with the application of processes compatible with the survival of the selected strains[8]. In scientific literature, a population of 10^6 – 10^7 Colony Forming Unit/gr (CFU/gr) is stated as the therapeutic amounts in processed food products. Various factors affect the organoleptic, rheological, textural, and microstructural characteristics of probiotic yogurt, including the fermentation process, type of milk, type of starter culture, probiotic species, formulation, and so on [9], which leads to the creation of a product that is qualitatively desirable and meets consumer demand. Thus, the survival and colonization in the gut environment are essential conditions for being classified as probiotics. Important probiotic species include *Lactobacillus* bacteria, such as *Lactobacillus acidophilus*, and *Bifidobacterium* bacteria, such as *Bifidobacterium bifidum*. Although dairy products are considered a suitable medium for delivering probiotic bacteria to the human body, technological barriers such as the selection of appropriate probiotic strains, salt content, type of packaging, presence or absence of oxygen, ripening time, and storage conditions can reduce the efficiency of producing and using these products. The sensory characteristics of probiotic products may negatively impact their market acceptability. Therefore, attention must be paid to improving the sensory characteristics of the product when selecting strains and formulating probiotic yogurt [10]. The aim of this research is to investigate the effect of fat content, solids-non-fat (SNF), and salt percentage on the physicochemical, microbial, and sensory characteristics of stirred probiotic yogurt, in order to produce a functional product of optimal quality from the consumer's perspective.

2-Materials and methods

2-1-Materials

The milk, cream, and skimmed milk powder were prepared from the Pegah Pasteurized Milk Company in East Azerbaijan. The mixed culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus* YC-X11 (Christian Hansen, Denmark), the probiotic bacterium *Lactobacillus acidophilus LA5* (Christian Hansen, Denmark), M17 agar medium (Quelab, Canada), and MRS agar medium (Liofilchem, Italy) were used.

2-2- Preparation of probiotic stirred yogurt

To produce probiotic stirred yogurt, standardized milks with different fat percentages, solids-non-fat (SNF), and salt (Table 1) were homogenized at a temperature of 60-70 ˚C under a pressure of 15-20 Mpa. It was then heated at a temperature of 85-90 ˚C for 15 minutes. After cooling to 45 ˚C, the yogurt starter culture YC-X11 (a mixed culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*) and the probiotic strain *Lactobacillus acidophilus LA5* were added and incubated at 45 ˚C for 2 to 3 hours. When the pH

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reached 4.6, the samples were removed from the incubator for stirring, cooled to 25 ◦C after stirring, and then packaged in plastic yogurt containers. Finally, they were transferred to a cold storage room at a temperature of 4 ◦C [11].

Table1 Coded and actual values of the independent variables used in the central composite design

Sample	Independent variables					
	Coded values			Actual values		
	Fat	Salt	SNF	Fat	Salt	SNF
	X_1	X_2	X_3	X_1	X_2	X_3
1	-1	-1	-1	2.82	0.28	**9.82**
2	0	0	0	4.75	0.55	**11.75**
3	0	0	0	4.75	0.55	**11.75**
4	0	0	+1.68	4.75	0.55	**8.50**
5	0	-1.68	0	4.75	1	**11.75**
6	-1	-1	+1	2.82	0.28	**13.68**
7	-1	+1	-1	2.82	0.82	**9.82**
8	-1.68	0	0	1.5	0.55	**11.75**
9	+1	+1	+1	6.68	0.82	**13.68**
10	+1	-1	+1	6.68	0.28	**13.68**
11	0	-1.68	0	4.75	0.10	**11.75**
12	+1	-1	-1	6.68	0.28	**9.82**
13	+1	+1	-1	6.68	0.82	**9.82**
14	-1	+1	+1	2.82	0.82	**13.68**
15	0	0	0	4.75	0.55	**11.75**
16	+1.68	0	0	8	0.55	**11.75**
17	0	0	0	4.75	0.55	**11.75**
18	0	0	+1.68	4.75	0.55	**15**

The unit of actual values is percentage.

2-3-Statistical analysis

To determine the optimal values of components in the formulation of probiotic stirred yogurt, a response surface methodology was employed using a central composite design with 18 treatments and 4 replicates at the central point (to determine experimental error). The effects of three independent variables, including fat percentage (8, 6.68, 4.75, 2.82, and 1.5 percent), salt (1, 0.82, 0.55, 0.28, and 0.10 percent), and solids-non-fat (SNF) (15, 13.68, 11.75, 9.82, and 8.50 percent), were evaluated on the physical, chemical, and sensory characteristics of the probiotic stirred yogurt samples (as shown in Table 1). To assess the model and select the optimal formulation, sensory evaluation responses were used, and data analysis was conducted using Design-Expert software version 7.0.0. In the second phase, the yogurt sample with the optimal composition (fat 5.45, salt 0.28, SNF 13.68 percent) was compared in terms of microbial characteristics with an industrial stirred yogurt sample (fat 1.4, salt 0, SNF 10 percent), used as a control sample, utilizing a factorial design. Data analysis for this phase was performed using SPSS software version 16.

3- Experiments

3-1-Physicochemical experiments

3-1-1-PH and acidity measurement

The pH of various samples of stirred probiotic yogurt was determined after 1, 7, 14, and 21 days of storage using a pH meter (NIK, Germany). After calibrating the device, the electrode was directly inserted into the homogenized yogurt matrix for measurement. Additionally, acidity was measured using the titration method with 0.1 N sodium hydroxide in the presence of phenolphthalein, and the results were ultimately reported in degrees Dornic according to the Iranian National Standard No. 2852 [12].

3-1-2-Fat measurement

The measurement of fat was performed using the Gerber method according to Iranian National Standard No. 695.

3-1-3- SNF measurement

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To measure the solids-non-fat, the moisture content of the milk was first determined using the oven drying method according to Iranian National Standard No. 637. Then, the amount of dry matter without fat was calculated by subtracting the fat content from the total dry matter obtained from the moisture content.

3-2-Microbial experiments

3-2-1- Enumeration of starter and probiotic bacteria

The counts of commercial yogurt starter bacteria (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*) and probiotics (*Lactobacillus acidophilus*) were performed at intervals of 1, 14, and 21 days of storage. *Streptococcus thermophilus* was cultured on M17 agar, and *Lactobacillus delbrueckii subsp. bulgaricus* was cultured on MRS agar using the pour plate method, and incubated under aerobic and anaerobic conditions for 72 and 48 hours, respectively, at a temperature of 37-42 ◦C. For counting *Lactobacillus acidophilus*, MRS agar containing clindamycin and ciprofloxacin was used, and it was cultured using the pour plate method and incubated anaerobically with a gas pack for 72 hours at a temperature of 37-42 ◦C. Plates containing 30-300 colonies were counted. [15 and 16].

4-Sensory evaluation of probiotic stirred yogurt samples

The sensory evaluation included the assessment of yogurt color, texture characteristics (consistency and mouthfeel), taste characteristics, sourness, moldiness, and overall acceptance of probiotic whipped yogurt, conducted using 15 sensory evaluators at 1, 7, 14, and 21 days post-production using a 5-point hedonic scale [17]. For this purpose, the samples were coded and provided to the evaluators along with a feedback form in which they rated the quality using a score of 5 for desirable quality and a score of 1 for undesirable quality. To achieve accurate results, the samples were allowed to reach room temperature before evaluation, and water was provided to the evaluators between each sample assessment.

5-Results and discussion

5-1- pH and acidity

Based on the results of the regression model and variance analysis, the effects of fat percentage, salt, and SNF on the changes in pH and acidity during the 21-day storage period showed that only the interaction effect of SNF and salt was significant on day 14 ($p < 0.05$). The high R^2 value (86.49%) on that day indicates that the regression model was able to demonstrate and predict the relationship between the independent and dependent variables. Additionally, all effects on changes in pH were found to be insignificant. Figure 1 illustrates the interaction of SNF and salt on changes in acidity. As shown in Figure 1, with an increase in SNF, the acidity also increased. The factor responsible for the reduction in pH during the storage period is the continuous fermentation of lactose by lactic acid bacteria. A similar effect was observed for salt, although this effect was less pronounced compared to SNF. It seems that the increase in SNF content enhances the buffering capacity, which is a key factor affecting pH changes in dairy products, and the additional acid produced by the starter culture is aimed at achieving the desired pH. It has also been reported that the type of microorganisms used, temperature, and ripening time have interactive effects on acidity development. Considering that in the production of yogurt samples in this study, probiotic strains were used simultaneously with a commercial starter culture, their fermentative effect on lactose and lactic acid production in the probiotic yogurt samples was greater than in the control samples. This result was consistent with the results of some researchers' experiments.

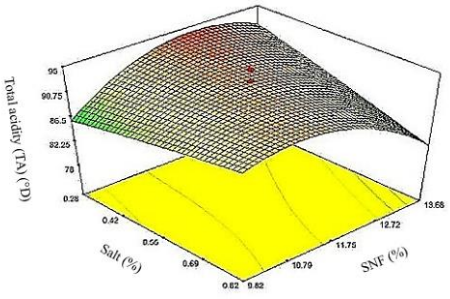

Figure 1 Three-dimensional response level of acidity versus SNF and salt percentage on day 14.

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5-2- Sensory evaluation features

The balance of flavor compounds in food products significantly determines their overall acceptability, which is generally perceivable by the consumer. Therefore, considering the importance of sensory characteristics, it is essential to examine and understand the factors influencing them in order to achieve desirable sensory attributes. [20]

5-2-1-Color

The results obtained from the evaluation of the color of the stirred probiotic yogurt samples indicated that with the increase in fat percentage and SNF, the lowest score (27.3) was assigned to color, which suggests that the increase in fat percentage has a negative effect on it. Additionally, according to the results of the regression model and analysis of variance for color, the interaction effect of the independent variables over the 21 days was not significant (Table 2).

Table 2 The results of variance analysis of the appearance properties of probiotic stirred yogurt samples during storage time

Source	Sum of Squares (SS)			
	Storage time (Day)			
	1	7	14	21
Model	2.97 ^{ns}	0.8 ^{ns}	0.43 ^{ns}	**0.88** ^{ns}
X_1	0.43 ^{ns}	0.02 ^{ns}	0.08 ^{ns}	**0.10** ^{ns}
X_2	0.038 ^{ns}	0.03 ^{ns}	0.06 ^{ns}	**0.11** ^{ns}
X_3	0.29 ^{ns}	0.02 ^{ns}	0.05 ^{ns}	**0.00** ^{ns}
X_1X_2	0.16 ^{ns}	0.1 1 ^{ns}	0.05 ^{ns}	**0.05** ^{ns}
X_1X_3	0.067 ^{ns}	0.11 ^{ns}	0.03 ^{ns}	**0.03** ^{ns}
X_2X_3	1.12 ^{ns}	0.06 ^{ns}	0.05 ^{ns}	**0.53** ^{ns}
X_1^2	0.57 ^{ns}	0.28 ^{ns}	0.09 ^{ns}	**0.07** ^{ns}
X_2^2	0.59 ^{ns}	0.07 ^{ns}	0.01 ^{ns}	**0.02** ^{ns}
X_3^2	0.15 ^{ns}	0.02 ^{ns}	0.01 ^{ns}	**0.00** ^{ns}
Residual	2.57 ^{ns}	0.2 ^{ns}	0.092 ^{ns}	**0.46** ^{ns}
Lack of fit	1.95 ^{ns}	0.11 ^{ns}	0.016 ^{ns}	**0.1** ^{ns}
Pure error	0.62 ^{ns}	0.09 ^{ns}	0.08 ^{ns}	**0.36** ^{ns}
Linear	4.15 ^{ns}	0.85 ^{ns}	0.26 ^{ns}	**0.78** ^{ns}
Intraction	2.8 ^{ns}	0.58 ^{ns}	0.14 ^{ns}	**0.18** ^{ns}
Quadratic	1.95 ^{ns}	0.11 ^{ns}	0.016 ^{ns}	**0.1** ^{ns}
Total	5.54 ^{ns}	1 ^{ns}	0.43 ^{ns}	**0.35** ^{ns}
R^2	0.5363	0.7964	0.8236	**0.6564**

Ns shows non-significance.

5-2-2- Textural characteristics

The results of the assessment of textural characteristics (consistency and mouthfeel) indicated that with an increase in the percentage of fat and SNF, the highest scores (4.53 and 4, respectively) were assigned to these characteristics. It is evident that with the increase of these two variables, the stability, viscosity, firmness, and mouthfeel of the samples increased. According to the results of regression modeling and variance analysis for textural characteristics (consistency), the interaction effect of fat and salt was significant at the 0.01 level, and the interaction effect of fat and SNF on day 21 was significant ($p < 0.05$). Figure 2 (a) shows the interaction effect of fat and salt on the desirability of consistency, while Figure 2 (b) illustrates the effect of fat and SNF. As seen in Figure 2 (a), with the increase in the percentage of fat and salt, the consistency also increased. Figure 2 (b) further indicates that at medium levels of fat and SNF, consistency increased. Based on the results of regression modeling and variance analysis for textural characteristics (mouthfeel), the interaction effect of salt and SNF was significant at the 0.05 level on day 7. Figure 3 shows the interaction effect of salt and SNF on the desirability of mouthfeel. As observed in Figure 3, with the increase in the percentage of salt and SNF, the mouthfeel received a higher score.

[DOI: 10.22034/FSCT.22.162.278]

(a)
(b)

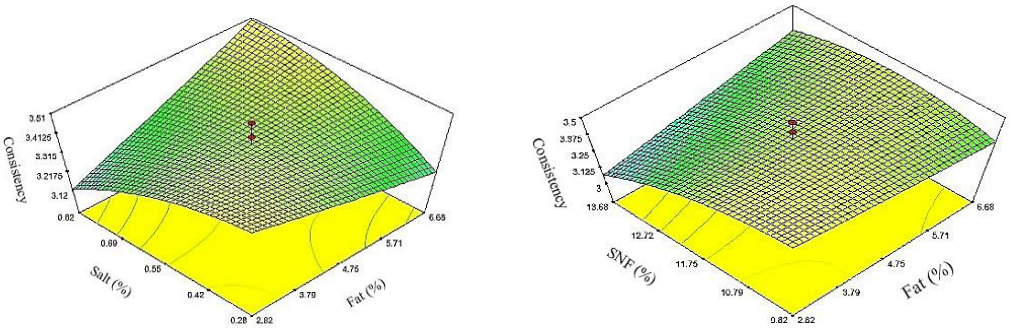


Figure 2 Three-dimensional response level of consistency versus fat and salt percentage (a), fat and SNF percentage (b) on day 21.

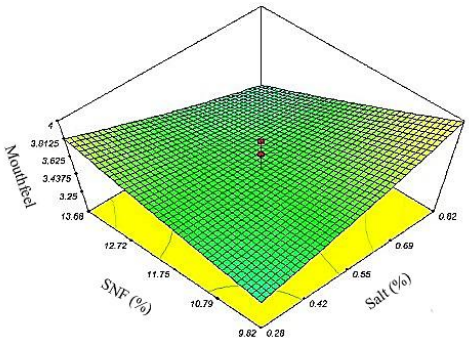


Figure 3 Three-dimensional response level of texture mouthfeel versus SNF and salt percentage on day 7.

5-2-3- Characteristics of teste

The results of the evaluation of taste characteristics indicated that with an increase in the percentage of SNF and consequently an increase in acidity due to the activity of starter bacteria over time, the lowest score (3) was achieved particularly on day 21. According to the results of the regression model and analysis of variance for taste, the interaction effect of fat and salt was significant at the 0.001 level on day 7. Figure 4 illustrates the interaction effect of fat and salt on the desirability of taste. As shown in the figure, when fat is present in low amounts and salt is in high amounts, the taste received a high score. The evaluation results for the old and musty taste also revealed that with an increase in the percentage of fat and SNF over time, this characteristic received the lowest score (2.60). Based on the results of the regression model and analysis of variance for the old and musty taste, the interaction effect of the independent variables over the 21 days was not significant.

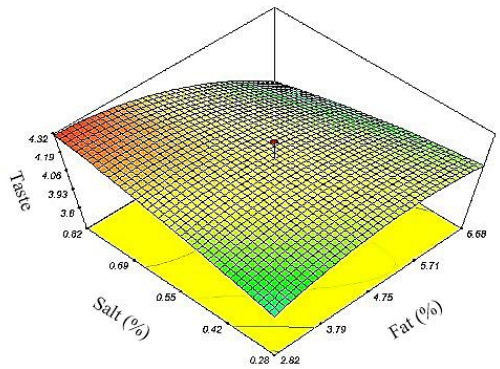


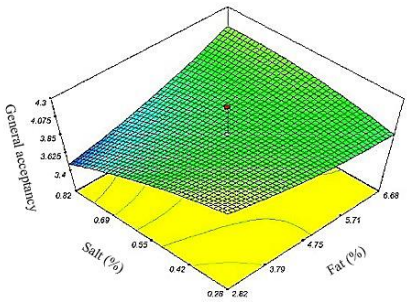
Figure 4 Three-dimensional response level of taste versus fat and salt percentage on day 7.

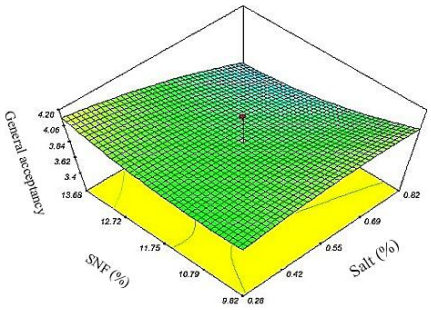
[DOI: 10.22034/FSCT.22.162.278]

5-2-4- General acceptancy

The results of the overall evaluation indicate that an increase in fat up to 8%, SNF up to 15%, and salt up to 1% is acceptable; however, exceeding these amounts leads to a decrease in the overall acceptability of the product. Based on the results of the regression model and analysis of variance for the overall evaluation, the interaction effect of fat and salt was significant at the 0.01 level, and the interaction effect of salt and SNF was significant at the 0.05 level on day 1. Figure 5 (a) illustrates the interaction effect of fat and salt, while (b) shows the interaction effect of salt and SNF on the overall evaluation. As can be observed, with an increase in the percentage of fat and salt, the overall evaluation also increases. In Figure 5 (b), it is also seen that when salt is at low levels and SNF is at high levels, the overall score is high.

(a)





(b)

Figure 5 Three-dimensional response level of general acceptancy versus fat and salt percentage (a), salt and SNF percentage (b) on day 1.

5-3- Optimization

To optimize the levels of independent variables, responses such as color, consistency, mouthfeel, taste, old and musty taste, along with overall evaluation with varying degrees of importance, which affect the acceptability of our probiotic yogurt, were determined. The criteria used and the optimal point are presented in Table 3.

Table 3 Criteria for optimizing process condition along with responses

Limitation	Target	Lower limit	Upper limit	Importance	Solution
Fat	In range	2.82	6.68	3	**5.45**
Salt	In range	0.28	0.82	3	**0.28**
SNF	Maximize	9.82	13.68	3	**13.68**
Color	In range	3.86	4.73	3	**4.50**
Consistency	Maximize	2.9	4.46	3	**3.79**
Mouthfeel	Maximize	2.9	4.46	3	**4.01**
Taste	In range	3.6	4.6	3	**4.14**
Old and musty taste	In range	4.06	4.8	3	**4.24**
General acceptancy	In range	3.4	4.5	3	**4.13**

5-4- The comparison of yogurt with the optimized formulation and control stirred yogurt in terms of microbial characteristics

5-4-1-The effect of composition and storage time on the count of *Streptococcus thermophilus*

The results of the variance analysis indicated that the combination, storage duration, and their interaction had a significant effect on the count of *Streptococcus thermophilus* ($p < 0.01$). Table 4 shows the effect of the combination and time on the count of *Streptococcus thermophilus* in the probiotic stirred yogurt samples. According to the table, there is a decreasing trend over time. Additionally, the highest

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count of *Streptococcus thermophilus* bacteria was recorded in the optimal sample (5.45% fat, 0.28% salt, 13.68% SNF) and the control sample (1.4% fat, 0% salt, 10% SNF) on the first day, while the lowest count was noted in the control sample on the 21st day. The genus *Streptococcus* is more sensitive to increased acidity compared to the genus *Lactobacillus*, and its growth and multiplication are more significantly reduced in acidic environments [21]. Therefore, it is likely that the increase in acidity in the control sample on the 21st day, along with the high sensitivity of *Streptococcus*, has led to a decrease in its count.

Table 4 Effect of composition and time on *Streptococcus thermophiles* count

Sample	***Streptococcus thermophiles* count during storage (Log cfu/ml)**		
	Storage time (day)		
	1	**14**	**21**
Control	7.62 ±0.07 [a]	7±0.10 [ab]	**5 ± 0.06 [c]**
Optimum	7.60± 0.11 [a]	7.07 ±0.16 [b]	**7.09 ±0. 43[b]**

Non-identical Latin letters indicate a significant difference ($p< 0.05$).

5-4-2- The effect of formulation and storage time on the count of *Lactobacillus bulgaricus*

The results of the analysis of variance indicated that both the type of formulation and storage duration ($p<0.01$) and their interaction effect ($p<0.05$) significantly influenced the count of *Lactobacillus bulgaricus*. Table 5 illustrates the effect of formulation and time on the count of *Lactobacillus bulgaricus* in samples of probiotic stirred yogurt. According to the table, the highest count of *Lactobacillus bulgaricus* was observed in the optimal sample (fat 5.45%, salt 0.28%, SNF 13.68%) on the first day, while the lowest count was found in both the optimal and control samples (fat 1.4%, salt 0, SNF 10%) on the 21st day. The reduction in the number of *Lactobacillus bulgaricus* in the control sample compared to the optimal sample is likely due to the increased acidity in this sample, which may inhibit the growth and activity of *Lactobacillus bulgaricus*.

Table 5. Effect of composition and time on *Lactobacillus bulgaricus* count

Sample	***Lactobacillus bulgaricus* count during storage (Log cfu/ml)**		
	Storage time (day)		
	1	**14**	**21**
Control	7.39 ± 0.06 [b]	6.97±0.09 [c]	**6.45 ±0.10 [d]**
Optimum	7.77 ±0. 13[a]	7.73 ±0.14 [a]	**6.56± 0.22 [d]**

Non-identical Latin letters indicate a significant difference ($p< 0.05$).

5-4-3- Effect of composition and time on *Lactobacillus acidophilus* count

The results of the variance analysis indicated that the type of formulation and storage duration had a significant effect on the count of *Lactobacillus acidophilus* ($p < 0.01$), as well as their interaction effect ($p \leq 0.05$). Table 6 shows the impact of formulation and time on the count of *Lactobacillus acidophilus* in probiotic stirred yogurt samples. According to the table, the highest count of *Lactobacillus acidophilus* was observed in the optimal sample (fat 5.45%, salt 0.28, SNF 13.68%) on the first day, while the lowest count was found in the control sample (fat 1.4%, salt 0, SNF 10%) on the 21st day. In low-fat yogurt, the increase in acidity over the storage period may be detrimental to probiotics, leading to a decrease in their viability compared to full-fat yogurt, which aligns with the findings of other researchers [22-24]. Changes in salt concentrations in dairy products affect the bacterial cell membrane, resulting in reduced growth and activity. However, there are few studies on the damage caused by salt to probiotic bacteria. According to the studies by Shah and Gandhi (2015), which examined the effect of salt on the viability and membrane permeability of *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium longum* using flow cytometry, *Lactobacillus acidophilus* is resistant at salt concentrations above 3.5%. Therefore, in this study, salt likely does not have a significant effect on the count of *Lactobacillus acidophilus*. Additionally, the conventional plate counting technique only reflects reduced cell growth and does not provide detailed information on metabolic activity, damage degree, and cell health. Generally, the number of inoculated bacteria in the product should be such that, upon consumption, their count is at a maximum to ensure the desired benefits of consuming probiotic products

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are met [25]. To achieve maximum probiotic benefits, the count of probiotic bacteria in a dairy product at the time of consumption should be at least 10^6 CFU/g, and this dairy product should be consumed regularly, daily, up to 100 grams [26].

Table 6. Effect of composition and time on *Lactobacillus acidophilus* count

Sample	***Lactobacillus acidophilus* count during storage (Log cfu/ml)**		
	Storage time (day)		
	1	**14**	**21**
Control	6.94 ± 0.67 [b]	6.87±0.13 [ab]	**4.72 ±0.17** [c]
Optimum	7.36 ±0. 05[a]	7.26 ±0.16 [b]	**6.42± 0.05** [b]

Non-identical Latin letters indicate a significant difference ($p< 0.05$).

6- Results and discussion

The results obtained from the physicochemical, microbial, and sensory characteristics of the produced probiotic stirred yogurts indicated that the percentages of salt and SNF and their interaction had a significant effect on some physicochemical properties of the probiotic stirred yogurt, including acidity. Overall evaluations regarding color, textural features, and taste of the probiotic stirred yogurt samples showed that, from the consumers' perspective, samples with compositions of (fat 4.75%, salt 1%, SNF 11.75%) and (fat 6.68%, salt 0.82%, SNF 13.68%) received the highest scores on the first day, while samples with compositions of (fat 8%, salt 0.55%, SNF 11.75%) and (fat 4.75%, salt 0.55%, SNF 15%) received the lowest scores on the 21st day. In general, samples with fat percentages ranging from 4.75% to 6.68% and SNF percentages from 11.75% to 13.68%, as well as higher salt percentages, were more favored. The results from the comparison of mean data indicated that the type of composition, storage duration, and the interaction effect of these factors significantly affected the counts of starter and probiotic bacteria. Overall, in the control sample and the optimal sample over a storage period of 21 days, a decreasing trend was observed in the counts of Streptococcus thermophilus and Lactobacillus delbrueckii, as well as in the count of Lactobacillus acidophilus due to the increase in acidity. The reduction in the counts of starter and probiotic bacteria in the control sample compared to the optimal sample could be attributed to the increase in acidity in this sample. In this study, salt did not have a significant effect on the count of *Lactobacillus acidophilus*, as this bacterium is resistant to salt concentrations up to 3.5%. Given the popularity of probiotic products, especially yogurt, due to their health benefits and therapeutic properties for consumers, including the control of intestinal infections, improvement of lactose intolerance, anti-cancer activity, impact on diabetes, etc., and also based on the summary of the results from various tests, the yogurt containing (fat 5.45%, salt 0.28%, SNF 13.68%) is introduced as the best formulation of probiotic stirred yogurt.

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بهینه‌سازی ترکیب ماست همزده پروبیوتیک حاوی سویه *لاکتوباسیلوس اسیدوفیلوس* ***LA5*** به روش سطح پاسخ

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چکیده

با استفاده از روش سطح پاسخ اثر سه متغیر مستقل شامل چربی (۸، ۶/۶۸، ۴/۷۵، ۲/۸۲، ۱/۵ درصد)، نمک (۱، ۰/۸۲، ۰/۵۵، ۰/۲۸، ۰/۱۰ درصد) و ماده خشک بدون چربی (SNF) (۱۵، ۱۳/۶۸، ۱۱/۷۵، ۹/۸۲، ۸/۵۰ درصد)، روی ویژگی‌های نمونه‌های ماست همزده پروبیوتیک مورد ارزیابی قرار گرفت. نتایج ارزیابی حسی نمونه‌ها نشان داد که نمونه‌های با ترکیب (چربی ۴/۷۵، نمک ۱، SNF ۱۱/۷۵ درصد) و (چربی ۶/۶۸، نمک ۰/۸۲، SNF ۱۳/۶۸ درصد) بیشترین امتیاز را در روز اول و نمونه‌های با ترکیب (چربی ۸، نمک ۰/۵۵، SNF ۱۱/۷۵ درصد) و (چربی ۴/۷۵، نمک ۰/۵۵، SNF ۱۵ درصد) کمترین امتیاز را در روز ۲۱ کسب کردند. با توجه به نتایج حاصل از بهینه‌سازی ارزیابی حسی، ماست همزده پروبیوتیک (چربی ۵/۴۵، نمک ۰/۲۸، SNF ۱۳/۶۸ درصد) به عنوان نمونه بهینه با نمونه تهیه شده در صنعت به عنوان نمونه کنترل (چربی ۱/۴، نمک ۰، SNF ۱۰ درصد)، برای انجام آزمون میکروبی مقایسه شد. نتایج نشان داد که درصد چربی، SNF و نمک اثر معنی‌داری بر pH نمونه‌ها نداشتند اما درصد SNF و نمک و اثر متقابل آن‌ها تأثیر معنی‌داری ($p<0/05$) بر اسیدیته آن‌ها نشان دادند. بررسی‌های میکروبی حاکی از تأثیر معنی‌داری ترکیب، مدت زمان نگهداری و اثر متقابل آن‌ها روی شمارش باکتری‌های آغازگر و پروبیوتیک بود ($p<0/01$). کاهش در شمارش باکتری‌های آغازگر و پروبیوتیک در نمونه کنترل به افزایش اسیدیته نسبت داده شد. با جمع‌بندی نتایج حاصل از آزمون‌های مختلف، چربی ۵/۴۵، نمک ۰/۲۸ و SNF ۱۳/۶۸ درصد به عنوان بهترین فرمولاسیون ماست همزده پروبیوتیک معرفی می‌شود.

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