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Effects of probiotic strains and prebiotic compounds on physicochemical, Microbiological and sensory properties of Buffalo synbiotic yogurt

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ABSTRACT

Synbioticyogurt is the most important food ingredient from the combination of probiotics and prebiotics, which increases the survival of probiotic bacteria during yogurt storage due to its prebiotic properties.The purpose of this studywasto develop synbiotic buffalo yogurt by addition of inulin and fructo-oligosaccharideas prebiotics compounds (0.5, 1 and 1.5%) on the survival of Lactobacillus acidophilus and Bifidiobacteriumbifidium bacteria and physicochemical and sensory properties of buffalo yogurt.The physicochemical characteristics (pH, acidity, dry matter, fat, protein, viscosity, syneresis) and sensory evaluation (taste and texture) during 21 days of storage and the survival rate of Lactobacillus-acidophilus and Bifidiobacteriumbifidium during 21 days were investigated.The results of the statistical analysis showed that with increase in the fructo-oligosaccharide level compared to the sample containing inulin and the control, the pH decreased, acidity, dry matter, water retention and viscosity increased ($p<0.05$).Also, the amount of fat and protein did not differ significantly during 21 days of shelf life ($p>0.05$).In terms of sensory evaluation, the samples of synbiotic buffalo yogurt containing high percentage of fructo-oligosaccharide and inulin were evaluated better than the control.Therefore, the use of fructo-oligosaccharide and inulin is recommended for the production of synbiotic buffalo yogurt with functionalproperties.

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1. Introduction

Yogurt is one of the most popular fermented dairy products, which is produced and marketed as the most important commercial probiotic product in the world. Various studies have been conducted to develop new formulations and increase functional properties of yogurt. Yogurt prepared from buffalo milk is one of the highest quality and strongest dairy products whose presence in the food basket can help increase the health of society. Buffalo milk contains more butyric, palmitic, and stearic fatty acids than caproic, caprylic, capric, and lauric fatty acids. Buffalo milk fat is without beta-carotene, colorless, and its vitamin A content is higher than cow's milk [1]. Probiotics and various prebiotic compounds, antioxidant compounds such as omega-3 fatty acids, vitamins, minerals, polyphenols and carotenoids, dietary fibers and various bioactive compounds are widely used to improve the nutritional and functional properties of yogurt. Probiotics are living and specific microorganisms that, when consumed in humans or animals, have beneficial effects on the host body's flora. Most of the probiotics belong to a large group of the main bacteria of the microbial flora of the human intestine and live there harmlessly [1 and 2]. The existing belief about the beneficial effects of probiotics is based on the fact that the intestinal microbial flora plays the role of protectors against various diseases, the main effect of probiotics is determined by the stabilization of the intestinal microbial flora [4, 3]. It has been observed that the constant use of probiotics is effective in reducing the incidence of various diseases, which is more evident in high-risk populations (such as hospitalized children, children who do not consume breast milk or live in deprived conditions) [5]. Probiotic products are sold in the commercial market in the form of tablets, capsules, powder, yogurt, milk and enriched cheese. Most of the probiotics that have been studied so far have been reported to be safe and have not shown any obvious side effects [6].

Probiotic bacteria (therapeutic starters) have the ability to tolerate stomach acid and bile salts and the ability to replace in the intestine. Continuous consumption of these bacteria is effective in performing their beneficial therapeutic roles [7]. Daily receipt of 10^8up to 10^9 Live bacteria have been proposed as the minimum acceptable number. Therefore, daily consumption of 100 grams of probiotic product with cfu^6 10 to $10^8$5 x live bacteria per gram of product can provide the desired optimal level [8 and 9].

Prebiotics are special compounds that are not digested by the host, but they have positive effects on the host by selectively stimulating, growing, or activating one or a limited number of bacteria in the digestive system, which mainly produce short-chain fatty acids [10]. Inulin, fructoligosaccharide and polydextrose are prebiotic food compounds that exist in many edible plants. They are indigestible polysaccharides and oligosaccharides and are classified as dietary fiber [11]. Today, products that have probiotic (beneficial bacteria) and prebiotic (probiotic food) properties are called synbiotics [12]. Until now, there have been fewer studies related to synbiotic buffalo botulismat. Therefore, in this research, the production of synbiotic buffalo yogurt with the addition of inulin and fructooligosaccharides along with probiotic bacteria (*Lactobacillus acidophilus*[1] *and Bifidobacterbifidium*[2]) and investigating its synbiotic properties during the storage period.

2- Materials and methods

To conduct this research, fructooligosaccharide and inulin from the company*Actilight*, France were purchased. Cultivation of starters containing single-strain probiotic bacteria*Lactobacillus acidophilus* And*Bifidobacterium bifidum*Freeze dried type*YOU*, from Christine Hansen Company (*Chr-Hansen*) Denmark, as well as all chemicals to perform the necessary tests were obtained from Merck and Sigma.

2-1- Production and preparation of synbiotic yogurt

Produced from buffalo milk with laboratory parameters such as protein (4.6 percent), fat (7.5 percent), ash (0.91 percent), lactose (4.5 percent), dry matter (16.5 percent) and pH (6.78) was used. The existing milk was heated up to a temperature of 45°C, prebiotic fructooligosaccharides and inulin were added in proportions of 0.5, 1 and 1.5% and at a temperature of 65°C, the contents of the container were homogenized by a laboratory homogenizer and in It was pasteurized at 85°C

[1]. Lactobacillusacidophilus

[2]. Bifidobacterium

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for 5 minutes. Further, after pasteurization, the temperature of milk is cooled to 38 degrees Celsius and the amount of probiotic starter (*Contains Lactobacillus acidophilus and Bifidobacterium bifidum)* According to the instructions of the manufacturer, it was added (25 grams for 100 kg of milk) and filled and sealed in sterile plastic cups. Incubation continued at 38°C until the pH reached 4.6, and then the yogurts were cooled to below 20°C, then kept at 4°C to perform the necessary tests on days 1, 14, and 21 [13].

2-2- Physicochemical tests

2-2-1- Measurement of acidity and pH

Measurement of acidity and pH of yogurt samples were measured according to Iranian national standard No. 2852 (Isiri 2852.2006).

2-2-2- Measurement of dry matter

The measurement of dry matter of yogurt samples was done according to the method of Misaghi et al. (2015).**[14].**

2-2-3- Measuring the percentage of irrigation (synersis)

In order to measure the water content, first 25 grams of yogurt sample was weighed in centrifuge tubes. Then it was centrifuged in a centrifuge (TC model, Shimatsu company) at 400 g for 30 minutes at a temperature of 10 degrees Celsius. The liquid separated from the samples collected in the upper part of the tube was taken out and the tube was weighed again. The water content was reported as water lost in 100 grams of yogurt (National Standard of Iran 2852).

2-2-4- Determination of viscosity in centipoise

Viscosity was measured using a rotary viscometer (Bohlin model, Visco 88, UK) equipped with a thermal circulator (Jolabo model 12MC-F, Germany). An appropriate volume of the prepared sample was transferred into the tank (Bob and Cobb) and Contact with the inner cylinder and thermal circulator was made. After reaching the desired temperature, a specific range of cutting speed was applied, which was increased on a logarithmic scale. The effect of cutting speed on the rheological behavior of the samples was investigated in the range of cutting from 14 to 400/s. [15].

2-2-5- Determining probiotic population count

Number of living cells*Lactobacillus acidophilus* And*Bifidobacterium* In synbiotic yogurt, production was counted after 21 days of production. To prepare the dilution series for the cultivation of bacteria, sterile normal silane was used. After homogenizing the yogurt samples, 1 milliliter of the sample was added to 9 milliliters of sterile normal silane and completely homogenized by shaking. After that, the next dilutions were prepared. An amount of 1 milliliter of the desired dilutions was cultured in the form of purple plates. counting*Lactobacillus acidophilus* (Merck, Germany) by cultivating in Agar-MRS medium with 10 mg/liter ciprofloxacin and 0.1 mg/liter clindomycin and counting*Bifidobacterium* It was done using Agar-MRS culture medium with 50 to 100 mg per liter of mupirocin antibiotic and 5 ml per liter of 10% cysteine hydrochloride [16].

2-2-6- Sensory evaluation

The sensory characteristic (taste, texture) was performed by 10 trained panelists in accordance with the national standard No. 2852 (2852, 2006). Sensory evaluation was performed based on the rating method, for each of the texture and taste variables, a score of 1 to 5 (very bad) =1, bad=2, average=3, good=4, very good=5) were considered [17].

2-3- Statistical analysis

In this research, a split design in time was used in the form of a completely randomized basic design with 3 repetitions and 4 treatments, and the comparison of treatment means was done using Duncan's multi-stage test by SAS 9.1 software and graphs were drawn using Excel 2013 software.

3. Results and Discussion

3-1- Measurement of acidity and pH

The results of statistical analysis in Tables 1 and 2 showed that the amount of pH and acidity changes of buffalo yogurt samples during shelf life was significant ($p<0.05$) and these changes are related to the production of lactic acid by lactic bacteria. The highest level of acidity during the storage period related to treatment B_6 (Buffalo yogurt contains 1.5% fructooligosaccharide). By increasing the percentage of fructo-oligosaccharide and inulin from 0.5 to 1.5%, the amount of acidity (stimulating effect in probiotic activity) increased.

Table 1 Acidity (based on Dornic degree)changes of yogurts produced by different prebiotics during storage

Days/Treatments	First day	14th day	21th day
A (Control)	80.02±0.01a	84.22±0.02d	96.22±0.05n
B (L. Acidophilus)	80.02±0.01a	87.02±0.05f	94.12±0.05m
C (B. Bifidum)	77.02±0.01a	78.54±0.01i	85.44±0.01It is
B1 (L. Acidophilus + Inulin (0.5%))	80.02±0.01b	89.22±0.02j	92.44±0.01l
B2 (L. Acidophilus + Inulin (1%))	80.02±0.01a	89.30±0.01i	92.45±0.01l
B3 (L. Acidophilus + Inulin (1.5%))	80.02±0.01a	89.65±0.05i	92.12±0.01l
C1 (B. Bifidum + Inulin (0.5%))	77.02±0.05b	79.54±0.01k	82.04±0.01r
C2 (B. Bifidum+ Inulin (1%))	77.02±0.03b	78.54±0.05i	82.14±0.05r
C3 (B. Bifidum + Inulin (1.5%))	77.02±0.05b	76.54±0.03k	82.54±0.05r
B4 (L. Acidophilus + fructooligosaccharide (0.5%))	8422±0.05c	92.25±0.05l	99.45±0.05s
B5 (L. Acidophilus + fructooligosaccharide (1%))	85.52±0.03d	94.27±0.05m	100.42±0.05t
B6 (L. Acidophilus + fructooligosaccharide (1.5%))	87.02±0.01It is	96.22±0.02n	102.22±0.01in
C4 (B. Bifidum+ fructooligosaccharide (0.5%))	77.02±0.05f	80.54±0.03O	88.54±0.01in
C5 (B. Bifidum+ fructooligosaccharide (1%))	77.12±0.05g	84.57±0.01p	90.27±0.03q
C6 (B. Bifidum+ fructooligosaccharide (1.5%))	78.22±0.01h	90.55±0.03q	94.05±0.03m

*Means followed by different lowercase letters are significantly different (p < 0.05).

Table 2 Comparison of pH changes of yogurts produced by different prebiotics during storage

Days/Treatments	First day	14th day	21th day
A (Control)	4.02±0.01a	4.02±0.01a	4.02±0.01O
B (L. Acidophilus)	4.02±0.02b	4.02±0.01f	4.02±0.01l
C (B. Bifidum)	4.12±0.03c	4.12±0.03d	4.12±0.02k
B1 (L. Acidophilus + Inulin (0.5%))	4.02±0.02b	4.02±0.02a	4.02±0.02f
B2 (L. Acidophilus + Inulin (1%))	4.02±0.02b	4.02±0.02a	4.02±0.02f
B3 (L. Acidophilus + Inulin (1.5%))	4.02±0.02b	4.02±0.02a	4.02±0.02f
C1 (B. Bifidum + Inulin (0.5%))	4.12±0.01c	4.12±0.01d	4.12±0.03a
C2 (B. Bifidum+ Inulin (1%))	4.12±0.01c	4.12±0.01d	4.12±0.01a
C3 (B. Bifidum + Inulin (1.5%))	4.12±0.01c	4.12±0.01d	4.12±0.01a
B4 (L. Acidophilus + fructooligosaccharide (0.5%))	4.02±0.02d	4.02±0.02a	4.02±0.02k
B5 (L. Acidophilus + fructooligosaccharide (1%))	4.02±0.02It is	4.02±0.03j	4.02±0.02a
B6 (L. Acidophilus + fructooligosaccharide (1.5%))	4.02±0.02f	4.02±0.02k	4.02±0.02p
C4 (B. Bifidum+ fructooligosaccharide (0.5%))	4.05±0.02g	4.12±0.01l	4.12±0.01q
C5 (B. Bifidum+ fructooligosaccharide (1%))	4.05±0.02h	4.12±0.01m	4.12±0.03r
C6 (B. Bifidum+ fructooligosaccharide (1.5%))	4.05±0.02i	4.12±0.01n	4.12±0.01s

*Means followed by different lowercase letters are significantly different (p < 0.05).

In general, the yogurt sample containing fructooligosaccharide had more favorable characteristics than the control and the samples containing inulin. The results of adding the amount of fructooligosaccharide in concentrations of 0.5, 1 and 1.5% showed that the sample containing 1.5% fructooligosaccharide provided the most suitable pH for the activity of probiotic bacteria. This study was consistent with Shain et al.'s (2000) studies on low-fat yogurt containing Bifidobacterium strains (Bf-6 and Bf-1) with different percentages of 0, 0.5, 1, 3 and 5 fructo-oligosaccharides, galactoligosaccharides and inulin, the results showed that the highest acidity (lowest pH) was related to the samples containing 5% prebiotic. Also, the pH reduction factor during the storage period is the continuous fermentation of lactose by lactic acid bacteria and different percentages of fructo-oligosaccharides in Synbiotic yogurt containing probiotic bacteria.*Lactobacillus brevis*KU200019 had a positive effect on the quality of produced yogurt[18].

2-3-Measurement of dry matter

The results of the statistical analysis in Table 3 showed significant changes in the dry matter of the synbiotic buffalo yogurt sample during shelf life (p<0.05), the addition of bacteria*Lactobacillus acidophilus* And*Bifidiobacterium bifidium* They did not have a significant effect on dry matter alone (p>0.05). But with the addition of fructooligosaccharide and inulin, the dry matter increased (p<0.05). According to the results of statistical analysis, synbiotic yogurt with 1.5% fructo-oligosaccharide and 1.5% inulin had the highest percentage of dry matter. The increase in the amount of dry matter is attributed to a significant decrease in the water volume of milk serum [19]. Similar results have been obtained by other researchers regarding probiotic low-fat yogurt formulated with inulin and fructooligosaccharides. Increasing the solids of yogurt increases the nutritional value, consistency and texture of the product. These characteristics are more evident when the increase in solids is based on carbohydrates. The addition of fructo-oligosaccharides compared to inulin had a significant effect on the amount of dry matter, which is due to the properties of fructo-oligosaccharides in absorbing water (about 3 times its own weight). 20]. Also, inulin causes an increase in the dry matter of yogurt due to the formation of a gel state during the storage of yogurt at refrigerator temperature and by retaining water [21].

Table 3 Dry matter changes(%) of yogurts produced bydifferent prebioticsduring storage.

Days/Treatments	**First day**	**14^{th} day**	**21^{th} day**
A (Control)	16.12±0.01^{a}	16.12±0.02^{a}	16.18±0.02^{O}
B (L. Acidophilus)	16.12±0.02^{b}	16.01±0.01^{b}	16.0±0.02^{p}
C (B. Bifidum)	16.12±0.02^{c}	16.10±0.02^{c}	16.04±0.01^{q}
B1 (L. Acidophilus + Inulin (0.5%))	17.02±0.02^{d}	16.52±0.03^{d}	16.12±0.02^{a}
B2 (L. Acidophilus + Inulin (1%))	17.32±0.01$^{It\ is}$	16.72±0.01$^{It\ is}$	16.12±0.02^{a}
B3 (L. Acidophilus + Inulin (1.5%))	18.62±0.01^{f}	18.62±0.01^{f}	16.84±0.03^{r}
C1 (B. Bifidum + Inulin (0.5%))	17.02±0.03^{d}	16.46±0.02^{j}	16.01±0.01^{s}
C2 (B. Bifidum+ Inulin (1%))	17.32±0.01$^{It\ is}$	16.52±0.01^{k}	16.46±0.01^{j}
C3 (B. Bifidum + Inulin (1.5%))	18.60±0.03^{f}	18.60±0.03^{f}	17.32±0.01$^{It\ is}$
B4 (L. Acidophilus + fructooligosaccharide (0.5%))	17.55±0.01^{g}	17.02±0.01^{d}	16.46±0.01^{j}
B5 (L. Acidophilus + fructooligosaccharide (1%))	17.7±0.01^{h}	17.2±0.01^{l}	16.64±0.03^{j}
B6 (L. Acidophilus + fructooligosaccharide (1.5%))	18.70±0.01^{i}	18.70±0.03^{i}	17.14±0.01^{t}
C4 (B. Bifidum+ fructooligosaccharide (0.5%))	18.22±0.01^{j}	17.13±0.01^{m}	16.24±0.03^{in}
C5 (B. Bifidum+ fructooligosaccharide (1%))	18.42±0.03^{k}	17.22±0.01^{n}	16.65±0.01^{in}
C6 (B. Bifidum+ fructooligosaccharide (1.5%))	18.8±0.01^{i}	18.7±0.01^{i}	16.54±0.01^{In}

*Means followed by different lowercase letters are significantly different ($p < 0.05$).

3-3- Measurement of watering

Watering is an undesirable feature of yogurt, which has a negative effect on its overall acceptance. The separation of the aqueous phase in fermented milk products occurs due to the accumulation of protein particles during storage and sedimentation under gravity, and some other factors such as stabilizers, acidity, powdered materials used, type of milk and culture can be effective on the separation of the aqueous phase of fermented sweet drinks. to be The results of statistical analysis showed that the addition of inulino-fructo-oligosaccharide with a concentration of 1.5% caused a significant decrease in the water level of the samples ($p<0.05$). In the samples containing 0.5% inulin, the amount of dehydration was significantly higher than the samples containing 1.5% inulin ($p<0.05$). In the samples containing fructo-oligosaccharide, with the increase in concentration from 0.5 to 1.5%, the water level in the samples decreased significantly ($p<0.05$). Research shows that active hydroxyl groups in inulin and fructo-oligosaccharides cause a reduction in hydration, also in inulin, due to the formation of a gel structure at low temperatures, significantly reduce hydration and increase the total solids, leading to firmness of the product texture [22]. With the passage of time and the increase in the percentage of prebiotics in the yogurt samples, the water content decreases. The reason for this is the decrease in the pH of the yogurt samples during the storage period, which affects the casein micelles and causes a decrease in the release of serum and, as a result, a decrease in syneresis. Decreasing water can also be related to the metabolic activity of starters and the reduction of pressure in the protein network [23]. The ability of fibers to connect to water molecules and interfere with milk components, especially proteins, and as a result, the stability of the protein network can prevent the free movement of water and lead to a decrease in syneresis [24].

Table 4 Syneresis changes(%) of yogurts produced by different prebioticsduring storage.

Days/Treatments	**First day**	**14^{th} day**	**21^{th} day**
A (Control)	24.12±0.03^{a}	29.88±0.02^{a}	34.18±0.01^{a}
B (L. Acidophilus)	24.12±0.01^{a}	29.81±0.03^{b}	34.18±0.02^{a}
C (B. Bifidum)	24.12±0.02^{a}	29.88±0.01^{c}	34.15±0.03^{b}
B1 (L. Acidophilus + Inulin (0.5%))	22.73±0.02^{d}	25.61±0.02^{h}	30.03±0.02^{h}

B2 (L. Acidophilus + Inulin (1%))	20.22 ± 0.02^{i}	23.72 ± 0.03^{k}	28.52 ± 0.02^{i}
B3 (L. Acidophilus + Inulin (1.5%))	18.72 ± 0.03^{k}	20.12 ± 0.01^{O}	24.8 ± 0.01^{l}
C1 (B. Bifidum + Inulin (0.5%))	23.02 ± 0.01^{c}	$26.46\pm0.02^{\text{It is}}$	33.01 ± 0.01^{d}
C2 (B. Bifidum+ Inulin (1%))	21.32 ± 0.01^{f}	26.92 ± 0.03^{d}	30.46 ± 0.01^{f}
C3 (B. Bifidum + Inulin (1.5%))	18.60 ± 0.01^{l}	21.60 ± 0.01^{m}	26.32 ± 0.01^{k}
B4 (L. Acidophilus + fructooligosaccharide (0.5%))	$22.53\pm0.02^{\text{It is}}$	25.52 ± 0.02^{i}	30.12 ± 0.02^{g}
B5 (L. Acidophilus + fructooligosaccharide (1%))	20.25 ± 0.02^{j}	23.92 ± 0.02^{j}	28.15 ± 0.03^{i}
B6 (L. Acidophilus + fructooligosaccharide (1.5%))	18.15 ± 0.03^{m}	20.09 ± 0.03^{n}	23.81 ± 0.01^{m}
C4 (B. Bifidum+ fructooligosaccharide (0.5%))	23.22 ± 0.01^{b}	26.13 ± 0.01^{g}	33.24 ± 0.01^{c}
C5 (B. Bifidum+ fructooligosaccharide (1%))	21.28 ± 0.01^{g}	26.22 ± 0.01^{f}	$30.55\pm0.01^{\text{It is}}$
C6 (B. Bifidum+ fructooligosaccharide (1.5%))	18.8 ± 0.01^{j}	21.7 ± 0.01^{l}	26.54 ± 0.01^{j}

*Means followed by different lowercase letters are significantly different ($p < 0.05$).

3-4-Viscosity value changes

Yogurt viscosity is an important feature that affects its quality[25]. Viscosity is influenced by various factors such as incubation temperature, fat and casein content, heat treatment of milk, acidity of milk in the whole period and the type of starter culture [26]. According to Table 4, the results of the statistical analysis of the effect of treatment, the effect of time and the mutual effect of time treatment on the viscosity of yogurt samples prepared from buffalo milk containing different percentages of prebiotic fructooligosaccharide and inulin were reported as significant ($p<0.05$).

Table 5 Viscositychanges (CP)of yogurts produced by different prebioticsduring storage.

Days/Treatments	First day	14th day	21th day
A (Control)	7500 ± 0.02^{O}	6900 ± 0.01^{c}	5300 ± 0.02^{n}
B (L. Acidophilus)	7520 ± 0.03^{m}	6950 ± 0.02^{b}	5310 ± 0.03^{m}
C (B. Bifidum)	7560 ± 0.02^{j}	7010 ± 0.03^{a}	5350 ± 0.01^{l}
B1 (L. Acidophilus + Inulin (0.5%))	7585 ± 0.02^{i}	6512 ± 0.02^{m}	6010 ± 0.02^{j}
B2 (L. Acidophilus + Inulin (1%))	$7650\pm0.02^{\text{It is}}$	6651 ± 0.01^{h}	6150 ± 0.02^{f}
B3 (L. Acidophilus + Inulin (1.5%))	7752 ± 0.03^{a}	$6755\pm0.01^{\text{It is}}$	6351 ± 0.03^{a}
C1 (B. Bifidum + Inulin (0.5%))	7535 ± 0.01^{k}	6501 ± 0.02^{O}	6008 ± 0.01^{k}
C2 (B. Bifidum+ Inulin (1%))	7610 ± 0.02^{g}	6625 ± 0.01^{i}	6339 ± 0.01^{b}
C3 (B. Bifidum + Inulin (1.5%))	7712 ± 0.01^{b}	6756 ± 0.01^{d}	6325 ± 0.01^{c}
B4 (L. Acidophilus + fructooligosaccharide (0.5%))	7525 ± 0.02^{l}	6528 ± 0.01^{l}	6025 ± 0.02^{i}
B5 (L. Acidophilus + fructooligosaccharide (1%))	7630 ± 0.02^{f}	6610 ± 0.02^{k}	$6150\pm0.02^{\text{It is}}$
B6 (L. Acidophilus + fructooligosaccharide (1.5%))	7702 ± 0.01^{c}	6689 ± 0.01^{g}	6295 ± 0.01^{d}
C4 (B. Bifidum+ fructooligosaccharide (0.5%))	7515 ± 0.02^{n}	6510 ± 0.01^{n}	6010 ± 0.01^{j}
C5 (B. Bifidum+ fructooligosaccharide (1%))	7600 ± 0.02^{h}	6610 ± 0.01^{j}	6115 ± 0.01^{h}
C6 (B. Bifidum+ fructooligosaccharide (1.5%))	7680 ± 0.01^{d}	6695 ± 0.01^{f}	$6250\pm0.01^{\text{It is}}$

*Means followed by different lowercase letters are significantly different ($p < 0.05$).

The viscosity value (centipoise) of the treatments showed an increasing trend during the shelf life of twenty one days. The treatments with inulin and fructooligosaccharides showed higher viscosity values. So that the highest amount of viscosity is related to the treatment containing 1.5% inulin and fructooligosaccharide and the lowest amount of viscosity is related to the control treatment. The increase in viscosity in the samples containing fructo-oligosaccharide and inulin is due to the increase in the amount of carbohydrates and dry matter of the treatments. A study on goat yogurt showed that the viscosity of goat milk increases with the increase of dry matter [27].

3-5 percent fat

The results of the statistical analysis showed that the percentage of fat remained unchanged (7.5%) during the 21st shelf life, and the amount of fat in the studied treatments was not significant ($p<0.05$). This is due to the sufficiency of nutrients and the lack of contamination in the environment of the use of probiotic bacteria and starters, which has caused the lack of fat hydrolysis in the samples [28].

6-3 percent of protein

The results of the statistical analysis showed that the percentage of fat remained unchanged (7.5%) during the 21st shelf life, and the amount of fat in the studied treatments was not significant ($p<0.05$). Of course, the possibility of a change in the amount of protein can be due to the proteolytic activity of the initiator bacteria and the binding of denatured soluble proteins bound to casein and the binding of caseins together and the reduction of water absorption, and the concentration of protein per

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unit volume shows an increase, and more serum leaving the clot helps to further increase the protein concentration. 29].

7-3- Viability of probiotics in yogurt during the storage period

Probiotic bacteria lack proteolytic activity and amino acids and peptides must be provided to them for their growth [30]. In synbiotic yogurts, due to proteolytic activity, these compounds are converted into amino acids and peptides, which are necessary for the growth of probiotics and increase their number in yogurt samples. Diagram (1 and 2) of the results of the survival rate of probiotic bacteria*Bifidobacterium bifidium* And*Lactobacillus acidophilus* shows during 21 days of storage. The results of the survival rate of probiotic bacteria*Lactobacillus acidophilus* And*Bifidobacterium bifidium* along with inulin and fructooligosaccharide showed that the initial number of these bacteria was 8.75 and 7.70 log cfu/ml on the first day of storage. By increasing the concentration of inulin and fructo-oligosaccharide from 0.5 to 1.5%, the survival rate of bacteria increased and the survival rate at high concentrations of fructo-oligosaccharide compared to inulin was significant ($p<0.05$).

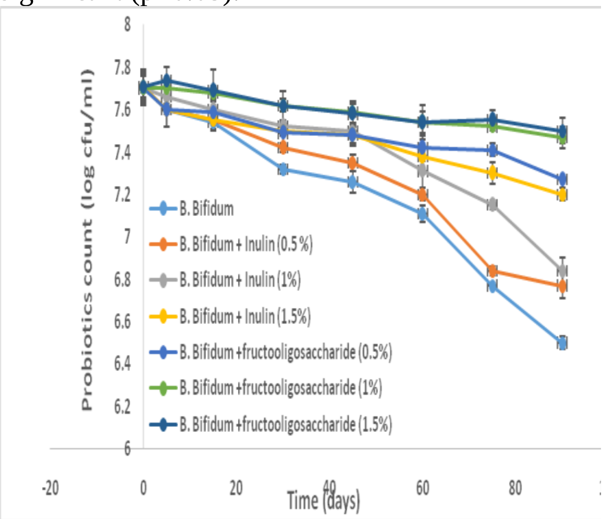

Fig 1 The rate of survivability of*B. bifidum*

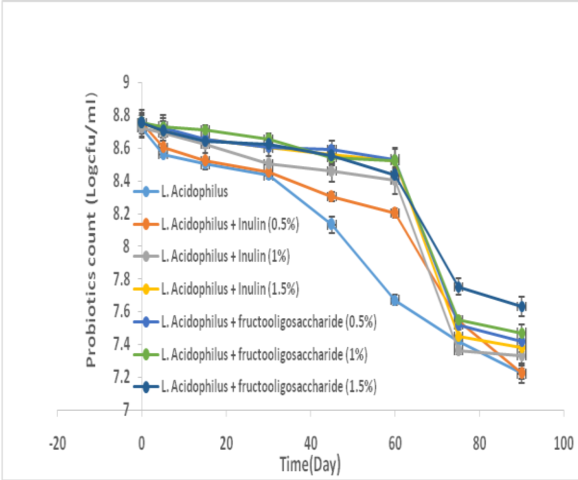

Fig 2 The rate of survivability of*L. Acidophilus*

Of course, it should be mentioned that the lifespan of probiotic bacteria is 21 days*Lactobacillus acidophilus*Than*Bifidobacterium bifidium* It was better. The increase in survival in the samples containing fructo-oligosaccharide and inulin is due to water absorption by fructo-oligosaccharide and the formation of a gel-like structure by inulin, which prevents recrystallization and with this method prevents shock and cell wall destruction [31 and 29]. This study is consistent with the results of the studies of Raskondiaz and colleagues [32] who investigated the protective effect of hydrocolloids on the survival of starter cultures in comparison with control yogurt. Among the treatments, pectin as a protective agent has improved their survival.

8-3-sensory characteristics

The first qualitative feature of food that is noticed by the consumer is its appearance. The appearance of a food product is an important factor that plays an essential and decisive role in the buyer's first encounter [33]. Other qualitative characteristics such as aroma, texture, etc. are criteria that are considered after the final consumption of the food product. Although there may not be a scientific relationship between the color and flavor of food in terms of the type of composition in the physicochemical properties, taste tests have shown that in most cases, the desired color has a significant effect on the feeling of the flavor of food. The tissue content of probiotic yogurt samples containing different percentages of fructo-oligosaccharide and inulin had a higher tissue sensory score during the storage period ($p<0.05$) compared to probiotic samples without fructo-oligosaccharide and inulin. The lowest tissue sensory score was related to the control treatment. The higher concentration of fructo-oligosaccharide and inulin (1.5%) due to the presence of hydroxyl active groups, fructo-oligosaccharide and the formation of inulin gel structure at low temperatures, caused a decrease in water absorption and firmness of the product [34].

Also, the taste of probiotic yogurt prepared from fructo-oligosaccharide increased during the storage period, and yogurt without fructo-oligosaccharide had the lowest taste ($p<0.05$). The higher concentration of fructo-oligosaccharide and inulin (1.5%) was better according to the evaluators, because it had a better taste and less wateriness than other treatments. The acidity of yogurt plays an important role in the aroma and taste of yogurt, and its role is probably greater than the concentration of acetaldehyde and diacetyl. In samples containing higher percentages of fructooligosaccharides, this aroma and taste is more noticeable due to the higher growth of starter bacteria and probiotic bacteria [27]. In the samples containing inulin and fructooligosaccharide, the addition of 0.5% to 1% of inulin had no significant effect on the taste characteristics of synbiotic yogurt ($p>0.05$).

Table 6 Organoleptic properties of yogurts produced by different prebioticsduring storage.

Days/Treatments	**Texture**	**taste**	**Appearance**	**General acceptance**
A (Control)	3.5 ± 0.02^{c}	$3.0\pm0.02^{\text{It is}}$	$2.5\pm0.01^{\text{It is}}$	$2.0\pm0.02^{\text{It is}}$
B (L. Acidophilus)	3.5 ± 0.02^{c}	4.0 ± 0.01^{c}	3.0 ± 0.02^{d}	3.0 ± 0.01^{d}
C (B. Bifidum)	3.5 ± 0.01^{c}	3.5 ± 0.02^{d}	3.0 ± 0.02^{d}	3.0 ± 0.01^{d}
B1 (L. Acidophilus + Inulin (0.5%))	4.0 ± 0.03^{b}	4.5 ± 0.02^{b}	4.0 ± 0.02^{c}	3.5 ± 0.02^{c}
B2 (L. Acidophilus + Inulin (1%))	4.0 ± 0.01^{b}	4.5 ± 0.03^{b}	4.5 ± 0.03^{b}	4.0 ± 0.02^{b}
B3 (L. Acidophilus + Inulin (1.5%))	5.0 ± 0.02^{a}	5.0 ± 0.01^{a}	5.0 ± 0.01^{a}	5.0 ± 0.03^{a}
C1 (B. Bifidum + Inulin (0.5%))	4.0 ± 0.02^{b}	4.5 ± 0.01^{b}	4.0 ± 0.02^{c}	3.5 ± 0.02^{c}
C2 (B. Bifidum+ Inulin (1%))	4.0 ± 0.03^{b}	4.5 ± 0.03^{b}	4.5 ± 0.03^{b}	4.0 ± 0.03^{b}
C3 (B. Bifidum + Inulin (1.5%))	5.0 ± 0.01^{a}	5.0 ± 0.02^{a}	5.0 ± 0.02^{a}	5.0 ± 0.01^{a}
B4 (L. Acidophilus + fructooligosaccharide (0.5%))	4.0 ± 0.02^{b}	4.5 ± 0.02^{b}	4.0 ± 0.03^{c}	3.5 ± 0.02^{c}
B5 (L. Acidophilus + fructooligosaccharide (1%))	4.0 ± 0.02^{b}	4.5 ± 0.01^{b}	4.5 ± 0.02^{b}	4.0 ± 0.02^{b}
B6 (L. Acidophilus + fructooligosaccharide (1.5%))	5.0 ± 0.02^{a}	5.0 ± 0.01^{a}	5.0 ± 0.03^{a}	5.0 ± 0.02^{a}
C4 (B. Bifidum+ fructooligosaccharide (0.5%))	4.0 ± 0.02^{b}	4.5 ± 0.02^{b}	4.0 ± 0.01^{c}	3.5 ± 0.01^{c}
C5 (B. Bifidum+ fructooligosaccharide (1%))	4.0 ± 0.02^{b}	4.5 ± 0.03^{b}	4.5 ± 0.02^{b}	4.0 ± 0.02^{b}
C6 (B. Bifidum+ fructooligosaccharide (1.5%))	5.0 ± 0.03^{a}	5.0 ± 0.02^{a}	5.0 ± 0.02^{a}	5.0 ± 0.01^{a}

*Means followed by different lowercase letters are significantly different ($p < 0.05$).

4 - Conclusion

However, the increase of prebiotics in probiotic yogurt increased the acidity of buffalo yogurt samples during storage (and in parallel, the pH decreased). Synbiotic yogurt samples containing fructo-oligosaccharide and inulin had the highest amount of dry matter at 1.5%. In terms of water level, viscosity and protein characteristics of the treatments, respectively, by increasing the concentration of fructooligosaccharide and inulin from 0.5 to 1.5%, the level of water level and protein decreased and viscosity increased. In terms of sensory evaluation, probiotic yogurt samples containing different percentages of fructo-oligosaccharide and inulin obtained a higher texture and taste sensory score during the storage period compared to the control sample. Also, by increasing the concentration of inulin and fructooligosaccharide from 0.5% to 1.5%, the survival rate of bacteria increased during 21 days. In general, the results showed that the use of fructooligosaccharide and inulin has a positive effect on the physicochemical characteristics, sensory evaluation and viability of probiotic bacteria, and synbiotic buffalo yogurt with beneficial properties can be produced by using them.

5- Resources

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تاثیر افزودن سویه های پروبیوتیک و ترکیبات پری بیوتیک روی ویژگی‌های فیزیکوشیمیایی، میکروبی و حسی ماست سین بیوتیک گاومیش

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چکیده

ماست سین‌بیوتیک یکی از مهم‌ترین مواد غذایی حاصل از ترکیب پروبیوتیکی و پری‌بیوتیکی است که ماده پری‌بیوتیک موجود در آن باعث افزایش زنده‌مانی باکتری‌های پروبیوتیک می‌شود. هدف از این مطالعه توسعه غذاهای لبنی عملگرا در قالب ماست گاومیش سین‌بیوتیک، بررسی تأثیر پری‌بیوتیکی اینولین و فروکتوالیگوساکارید در هرکدام در سه سطح ۰/۵، ۱ و ۱/۵ درصد بر زنده‌مانی باکتری‌های *لاکتوباسیلوس اسیدوفیلوس* و *بیفیدیوباکتریوم بیفیدیوم* و نیز خواص فیزیکوشیمیایی و حسی ماست گاومیش بود. ویژگی‌های فیزیکوشیمیایی (**pH**، اسیدیته، ماده خشک، چربی، پروتئین، ویسکوزیته، آب اندازی) وارزیابی حسی (طعم و بافت) طی ۲۱ روز نگهداری و میزان زنده‌مانی *لاکتوباسیلوس اسیدوفیلوس* و *بیفیدیوباکتریوم‌بیفیدیوم* طی ۲۱ روز بررسی شد. نتایج حاصل از آنالیز آماری نشان داد، با افزایش سطح فروکتوالیگوساکارید در مقایسه با نمونه حاوی اینولین و شاهد، میزان **pH** کاهش و میزان اسیدیته، ماده خشک، آب‌اندازی و ویسکوزیته افزایش بیشتری یافتند. همچنین مقدار چربی و پروتئین در طی ۲۱ روز ماندگاری تفاوت معنی‌داری نداشتند (**p>۰/۰۵**). از لحاظ ارزیابی حسی، نمونه ماست گاومیش سین‌بیوتیک حاوی درصد بالای فروکتولیگوساکارید و اینولین نسبت به شاهد بهتر ارزیابی شدند. همچنین در طی ۲۱ روز ماندگاری میزان زندمانی باکتری‌های پروبیوتیک *لاکتوباسیلوس اسیدوفیلوس* (**۸/۲log cfu/ml**) نسبت به *بیفیدوباکتریوم بیفیدیوم* (**۷/٦٢log cfu/ml**) بهتر بود. بنابراین، استفاده از فروکتوالیگوساکارید و اینولین برای تولید ماست گاومیش سین‌بیوتیک با ویژگی‌های فراسودمند توصیه می‌شود.