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Effects of Partial Replacement of Sugar with Fig Syrup on *Bacillus coagulans* Survival and Physicochemical Properties of Probiotic Ice Cream

Running head: Partial replacement of sugar with fig syrup in probiotic ice cream

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SUMMARY

Research background. Various sectors of the food industry demand the enrichment of food with functional compounds. Probiotic products with valuable nutritional and therapeutic properties have attracted great attention in the fields of industry, nutrition, and medicine. The aim of the present study was to investigate the sensory and physicochemical properties of probiotic ice cream containing fig syrup and to evaluate the survival of *Bacillus coagulans* after 90 days of storage at -18 °C.

Experimental approach. In this study, four experimental groups of ice cream were produced as follows: plain dairy ice cream (without additives), ice cream containing 10^9 CFU/g *B. coagulans*, ice cream containing 25 % fig syrup substitute, and ice cream containing 25 % fig syrup substitute and 10^9 CFU/g *B. coagulans*. They were stored at -18 °C for 3 months. Texture analysis, pH, acidity, viscosity determinations, and microbial counts were performed at 1, 30, 60, and 90 days of storage. Organoleptic evaluation was performed at days 1 and 90.

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Results and conclusions. The results showed that during the initial freezing process and the transformation of the mixture into ice cream, the number of *B. coagulans* decreased from 10^9 CFU/g to 10^7 CFU/g, with no significant changes observed over the 90-day period. No significant changes were also observed in the sensory and textural properties of the samples. Replacement of fig syrup with 25 % sugar reduced the pH, increased the acidity of ice creams and, improved their viscosity. In conclusion, the production of functional ice cream using fig syrup and *B. coagulans* was recommended due to their health benefits.

Novelty and scientific contribution. The findings of this study can be used to prepare functional and healthy foods. Our results suggest that fig syrup has the potential to be used as a natural sweetener or sugar substitute in various products.

Keywords: dairy ice cream; *Bacillus coagulans*; fig syrup; sugar replacement

INTRODUCTION

Nowadays, the introduction and promotion of functional foods have attracted more attention. Functional foods are considered to have health-promoting effects that extend beyond those of a basic diet. These foods have demonstrated physiological benefits when consumed in the usual diet (*1,2*). Probiotics and prebiotics are functional ingredients used to enhance the health benefits of foods (*3*). Granato *et al*. (*4*) define probiotics as live microorganisms that are beneficial to the host when ingested in large enough quantities. They have the potential to improve the immune system, modulate the intestinal microflora, and inhibit the growth of pathogenic bacteria (*4,5,6*). Probiotic-enriched products should contain at least 10^6 CFU/g of viable organisms to be considered health foods (*7*).

B. coagulans is a spore-forming bacteria and belongs to a new family of probiotics. It has been compared to other probiotic bacteria such as *Lactobacillus* and *Bifidobacteria*. *B. coagulans* grows as a Gram-positive rods, motile, single, or rarely in short chains of variable length. Their optimal pH and temperature for growth are 5.5-6.5 and 30-50 °C, respectively. From a metabolic perspective, they are facultative anaerobic microorganisms that produce acid by fermenting maltose, mannitol, raffinose, sucrose, and trehalose, without producing gas. The potential applications of Bacillus probiotics are not limited to dietary supplementation; they can also be used as a clinical therapy for gastrointestinal and urinary tract infections. Their therapeutic effect is mainly due to their ability to produce bacteriocins such as coagulin, which have a broad spectrum of activity against enteric pathogens (*8*).

Dairy products are the most common probiotic carriers (*4*). In this regard, ice cream is a suitable choice for formulating probiotic-enriched foods; because it has a near-neutral pH, which does not affect probiotics (*9*,*10*). Ice cream is rich in carbohydrates, milk proteins, essential amino acids, vitamins, and minerals, and its ingredients are well absorbed by the body (*11*). However, consumer acceptance and survival of probiotics during the storage period should be considered when preparing a probiotic or symbiotic product.

Two important criteria for the efficacy and success of probiotic and prebiotic products are consumer acceptance and survival of probiotic microorganisms during manufacture and storage (*12, 13*). Sucrose is an ingredient in ice cream that makes it more delicious and popular. Excessive sugar consumption increases the incidence of many diseases, such as dental problems, obesity, diabetes, high cholesterol, and cardiovascular diseases. Therefore, consumers prefer alternative sweeteners to sucrose (*14*).

The fig is a sweet and nutritious fruit with numerous therapeutic properties. Figs are among the most valuable and energy-rich fruits consumed in various forms, such as fresh, dried, canned, jam, syrup, concentrate, fruit jelly, or chocolate and nut cookies (*15*). This fruit contains a variety of beneficial substances, including essential vitamins, antioxidants, and antimicrobial and anticancer compounds. It helps to lower blood sugar and fat levels (*16*). Therefore, figs can add special value to foods and be used in special diets such as low-fat, low-sodium, high-fiber, diabetic, and weight-loss diets. Fig syrup has the potential to be used as a natural sweetener or sugar substitute in baked and cooked products. Compared with most fruits and vegetables, it is a prime source of dietary fiber that aids digestion (*15*). Therefore, the aim of the present study was to investigate the characteristic changes of ice cream after *B. coagulans* was added and partially replaced fig syrup instead of sugar.

MATERIALS AND METHODS

Before we started this project, we conducted a pilot study. In the production of ice cream, 25, 37.5, and 50 % of sugar was replaced with fig syrup. Ice cream with 37.5 and 50 % replacements did not have acceptable organoleptic and textural characteristics. Therefore, while 25 % replacement was suitable 25 % fig syrup was used instead of sugar.

In this study, four experimental groups of ice cream were produced as follows: plain milk ice cream (control), ice cream with 10^9 CFU/g *B. coagulans* as probiotic bacteria, ice cream with 25 % fig syrup replacement, and ice cream with 25 % fig syrup replacement and 10^9 CFU/g *B. coagulans*. All productions were performed in triplicate.

Equalizing the sweetness of sugar with the fig syrup

A refractometer (Model: Dr-101, Cosecta S.A. Barcelona, Spain) was used to determine the Brix of the fig syrup at room temperature. It was 16° Brix. According to the results of HPLC (Agilent 1100 series, Waldbronn, Germany), in 100 mL of fig syrup with Brix 16, contained 6.09 g of fructose, 5.10 g glucose, and 0.44 g sucrose. According to their sweetness coefficient (1.4 for fructose, 0.75 for glucose, and 1 for sucrose), the sucrose equivalent of fig syrup sugar (100 mL) was 12.8. To make 1 kg of ice cream with 25 % sucrose replacement by fig syrup, 351.5 mL of fig syrup is needed to achieve the same sweetness as sucrose.

Ingredients and probiotic strain

Khoshmaze Co. (Shiraz, Iran) supplied us with all the ingredients needed to make ice cream, including skim milk powder (34 % protein, 1 % fat) (Pegah Infant Formula Co., Shahrekord, Iran), hydrogenated vegetable oil (Narges, Shiraz, Iran), commercial sugar (Bally, Isfahan, Iran), fig syrup as sweetener, vanilla (Polar Bear, Shanghai, China), carboxymethylcellulose (E 466; Sunrose ,Tokyo, Japan), Panisol) E 471; Danisco, Copenhagen, Denmark), cellulose gum (E 410), guar gum (E412), carrageenan (E 407), tocopherol-rich extract (E 306), and ascorbyl palmitate (E304) (Ramak Co, Shiraz, Iran). The probiotic *B. coagulans* strain was kindly add in lyophilized form by Pardis Roshd Mehregan, Iran. Further details on the composition of the different experimental groups are given in Table 1.

Probiotic culture activation

Lyophilized probiotic *B. coagulans* was obtained from the Pardis Roshd Mehregan Company, Iran. The activation was done by inoculating the nutrient yeast salt agar plates (NYSM) broth culture at 37 °C for 24 h. The probiotic cells were centrifuged (75005286 EA, Thermo Fisher Scientific Inc. USA) and thereafter washed in a sterile saline solution with the same centrifugation process. Probiotic bacteria were inoculated into the ice cream. The cells concentration was adjusted at 10^9 CFU/mL. The ice cream mix was frozen at − 4 to − 5 °C and stored at − 20 °C for hardening (*2*).

Ice cream production

The experimental mixtures were prepared in 3 kg batches. According to the recipes provided by the ice cream company, the water was measured with a scaled cylinder then, the milk powder was then added to it and it was heated to 40-45 °C. The other ingredients were then incorporated into the reconstituted milk. After pasteurization (85 °C, 15 min), the mixtures were stirred at 45 °C for 5 min using a simple mixer (Model 6790; Tefal, Rumilly, France), cooled to room temperature, and ripened overnight at 4 °C. They were then fortified with vanilla and a probiotic strain before freezing. Whipping and freezing of the mixtures for 20 min at 52 rpm in a batch ice cream machine (Model: BQL-12Y; ShanghahiLisong, Shanghai, China) was performed. The final products at a temperature of approximately - 5 °C were packed in plastic cups, cured at -30 °C for 2 h, and stored at -18 °C (*17*).

Physicochemical analyses

The overrun (%) of the samples was measured using the following formula (*11*):

$$\text{Overrun (\%)} = \frac{\mathit{Weight\ of\ a\ known\ volume\ of\ \ mix - weight\ of\ an\ equal\ volume\ of\ ice\ cream}}{\text{Weight of equal volume of ice cream}} \times 100 \qquad /1/$$

After melting, pH was determined at room temperature using a digital pH meter (Model 350 pH meter, Jenway, Dunmow, UK).

For determination of total titratable acidity, the sample was titrated with 0.1 N sodium hydroxide (NaOH) and a phenolphthalein indicator. Results were expressed as a percentage of lactic acid (*18*).

Viscosity was measured using a digital viscometer (Model RVT; Brookfield Engineering Laboratories, Stoughton, MA, USA) at 25 °C.

The hardness, cohesiveness, and adhesiveness of the samples were determined using a CT3 4500 Texture Analyzer (Brookfield Engineering Laboratories) equipped with a stainless cylindrical probe (6.0 mm diameter, 35 mm height). Each sample was compressed twice to 50 % of its original height at a test speed of 2 mm/s.

Probiotic enumeration

The *B. coagulans* concentration of probiotic samples was counted at days 1, 30, 60, and 90 of storage. Serial dilutions of the samples were plated on nutrient yeast salt agar plates (NYSM agar; Merck, Darmstadt, Germany), which were then incubated at 37 °C for 24 hours.

Sensory evaluation

A 30-member untrained panel conducted the sensory analysis of the samples after 1 and 90 days of storage at -18 °C. The sensory attributes of the samples, including flavor, texture, color, and mouthfeel, were rated on a five-point scale where 0 and 4 mean “unacceptable” and “excellent”, respectively (*19*).

Statistical analysis

All measurements were performed in triplicate. Data were analyzed with the statistical package SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was performed for the comparison of physicochemical parameters and probiotic concentrations among the different experimental groups. Duncan’s multiple range test was used to determine significant differences. Non-parametric comparisons, including the Kruskal–Wallis test and Mann-Whitney *U* test, were used for the comparison of sensory values. The significant difference was assessed at $P < 0.05$.

RESULTS AND DISCUSSION

Physicochemical properties

The results presented in Table 2 and Table 3 show that the addition of *B. coagulans* did not significantly change the pH and acidity of the different types of ice cream. On the other hand, the addition of fig syrup to ice cream resulted in a decrease in the pH and an increase in the acidity of the ice cream, which is consistent with the results of Salama (*20*) and Tammam (*21*).

In agreement with Abghari *et al.* (*22*) and Akalin and Erisir (*23*), the addition of probiotics did not affect the overrun of the probiotic samples. Ghorbani *et al*. (*24*) also reported similar results. Similarly, the replacement of 25 % sugar with fig syrup also did not result in a significant difference in the overrun of the different ice cream varieties. Tammam *et al*. (*21*) reported a significant reduction in overrun by replacing 60 % of the ice cream sugar with date syrup. They stated that the reduction in overrun could be attributed to inappropriate excessive viscosity, i.e., a reduction in viscosity causing a reduction in the whipping ability of the ice cream mix. Honey, high-fructose corn syrup (HFCS), corn syrup, sucralose, and maltitol have also been reported to decrease overruns (*25*,*26*).

The overrun values obtained in the present study were considerably lower than those of commercial ice cream, which may be, at least in part, due to the ice cream machine used (Fig. 1).

Apparent viscosity is referred to as the force required to move one layer of fluid over another (*27*). In the present study, the addition of probiotic bacteria to ice cream had no effect on viscosity (Fig. 2). However, replacing sugar with fig syrup resulted in a significant increase in apparent viscosity, which is consistent with previously published results (*21,22,28*). Compared to sugar, fig syrup has a higher water-binding capacity, which could be due to the pectin content, which has hydrocolloidal properties. Replacement of sugar with High-fructose corn syrup (HFCS), honey, and glucose syrup has been shown to have similar effects on the apparent viscosity of ice cream (*25*). In a study conducted by Akalin and Erisir (*23*), the addition of inulin and oligofructose to probiotic ice cream resulted in higher viscosity, which could be due to the interactions between the dietary fiber and liquid components of the ice cream mixture.

The replacement of fig syrup with 25 % sugar had no effect on the textural properties (except adhesiveness) of ice cream (Fig. 3). In the study by Hashim and Shamsi (*29*), the addition of date syrup at 50 % and 100 % reduced the hardness values of ice cream samples. While 25 % date syrup had no significant effect on texture. A harder texture was found in the case of the substitution of sucrose by maltitol and sucralose.

Viable counts of probiotic microorganism

The viability of probiotic bacteria can be affected by their initial concentration, temperature, type of food carrier, and storage conditions. Other parameters that may affect probiotic viability include probiotic strain, pH, and freezing and thawing conditions (*30*).

According to the results of bacterial counting Fig. 4, initial freezing of the mixture by a freezer resulted in a significant decrease in probiotic concentration in all samples, whereas storage at -18 °C for three months had no significant effect on their survival. Our results are in line with numerous recent studies on the fortification of dairy products with various microbial strains (*2,13,24*). Ghorbani *et al*. (*24)* found similar results, which demonstrated the effect of iron and *L. casei* fortification on probiotic ice cream properties. Their results showed that the bacterial count in the ice cream samples decreased during storage.

The reduction in probiotic bacterium was only partially related to ice crystal formation and freezing damage; the possible deleterious effects of aeration and mechanical stress during initial freezing should not be ignored (*31-33*). Saccharides, particularly sucrose, are one of the main ingredients in the production of ice cream. Their cryoprotective properties may enhance the viability of probiotics in frozen products (*27*). The contact of probiotics with the cryoprotective components of the ice cream mixture, such as proteins and sugars, during overnight ripening at 4 °C could play a role in protecting bacteria during the freezing process (*22*).

Sensory evaluation

The results of the sensory analysis are shown in Fig. 5. The addition of *B. coagulans* to ice cream had no negative effects on its organoleptic properties. Addition of fig syrup instead of 25 % sugar yielded similar results. All sensory properties (color, texture, flavor, and mouthfeel) were rated above three points in all treatments. The 90-day storage of the samples in the freezer did not lead to any organoleptic complaints. Crumbly, weak, greasy, or sandy texture was not observed in any of the treatment groups.

Salama (*20*) and Tammam *et al* (*21*) used date syrup as a sweetener and flavoring ingredient in the manufacture of ice creams. The substitution of date syrup with 40 % sugar resulted in an acceptable product.

Salem *et al* (*34*) showed that enrichment of ice cream with probiotic strains did not affect the acceptability or flavor of the product. In another study, sensory analysis showed that date syrup-containing ice cream was not preferred over sugar-containing control samples; although the flavor and texture of both groups were not particularly appealing, higher date syrup concentrations increased viscosity and decreased overrun, resulting in an undesirable moist and hard texture (*35*).

CONCLUSIONS

The development of ice cream fortified with fig syrup and a probiotic such as *B. coagulans* could be an effective means to boost the nutritional and functional values of ice cream. In the present study, 4 formulae for ice cream containing fig syrup and a probiotic strain (*B. coagulans*) were developed. The presence of these functional ingredients did not exhibit any adverse effects on ice creams' physicochemical, rheological, and sensory properties. The microbial population of *B. coagulans* in ice creams maintained above the threshold value of 10^6 CFU/g even after 90 days of storage at -18 °C. Owing to the health benefits of fig syrup and *B. coagulans* probiotic bacteria, the production and consumption of functional ice cream are recommended.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

R. Janipour conceived and designed the evaluation and collected the data. SS. Shekarfrush participated in designing the evaluation, re-evaluating the data, and revising the manuscript. S. Ghorbani helped drafted the manuscript and performed parts of the statistical analysis, and revised the manuscript. H. Ghaisari conceived and designed the evaluation, re-evaluated the data, and revised the manuscript.

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Table 1. The composition of ice cream formulations (*w*/%)

	Treatment groups			
Ingredients	Control	Fig	Bc	Fig+ Bc
Water	63.8	33.2	63.8	33.2
Skim milk powder	8.5	8.5	8.5	8.5
Carboxy methyl cellulose	0.2	0.2	0.2	0.2
Stabilizer-Emulsifier	0.4	0.4	0.4	0.4
Vanilla	0.1	0.1	0.1	0.1
Hydrogenated vegetable oil	9	9	9	9
Sugar	18	13.5	18	13.5
Fig syrup	0.0	35.1	0.0	35.1
Bacillus coagulans (10^9 CFU/g)	-	-	+	+

Control, (Fig): Ice cream with 25 % fig syrup replacement, (Bc): ice cream with 10^9 CFU/g *B. coagulans* as a probiotic bacteria, (Fig + Bc): ice cream with 25 % fig syrup replacement and 10^9 CFU/g *B. coagulans*

Table 2. Mean ± SD of pH (*N*=3) in ice cream samples stored at -18°C for 90 days

	t(Storage) / day			
Trials	1	30	60	90
Control	$(6.87 \pm 0.03)^{a}$	$(6.65 \pm 0.05)^{a}$	$(6.67 \pm 0.05)^{ab}$	$(6.54 \pm 0.04)^{a}$
Fig	$(6.60 \pm 0.06)^{b}$	$(6.35 \pm 0.01)^{b}$	$(6.52 \pm 0.01)^{b}$	$(6.23 \pm 0.07)^{b}$
Bc	$(6.86 \pm 0.04)^{a}$	$(6.67 \pm 0.04)^{a}$	$(6.80 \pm 0.07)^{a}$	$(6.51 \pm 0.04)^{a}$
Fig + Bc	$(6.57 \pm 0.02)^{b}$	$(6.40 \pm 0.01)^{b}$	$(6.60 \pm 0.07)^{b}$	$(6.30 \pm 0.04)^{b}$

Means in the same column followed by different small letters were significantly different ($P < 0.05$).

Table 3. Mean ± SD of titratable acidity (g/L) (*N*=3) in ice cream samples stored at -18 °C for 90 days

	t(Storage) / day			
Trials	1	30	60	90
Control	$(1.62 \pm 0.02)^{b}$	$(1.71 \pm 0.01)^{b}$	$(1.66 \pm 0.06)^{c}$	$(1.72 \pm 0.06)^{c}$
Fig	$(1.75 \pm 0.01)^{a}$	$(2.01 \pm 0.06)^{a}$	$(1.98 \pm 0.03)^{a}$	$(2.11 \pm 0.06)^{a}$
Bc	$(1.60 \pm 0.04)^{b}$	$(1.65 \pm 0.01)^{c}$	$(1.62 \pm 0.02)^{c}$	$(1.68 \pm 0.01)^{c}$
Fig+ Bc	$(1.72 \pm 0.05)^{a}$	$(1.98 \pm 0.02)^{a}$	$(1.75 \pm 0.01)^{b}$	$(2.00 \pm 0.03)^{b}$

Means in the same column followed by different small letters were significantly different (P < 0.05).

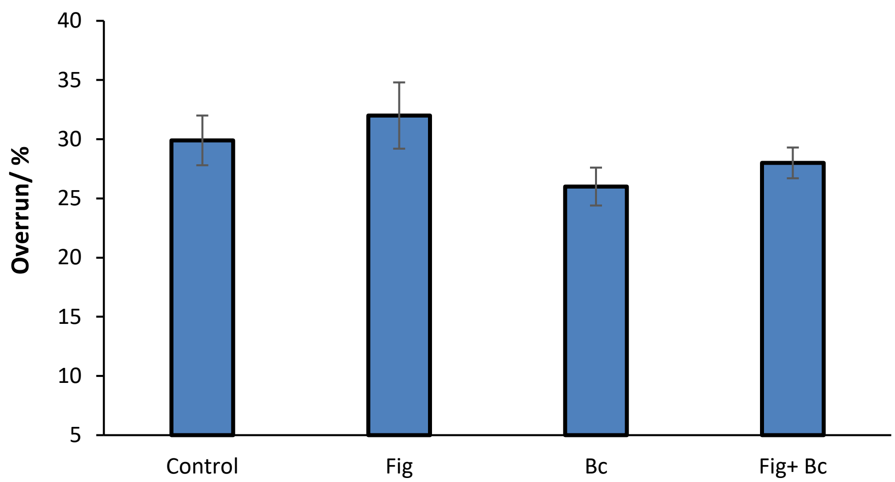


Fig. 1. Mean ± SD of overrun in ice cream samples immediately after first production (*N*=3)

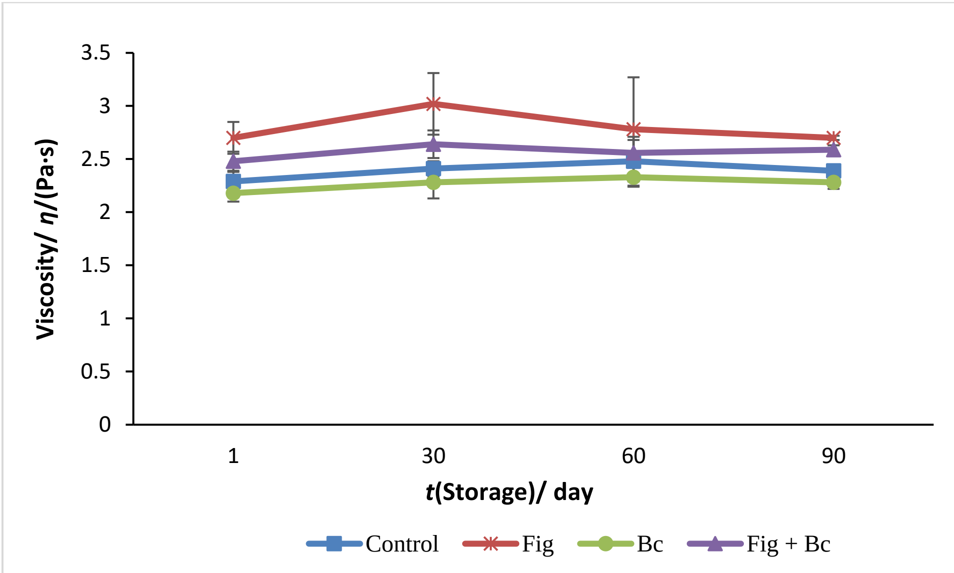
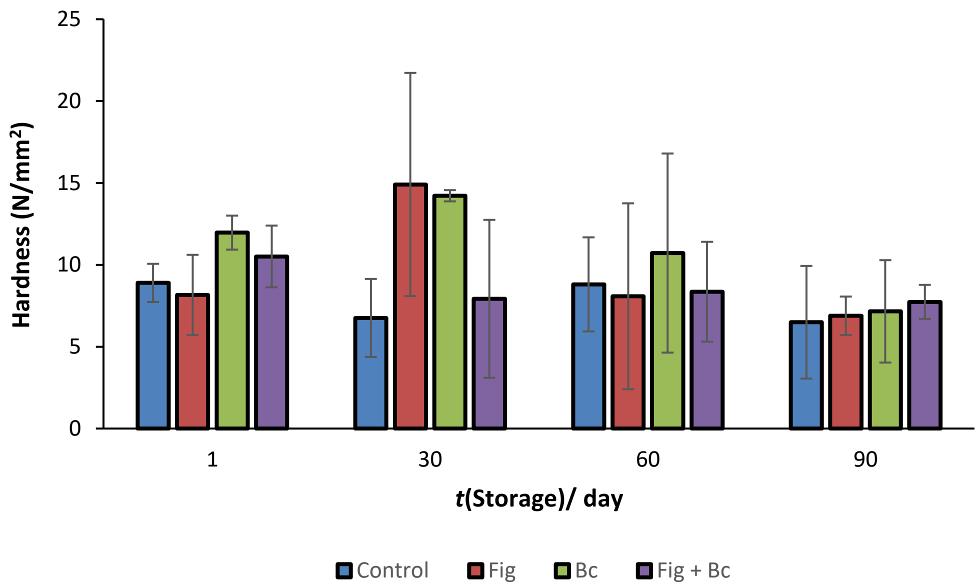
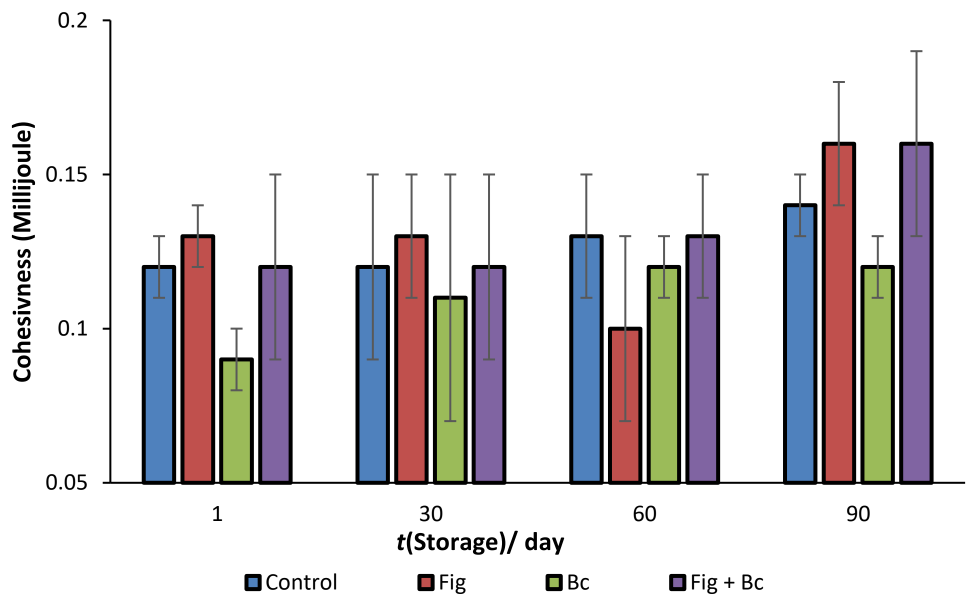


Fig. 2. Viscosity value (Pa·s) of ice cream samples: Control, Fig: (ice cream with 25 % fig syrup replacement), Bc: (ice cream with 10^9 CFU/g B. coagulans), Fig + Bc: (ice cream with 25 % fig syrup replacement and 10^9 CFU/g B. coagulans)

a)


Hardness (N/mm²)
25
20
15
10
5
0
1
30
60
90
t(Storage)/ day
Control
Fig
Bc
Fig + Bc

b)


Cohesivness (Millijoule)
0.2
0.15
0.1
0.05
1
30
60
90
t(Storage)/ day
Control
Fig
Bc
Fig + Bc

c)

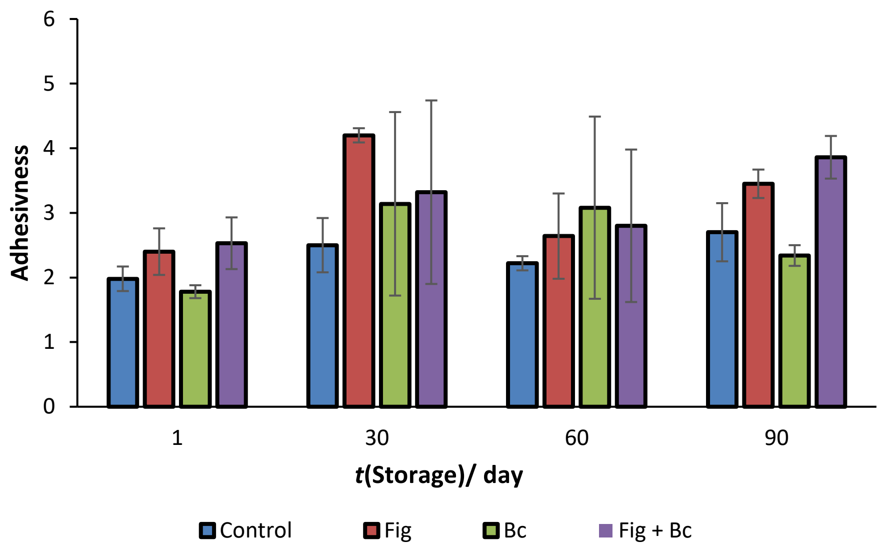


Fig. 3. The textural characteristics of the ice creams samples: a) hardness, b) cohesiviness and c) adhesiveness. Control, Fig: (ice cream with 25 % fig syrup replacement), Bc: (ice cream with 10^9 CFU/g *B. coagulans*), Fig + Bc: (ice cream with 25 % fig syrup replacement and 10^9 CFU/g *B. coagulans*)

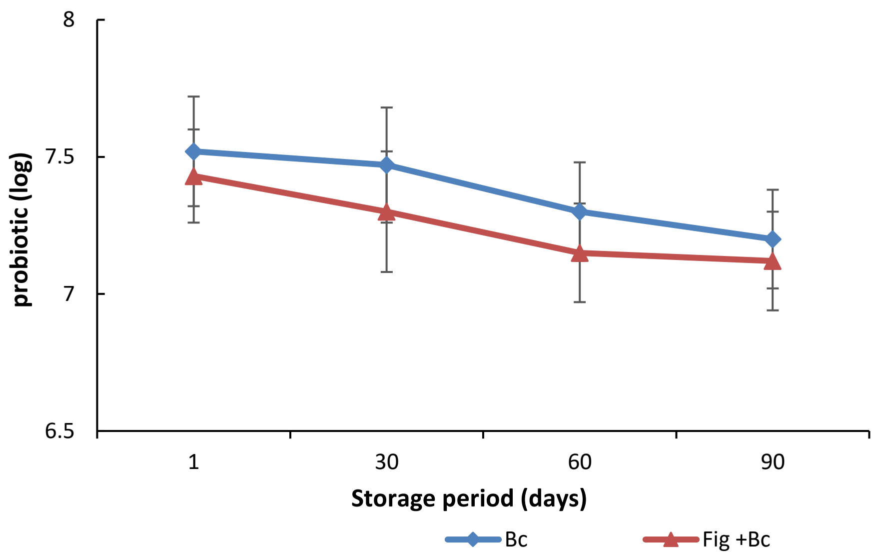


Fig. 4. Survival of *B. coagulans* in ice cream samples: Control, Fig: (ice cream with 25 % fig syrup replacement), Bc: (ice cream with 10^9 CFU/g *B. coagulans*), Fig + Bc: (ice cream with 25 % fig syrup replacement and 10^9 CFU/g *B. coagulans*)

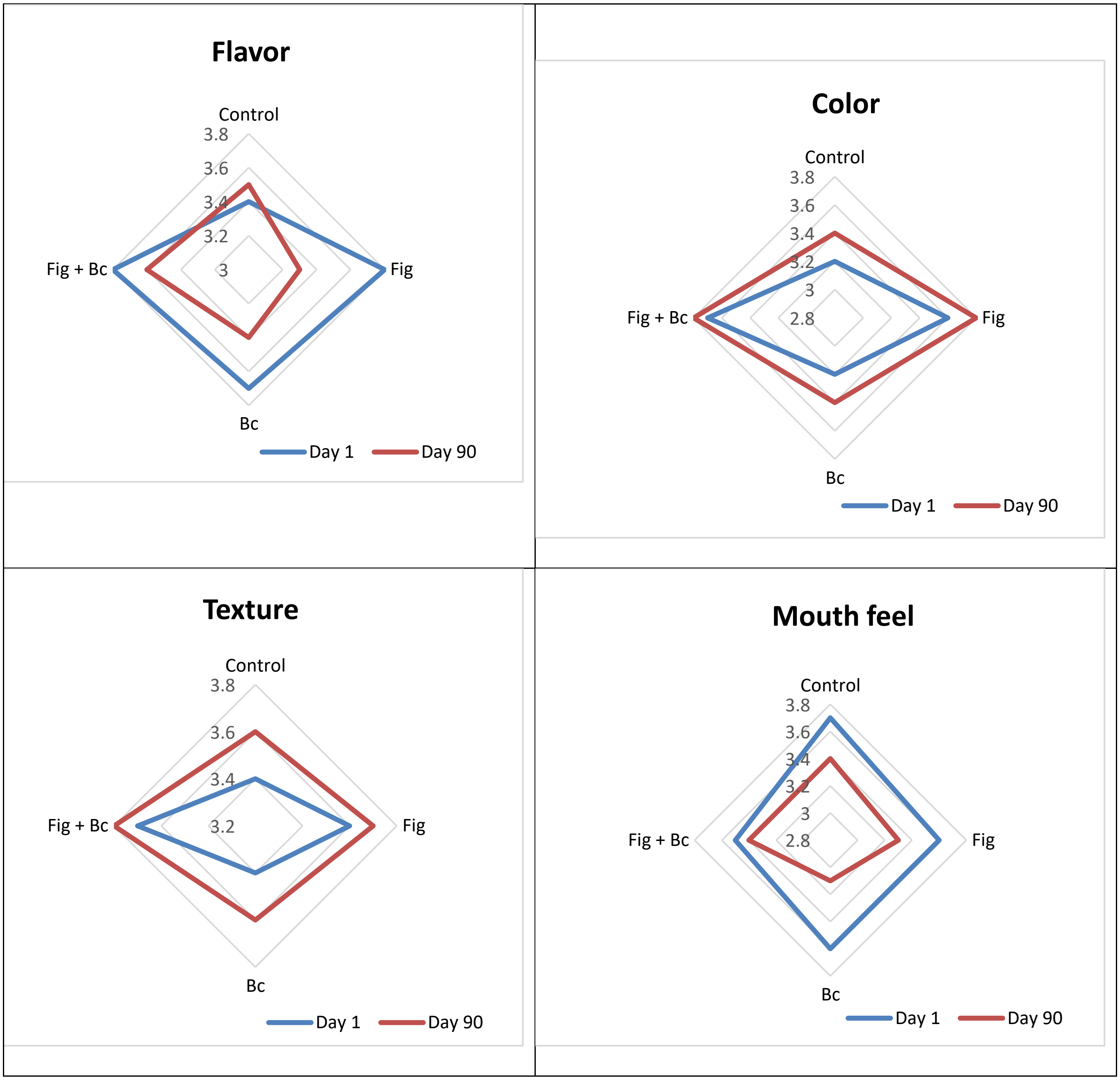


Fig. 5. Sensory evaluation of different treatments of ice creams on days 1 and 90 of storage. Control, Fig: (ice cream with 25 % fig syrup replacement), Bc: (ice cream with 10^9 CFU/g *B. coagulans*), Fig + Bc: (ice cream with 25 % fig syrup replacement and 10^9 CFU/g *B. coagulans*)