



## Scientific Research

### Investigation of the rheological and physicochemical properties of ice cream enriched with kefir and vegetable stabilizers using the Mixture Design method

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## ABSTRACT

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In this study, kefir, after being cultured from kefir grains and extracted, was added to ice cream in combination with aloe vera gel and guar gum at various levels (2.83%, 5.66%, 8.5%, 11.33%, and 17%) by weight, along with gum arabic (constituting 50% of the total gums). The effects on the rheological, physicochemical, and organoleptic properties of the ice cream were investigated. Data analysis was performed using analysis of variance (ANOVA) with an appropriate follow-up model at a 5% probability level, employing Design Expert 12 software. The results of the physicochemical tests on the ice cream samples revealed that the addition of hydrocolloids led to an increase in viscosity, melting rate, and adhesiveness. Specifically, treatments containing 17% kefir exhibited the lowest values (0.25 Pa·s, 42% decrease, 17.31 MJ), while those with 17% guar gum showed the highest values (0.282 Pa·s, 80% decrease, 18.11 MJ). Increasing the gum content resulted in higher hardness of the ice cream treatments. The treatment with 17% kefir had the lowest hardness (1834.11 g/cm<sup>2</sup>), whereas the treatment with 17% guar gum exhibited the highest hardness (1989.6 g/cm<sup>2</sup>). Additionally, the incorporation of gums led to an increase in the overrun of the ice cream treatments. FE-SEM images of treatments containing 17% kefir and 17% guar gum indicated that the 17% guar gum treatment featured spherical particles with minimal wrinkling and no tearing, presenting a uniform surface. DLS results demonstrated that the particles in the 17% kefir treatment had an average diameter ranging from 1 to 3.5 microns. Organoleptic evaluation of the ice cream treatments revealed that samples with a higher proportion of kefir received higher scores for color and overall acceptability compared to those containing guar gum.

## 1- Introduction

Ice cream is fundamentally a complex colloidal system or a type of "frozen foam," in which air cells, fine ice crystals, and fat droplets are uniformly distributed and intermixed within a dense, viscous liquid phase (the serum phase). Various structural elements, including ice content and ratio, fat content, degree of fat aggregation, and mix viscosity, significantly influence the structure of ice cream and consequently impact sensory perception [1, 2]. Stabilizers are a group of biopolymers that, when incorporated into ice cream formulations, impart softness to the texture, reduce melting rate, and inhibit ice crystal growth, thereby playing a crucial role in product quality [1, 2]. A wide range of hydrocolloids from diverse origins have been identified for use in the food industry; among these is kefir. Kefir, or glucogalactan, is a constituent of kefir grains, produced by the grains' microbial flora [3]. Kefir consists of repeating units of glucose and galactose in a 1:1 ratio, forming a polymer of D-glucose and D-galactose [4, 5]. According to some researchers, kefir is produced by several kefir microorganisms (e.g., *Lactobacillus brevis*, *Lactobacillus kefirifaciens*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus helveticus*, *Streptococcus thermophilus*, and *Lactococcus lactis*) [4]. In recent decades, this polysaccharide has found extensive application in improving the properties and enrichment of food products. Reports on the use of hydrocolloids in ice cream are summarized below. Zarei et al. (2020) added various levels of phytosterols to ice cream mix for enrichment and stored the samples at -18°C for 60 days. They reported that increasing the phytosterol percentage led to significant increases in acidity, total solids, fat content, melting resistance, and viscosity [6]. Kasemtan et al. (2011) investigated the effect of hydrocolloids on ice cream storage quality, reporting that some stabilizers reduce or delay ice crystal growth, and that a combination of guar and xanthan gums had a better stabilizing effect on texture and flavor

improvement, while a combination of locust bean gum and carrageenan considerably reduced shelf life [7]. Rincon et al. (2006) examined a mixture of gums (*Acacia glomerosa*, *Enterolobium cyclocarpum*, and *Hymenaea courbaril*) as stabilizers in ice cream formulation. They reported that ice creams containing the gum mixture exhibited more suitable viscosity and desirable texture compared to treatments with individual gums. Furthermore, treatments containing the commercial gum mixture demonstrated better foaming, aeration properties, and superior sensory characteristics. They concluded that gum mixtures performed more effectively in ice cream preparation than single gums [8]. Javidi et al. (2012) studied the effects of guar gum, basil seed gum, and their 50:50 mixture on the properties of low-fat and medium-fat ice cream compared to full-fat samples. They reported that reducing fat decreased apparent viscosity and increased melting rate and overrun; however, adding gum as a fat replacer and increasing its concentration reversed these trends [9]. Considering the aforementioned studies and the lack of research on the combined use of kefir with plant-based hydrocolloids (aloe vera gel and guar gum) in ice cream formulation, this study was designed to assess the feasibility of applying a combination of kefir and plant-based hydrocolloids in ice cream formulation and to investigate its physicochemical, organoleptic, and rheological properties.

## 2-Materials and Methods

### Cultivation of Kefir Grains

In this research, kefir grains (a consortium of lactic acid bacteria and yeasts) were cultured for 9 months in pasteurized skim milk (at a 1:5 ratio), supplemented with milk powder (East Azerbaijan origin) and yogurt or buttermilk (Pegah Golestan) to lower the pH. The culture was incubated at 25°C in an incubator equipped with a stirrer. Subculturing of the kefir grains was

performed every 24 hours under sterile conditions in sterile containers. The addition of milk powder, yogurt, or buttermilk was done empirically to accelerate the growth of the kefir grains in the skim milk [10].

### **Extraction of Kefiran from Kefir Grains**

For kefir extraction, the method described by Soleimani Fard et al. (2017) was used with minor modifications. A specified amount of kefir grains was stirred in boiling water (1:3 ratio) for 3 hours. The resulting mixture was centrifuged using a refrigerated centrifuge (Hanel model, South Korea) at 10,000 rpm for 20 minutes. Soluble kefir was precipitated by adding cold ethanol and separated after 24 hours of storage at refrigerator temperature. The solution was centrifuged again at 10,000 rpm for 20 minutes at 4°C. The resulting precipitate was mixed with boiling water at a 1:5 ratio and washed twice. Finally, the solution (kefir) was freeze-dried using a lyophilizer (Operon model, South Korea) to obtain a powder [11].

### **Preparation of Aloe Vera Gel Powder**

Mature and healthy aloe vera leaves were obtained from the market, washed, and the fleshy gel tissue between the upper and lower epidermis was carefully separated layer by layer manually (Agarry, O.O., Olaleye, M.T., Bello, C.O. 2005). Subsequently, the aloe vera gel was chopped with a sharp knife and thoroughly blended using a mixer. The gel was then filtered through a cloth to obtain a clear, uniform aloe vera gel. Finally, the prepared gel was dried in an oven at 50°C for 6 hours until a constant weight was achieved. The dried powder was milled using a Retsch sieve shaker (Germany), and coarse particles were removed using sieves with mesh sizes of 125 micrometers, 250 micrometers, 500 micrometers, and 1 mm [12].

### **Production Process of Enriched Ice Cream**

To optimize the ice cream formulation, Design Expert 12 software and relevant literature were utilized. The base formulation consisted of 78.5% milk (10% fat, achieved by blending 2.5% and 10% fat milk/cream using Pearson's square), 18% sugar, 0.4% stabilizer (49% gum arabic, 51% total of kefir, aloe vera gel, and guar gum), 2.3% milk powder, and 0.8% orange extract powder (Table 1). To prepare the samples, the required amounts of raw materials were calculated and weighed using mass balance. First, the liquid components of the ice cream mix (cream and milk) were mixed at 25°C, and the temperature was raised to 45°C using a water bath. The dry ingredients were then mixed together and heated to 45°C. These dry ingredients were added to the 10% fat milk and stirred for an additional 2 minutes. For complete homogenization, the mixture was processed at 5000 rpm for 5 minutes. Pasteurization was carried out discontinuously in a water bath at 85°C for 5 minutes. Immediately after pasteurization, the mix was rapidly cooled to 4°C using an ice-water bath. Orange extract powder was added at this temperature, and the ice cream mix was aged for 24 hours in a refrigerator at 4°C to complete the maturation process (Marshall and Arbuckle, 1996). Aging increases viscosity, melting resistance, overrun, improves texture, and enhances whipping ability. After aging, a portion of the mix was set aside for rheological tests. The remaining mix was frozen in an ice cream maker (Feller, Germany) at 0°C for 20 minutes. The prepared samples were then packaged in polypropylene containers, coded, and stored at -18°C for subsequent analyses [12].

Table 1. Formulation of ice cream treatments

Sugar	Milk powder	Orange extract powder	Stabilizers (0.4 %)				شیر ۱۰٪ چربی	Treatment
			Guar	Aloe vera gel	Kefiran	عربی		
18	2.3	0.8	11.3333	2.83333	2.83333	0.196	78.5	1
18	2.3	0.8	0	17	0	0.196	78.5	2
18	2.3	0.8	5.66667	5.66667	5.66667	0.196	78.5	3
18	2.3	0.8	8.5	8.5	0	0.196	78.5	4
18	2.3	0.8	0	8.5	8.5	0.196	78.5	5
18	2.3	0.8	2.83333	2.83333	11.3333	0.196	78.5	6
18	2.3	0.8	17	0	0	0.196	78.5	7
18	2.3	0.8	2.83333	11.3333	2.83333	0.196	78.5	8
18	2.3	0.8	8.5	0	8.5	0.196	78.5	9
18	2.3	0.8	0	17	0	0.196	78.5	10
18	2.3	0.8	0	0	17	0.196	78.5	11

### Apparent Viscosity

The apparent viscosity of the ice cream samples was measured before the freezing operation and after the aging process using a Brookfield DVII viscometer at 5°C (temperature controlled with an ice-water mixture) and a shear rate of 1.7 s<sup>-1</sup>, in a 500 ml container. Spindles L1, L2, or L3 were selected based on the mix viscosity. The ice cream mix, after completing the aging period, was poured into the viscometer cup. The viscosity measurement was performed at a rotational speed of 100 rpm [13].

### Specific Gravity

The specific gravity of the ice cream mix samples was measured using a pycnometer at 25°C. The procedure was as follows: the weight of the empty, dry pycnometer (G), the weight of the pycnometer filled with distilled water (G<sub>1</sub>), and the weight of the pycnometer filled with the mix (G<sub>2</sub>) were recorded. The specific gravity was then calculated using the following formula [14]:

$$\text{Specific Gravity} = \frac{G_2 - G}{G_1 - G} \quad \text{Equation 1}$$

### pH

The pH of the ice cream treatments was evaluated before freezing using a digital pH meter (EDT, Model GP353) [15].

The overrun was measured by comparing the weight of the ice cream mix (before freezing) and the ice cream (after freezing) in a fixed, specified volume. The percentage overrun was calculated using Equation 2 [16]:

### Overrun

$$\text{Overrun} = \frac{\text{weight of mix} - \text{weight of ice cream}}{\text{weight of ice cream}} \times 100 \quad \text{Equation 2}$$

### Texture Analysis Test

To measure texture hardness, a penetrometer (Precision Penetrometer, Precision Scientific Company, Chicago) was used to determine ice cream firmness at  $-15^{\circ}\text{C}$ . Samples were quickly transferred to the penetrometer device and held at room temperature; the analysis of treatments was then completed within 30 seconds to minimize textural changes. The penetrometer was equipped with a stainless-steel probe (a 60-degree cone with a maximum diameter of 6.5 cm), positioned aligned with and in contact with the surface of the sample. A gravitational force was then applied for 5 minutes to allow the probe to penetrate the sample. The penetration depth was inversely related to the sample's hardness [13].

### Melting Rate

A sample from each ice cream treatment was cut from the end of a rectangular container to prepare a rectangular ice cream block weighing  $100 \pm 1$  g. The dimensions of the sample (typically  $6\text{-}7\text{ cm} \times 6\text{-}7\text{ cm} \times 3\text{ cm}$ ) varied slightly between ice creams due to differences in physical properties (Overrun). The ice cream sample (initially at  $-15^{\circ}\text{C}$ ) was placed on a wire screen (6 holes per cm) atop a funnel leading to a graduated cylinder. The setup was placed in an incubator at  $25^{\circ}\text{C}$  (50% RH). The volume of dripped melt was recorded at 5-minute intervals over a total period of 3 hours (90 minutes). The time was plotted against the drip volume, and the slope of the main melting phase was considered the melting rate [13].

### Dynamic Light Scattering Test

A particle size analyzer (Zetasizer) was used to examine particle size. For this purpose, spray-dried ice cream powder was dissolved in ethanol and thoroughly homogenized prior to injection into the device. Measurement was then performed at ambient temperature using a laser diffraction particle size analyzer. The

average particle size was determined based on the volume-weighted mean diameter [17].

### Coating/ Morphology Examination Test

The morphological characteristics of spray-dried ice cream treatments were determined using a Field Emission Scanning Electron Microscope (FE-SEM). Approximately 10 mg of the sample was mounted on a metal stub and sputter-coated with gold. The surface features and shape of the samples were then observed using FE-SEM [18].

### Organoleptic Test

The organoleptic properties of the ice cream treatments were evaluated by a panel of 12 assessors. Panelists were trained to recognize target attributes in the final product, including softness, texture uniformity, absence of sandiness or clumping, fresh (non-cooked) flavor, appropriate aroma, final mix color, and overall acceptability. A 5-point hedonic scale was used for this test, where products were scored as follows: Excellent=5, Good=4, Fair=3, Poor=2, and Very Poor=1. Bread and water were provided between samples for palate cleansing.

### Statistical Analysis

In the first stage, Design Expert 12 software and the Mixture Components method were used to optimize the ice cream formulation production. Three components—kefiran, aloe vera gel, and guar gum—were used as independent variables at several levels suggested by the software (Table 1), and the effect of each independent variable on the research's target factors was investigated. In the second stage, Design Expert 12 software was also employed for the statistical analysis of the results. During the analysis phase, after reviewing Diagnostic plots (Normal Plot,

Residual vs. Prediction, Cook's Distance, & Box-Cox Plot) and Analysis of Variance & Fit Statistics tables, if the initial model was non-significant for any factor, the best model suggested by the software (after comparing models: Linear vs. Mean, Quadratic vs. Linear, Special Cubic vs. Quadratic, Cubic vs. Special Cubic, Quadratic vs. Cubic, Special Quadratic vs. Quadratic, Quadratic vs. Special Quadratic) was applied with a 95% confidence level ( $p \leq 0.05$ ). All tests were performed in two blocks and with three replications to eliminate environmental effects and conditions. Among the resulting plots, 3D Surface and Real Contour plots were selected and presented.

### 3-Results and Discussion

#### Viscosity or Resistance to Flow

Viscosity is a key parameter for air retention in the ice cream mix during whipping and is a fundamental criterion for selecting the appropriate mixer, transfer pump, and related equipment [19]. Furthermore, viscosity is a significant parameter affecting the rate of creaming, heat transfer and melting rates, mass transfer, and the flow behavior of milk and its related products [19]. It is important to note that several key factors influence the viscosity of the ice cream mix, including its compositional elements, particularly the type and amount of fats and stabilizers (Bahram Parvar et al., 2008). Figure 1 shows the viscosity values of ice cream treatments containing different stabilizer levels. For evaluating the viscosity of the ice cream samples, the software suggested the Linear vs Mean model as the significant ( $p \leq 0.05$ ) model. The results indicated that the addition of hydrocolloids increased the viscosity of the ice cream mix, a property attributed to water absorption and the formation of a gel-like

network. Comparing the treatments revealed that kefiran produced the lowest viscosity (0.25 Pa·s), while guar gum produced the highest (0.282 Pa·s) in the ice cream mixes, which was dependent on their water absorption capacity. Amiri et al. (2012), by adding mucilage from basil and psyllium seeds to the ice cream mix, reported that increasing the concentration of natural gums leads to an increase in mix viscosity [20]. Javidi et al. (2015), by adding guar and basil seed gums as fat replacers, reported that increasing gum concentration increases viscosity; however, compared to the impact of fat on increasing viscosity, this increase is significantly and substantially lower [9]. Some researchers have reported the positive effect of fibers such as inulin, barley and wheat fiber, pectin from pumpkin powder, and isolated pectin on ice cream mix viscosity (due to increased serum phase solids concentration and gel network formation), as well as the effect of wheat germ protein isolate [21-25]. Among the reasons for changes in ice cream mix viscosity induced by hydrocolloids are their high molecular weight, limited flexibility between the constituent monomers of the polymer chain, and specific interactions with other polymer chains within the mix [26]. Finally, despite the research conducted in this area, a precise and optimal amount of hydrocolloid consumption for achieving suitable viscosity has not yet been established. This reality is likely due to factors such as the different final mix concentrations achieved by different stabilizers at equal weights, their varying absorption capacities, the different functional groups in stabilizer structures affecting their binding or reaction with water or the ice cream mix, the differing stabilizing powers of native and synthetic hydrocolloids, the stability of the formed gel network, the presence of ions and compounds affecting network stability, and similar considerations.

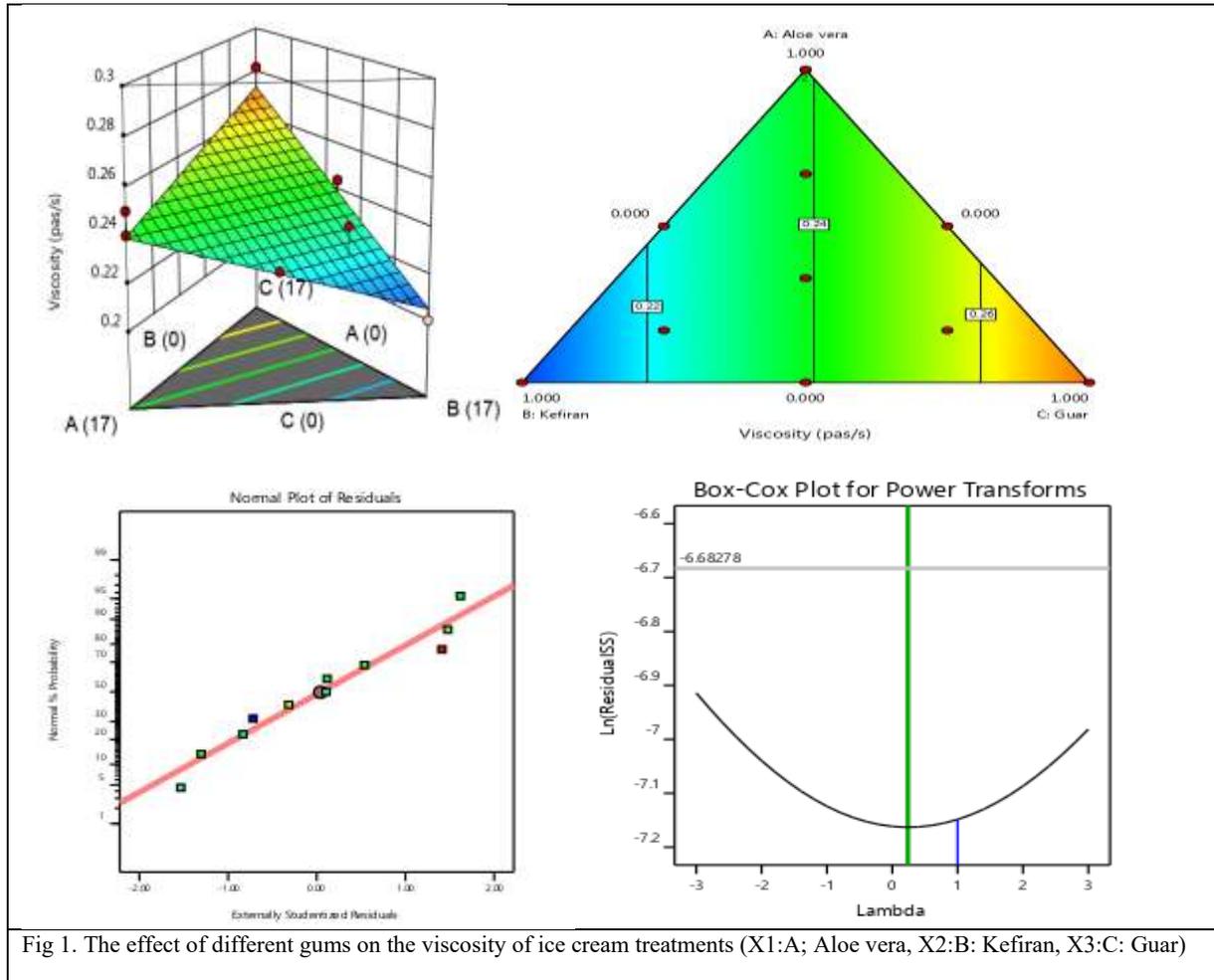


Fig 1. The effect of different gums on the viscosity of ice cream treatments (X1:A; Aloe vera, X2:B: Kefiran, X3:C: Guar)

## Melting Rate

The structure and network of ice cream have a significant and specific influence on its melting rate characteristic. This parameter depends on factors such as the amount of air incorporated into the ice cream network, the nature of the ice crystals, and the network of fat globules formed during the freezing process [23, 27]. For evaluating the melting rate of the ice cream samples, the software suggested the Quadratic vs Linear model as the significant ( $p \leq 0.05$ ) model. Figure 2 shows the results of the effect of adding gums on the melting rate or melting resistance of the ice cream.

Based on a comparison of the results from the melting rate and overrun sections, it was determined that treatments with lower overrun exhibited greater melting resistance, or in other words, a lower melting rate. According to the results, the formulation containing kefiran, due to its higher overrun, showed a lower melting rate (42% decrease), while the formulation containing guar gum, due to its increased viscosity, lower air incorporation (resulting in reduced insulating effect and increased heat transfer rate), showed a higher melting rate (80% decrease).

Based on reviewing researchers' findings regarding the effect of adding gums on

melting resistance or melting rate, several perspectives exist. The first perspective is as follows: adding gums, due to increased viscosity, leads to reduced air incorporation during the whipping and aeration process. Consequently, with less air in the ice cream mix, the insulating role of air diminishes (increasing heat transfer rate), leading to a higher ice cream melting rate [20, 28].

The second perspective pertains to the viscosity of the ice cream mix. It is possible that with increased concentration of hydrocolloids, gums, or water-absorbing and gel-forming powders, due to water absorption and increased mix viscosity, more time is required for the distribution of melted ice (water from melting) within the ice cream network. The final result of this shows an inverse relationship between hydrocolloid concentration and ice cream melting rate [20, 28].

The third perspective refers to the gel network of macromolecules. This view states that an increase in macromolecules and their water absorption leads to the formation of a gel-like network, and water particles become trapped as droplets within this network. However, this water is considered free water and remains in the product. Therefore, during heat transfer and warming, treatments containing a gel network melt faster compared to the control sample [29].

Despite the existence of different and contradictory perspectives, and based on researchers' studies, it can be concluded that, in general, increasing air volume and overrun (reducing heat transfer rate) and hydrocolloid concentration (due to gel network formation and water entrapment), as well as using hydrocolloids with lower shear strength, leads to a decrease in melting rate.

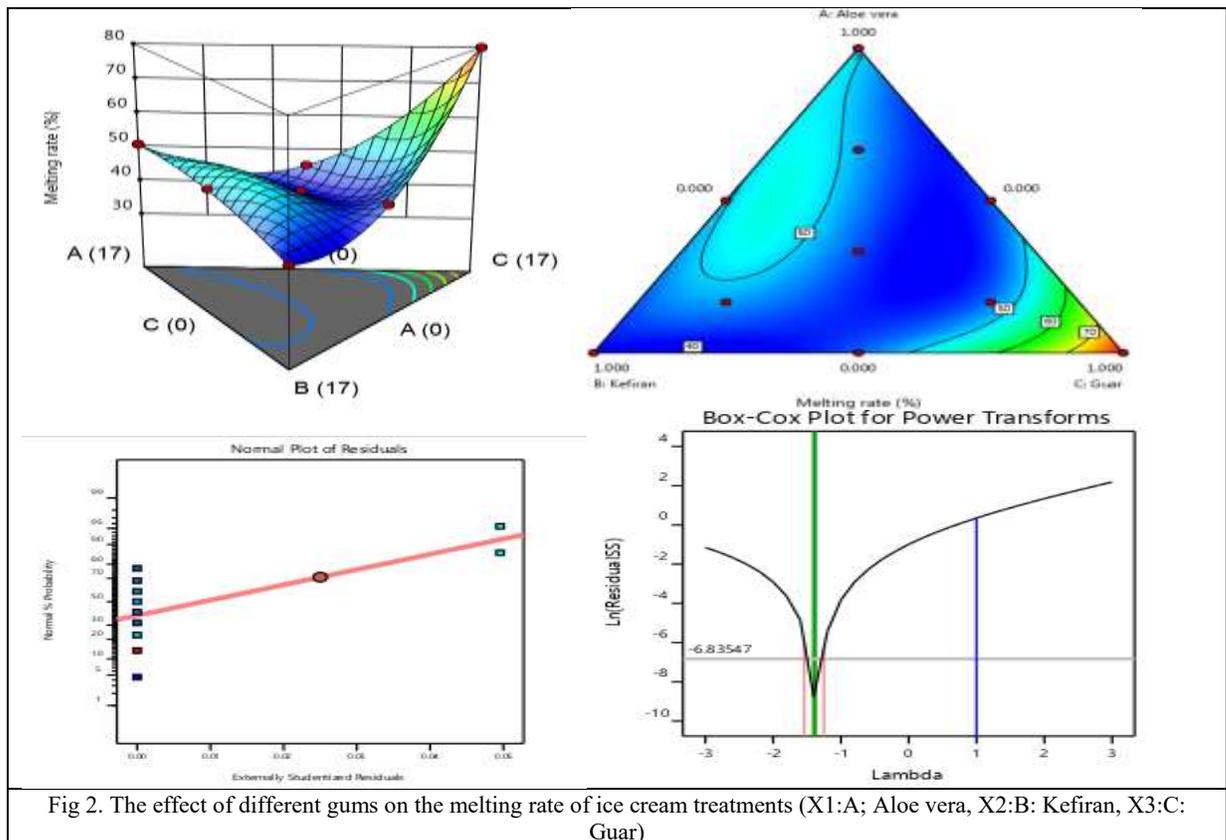


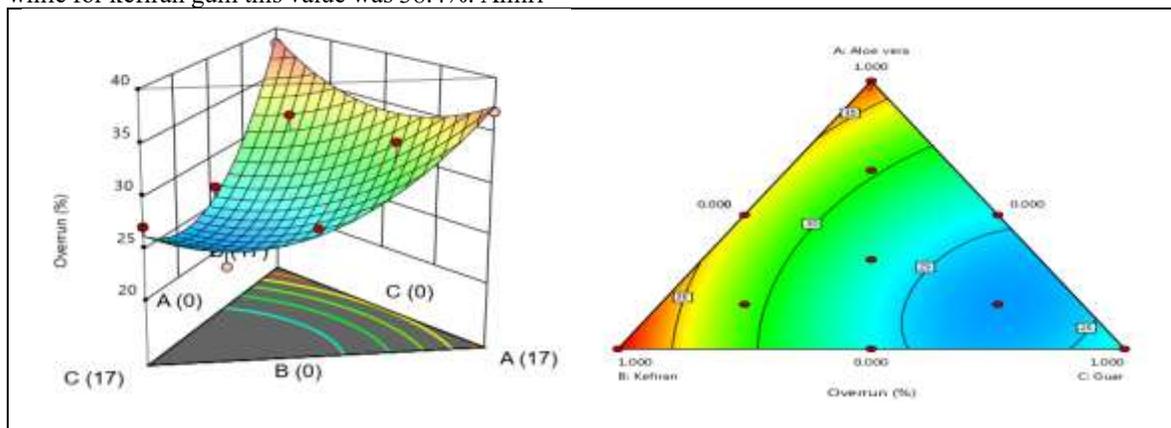
Fig 2. The effect of different gums on the melting rate of ice cream treatments (X1:A; Aloe vera, X2:B: Kefiran, X3:C: Guar)

## Overrun

Overrun is of particular importance due to its positive impact on production efficiency and its specific influence on the texture and body of ice cream [30]. Figure 3 shows the results of significant increases in the overrun changes of the ice cream treatments. Some researchers have reported that this level of overrun is related to the total solids content, mix viscosity, and the concentration and combination of its constituent components [25]. For evaluating the overrun of the ice cream samples, the software suggested the Quadratic vs Linear model as the significant ( $p \leq 0.05$ ) model. In general, with an increase in gum concentration, a significant decrease in the overrun of ice cream treatments is observed [25]. It is likely that among the reasons for the reduced overrun in ice cream treatments are a sharp increase in viscosity and, consequently, reduced air incorporation into the ice cream mix during the whipping and aeration process. However, this decreasing trend was not observed for the two gums, kefiran and aloe vera gel.

In this research, with an increase in the amounts of guar gum and aloe vera gel compared to kefiran gum in the ice cream formulation, a more significant increase in the reduction of overrun was observed (due to the higher concentration of guar gum compared to kefiran and the increased viscosity of the ice cream network). The overrun for ice cream treatments containing guar gum and aloe vera gel was 27.11% and 36.8%, respectively, while for kefiran gum this value was 38.4%. Amiri

et al. (2012), by adding psyllium seed and basil seed mucilage to the ice cream mix, reported that with an increase in psyllium seed mucilage concentration, the ice cream overrun decreases, whereas with an increase in basil seed mucilage concentration, the opposite is true, which is consistent with the results of this study. They also reported that with the addition of a combined gum (mucilage-salep), increasing the stabilizer concentration leads to a decrease in the overrun of ice cream treatments [20]. Zarei et al. (2020), by adding phytosterols to ice cream, reported that with an increase in phytosterol amount, the overrun of ice cream treatments increased significantly [6]. Faraji et al. (2014), by replacing sugar with grape must in ice cream, reported an increase in the overrun of ice cream treatments [31]. Mahpour et al. (2020), by adding wheat protein isolate and investigating its effect on the physicochemical properties of ice cream, reported that adding protein isolate, due to increased mix viscosity, led to a significant decrease in the overrun of ice cream treatments [25]. Ashourmohammadi & Hosseini Qaboos (2017), by adding pumpkin powder to the ice cream mix, reported that with an increase in pumpkin powder, a sharp and significant decrease in the overrun of ice cream treatments is observed. They reported that with increased viscosity, greater shear force is required for aeration and freezing, and bubbles, due to bursting, become distributed asymmetrically and non-uniformly in the ice cream texture.[۲۲]



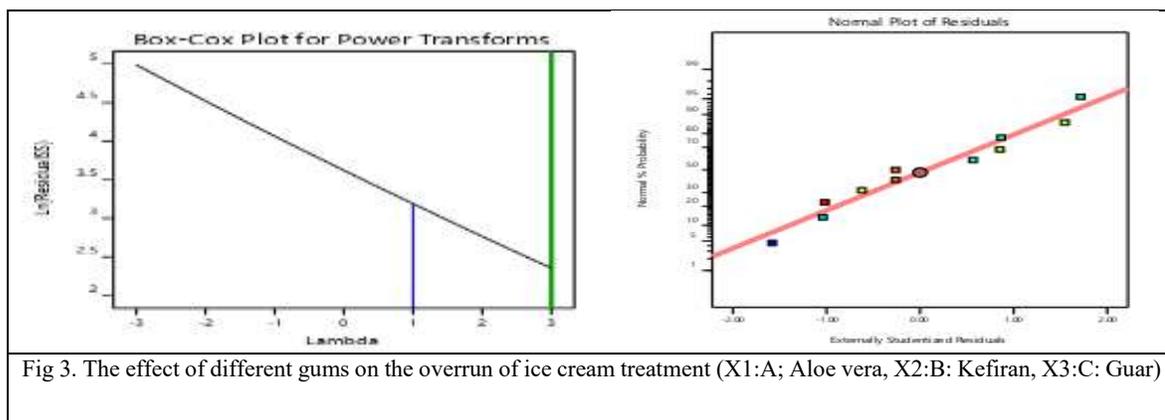


Fig 3. The effect of different gums on the overrun of ice cream treatment (X1:A; Aloe vera, X2:B: Kefiran, X3:C: Guar)

### Texture Hardness

Ice cream hardness is measured as the resistance of the ice cream to deformation when an external force is applied. Hardness is influenced by factors such as overrun, ice crystal size, ice phase volume, and the degree of fat destabilization. An inverse relationship between hardness and overrun has been noted by many researchers [23, 32, 33, 34]. Two factors, ice crystal size and ice phase volume, have a positive effect on ice cream hardness [23]. Sakurai et al. (1996) found that ice creams with larger ice crystals are harder than those with fewer coarse ice crystals [23]. Wilbey et al. (1998) found that ice cream hardness is exponentially related to the ice phase volume, and the fat network also influences hardness [34]. Tharp et al. (1998) found that ice cream hardness increases with higher levels of Polysorbate 80 (PS80) or fat content [35].

For evaluating the texture firmness of the ice cream samples, the software suggested the Linear vs Mean model as the significant ( $p \leq 0.05$ ) model. The results of the hardness variations in the ice cream treatments are presented in Figure 4. The application of hydrocolloids in ice cream leads to a significant increase in the texture hardness of the treatments. The highest and lowest hardness values

were related to the treatment with the highest amount of guar gum (1989.6 grams per square centimeter) and kefiran (1834.11 grams per square centimeter), respectively (due to differences in high or low water absorption capacity). This change is likely due to the fact that hydrocolloid powders, in addition to absorbing water present in the ice cream system and interacting with other components in the mix, act as nuclei for the growth of large ice crystals; this action leads to increased texture firmness in the ice cream treatments. Mahpour et al. (2020), by adding wheat germ protein isolate to ice cream, reported that adding protein isolate leads to an increase in ice mass volume and the enlargement of ice crystals [25]. Asadi Nejad et al. (2004), by investigating the effect of whey protein concentrate on ice cream, reported that the protein concentrate leads to an increase in the hardness index of ice cream, which is consistent and in agreement with the results of the present study.[36]

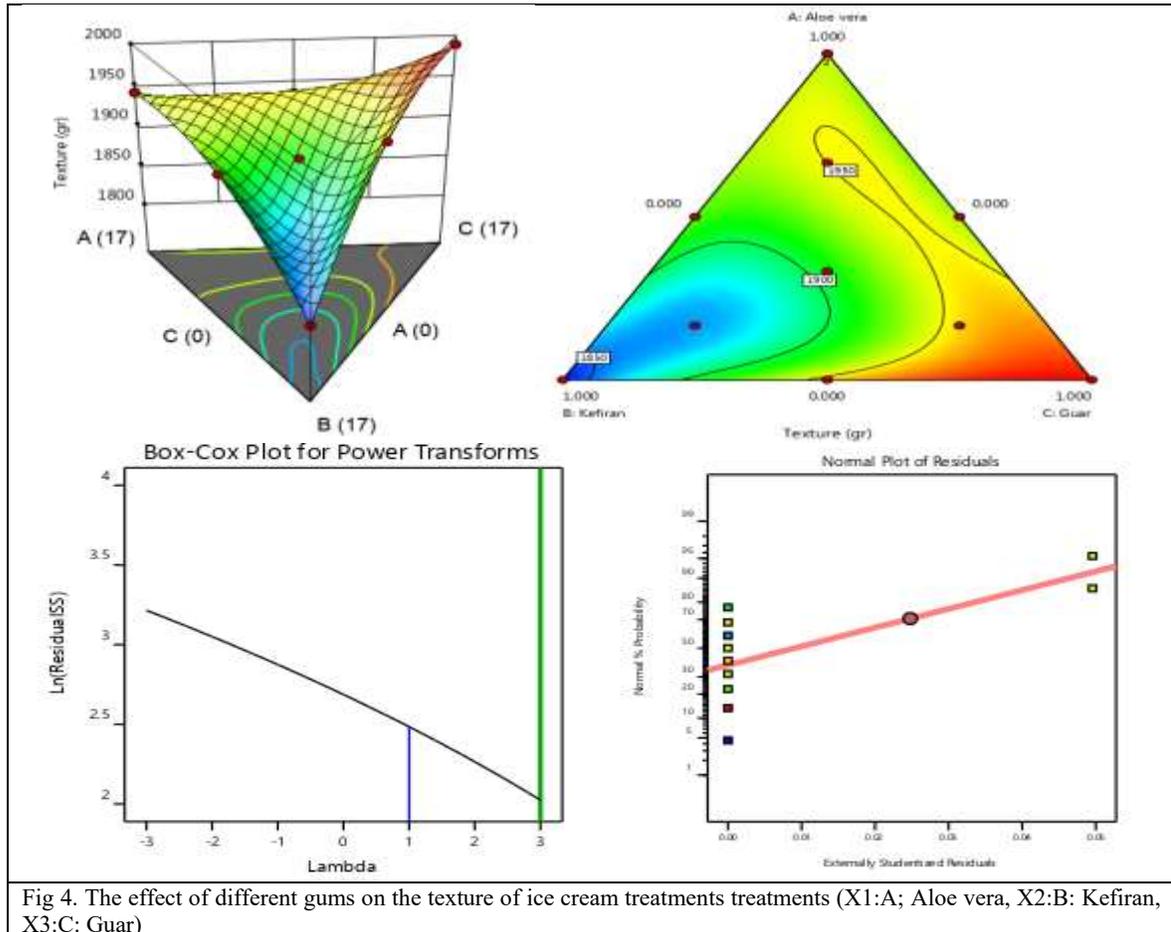


Fig 4. The effect of different gums on the texture of ice cream treatments treatments (X1:A; Aloe vera, X2:B: Kefiran, X3:C: Guar)

### Adhesiveness

Adhesiveness refers to the amount of work required to overcome the force at the common interface between the food material and the surfaces in contact with it [21]. Essentially, it is a parameter indicating the force between the food and the tongue, teeth, and mouth. For evaluating the adhesiveness of the ice cream samples, the software suggested the Special Quartic vs Quadratic model as the significant ( $p \leq 0.05$ ) model. In general, the addition of gum to the ice cream treatment leads to an increase in the adhesiveness of the ice cream mix. According to

Figure 5, a significant difference was observed in the adhesiveness characteristic among the ice cream treatments, such that the treatment containing the highest concentration of guar gum and kefiran showed the lowest (17.31 MJ) and highest (18.11 MJ) adhesiveness, respectively. Mahpour et al. (2020), by adding wheat germ protein isolate, reported that the addition of macromolecules forming the gel network leads to an increase in adhesiveness in ice cream treatments [25], which is consistent with the results of this research.

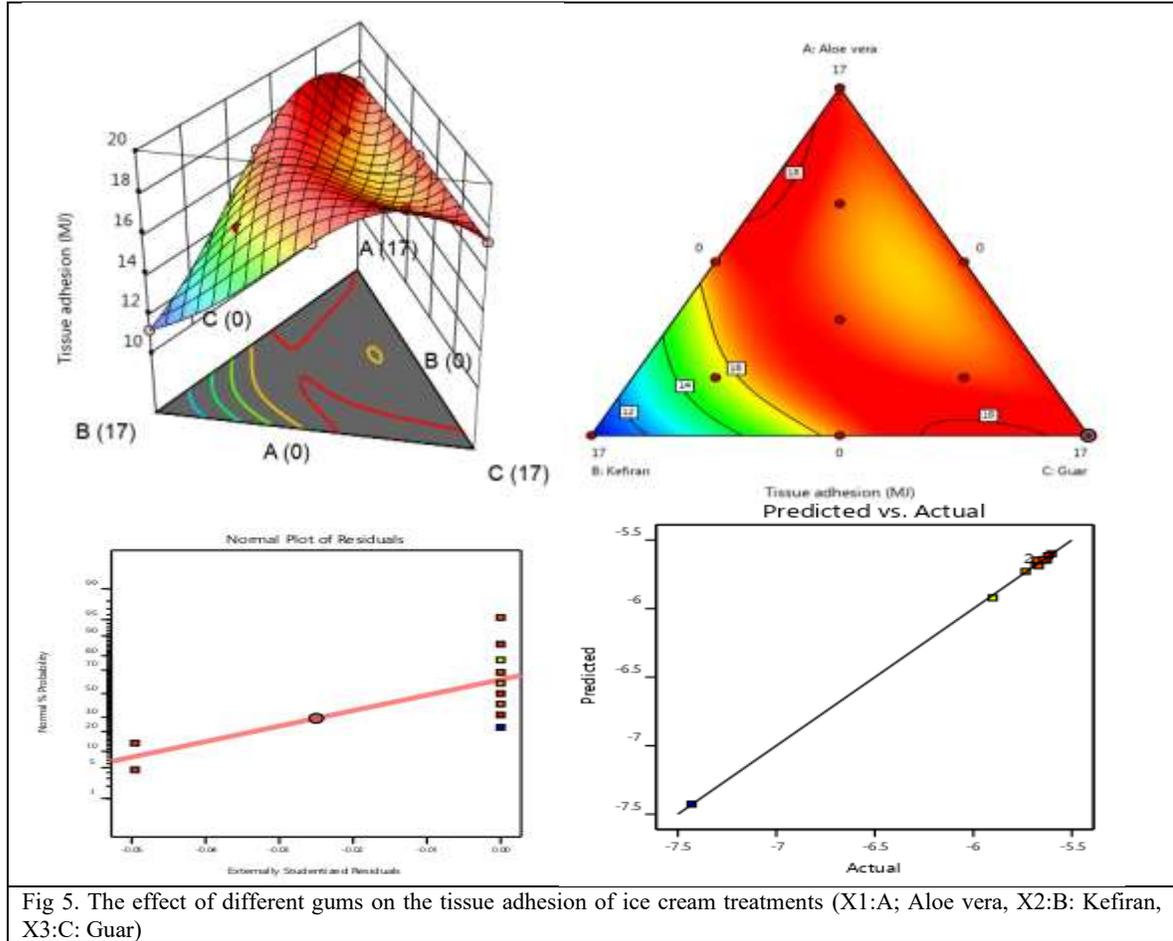
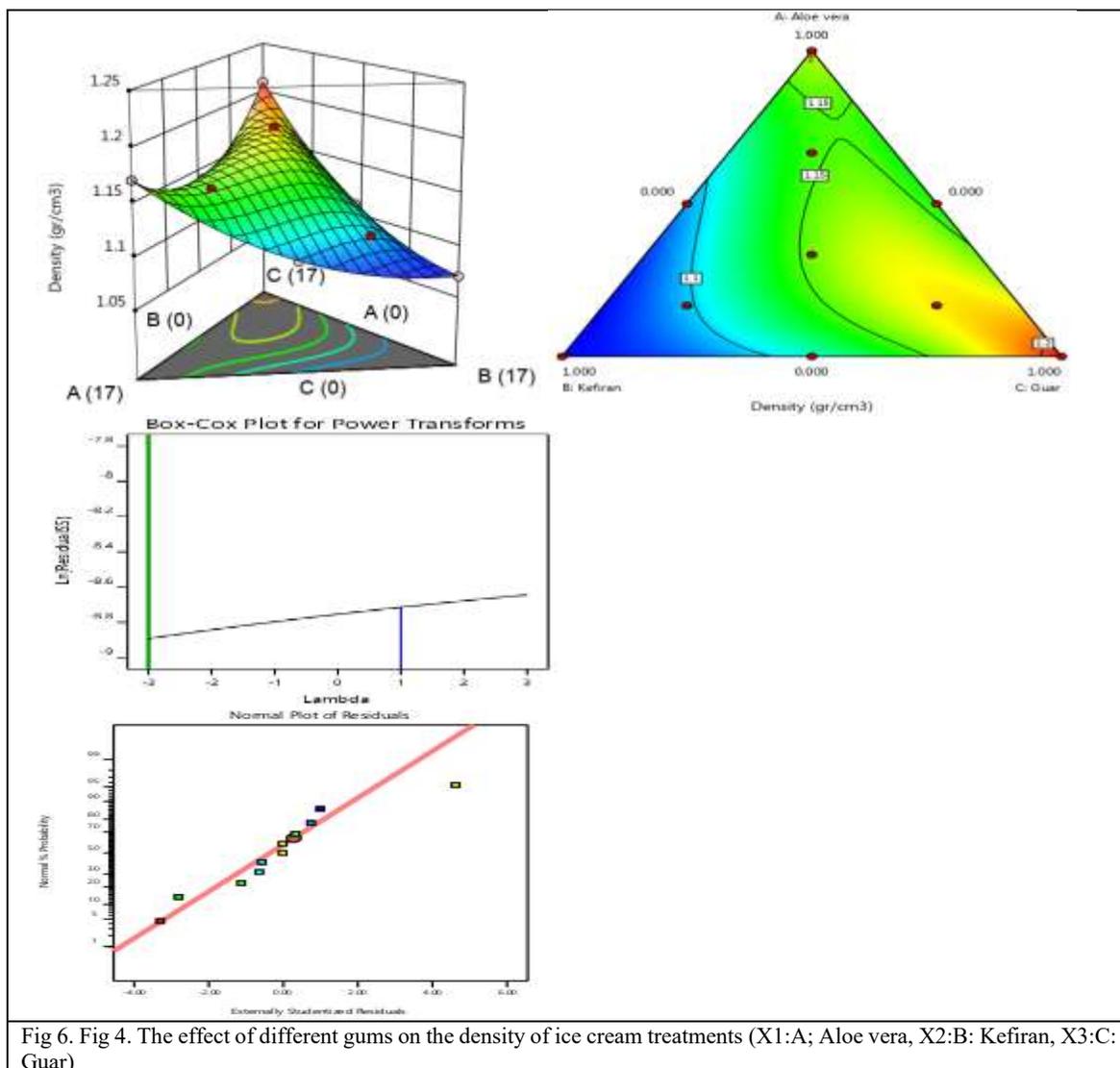


Fig 5. The effect of different gums on the tissue adhesion of ice cream treatments (X1:A; Aloe vera, X2:B: Kefiran, X3:C: Guar)

**Density**

For evaluating the density of the ice cream samples, the software suggested the Special Cubic vs Quadratic model as the significant ( $p \leq 0.05$ ) model. Figure 6 shows the results of the effect of adding gums to the mix of ice cream treatments on their density. As can be observed, the use of gums in the ice cream mix, due to the increase in the mass of the ice cream and the subsequent water absorption

and further increase in the final mass, leads to an increase in density. Accordingly, the treatment containing the highest level of guar gum and kefiran exhibited the highest ( $1.21 \text{ g/cm}^3$ ) and lowest ( $1.07 \text{ g/cm}^3$ ) density values, respectively. Mahpour et al. (2020), by adding wheat germ protein isolate, reported that the addition of macromolecules forming the gel network leads to an increase in adhesiveness in ice cream treatments [25], which is consistent with the results of this research.



### pH Measurement

For evaluating the pH of the ice cream samples, the linear model was suggested as the significant model ( $p \leq 0.05$ ) by the software. The results of the pH measurement test for ice cream treatments containing different levels of stabilizers are presented in Figure 7.

The results showed that adding the microbial exopolysaccharide kefiran led to a significant decrease in the pH of the ice cream samples, whereas adding guar gum did not result in a

significant or remarkable increase in pH, although an upward trend was observed. Meanwhile, increasing the amount of aloe vera gel kept the pH within the range of 5.25. The highest and lowest pH values were observed in samples with the highest level of guar gum (Treatment 3) and the highest level of kefiran (Treatment 2), respectively. Hwang et al. (2009) added grape juice to ice cream formulation and reported that grape juice leads to a decrease in the pH of ice cream samples [37], which is consistent with the results of this study. In contrast, Amiri et al. (2012) reported no significant change in the pH and

acidity of ice cream upon adding basil and psyllium seed gums [20]. Javidi et al. (2013) reported that no significant change in pH was observed upon adding guar and basil seed gums to ice cream.[<sup>9</sup>]

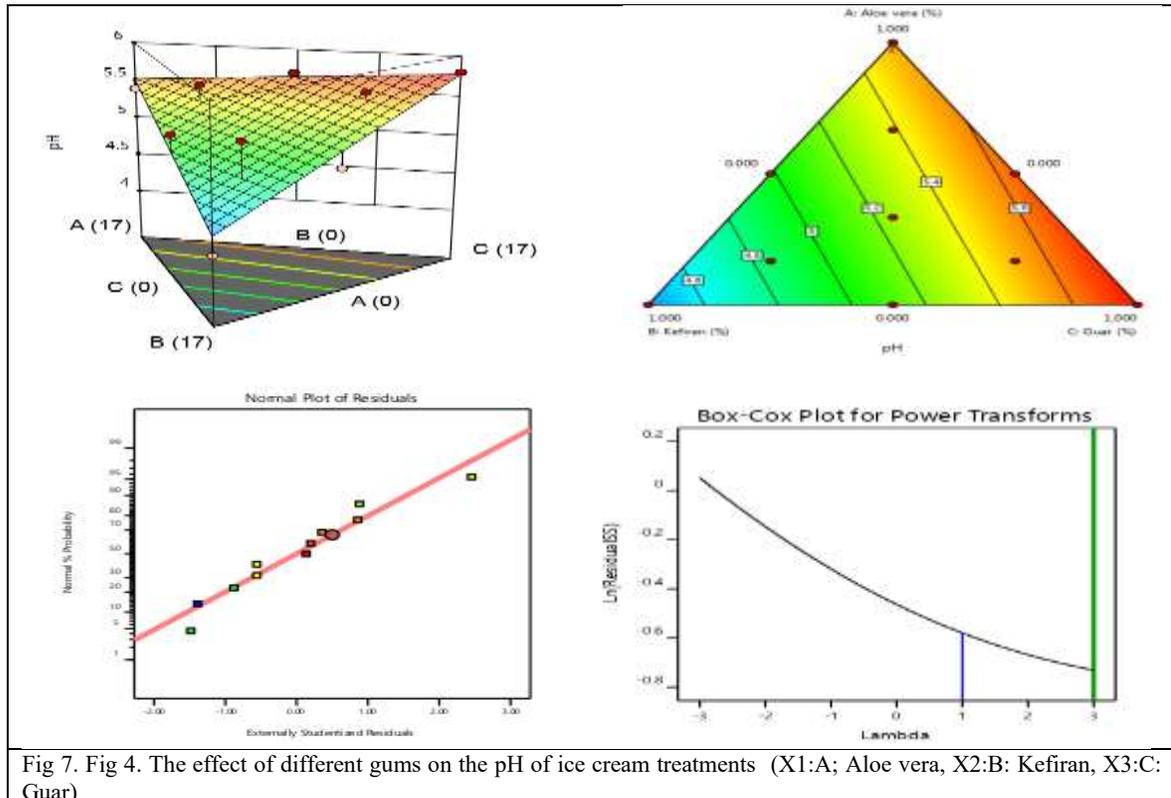


Fig 7. Fig 4. The effect of different gums on the pH of ice cream treatments (X1:A; Aloe vera, X2:B: Kefiran, X3:C: Guar)

### Scanning Electron Microscopy

One of the methods for material analysis and identification is the use of FE-SEM. The morphology of the optimized ice cream samples was examined using an FE-SEM device. The objective of this test was to investigate the structure of the optimized ice cream samples using this instrument.

As observed in Figures 8, images of the spray-dried ice cream samples at a magnification of 10 microns are presented. Based on the provided figures, it can be observed that the optimized ice cream samples containing 17% kefiran and the treatment with 17% guar gum had

a spherical, wrinkled structure. These images revealed that the 17% guar gum sample, compared to the optimized 17% kefiran treatment, possessed a homogeneous structure with regular, continuous chains of spherical particles and low inter-particle porosity. Furthermore, the carbohydrate chains exhibited an adhesive structure with intact, spherical walls that were wrinkled, and no evidence of cracking was observed.

Otálora et al. (2015) stated that microparticles containing carrier materials with higher molecular weight experience less particle wrinkling and breakage. This

aligns with the comparison of the molecular weight of guar gum ( $C_{35}H_{49}O_{29}$ ) versus kefiran ( $C_{12}H_{24}O_{11}$ ) and is consistent with the present results. From this examination and comparison of the obtained electron micrographs, it was determined that treatments containing 17% kefiran, compared to those containing 17% guar gum, exhibited greater wrinkling after drying due to their

lower viscosity and higher overrun. It is likely that this characteristic, in addition to being influenced by molecular weight, is also affected by other factors such as adhesiveness and viscosity, which aligns with the results presented in the respective sections of the current research[38].

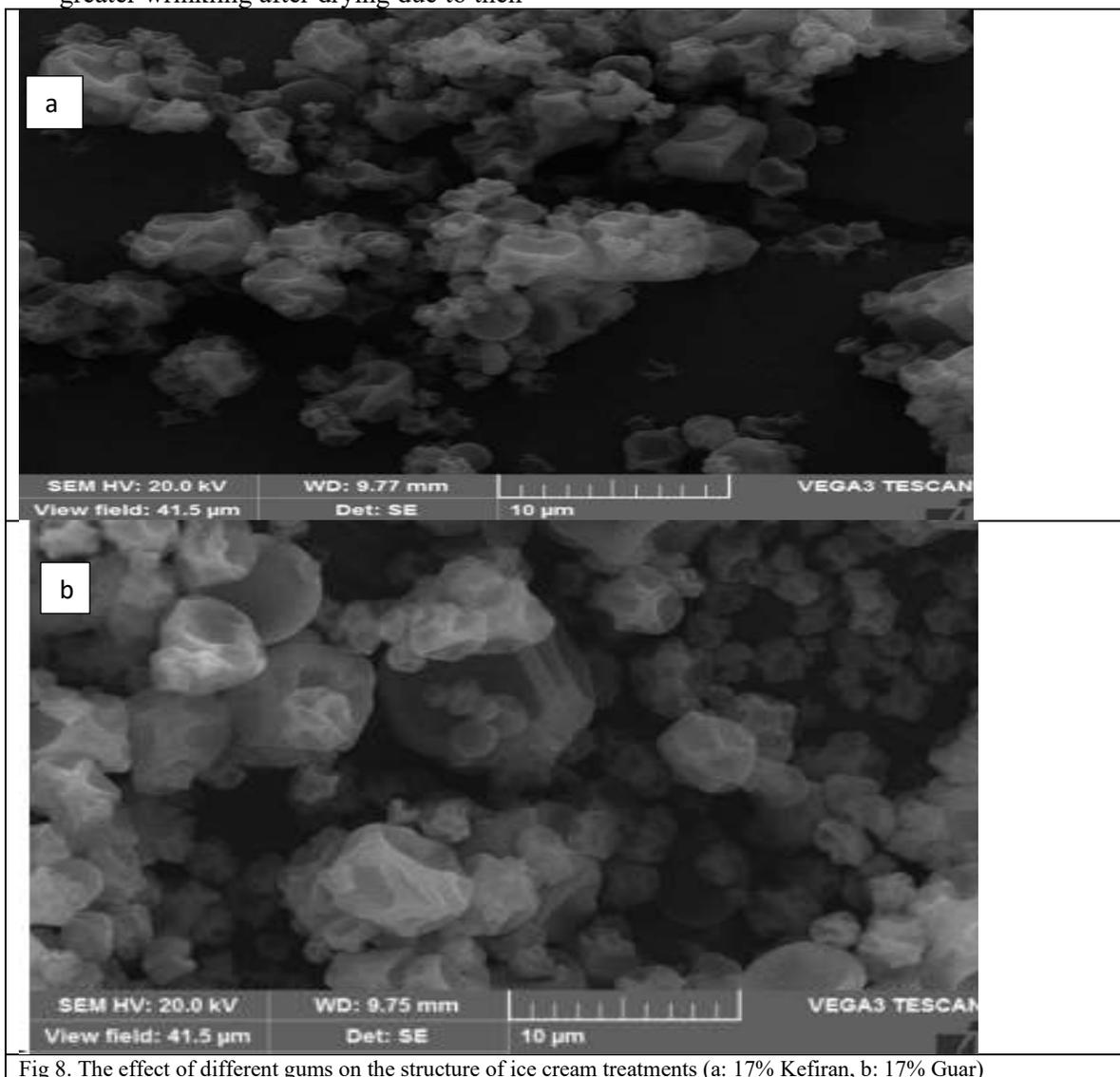


Fig 8. The effect of different gums on the structure of ice cream treatments (a: 17% Kefiran, b: 17% Guar)

### Dynamic Light Scattering Test

The results of the dynamic light scattering test for treatments 2 and 3 are presented in Figure

9. Despite differences in the texture and mouthfeel of the ice cream treatments, which were expected due to reduced adhesiveness

and increased overrun in treatments containing kefir and aloe vera gel, it was anticipated that the sample with a higher percentage of guar gum would exhibit greater particle dispersion.

displayed a broader particle dispersion, ranging from 1800 to 4200 nanometers (1.8 to 4.2 microns), with the highest concentration of particles observed between 1800 and 3800 nanometers (1.8 to 3.8 microns).

However, the mean particle diameter for the treatment with the highest kefir content (17%) fell within the range of 1000 to 3500 nanometers (1 to 3.5 microns), with the highest dispersion observed in the 1500 to 3000 nm region. In contrast, the treatment with the highest guar gum content (17%)

This increase in particle size for the treatment containing the higher guar gum level (11.33%) is attributed to the increased adhesiveness and final viscosity of the treatment, which aligns with the results obtained from the electron microscopy images (indicative of particle aggregation).

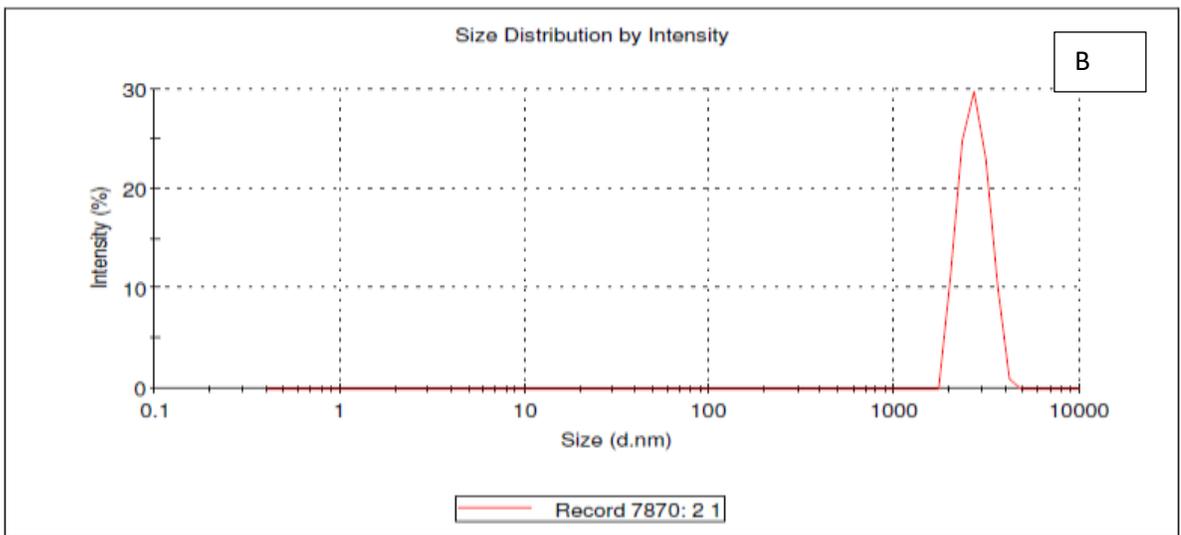
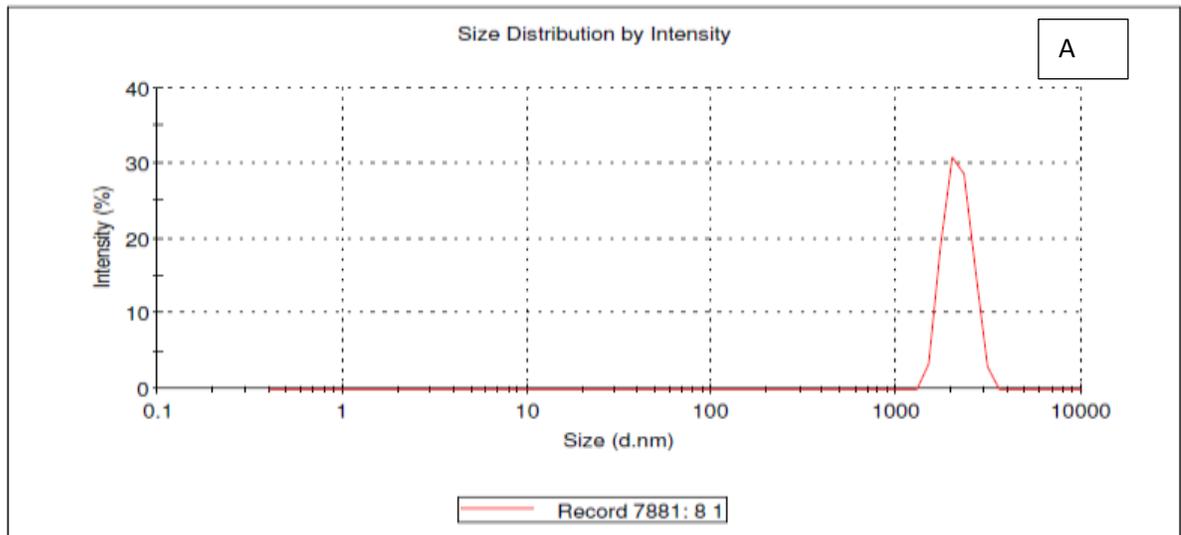


Fig 9. The effect of different gums on the particle size of ice cream treatments (A: 17% Kefiran, B: 17% Guar)

### Sensory Analysis

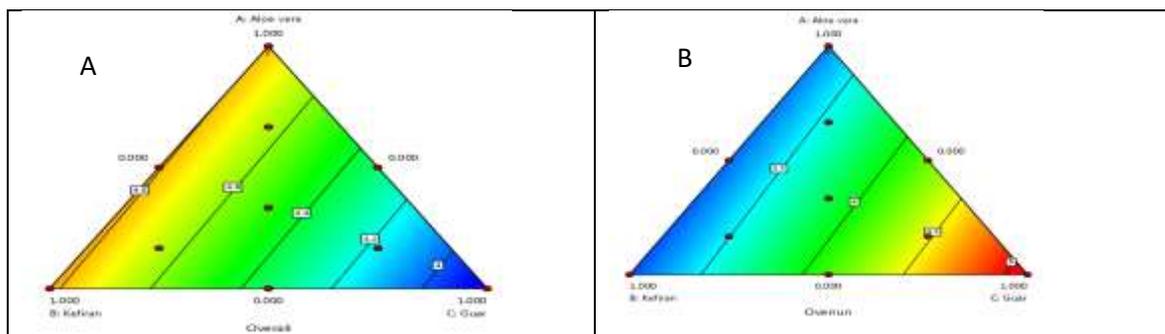
For evaluating the organoleptic properties of the ice cream samples, the Linear vs Mean model was suggested as the significant ( $p \leq 0.05$ ) model by the software. The results of the sensory characteristics of ice cream treatments containing plant and microbial compounds are presented in Figure 10 (a, b, ...).

Among the most prominent and important features for ice cream acceptance from a consumer perspective are factors such as overrun, ice cream texture, aroma and flavor, and the overall acceptability of the final product. According to Figure 10 related to the texture of the ice cream samples, it was found that with an increase in the amount of guar gum, the adhesiveness of the ice cream increased, and with a decrease in the amounts of kefiran and aloe vera gel, the adhesiveness also increased. For this reason, samples containing more guar gum had lower overrun compared to samples containing kefiran and aloe vera gel, or samples with a higher proportion of kefiran and aloe vera gel. However, treatments with a higher proportion of kefiran and aloe vera gel received higher scores for aroma and flavor, color, and overall acceptability compared to samples containing guar gum.

The application of high levels of guar gum (increased guar levels) led to a reduction in the aroma and flavor of the ice cream. This is attributed to the increased particle diameter in ice creams containing guar gum, along with increased overrun and water retention in this compound and the final product. Ultimately, this reduces the contact surface area of the particles with the taste receptors on the tongue, leading to a decrease in the product's aroma and flavor. This finding is consistent with the results obtained from the dynamic light scattering particle size analysis and the examination of the morphological and structural properties of the ice cream.

Amiri et al. (2011), by investigating the effect of psyllium on ice cream, reported that using psyllium in ice cream formulation should be done in combination with other stabilizers to achieve positive effects on ice cream properties [20]. Mahpour et al. (2019), by examining wheat germ protein isolate on ice cream properties, reported that the mouthfeel of ice cream can be influenced by viscosity, aeration level, and melting resistance index. Accordingly, based on the obtained results, the ice cream sample with a higher amount of kefiran, due to its lower viscosity and higher overrun, had a lower melting rate and therefore received a higher mouthfeel score compared to other ice cream samples [25].

Table 2 presents the results for  $R^2$ , Adjusted  $R^2$ , Predicted  $R^2$ , and Adeq Precision.



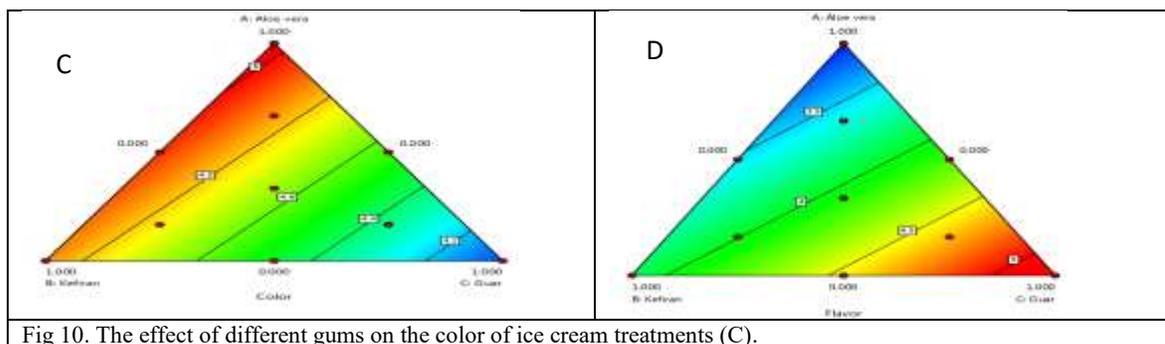


Fig 10. The effect of different gums on the color of ice cream treatments (C).

Table2. Fit Statistics

	pH	Overrun	Melting rate	Viscosity	Texture	Tissue adhesion	Density	Overall	Flavor	Color
R <sup>2</sup>	0.7577	0.9253	0.9986	0.8152	0.6184	0.9992	0.9943	0.6710	0.9203	0.8818
Adjusted R <sup>2</sup>	0.6971	0.8505	0.9932	0.7690	0.5230	0.9962	0.9714	0.5887	0.9003	0.8523
Predicted R <sup>2</sup>	0.6010	0.1222	0.2159	0.6217	0.2463	0.5614	-1.3164	0.3971	0.8561	0.7493
Adeq Precision	10.7705	9.2361	49.8548	13.1365	7.3066	61.4841	21.5363	7.5121	18.8205	14.8433

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#### 7-Final Conclusion

In this research, the addition of stabilizers led to significant changes in the ice cream. Specifically, the addition of gums resulted in an increase in viscosity (Treatment with 17% kefiran: 0.25 Pa·s and Treatment with 17% guar gum: 0.282 Pa·s) and adhesiveness (Guar gum: 18.11 and Kefiran: 17.31 g/cm<sup>2</sup>) of the ice cream treatments. Furthermore, the density of the treatments increased with higher gum content and water absorption (Guar gum: 1.21 and Kefiran: 1.07 g/cm<sup>3</sup>). In general, as viscosity increases, the incorporation of air into the ice cream mix decreases. This leads to a reduction in the volume of air trapped within the ice cream network, diminishing its insulating properties and consequently increasing the rate of heat transfer. Ultimately, this resulted in a higher melting rate for these treatments (Treatment with 17% kefiran: 42% and Treatment with 17% guar gum: 80%). In line with this, the increase in gums and the subsequent rise in viscosity, followed by reduced air incorporation, led to a significant decrease in the overrun of the treatments. For equal weights, the gum that created a higher concentration caused a more pronounced reduction in this factor (Treatment with 17% kefiran: 38.4% and Treatment with 17% guar

gum: 27.11%). Overall, an inverse relationship exists between texture hardness and the overrun factor in ice cream. The increase in gums, due to higher solids content and absorption, leads to a larger volume of ice crystals within the ice cream network, consequently increasing its hardness. In this study, the highest value was observed in the treatment with 17% guar gum. Finally, the addition of gums did not dramatically alter the pH of the treatments; however, adding a higher amount of guar gum compared to kefiran increased the pH.

#### Data Availability

The data used to support the finding of this study are available from the corresponding author upon request.

#### Conflict Of Interest

The authors have no conflicts interest to report.

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بررسی ویژگی‌های رئولوژیکی و فیزیکوشیمیایی بستنی غنی‌سازی شده با کفیران و پایداری‌کننده‌های گیاهی به روش طرح

Mixture Design مخلوط

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در این تحقیق، کفیران پس از کشت دانک‌های کفیر و استخراج از آن، به صورت ترکیبی با ژل آلونه ورا و گوار در سطوح مختلف (۲.۸۳٪، ۵.۶۶٪، ۸.۵٪، ۱۱.۳۳٪ و ۱۷٪) (درصد وزنی) و عربی (۵۰٪ صمغ‌ها) به بستنی افزوده و اثرات آن بر ویژگی های رئولوژیکی، فیزیکوشیمیایی و ارگانولپتیکی بستنی مورد بررسی قرار گرفت. تجزیه و تحلیل داده ها با استفاده از روش آنالیز واریانس توسط مدل مناسب تعقیبی در سطح احتمال ۵٪ با استفاده از نرم افزار Design Expert 12 انجام گرفت. نتایج آزمون های فیزیکوشیمیایی بستنی مورد مطالعه نشان داد افزودن هیدروکلونیدها منجر به افزایش ویسکوزیته، سرعت ذوب و چسبندگی تیمارهای بستنی می‌شود به طوری که تیمارهای حاوی ۱۷٪ کفیران کمترین (۰.۲۵ - ۴۲٪ - ۱۷.۳۱MJ) و ۱۷٪ گوار (۰.۲۸۲pas/s - ۱۸.۱۱MJ) بیشترین مقدار را دارا بودند. با افزایش صمغ‌ها سختی تیمارهای بستنی افزایش یافت به طوری که این مقدار برای تیمارهای ۱۷٪ کفیران کمترین (۱۸۳۴.۱۱gr/cm<sup>2</sup>) و ۱۷٪ گوار (۱۹۸۹.۶gr/cm<sup>2</sup>) بالاترین مقدار را دارا بودند. افزایش صمغ‌ها منجر به حجم‌افزایی تیمارهای بستنی می‌شود. تصاویر FE-SEM تیمارهای حاوی سطح ۱۷٪ کفیران و گوار نشان داد که تیمار ۱۷٪ گوار دارای ذراتی کروی با چروکیدگی کم و بدون پارگی بود و سطح بکنواختی را نشان داد. نتایج DLS نشان داد اندازه ذرات تیمارهای دارای کفیران ۱۷ دارای میانگین قطر ۱ تا ۳.۵ میکرون بودند. نتایج آزمون ارگانولپتیکی تیمارهای بستنی نشان داد تیمارهایی که نسبت بیشتری کفیران داشتند از نظر فاکتور رنگ و پذیرش کلی امتیاز بالاتری نسبت به نمونه های حاوی گوار داشتند.