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The study of the Survival of *Lactobacillus acidophilus* in synbiotic stirred yogurt using lemon fiber

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ABSTRACT

The aim of this study was to measure the physicochemical, microbial and sensory properties of synbiotic stirred yogurt containing lemon fiber and probiotic bacterium Lactobacillus acidophilus during 20 days of storage. Sensory characteristics on days 0, 10 and 20 were evaluated by 5 trained panelists with 5 treatments (0.25, 0.5, 0.75 and 1 percentage of lemon fiber) The observed data were analyzed by SPSS.21 software. All experiments were performed in triplicate and Duncan multiple range tests with a confidence interval of 95% was used to compare the means. Excel software was used to draw the charts. According to the results, the effect of sample type and storage time on acidity, pH, synersis, dry matter, viscosity, bacterial viability and sensory indices (except for the effect of storage time on the overall acceptance) was significant ($p \leq 0.05$). Physicochemical, microbial and sensory results showed that with increasing lemon fiber content, pH, synersis and sensory indices decreased and acidity, dry matter, viscosity, and survival of probiotic bacteria increased. Also, with increasing storage time, pH, viscosity, probiotic bacterial viability and sensory indices decreased and acidity, synersis and dry matter content increased. The fat content of all treatments within all storage periods was 0.5%. According to the results of sensory evaluation and also considering probiotic bacterial viability, the treatment containing 0.25% lemon fiber was introduced as the superior treatment.

1-Introductin

Yogurt is a widely consumed dairy product that has received a lot of attention worldwide due to its high nutritional value, and its nutritional value can be increased by adding functional food compounds such as fibers. Yogurt is a dairy product obtained from lactic fermentation of pasteurized milk *Lactobacillus delbruecki* subspecies *Bulgaricus*[1]and *Streptococcus thermophilus*[2] is In the process of yogurt production, during fermentation due to the production of lactic acid and the decrease in pH, casein becomes unstable and coagulates, which leads to the formation of a soft gel, connecting the strands of casein micelles and forming a matrix network, and as a result, whey proteins are trapped in the produced matrix network. The structure of yogurt is the result of disulfide bonds between kappa casein and whey proteins and the formation of casein mass due to the reduction of pH to its isoelectric point during fermentation. Due to high protein and calcium and special properties, yogurt is a useful food product and its consumption is increasing in the world [1]. Yogurt is considered as a source of energy, a source of protein and all kinds of vitamins and minerals, and it prevents the growth of pathogenic microorganisms that cause diarrhea and tumors, and it also reduces serum cholesterol and blood pressure. Therefore, due to the very favorable effects of this product on human health, its widespread production has received much attention in recent years. From the point of view of consumers, good yogurt has characteristics such as mouthfeel and pleasant aroma, mild acidity, firmness and proper consistency with minimal wateriness. In general, the production of yogurt in the industry is done in two forms: molded and stirred [2].

In recent years, the quality and healthiness of food has attracted the attention of consumers. Today, most consumers pay attention not only to the healthiness of food and its nutritional value, but also to its health benefits. Such characteristics can be found in a new group of foods called functional foods, which contain probiotics and prebiotics, and as a result of the combination of these two, a food product called synbiotics is produced. Functional foods lead to a reduction in the risk and protection of high blood pressure, diabetes, cancer, osteoporosis, and heart diseases [3].

Probiotics are live microorganismsare (mainly lactobacillus and bifidobacteria) which, if consumed in sufficient quantities, create health-giving properties in the host. There is no doubt that dairy products are the main means of transmission for probiotic supplements [4]. Hence, it is not surprising that there are a large number of clinical studies reporting the health effects of probiotic strains. In fact, the consumption of probiotic yogurt and fermented dairy drinks and cheese have been recommended to promote health benefits [5] Probiotics' resistance to the acidic conditions of gastric juice compared to other microorganisms has caused them to easily settle in the intestine [6]. The most important effect of probiotics is their replacement in the small intestine, which stimulates the intestine and cleans it, thus preventing the attachment of pathogens and inhibiting the toxic effect of toxins. [7, 8]. The most important beneficial effects of probiotics are related to the properties of anti-infections of the gastrointestinal tract, reduction of serum cholesterol, improvement of lactose metabolism, improvement of the immune system, anti-cancer, anti-mutagenic and anti-diarrheal properties, improvement of intestinal inflammation and stopping the growth of Helicobacter pylori bacteria [9, 10].

The use of compounds that enhance the growth of probiotics leads to the stimulation and growth and activity of these bacteria in the digestive system, especially the intestines. These components often have a

1- *Lactobacillus delbrueckii subsp bulgaricus*

2- *Streptococcus thermopilus*

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carbohydrate composition and are called prebiotics. Among these compounds, we can mention dietary fibers that increase the viability of probiotics and can be useful as a special product in the food industry [11]. Wastes from the processing of cereals and fruits are important sources of dietary fibers that can be used in food products [12].

Dietary fibers promote beneficial physiological effects such as reducing fatigue, reducing cholesterol and blood glucose. It also reduces cardiovascular diseases and intestinal insufficiency, especially colon cancer, and diabetic patients will need less insulin if they consume these substances. For its beneficial effect on the disease, the daily intake of fiber should be 38 grams for men and 25 grams for women. The beneficial effects of dietary fibers for human health have been widely reported [13].

Lime with scientific name (*Citrus aurntifolia, often given as C. limon*), the fruit of the lemon tree and citrus fruits. In fact, lime is a shrub whose height reaches four meters and its cultivation is possible only in tropical regions. Its fruit is oval in shape, which is juicier in late autumn and early winter [14]. The residues obtained from lemon processing are rich in fiber, which can be used as edible fiber in the food industry. One third of the produced lemon is used in the preparation of lemon juice, which is obtained as a residue in about 50% of the total primary fruit [15]. Compound fiber significantly reduced the amount of HDL cholesterol in the blood and its protective role against diet-induced arteriosclerosis and thyroid disorders in laboratory mice has been reported [16]. The use of citrus fiber containing bioactive compounds such as polyphenols in meat products has a significant effect in preventing the oxidation of fats and increases its storage time [17]. Several researches have been conducted in the field of using fiber in different types of yogurt, and some of them are mentioned as a great example, Azimi Mahaleh et al. (2016) investigated the effect of orange fiber on the physicochemical, rheological, and sensory properties of strawberry fruit yogurt. By increasing the amount of fiber, dry matter and viscosity increased significantly and syneresis decreased. While its effect on the acidity and pH of the samples was not significant. During the storage time, the percentage of acidity and syneresis increased significantly, and pH and viscosity decreased [18]. Mehdian et al., (2016) investigated the effect of adding fiber from sugar beet waste on rheological and physicochemical properties of probiotic yogurt. The results showed that the use of sugar beet fiber led to a decrease in the pH of the samples during the storage period [19]. The investigation of the rheological and sensory properties of fruit yogurt enriched with wheat fiber showed that with an increase in the amount of fiber, synergism decreased and dry matter increased and the meaning changed.It was not observed in the pH of the samples [20]. In the study of the effect of apricot fiber on the physicochemical, sensory and survival properties of probiotic bacteria in fat-free probiotic yogurt, researchers reported that the increase in fiber decreased pH and increased acidity, and the addition of apricot fiber increased the b index.* and reducing L indices* and a* became. The control sample had the highest sensory evaluation scores, and then the sample containing 1% apricot fiber had the best sensory evaluation scores [21]. during a study They investigated the effect of a number of dietary fibers of apple, orange, oat and wheat on the physicochemical, rheological and sensory properties of probiotic ice cream. The results showed that apple and orange fibers increased acidity and decreased brightness and increased redness and yellowness of ice cream samples. Compared to the control sample, the consistency and apparent viscosity of all samples increased except for the samples containing oat fiber, and the highest viscosity belonged to the samples containing apple fiber. The addition of apple and orange fibers increased the melting point of the samples [22]. According to the studies,

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the purpose of this research was to investigate the effect of lemon fiber on the physicochemical, microbial and sensory properties of synbiotic stirred yogurt.

2- Materials and methods

To prepare soluble lemon fiber according to the method of Azimi Mahaleh et al. (2013), the lemon peel was first cut into smaller pieces and then washed in hot water at 90°C for 5 minutes. became Also, according to the research results, lemon powder can cause a feeling of sand in the mouthTherefore, the size of fiber particles was chosen between 0.5-1 [18]. It should be mentioned Contains lemon fiber WHC[3] (g/g) was 12, moisture was 4.26%, ash was 3.24%, pH was 5.48 and acidity was 0.13.

1-2- Preparation of bacterial suspension

bacteria *Lactobacillus acidophilus*PTCC (1643) which was prepared in lyophilized form from the collection center of industrial fungi and bacteria of Iran Scientific-Industrial Research Organization And in order to activate the bacteria, after sterilizing the medium, the vial containing the microorganism was transferred to the Broth MRS medium, which was previously sterilized in an autoclave at 121°C for 15 minutes, and after inoculation, it was transferred to the incubator. 37°C in anaerobic conditions and kept in a greenhouse for 24 hoursAfter the above time passed, the broth environment changed from clear to turbid, and some cream-colored sediment was observed at the end of the tubes, which indicates the growth of microorganisms in the respective environment. At this stage, the microorganisms were transferred to the MRS-Agar medium (Merck, Germany) [23].

2-2- Preparation of strains for inoculation

For this purpose, 66 grams of MRS Agar culture medium was weighed and distilled water was added accordingly, and the lid of the container containing the culture medium was completely covered with cotton and foil and heated on a flame until it became completely transparent and sterilized in an autoclave at a temperature of 121 degrees Celsius for 15 minutes. The solid is then removed from the liquid MRS media (Merck, Germany) which contained microorganisms and was well mixed by a shaker, was removed from the microbial suspension by a sterile lance and cultured on the surface of the solid medium and then placed in an incubator at 37°C for 48 hours. 0.5 McFarland on the level *cfu/ml*$10^7$1.5 x were used [24].

2-3- Method of preparation of samples of synbiotic stirred yogurt containing lemon fiber

In order to produce yogurt, first the prepared raw milk was heated to 50 degrees Celsius and then its dry matter was adjusted by adding non-fat dry milk. Then, lemon fiber was added to yogurt milk with concentrations of zero (control), 0.25, 0.5, 0.75 and 1). In order to produce yogurt, it was pasteurized in a hot water bath at a temperature of 90 degrees Celsius for 15 minutes while gently stirring due to the evaporation of tap water and the reduction of dilution and the removal of undesirable microorganisms. After cooling the milk to 42 degrees Celsius, commercial yogurt starter (Hansen, Denmark) according to the instructions of the manufacturer of the samples along with the probiotic bacteria Lactobacillus acidophilus (Caminox, Spain) on the surface *cfu/ml* 10^8 added to treatments. Then the samples were placed in the greenhouse until the pH reached 4.5-4.65, for this purpose, the pH of the samples was continuously controlled during the greenhouse. After that, the samples were gently stirred for 1 minute and packed. Sample temperaturewere quickly reduced to 4°C by ice water and the samples were kept at this temperature for testingwere kept [18].

3 -WHC: Water Holding Capacity

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2-4- Research tests

In order to measure the pH and acidity of the yogurt sample, the Iranian national standard method No. 2852 and a pH meter device (Switzerland, Metrohm) were used. size directionAcidity was measured by titration with 0.1 normal interest (Merck, Germany) [25]. Also for sizeThe dry matter was measured according to the Iranian national standard method number 9874, based on heating, drying and weighing the samples [26]. To measure fat, Iran's national standard method No. 695 was used. In this method, dissolving the sample in 10 ml of 90% sulfuric acid (Merck, Germany) and using a centrifuge (Sigma, Germany) was used to separate the fat [27]. for sizeViscosity was measured according to the method of Sahan et al. (2008) using a viscometer (Brookfield, USA) with spindle number 4 and 60 rpm, and to measure the water holding capacity of the yogurt samples, a centrifuge was used at 4500 rpm for 30 minutes and equation (1) was used [28].

$$WHC = \left(1 - \frac{Wt}{W1}\right) \times 100$$

relationship (1)

Wt: weight of released liquid W_1: Our initial weight

To count probiotic bacteria after homogenizing the sample and diluting it in dilutions[3-] 10،[4-] 10[5-] 10, one milliliter of the desired dilution was cultured in MRS-sorbitol agar culture medium by porplate method and finally after 72 hours of greenhouse at 37 degrees CelsiusGrad Colonywere counted in each plate [18].

The sensory evaluation of the yogurt samples was carried out according to the method described by the Iranian National Standard No. 695. For this purpose, measuring the sensory characteristics of yogurt (color, appearance, taste, and overall acceptance) was done using a five-point hedonic scoring method (least important: 1 and most important: 5) [27, 29].

5-2- Statistical analysis

A completely randomized design was used to analyze the data. Finally, 4 treatments were performed along with a control treatment in three repetitions according to the treatment table. SPSS 21 software was used to analyze the data, and Duncan's multi-range test was used at the 95% level to analyze and compare the averages, and Excel software was used to draw graphs.[4] 2013 was used.

3-Results

1-3- pH and acidity

The results of measuring pH and acidity according to Dornick of yogurt samples containing lemon fiber are shown in Tables 1 and 2. After twenty days of storage, the highest pH value (4.46) and the lowest acidity value (93.83) belonged to the control sample, and the lowest pH value (4.32) and the highest acidity value (101.17) belonged to the T treatment.5 was Finally, the comparison of the average data changes showed that with the increase of lemon fiber and storage time, the pH and acidity of the samples significantly increased. ($P<0.05$) decreased and increased respectively.

Table 1- pH changes of stirred yogurt* samples of synbiotic containing lemon fiber during 20 days of storage**

Treatments		1th day	10th day	20th day
T_1 (control)	0% Lemon fiber	4.57 ± 0.05^{aA}	4.56 ± 0.01^{aA}	4.46 ± 0.01^{BC}

4-Excel

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T_2	0.25% Lemon fiber	4.57±0.01^{aA}	4.53±0.01^{bB}	4.42±0.01^{bC}
T_3	0.5% Lemon fiber	4.51±0.01^{not}	4.46±0.01^{cB}	4.40±0.01^{cC}
T_4	0.75% Lemon fiber	4.48±0.01^{that}	4.42±0.02^{dB}	4.35±0.02^{dC}
T_5	1% Lemon fiber	4.44±0.02^{and}	4.41±0.01$^{as B}$	4.32±0.02^{eC}

*Identical lowercase letters indicate no significant difference p>0.05 in each column.
**Different uppercase letters indicate a significant difference of p<0.05 in each row.

Table 2- Changes in the acidity of stirred yogurt* samples of synbiotic containing lemon fiber during 20 days of storage**

Treatments		1th day	10th day	20th day
T_1 (control)	0% Lemon fiber	85.33±0.57^{eC}	88.03±0.76^{eB}	93.83±0.46$^{of\ A}$
T_2	0.25% Lemon fiber	88.33±0.28^{dC}	89.67±0.58^{dB}	95.50±0.76^{and}
T_3	0.5% Lemon fiber	90.50±0.50^{cC}	93.83±0.46^{cB}	97.17±0.61^{that}
T_4	0.75% Lemon fiber	94.00±0.54^{bC}	97.17±0.75^{bB}	100.17±0.76$^{no t}$
T_5	1% Lemon fiber	96.67±0.52$^{B C}$	98.33±0.57^{aB}	101.17±0.34^{aA}

*Identical lowercase letters indicate no significant difference p>0.05 in each column.
**Different uppercase letters indicate a significant difference of p<0.05 in each row.

By consuming sugar and producing organic acids, microorganisms can decrease pH and increase acidity [2]. The reason for the decrease in pH of yogurt samples can be attributed to the secondary metabolic activities of yogurt starters as a result of lactose decomposition and lactic acid production [30]. during maintenance *Lactobacillus bulgaricus and Streptococcus thermophilus* They are active even at refrigerator temperature and by fermenting lactose, they produce lactic acid and increase acidity and decrease pH. Also the presence of probiotic bacteria *Lactobacillus acidophilus* In yogurt samples, due to the consumption of nutrients and creation of organic acids, it can increase the acidity of probiotic mixed yogurt samples. It has also been shown in some research that adding fiber to low-fat yogurtIt can decrease the pH and increase the acidity in yogurt samples [31]. According to the national standard of Iran No. 695, the pH of yogurt should not exceed 4.6, according to which, the pH of the yogurt samples in this research was within the range determined by the standard [25].

Mehdian et al., (2016) The effect of adding fiber from sugar beet waste on rheological, physicochemical properties and bacterial viability. *Lactobacillus acidophilus* In frozen yogurt, probiotics for 60 days at temperature ˚C 18- checked. The results showed that the use of sugar beet fiber led to a decrease in the pH of the samples during storage [19]. Tavakoli (2012), the effect of adding probiotic compounds (wheat and barley fibers) on the propertiesPhysicochemical and livingManny examined Lactobacillus acidophilus dermaset. The results showed that during the storage period, the pH decreased significantly [32]. Tohidzadeh et al., (2012) investigated the effect of carrot fiber on the survival of Lactobacillus casei and the quality of apricot yogurt using the response surface method. The amount of carrot fiber was in the range of 0.5-1.5% and the storage

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time was in the range of 2-20 days. During the storage time, the percentage of acidity increased significantly ($p<0.05$) [33]. In 2017, Haddad investigated the viability of probiotic bacteria in probiotic fermented dairy products in Oman. pH for all samples decreased during storage and was between 4.5-4.1 until the end of the study period [34]. Samadi et al. (2013), in the study of the effect of different levels of beta-glucan (0.25 and 0.5%) on the qualitative characteristics of low-fat yogurt, observed that the addition of beta-glucan to the yogurt formulation increased the acidity [35].

3-2- Synergy

The results of syneresis measurement of yogurt samples containing lemon fiber are shown in Table 3. The comparison of the average changes of syneresis showed that with the increase of lemon fiber, the amount of syneresis significantly increased ($P<0.05$)decreased Also, by increasing the retention time, the amount of syneresis increases significantly ($P<0.05$) increased

Table 3- Changes in the synersis of stirred yogurt* samples of of synbiotic containing lemon fiber during 20 days of storage**

Treatments		1th day	10th day	20th day
T_1 (control)	0% Lemon fiber	7.83 ± 0.05^{BC}	8.10 ± 0.00^{aB}	8.15 ± 0.00^{aA}
T_2	0.25% Lemon fiber	7.20 ± 0.11^{bC}	7.57 ± 0.05^{not}	7.57 ± 0.05^{not}
T_3	0.5% Lemon fiber	6.45 ± 0.05^{cC}	6.80 ± 0.00^{cB}	7.17 ± 0.00^{that}
T_4	0.75% Lemon fiber	5.10 ± 0.10^{dC}	5.47 ± 0.05^{dB}	5.62 ± 0.05^{and}
T_5	1% Lemon fiber	5.10 ± 0.10^{dB}	5.10 ± 0.10^{eB}	5.40 ± 0.10^{ofA}

*Identical lowercase letters indicate no significant difference p>0.05 in each column.
**Different uppercase letters indicate a significant difference of p<0.05 in each row.

The structure of yogurt can be explained as a three-dimensional network of chains and clusters of casein micelles that have maintained their spherical shape. Dehydration and reconstruction of the protein network in yogurt is basically due to the shrinkage of the structure and as a result, the reduction of the binding power of whey proteins to the casein network during storage. Research has shown that water retention in yogurt is related to the amount of casein compounds in milk and the addition of stabilizers [36]. The reason for the decrease in the hydration of the samples compared to the control sample can be the ability of fibers to bind 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 [37]. Some researchers investigated the rheological and sensory properties of fruit yogurt enriched with wheat fiber and the results showed that syneresis decreased with increasing fiber content, on the other hand, syneresis significantly increased with increasing storage time [20]. Mejri et al. (2014), stated that with the increase in storage time, the water holding capacity of the samples, except for the samples containing zero and 0.5% beta-glucan, decreased and synergism increased [38].

3-3- Dry matter

The results of dry matter measurement of yogurt samples containing lemon fiber are shown in Table 4. After twenty days of storage, the highest amount of dry matter (15.90) belongs to T treatment$_5$ and the lowest amount of dry matter (14.28) belongs to the control sample and the comparison of the average changes of the data showed that with the increase of lemon fiber, the amount of dry matter increased significantly ($P<0.05$)

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increased Also, increasing the storage time caused a slight increase in dry matter in the samples.

Table 4 - Dry matter changes of stirred yogurt* samples of synbiotic containing lemon fiber during 20 days of storage**

Treatments		1th day	10th day	20th day
T_1 (control)	0% Lemon fiber	14.27±0.05^{dB}	14.25±0.00^{eB}	14.28±0.01$^{of\ A}$
T_2	0.25% Lemon fiber	14.60±0.00^{cB}	14.60±0.00^{dB}	14.64±0.01^{and}
T_3	0.5% Lemon fiber	14.70±0.05^{bB}	14.68±0.00^{cB}	14.71±0.00^{that}
T_4	0.75% Lemon fiber	15.75±0.04^{BC}	15.85±0.00 ^{bB}	15.86±0.01^{not}
T_5	1% Lemon fiber	15.75±0.04^{BC}	15.87±0.00^{aB}	15.90±0.00^{aA}

*Identical lowercase letters indicate no significant difference p>0.05 in each column.
**Different uppercase letters indicate a significant difference of p<0.05 in each row.

The reason for the increase in the dry matter of the samples can be the addition of lemon fiber to the samples. Also, during the storage period, the moisture content of the samples decreases and the amount of dry matter of the samples increases [38]. According to the Iranian National Standard No. 695, the minimum number of fat-free solids in low-fat yogurt should be at least 9.5%. According to the results obtained in the present study, the number of solids of all the treatments examined in this research was within the standard range [26]. Badari and Alizadeh (2015) showed in research that increasing the amount of beta-glucan up to 0.1% increased the viscosity, dry matter and hardness of the samples during storage compared to the control sample [30]. Shafiei, (2014) the process of physicochemical and sensory changes of probiotic yogurt containing Lactobacillus They examined the micro-encapsulated free plantarum during eight weeks of storage. The results showed that the dry matter of all samples increased significantly [39]. Sahana et al. (2008) investigated the physicochemical and sensory properties of fat-free yogurt containing hydrocolloid beta-glucan during the storage period and the results showed that the dry matter of yogurt increased with the increase of beta-glucan, which was consistent with the results of this research [28]. Yazici and Akgun, (2004) reported that with increasing concentration of substituteCommercial fats based on protein (Deiry Low[5] and Simples[6]) in low-fat abstract yogurt, the number of total solids has increased, which was consistent with the results of the present research [40].

3-4- fat

In all maintenance periods, the amount of fat for all treatments was a constant value of 0.5% and no significant difference (P>0.05) was observed. The reason for the constant amount of fat can be the lack of influence and the very low amount of lemon fiber in the samples.Alirezalu et al., (2014) in the study of the qualitative characteristics and durability of ultra-beneficial yogurt enriched with sugar beet, spinach, and tomato extracts showed that the percentage of fat for different yogurt samples did not change significantly over time (p>0.05) [44]. Sahana et al. did not have [41].

3-5- Viscosity

5 -Dairy-Lo

6 -Simplesse

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The results of measuring the viscosity of yogurt samples containing lemon fiber are shown in Table 5. After twenty days of storage, the highest amount of viscosity (1441/00) belongs to T treatment$_5$ and the lowest viscosity value (636.33) belongs to the control sample and the comparison of average changes of the data showed that with the increase of lemon fiber, the amount of viscosity was significant (P<0.05) increased Also, increasing the storage time causes a significant reduction (P<0.05) The viscosity of the samples was

Table 5 - Viscosity changes of stirred yogurt* samples of synbiotic containing lemon fiber during 20 days of storage**

Treatments		1th day	10th day	20th day
T_1 (control)	0% Lemon fiber	644.67±0.05$^{of A}$	641.00±0.00^{aB}	636.33±1.52^{BC}
T_2	0.25% Lemon fiber	683.00±0.57^{and}	680.33±0.57^{aB}	671.33±1.15^{BC}
T_3	0.5% Lemon fiber	726.33±2.54^{that}	710.00±0.00^{aB}	705.33±0.57^{BC}
T_4	0.75% Lemon fiber	1214.67±3.51^{not}	1151.33±1.15^{aB}	1148.33±3.21^{aAB}
T_5	1% Lemon fiber	1699.00±4.58^{aA}	1481.00±0.00^{aB}	1441.00±0.00^{BC}

*Identical lowercase letters indicate no significant difference p>0.05 in each column.
**Different uppercase letters indicate a significant difference of p<0.05 in each row.

Yogurt viscosity is an important feature that affects its quality. Viscosity is influenced by various factors including incubation temperature, dry matter content, heat treatment of milk, acidity of milk and type of starter culture [42]. Yogurt is a protein gel in which denatured soluble proteins form connections with casein, creating a porous and network-like structure that is able to retain water [43]. The reason for the increase in viscosity in the samples can be attributed to the addition of lemon fiber to the samples, the increase in dry matter and, as a result, the increase in viscosity [44]. Amiri Eghdaei et al. (2009) stated the increase in the viscosity of yogurt during the storage period as a result of rearrangement of proteins and increased hydration [45].

6-3-Counting probiotic bacteria

The results of counting probiotic bacteria of yogurt samples containing lemon fiber are shown in Table 6. After twenty days of storage, the highest bacterial count (7.53) belongs to the treatment of T_5 and the lowest bacterial count (7.22) after the control sample belongs to the treatment of T_2 and the comparison of the mean changes of the data showed that the number of probiotic bacteria was significantly increased with the increase of lemon fiber (P<0.05) increased Also, increasing the storage time caused a slight decrease in the number of probiotic bacteria.

Table6 – Counting Probiotics bacteria of stirred yogurt* samples of synbiotic containing lemon fiber during 20 days of storage**

Treatments		1th day	10th day	20th day
T_1 (control)	0% Lemon fiber	0.00±0.00$^{of A}$	0.00±0.00$^{of A}$	0.00±0.00$^{of A}$
T_2	0.25% Lemon fiber	7.28±0.10 ^{and}	7.31±0.01^{DB}	7.22±0.02^{dC}
T_3	0.5% Lemon fiber	7.50±0.00^{that}	7.43±0.02^{cB}	7.34±0.01^{cC}

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T_4	0.75% Lemon fiber	7.60 ± 0.02^{not}	7.48 ± 0.02^{bB}	7.43 ± 0.00^{bC}
T_5	1% Lemon fiber	7.75 ± 0.01^{aA}	7.60 ± 0.01^{aB}	7.53 ± 0.01^{BC}

*Identical lowercase letters indicate no significant difference p>0.05 in each column.
**Different uppercase letters indicate a significant difference of p<0.05 in each row.

The presence of saturated compounds Because of stimulating the growth and activity of probiotics, biotics are one of the most important reasons for the survival of most bacteria. fairy Biotics may provide some nutrients needed by microorganisms or moderate adverse and negative environmental conditions, including acid damage. The survival of probiotic bacteria in probiotic yogurt depends on factors such as the species used, the reaction between existing species, cultivation conditions, chemical composition of the culture medium, final acidity, temperature, inoculation levels, etc. [47 and 46]. Also, the decrease in pH is always one of the most important reasons for the decrease in the number of probiotic bacteria. In fermented products such as yogurt, low pH and high acidity of the product on the one hand and the cultivation of bacteria non-probiotic lactic acid with probiotic starters, on the other hand, cause the probiotic to die faster during the storage period, especially in the final stages, and this is the reason for the reduction of the number of probiotic bacteria during the storage period of yogurt [2]. Pre-acidification is basically due to the uncontrolled growth of Lactobacillus bulgaricus in low pH values and refrigerator temperature, and it causes the production of hydrogen peroxide by Lactobacillus bulgaricus bacteria during the production and storage of yogurt samples, and as a result, it causes an increase in acidity and a decrease in pH [5]. In general, the results showed that the count of bacteria after the end of the storage period has changed slightly compared to the time of production, and the reason for this isIt could be the presence of lemon fiber [46].

Mehdian et al., (2016) in investigating the effect of adding fiber from sugar beet waste in probiotic frozen yogurt, found that the bacteria population decreased significantly with the passage of time, but samples containing fiber were more viable than the control sample [19]. Tohidzadeh et al., (2012) in investigating the effect of carrot fiber on the survival of Lactobacillus casei and the quality of apricot fruit yogurt, showed that by increasing the amount of fiber, the number of colonies *Lactobacillus casei* increased [35]. Zomorodi et al. (2015) Bacterial viability *Lactobacillus acidophilus* They investigated symbiotic yogurt containing apple fiber and wheat bran. The results showed that the number of probiotic bacteria decreased by one logarithm during the storage period in the control sample, but this value was 0.25 logarithms in the samples containing wheat bran and apple fiber [20].

3-7- Sensory evaluation

Comparing the mean changes of sensory evaluation scores showed that with the increase of lemon fiber, except in the treatment of T_2 Color, appearance, taste and overall acceptance index significantly ($P<0.05$) It also decreased with increasing storage time until the 20th day of storage, sensory evaluation scores decreased (Figures 1, 2, 3 and 4).

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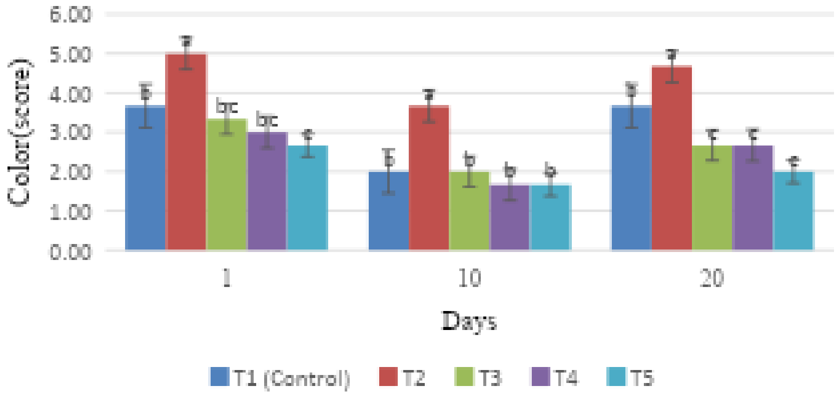

Figure 1- Changes in sensory color evaluation of stirred yogurt samples of synbiotic containing lemon fiber during 20 days of storage

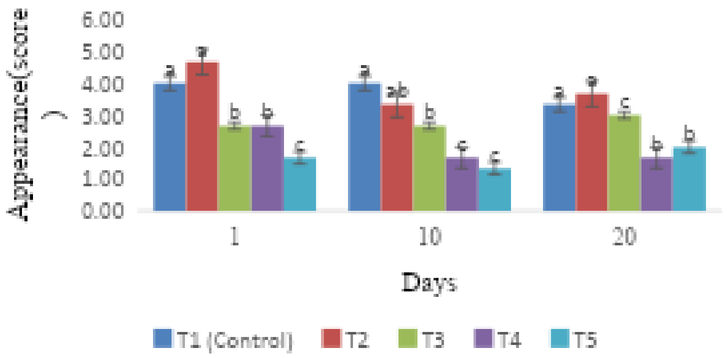

Figure 2- Changes in sensory evaluation of the appearance of stirred synbiotic yogurt samples containing lemon fiber during 20 days of storage

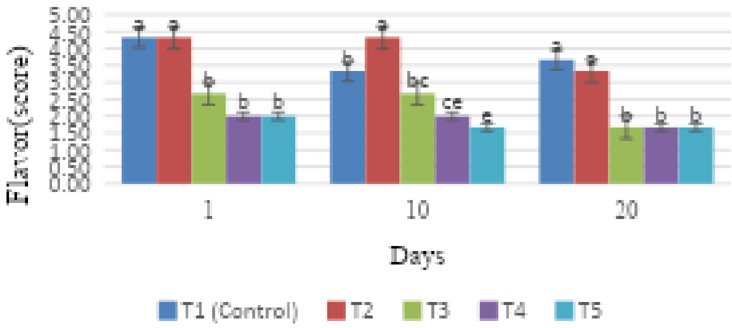

Figure 3- Changes in sensory evaluation of the taste of stirred synbiotic yogurt samples containing lemon fiber during 20 days of storage

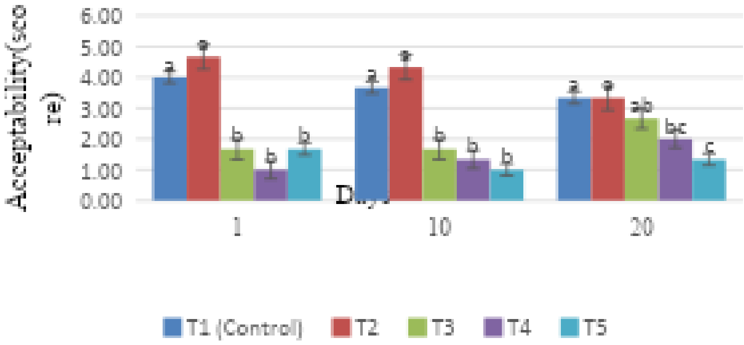

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Figure 4- Changes in sensory evaluation of the overall acceptance of stirred synbiotic yogurt samples containing lemon fiber during 20 days of storage

The sensory parameters of yogurt play an important role in the acceptability and acceptance of consumers, and in particular, the color of yogurt will affect the sensory evaluation and acceptance of consumers. Also, in probiotic yogurt, the sensory characteristics of the produced product should be as close as possible to the traditional sample or without probiotic bacteria [48]. The flavor of yogurt is generally due to acetaldehyde produced from threonine by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* It depends. In addition, other volatile organic aromatic compounds, such as diacetyl and 3,2-pentane-den[7] They also contribute to the flavor of yogurt. 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 [49]. Research has shown that the use of fiber caused a layer of fiber to be seen in the yogurt samples, which reduced the sensory evaluation scores of the samples [50]. Some researchers reported that the firmness of the yogurt texture completely depends on the amount of dry matter of the product, the amount of fat and especially its protein. Increasing the amount of dry matter and protein by increasing the transverse connections in the yogurt gel network leads to the formation of a three-dimensional network and a strong gel structureIt gets hotter. [51]. In general, the results of this research showed that increasing the fiber from 0.25% decreases the sensory evaluation scores, so the appropriate amount of lemon fiber to maintain the sensory properties and closeness to the control sample and even a higher score than the control sample was 0.25%. Azimi Mahaleh et al. (2013) during a research showed that the increase of orange fiber in strawberry and orange fruit yogurt decreased the sensory evaluation scores of color and taste in the samples, but their scores were in the good and acceptable range [18]. Zomorodi *and* Colleagues (2015), during a research, showed that with the increase in the percentage of wheat and apple fiber, the scores of sensory characteristics of probiotic yogurt samples decreased [20]. Narender and Dharam, 2012 investigated the effect of dietary fibers on the physicochemical, sensory and textural properties of yogurt. The results showed that the use of fiber in the samples decreased the sensory evaluation scores [50]. Staffolo et al. (2004) investigated the effect of dietary fiber addition on the sensory and rheological properties of yogurt and the results showed that increasing the amount of fiber in normal yogurt samples reduces the sensory properties of normal yogurt samples by consumers [37].

4- conclusion

Adding lemon fiber to yogurt samples improved the sensory properties and some physicochemical properties such as viscosity, syneresis and dry matter compared to the control sample and also increased the viability of probiotic bacteria in samples containing fiber. Also, the synbiotic stirred yogurt sample with 0.25% lemon fiber had better physicochemical, microbial (in standard) and sensory properties compared to the control sample.

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بررسی زنده مانی *لاکتوباسیلوس اسیدوفیلوس* در ماست هم زده سین بیوتیک با استفاده از فیبر لیمو

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چکیده

این تحقیق با هدف اندازه‌گیری خواص فیزیکوشیمیایی، میکروبی و حسی ماست همزده سین‌بیوتیک حاوی فیبر لیمو و باکتری پروبیوتیک لاکتوباسیلوس اسیدوفیلوس طی ۲۰ روز نگهداری انجام شد. ویژگی‌های حسی در روز تولید، روز دهم و روز بیستم توسط ۵ ارزیاب آموزش دیده با ۵ تیمار (۰/۲۵، ۰/۵، ۰/۷۵ و ۱ درصد فیبر لیمو) و سه تکرار ارزیابی و برای تجزیه‌وتحلیل داده‌ها از آزمون چند دامنه‌ای دانکن، از نرم افزار آماری **SPSS21** در سطح احتمال ۹۵ درصد **(P<0.05)** و برای ترسیم نمودارها از نرم‌افزار اکسل استفاده شد. مطابق با نتایج اثر نوع نمونه و زمان نگهداری بر اسیدیته، **pH**، سینرزیس، ماده‌خشک، ویسکوزیته، زنده‌مانی باکتری و شاخص‌های ارزیابی حسی (به جز اثر زمان نگهداری بر پذیرش‌کلی) معنی‌دار بود (**p**≤۰/۰۵). نتایج فیزیکوشیمیایی، میکروبی و حسی نشان داد با افزایش فیبر لیمو **pH**، سینرزیس و شاخص‌های ارزیابی حسی کاهش و اسیدیته، ماده خشک، ویسکوزیته، زنده مانی باکتری پروبیوتیک افزایش یافت. همچنین با افزایش زمان نگهداری **pH**، ویسکوزیته، زنده‌مانی باکتری پروبیوتیک و شاخص‌های ارزیابی حسی کاهش و اسیدیته، سینرزیس و ماده‌خشک افزایش یافت. مقدار چربی در تمامی تیمارها و دوره‌های نگهداری مقدار ۰/۵ درصد بود. مطابق با نتایج ارزیابی حسی و همچنین با توجه به زنده‌مانی باکتری پروبیوتیک تیمار حاوی ۰/۲۵ درصد فیبر لیمو به عنوان تیمار برتر معرفی گردید.