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ORIGINAL ARTICLE

Effect of Inulin on Physicochemical and Sensory properties of synbiotic yogurt under different storage times

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ABSTRACT
The aim of this study was prepare low-fat synbiotic yogurt using the constant values of probiotic bacteria Lactobacillus rhamnosus and inulin at 0, 1 and 2% (w/w). in this study, synbiotic yogurt samples were produced in different storage times (1, 7, 14 and 21 days) with three replications. In during the storage times, physicochemical properties, sensory and microbial properties of synbiotic yogurt samples, were investigated. The results showed that pH of yogurt samples decreased with over time, and acidity and synergies significantly increased. In all days, synergistic treatments of inulin (1 and 2%) was significantly lower and viscosity and all of the sensory factors studied in these treatments were significantly higher than without inulin treatment (control). The highest synergy and lowest sensory score were observed in the treatment without inulin in the first day and the lowest synergy and the highest sensory score was observed in 2% inulin treatment in the 14th day of storage. Viability of Lactobacillus rhamnosus decreased with over time. As time passed, its number decreased in all treatments, but the addition of inulin caused reduction this process, so that with addition of inulin (1 and 2%), number of Lactobacillus rhamnosus in 14 and 21 days of storage, there was not significant decrease.
***Keywords**: Inulin, Lactobacillus rhamnosus, Synergist, Sensory factors*

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INTRODUCTION

Obesity has seriously threatened the health of people in developing and industrialized countries. Today, one third of the world's population is considered obese [1]. Obesity leads to diseases like type 2 diabetes, arrhythmia, and certain types of cancer. Lowering fat in the diet is a good way to manage your fat intake. Accordingly, demand for low-fat products is steadily increasing [2]. Accordingly, the production and development of dairy products with reduced fat content, such as yogurt, is of particular importance. However, consumers often dropped products with reduced fat content as a product of poor satisfactory quality. Because fat substitutes influence on product characteristics such as taste, oral sensation, texture, viscosity and other organoleptic properties [8]. One of the fat replacement compounds is hydrocolloids, which produce tissue and consistency, increase stability, form gels, and improve the oral health of food products. Hydrocolids create a similar fatty and oily body shape by their ability to absorb and bind water in low-fat products. Water absorption capacity of hydrocolloids is widely used in the production of low-fat dairy products [6]. Some of these compounds, in addition to the role of fat substitutes, as a perbiotic compound, stimulate the growth and proliferation of probiotic bacteria such as bifidobacterium and lactobacillus in the large intestine and thus improve host's health [29, 43], including these peribiotic carbohydrates is inulin [41].

Research shows that at least 5 g of inulin per day for 21 days, increases the logarithmic cycle in the number of bifidobacteria [20]. The production of short chain fatty acids by bifidobacteria significantly reduces the pH of the intestine, which leads to a significant reduction in gastrointestinal infections. On the other hand, these fatty acids are considered as the source of energy for colon muscles. Thus, the production of these substances increases the colon movements [19, 44]. Other research results show synergistic effects and increasing the adsorption of minerals, especially calcium and magnesium, was in

the inulin-based diet. Griffin *et al.* [12] showed that daily use of 8 g of inulin would increase calcium uptake by about 33%. The use of inulin-rich foods in the long term will increase bone density and thus reduce the risk of osteoporosis. Various mechanisms have been proposed about the effect of inulin on increasing the absorption of salts, including the reduction of intestinal pH, followed by increased dissolution of calcium salts, the effect on the osmotic capacity of the intestinal environment, the indirect effect of SCFA, especially butyric acid, on the stimulation of protein production Ca-bonding agents and increased intestinal mucosal cells [21, 34, 3, 45]. Glycemic index reduction is one of the other benefits of inulin, which can be used to produce good food for people with type 2 diabetes and dietary foods [7].
Accordingly, the present study was conducted with the aim of "effect of inulin on the physico-chemical and sensory properties of synbiotic yogurt under different storage times".

MATERIAL AND METHODS

in this study, yogurt samples were produced in time storage (1, 7, 14 and 21 days) and inulin at 0, 1 and 2% (w/w) with three replications. In during the storage times, physicochemical, sensory and microbial properties of yogurt samples, were investigated. the bacteria used include *Lactobacillus rhamnosus* as probiotic and commercial culture of yogurt containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Hansen, Denmark) as starter, which was dried on freezing (DVS) at the time of purchase and were maintained based on the manufacturer's advice until consumption in freezing conditions (freezer -18°C). Also inulin was used with high-performance inulin (BeneoTM HP) with an average degree of polymerization ≥ 23, which was obtained from Foodyar Company in Iran.
In order to produce yoghurt, sterilized milk and homogeneous (1.5% fat, prepared from Pegah Khorasan Company in Iran) were divided into two equal parts. A portion of the milk was heated to about 60°C. Then, 1 and 2% (w/w) inulin was added to it, and the milk was well distilled until the inulin was completely dissolved (inulin in the cold milk was not difficult to dissolve, because the milk sample was 60°C Warmed up). Insulin was not added to the other sample. In the next step, both milk samples were heated at 85°C for 30 minutes. After that, each milk sample was divided into two equal parts. For inoculation of bacteria, all four milk samples were cooled to 40°C and kept until the end of inoculation of the bacteria in a water bath.
Finally, the samples were inoculated with yoghurt starter (0.05% w/v) and probiotic bacteria (*Lactobacillus rhamnosus)* (0.05% w/v). Then, milk samples were poured into 250g containers and incubated at 40°C to reach pH= 4.6. After that, the samples were transferred to a refrigerator at a temperature of 4°C and analyzed in the 1 (24 hours after the preparation of yogurt), 7, 14 and 21 days [38].

Measurement of chemical properties of yogurt samples

The fat content of the yogurt samples produced by the Gerber method was measured (Standard No. 627 of Iran). Moisture, total dry matter and ash content of yogurt samples were measured by the Association of Official Agricultural Chemists Association (AOAC) (1999) and protein content using the Kjeldahl method. PH was measured by pH meter (Methrohm, Switzerland), in a Beaker containing yoghurt at 25°C. At each step, the pH was determined three times from each of the yogurt samples [17, 33].
The total carbohydrate content of each of the yogurt samples was obtained from the following equation.
Total carbohydrate= dry matter - (fat + protein + ash)

Measurement of physical properties of yogurt samples

To measure the water holding capacity of the samples (WHC), about 5 grams of sample (Y) were centrifuged for 30 minutes at 4500 rpm, and serum (W) weighed [25, 32]. Then the water holding capacity of the yogurt samples was obtained from following equation [36].
WHC= (Y-W)/Y × 100
For the synthesis of yogurt synthesis, yogurt samples were well mixed and uniformly, 50 ml of yogurt samples were placed in a 150 ml tube. Then 25 g of yogurt (w1) weighed and was shed on a Watten No. 43 filter paper. After that, placed in a refrigerator at 4°C for 120 minutes and then The weight of the liquid collected in a glass container was deducted from the glass bottle weight (w2) and the results were reported using following equation [32].
Synthesis= (w2/w1) × 100
The viscosity of yogurt samples was measured using Brockfield viscometer (RV-DVII). In this test, after the initial tests, Spindle No. 6 was selected as a suitable spindle for viscosity measurements. All tests were carried out at 5°C and under identical conditions. The viscosity of the samples was read at 30 rpm and after 15 seconds of spindle rotation [39].
To calculate the total bacteria in the samples, MRS agar and Pellet technique were used. After finishing the cultivation, the plates were transferred to the oven at 37 °C. Plates were incubated for 48 hours under

pressure of 5% carbon dioxide gas. After removing the plates from the incubators, the bacterial population was determined using colony counter [23].

Sensory evaluation of yogurt samples

In this section, the most important organoleptic characteristics of low-fat yoghurt samples, including color, appearance, aroma, taste, taste, consistency, and total acceptance of the product, were evaluated by 8 evaluators in three replications Was evaluated. In fact, the samples were compared with 5-point hedonic scale (1 very bad and 9 excellent) [5].

Statistical Analysis

Data were analyzed in a factorial design based on completely randomized design (CRD) with three replications, with using SPSS software (version 17, SPSS Inc., USA) and MSTAT-C (version 2.10, The Crop and Soil Sciences Department of Michigan State University, USA). The mean of treatments was compared with Duncan 5 % test.

RESULTS AND DISCUSSION

The results of analysis of variance of the effects of treatments on the studied traits and also the sensory evaluation of the samples are presented in tables 1 and 2, respectively.

acidity changes

According to the analysis of variance (Table 1), the inulin and storage time interaction had a significant effect on the acidity changes of the yoghurt produced at 1% probability level. As shown in Fig. 1, with over time, the acidity of the yoghurt samples was increased, so that the least acidity changes was on the first day and 0% inulin treatment and the most changes related to day 14 and 2% inulin treatment.

Table 1. variance Analysis of physicochemical traits of yogurt samples

SOV	df	Mean of Squares (MS)				
		Acidity	Dry matter	pH	Ash	Total Carbohydrates
Inulin (A)	2	45.74**	5.18**	0.078**	7.25ns	61.10**
Storage time (B)	3	7.36ns	3.67*	0.064**	6.71ns	12.10ns
A×B	6	38.05**	7.87**	1.24**	1.25ns	8.38ns
Error	24	9.33	1.07	0.009	9.46	13.33
C.V (%)		4.14	3.22	9.20	2.10	4.15

Table 1. Continue

SOV	df	Mean of Squares (MS)			
		Viscosity	WHC	Synergies	Number of bacteria
Inulin (A)	2	95.30**	73.98*	87.64**	6.03**
Storage time (B)	3	31.26*	91.56**	73.14**	7.90**
A×B	6	107.71**	18.41ns	92.67**	4.24**
Error	24	8.36	21.14	11.31	0.46
C.V (%)		6.21	8.74	5.97	8.10

**, * and ns: Significant at 1, 5% probability level and insignificant, respectively.

According to Fig. 1, there is no statistically significant difference between 0, 1 and 2% inulin on days 1, 14 and 21 in the production of yogurt samples, and only this difference on the seventh day is statistically significant Has been. Guven *et al*. [14] stated that the acidity of the samples was not dependent on the concentration of inulin on the quality of inulin as a fat substitute in the quality of low-fat yogurts. On the other hand, the acidity of the samples was significantly Increased on the fifteenth day, with the lowest acidity. Sahan *et al*. [32] in their studies on the addition of hydrochloride to low-fat yogurt stated that the addition of various hydrocolloids in different amounts did not significantly alter the acidity of the samples. Aghazadeh Meshgi *et al*. [1] in the study of unbroken omnipotent yoghurt using corn starch and gelatin stated that acidity changes at different time levels were not the same and the highest acidity was related to lean yoghurt with 5% gelatin during For 15 days there was a slight acidity in the sample containing 1% gelatin in one day. The results of this study are consistent with our results.

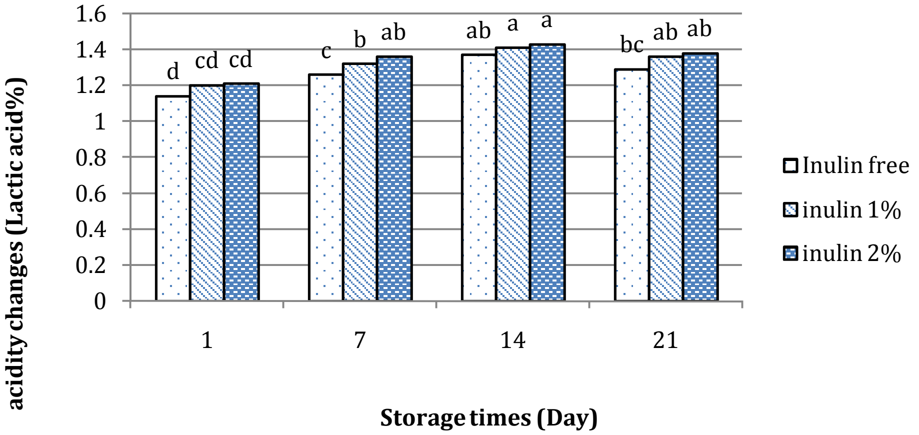
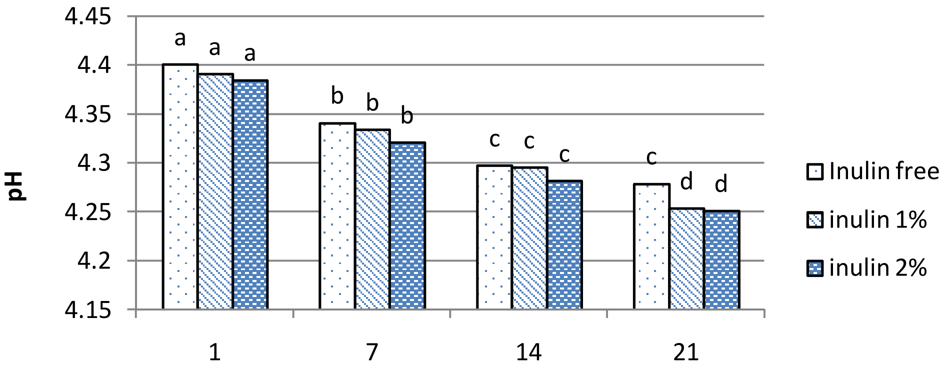


Figure 1. Mean comparison for interaction of inulin and storage time on acidity changes of synbiotic yoghurt

pH

Interaction of inulin and storage time of yogurt samples were significant at 1% probability level (Table 1). As time passed, pH was decreased at all levels of inulin. The highest pH was observed in the first day and in control treatment (0% inulin) and the lowest pH was observed on day 21 and 2% inulin concentration (Fig. 2). In a study conducted by Kailasapathy [16], the pH of our yogurt samples during storage was due to the continuation of the process of fermentation of lactose and the production of small amounts of lactic acid. On the other hand, acidity also increases during this period. This re-acidification is due to the activity of the β-galactosidase enzyme, which is still active at 0-5 degrees Celsius. In this case, the pH is further reduced by the production of more hydrogen ions than lactate ions. Also, Unal *et al*. [46] also showed that the pH of the yogurt samples contained low-fat and hydrocolloid content during storage. Regarding the comparison of the mean, it can be seen that there is no statistically significant difference between inulin levels on days 1, 7 and 14, and only on the 21st day this difference is significant (Fig. 2). Dello Staffolo *et al*. [40] showed that the pH of the yoghurts containing different amounts of inulin remained unchanged. Also, Rinaldony *et al.* [27] investigated the addition of inulin on the physico-chemical and sensory properties of soybean yogurt, which showed that the pH of the samples containing inulin was almost constant and different amounts of inulin in the yogurt samples did not show a significant difference. The results of this research are consistent with the results obtained by these researchers. It should be noted that according to Girard and Schaffer-Lequart [11], the presence of hydrocolloids in the milk environment has an effect on the changes in pH during incubation, and adding them leads to a change in the time to reach the isoelectric point but has an effect on the process Reduced or augmented pH does not produce yogurt.



Storage times (Day)

Figure 2. Mean comparison for interaction of inulin and storage time on pH of synbiotic yoghurt

Dry matter

Table 1 shows the interaction between two inulin variables and storage time on the amount of dry matter of manufactured yoghurt samples. As can be seen, there is a significant interplay between the two inulin variables and the storage time on the dry matter of the samples. The highest amount of dry matter was observed for samples with 2% inulin concentration on day 21 and the lowest amount was for the first day of storage and the sample without inulin (Fig. 3). Results of Rinaldony *et al*. [27] showed that increased inulin concentration increased the amount of dry matter of yogurt but did not affect the amount of fat and protein in the samples. It can be concluded that increasing the dry matter content of yoghurts by adding inulin is a high water inulin storage capacity, which improves the viscosity of yogurt [5].

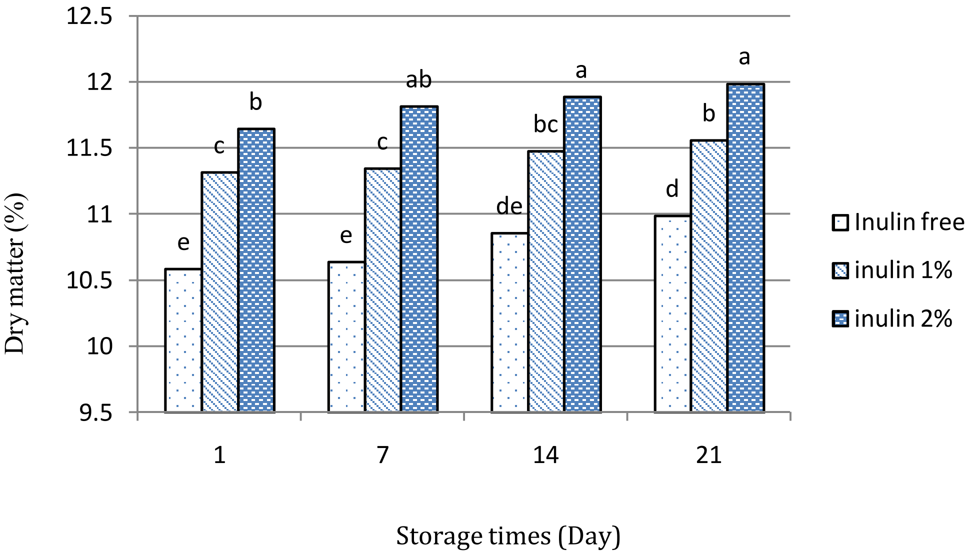


Figure 3. Mean comparison for interaction of inulin and storage time on dry matter of synbiotic yoghurt

Total carbohydrates

The effect of inulin on total carbohydrates was significant at 1% level (Table 1). There was a statistically significant difference in total carbohydrate between yolk samples without inulin and inulin 1 and 2%, but there was no statistical difference in the total carbohydrate content between 1 and 2% inulin (Fig. 4) . The reason for these differences can be added to the addition of inulin, which, by increasing the dry matter of milk used, reduces the amount of moisture and increases the total carbohydrate content. The ability of inulin to increase carbohydrates has been proven by Tudorica *et al*. [40] and Brennan *et al.* [7].

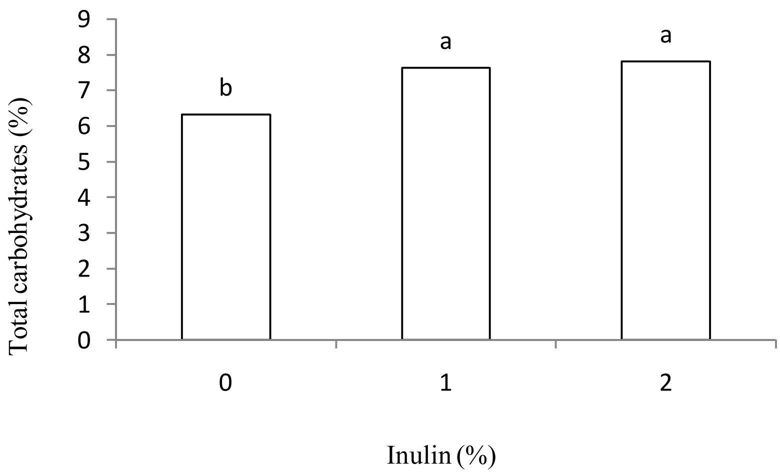


Figure 4. Mean comparison of symbiotic yogurt total carbohydrates in different inulin levels

WHC

According to the variance analysis table (Table 1), it was found that time had a significant effect at 1% probability level on the water holding capacity of yogurt samples during storage. The results show that there is a significant difference between the first and seventh days, while between 14 and 21 this difference was not statistically significant and day 14 had the highest water holding capacity (Fig. 5).
Also, the effect of inulin on water holding capacity was significant at 5% probability level (Table 1). There was a significant difference between non-inulin and inulin 1 and 2% yoghurt samples, but there was no statistically significant difference between inulin 1 and 2% concentration in terms of water holding capacity (Fig. 6). The percentage of fat, the characteristics of primer bacteria, the amount of dry matter without fat, the production of exopolysaccharides, the addition of fibers and stabilizers, fermentation temperature and pH of the product are one of the most important factors affecting the watering of yogurt. Ways of increasing water holding capacity can be the enrichment of dry matter or the amount of protein by adding hydrocolloids such as inulin. But excessive use of these stabilizers can have a negative effect on our sensory features by creating an abnormal taste and excessive tissue stiffness [20]. With the prolongation of the maintenance period of ordinary yogurt and probiotic, the water content is increased, but according to studies, this trend is lower in probiotic yogurt containing perbiotic compounds [15]. Because hydrocolloids can interfere with the water molecules and interfere with the components of milk and thus the stability of the network of proteins, hydrocloids can prevent the free movement of water and lead to an increase in water holding capacity in yogurt [37, 38].

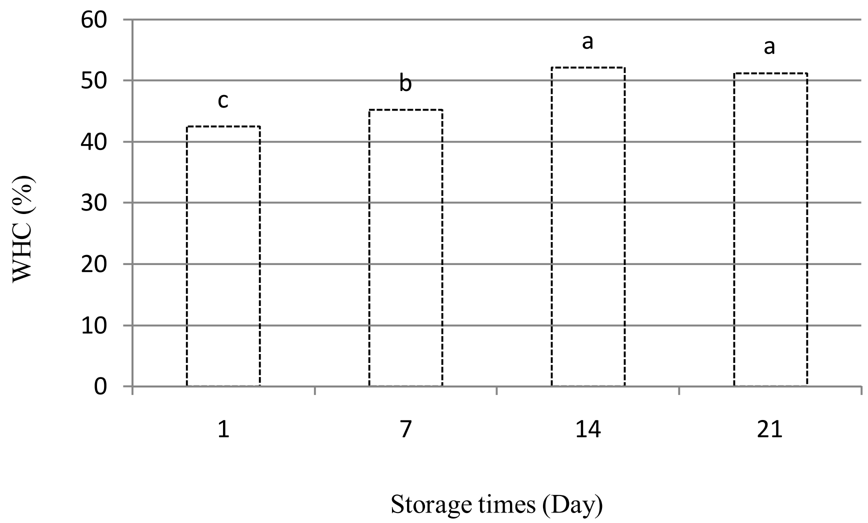


Figure 5. Mean comparison of symbiotic yogurt WHC in different storage times

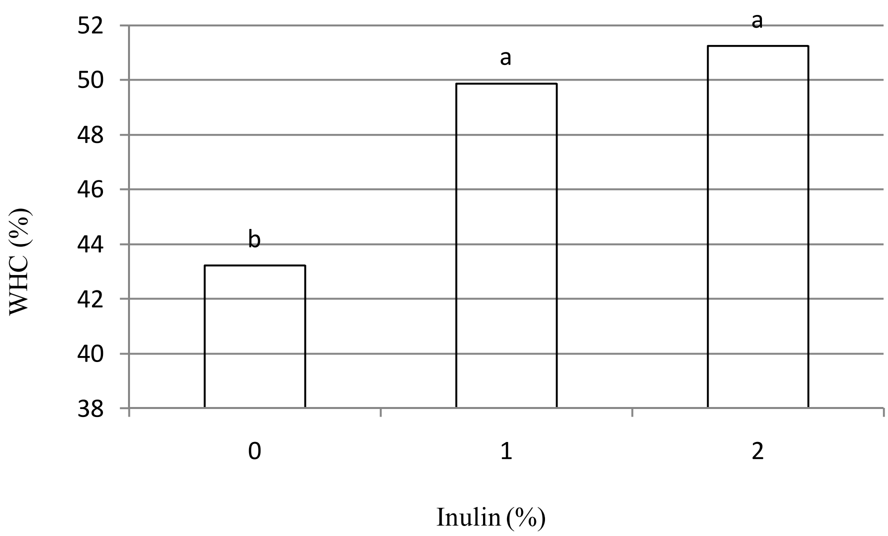


Figure 6. Mean comparison of symbiotic yogurt WHC in different inulin levels

Synergies
According to the analysis of variance of the research data (Table 1), the interaction effect of time in inulin on the water content was significant at 1% probability level. So that the highest water content was observed on the first day and treatment without inulin and the lowest was observed on day 21 and 2% inulin treatment. There was a significant difference between the storage time of yogurt samples in non-inulin samples, but by adding inulin to samples of yogurt produced at 1% inulin concentration between days 1 and 7 at 2% concentration Inulin, between 1 and 21, showed a significant difference in water uptake of the samples (Fig. 7). One of our major disadvantages is watering, which in fact refers to the appearance of serum or whey in our yogurt. Watering in yogurt occurs due to the shrinkage of the three-dimensional structure of the protein network, which leads to a reduction in the binding power of whey proteins and its removal from yogurt [20]. In general, we can describe the structure of yogurt as a three-dimensional network of chains and clusters of casein micelles that retain their spherical shape [22]. Watering and rebuilding the protein network in yogurt is mainly due to the structure's wrinkling and, consequently, the reduction of the binding power of protein proteins to the casein network during storage. Fluidization seems to be highly correlated with the amount of Casino compounds in milk or the addition of stabilizers [47]. Guggisberg *et al*. [13] acknowledged that the addition of 2% inulin has led to a significant reduction in synergy of our samples. It was also reported that inulin formed the formation of a secondary gel in the accumulation of casein micelles. This is while fructans tend to be casein-based.
Based on the results shown by Fiszman [11], the addition of hydrocloid as a stabilizer to the yoghurt formulations by forming the surfaces attached to the casein matrix causes its microscopic structure to change, and binding of the granulomas and the protein chains, thus forming The dual network structure forms an almost endless homogeneous structure, which can continuously maintain a blue phase and thereby reduce the yogurt's water content. Tamime *et al*. [37] have stated that watering, which is one of our undesirable properties, is due to the rearrangement of the gel network, which leads to an increase in the number of particle joints, and therefore the network tends to become wrinkled and the fluid The interior is extruded out. Also, hydrocolloids such as inulin, also increase the apparent viscosity due to colloidal interactions such as intrathecal (stearic) and electrostatic discharges, cause the stability of fermentation systems. The hydrocloid joints alone or in binding with proteins result in the formation of a three-dimensional network and, by trapping the protein particles and fiber in the network, leads to product stability [18]. As shown in Figure 7, the addition of inulin to yogurt decreased the water content of the samples. By increasing the inulin concentration in our samples, the rate of water absorption was also a downward trend due to the formation of a dense gel network Compared to the control samples.

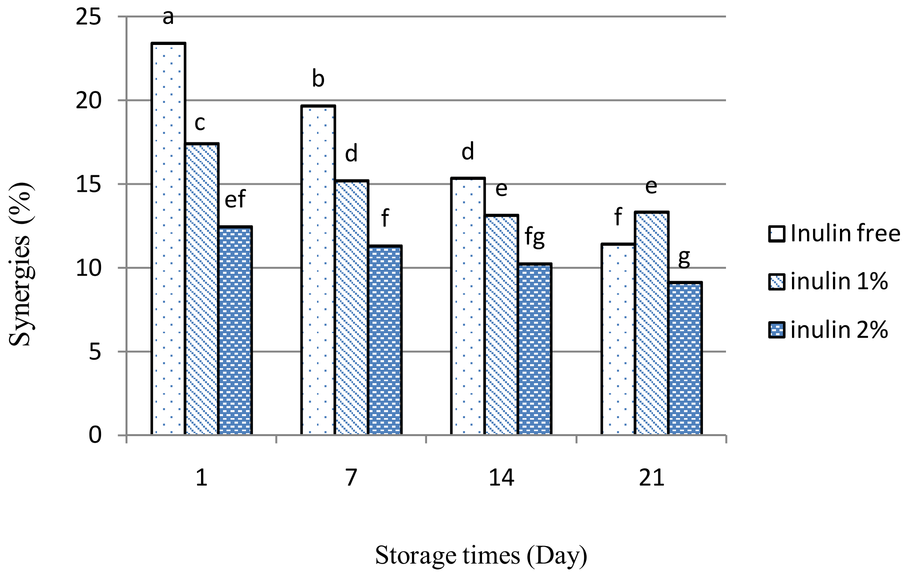


Figure 7. Mean comparison for interaction of inulin and storage time on synergies of synbiotic yoghurt

Viscosity
The results of variance analysis (Table 1) showed that interaction inulin at time on the viscosity of yoghurts is significant at 1% probability level. According to Fig. 8, in all of the inulin concentrations used, with over time, the viscosity of the samples increased, but there was no statistically significant difference between the 14 and 21 days. Also, the results showed that inulin samples had higher viscosity than inulin-

free samples, and there was a significant difference between all of these concentrations except in the 21st day. The highest viscosity was associated with 2% inulin treatment in day 21 and the lowest amount was for inulin-free sample in 1 day (Fig. 8). Rezaei *et al.* [2], studying the effect of inulin on the physico-chemical and rheological properties of frozen yogurt, acknowledged that, although with inulin addition, viscosity increased significantly compared to the control sample. The increase in viscosity due to the addition of inulin has been attributed to the inulin absorbing properties of the inulin and the ability to bond water [9]. When inulin is mixed with water or any other liquid, the inulin crystals of the inulin particles form a 3D gel network, which causes large amounts of water to remain in the network and stabilized physical state of the solution [29].

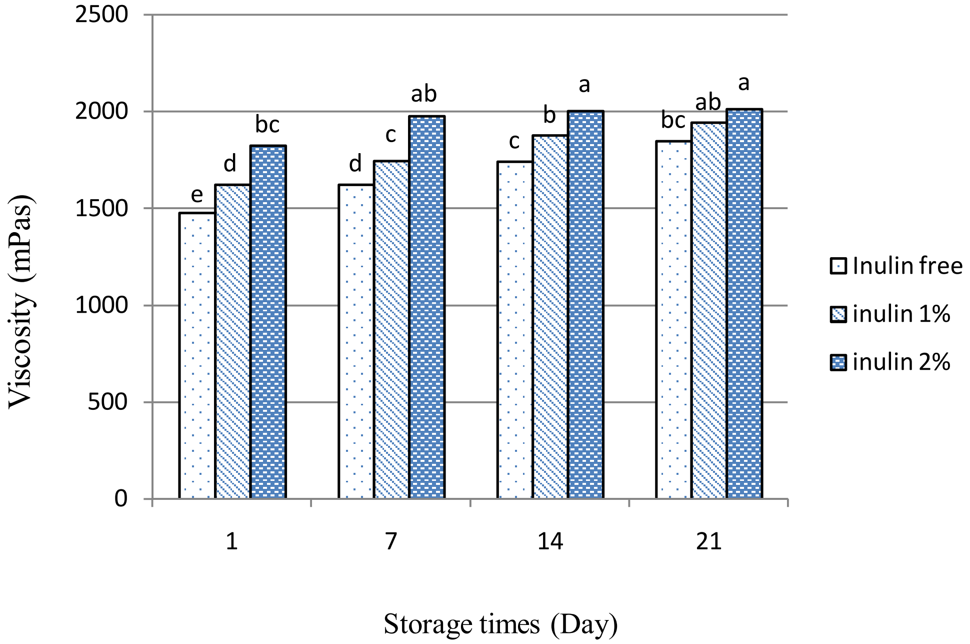


Figure 8. Mean comparison for interaction of inulin and storage time on viscosity of synbiotic yoghurt

***Lactobacillus rhamnosus* bacteria count**

According to the data analysis table (Table 1), the inulin interaction in time was statistically significant for *Lactobacillus officinalis* count at 1% probability level. As shown in Fig. 9, different inulin treatments had a significant difference in the number of *Lactobacillus rhamnosus* bacteria during different days, so that the highest number of Rameniosus in the treatment of 2% inulin and the first day of storage and the lowest number It was seen in unrolled treatment on the 21st day. According to Fig. 9, while increasing the storage time of the produced samples, the number of Ramanosus bacteria decreased, but with the addition of inulin, the trend in 1 and 2 percent inulin showed a significant decrease, so that between On days 14 and 21, there is no statistical difference in the concentrations mentioned. In their studies on the effect of inulin on the number of probiotic bacteria, Shin *et al*. [35] stated that the addition of inulin to 5% resulted in an increase in viability and concentrations of more than 5% had a negative effect and a decrease The donor is responsible for the survival rate of probiotic species. He also stated that the addition of inulin to a concentration of 5% would reduce the proliferation time and increase the viability of bifidobacterium. Saarela *et al*. [30] showed that inulin increased *lactobacillus rhamnosus* in vivo. Also, Akalin *et al*. [4] reported the increase in the number of these species in the presence of hydrocloids in the study of the effects of hydrocloids on different species of Lactobacillus bacteria. On the other hand, the researchers announced a decrease in the number of microorganisms when stored in the presence or absence of hydrocloids. Nighswonger *et al*. [24] also reported a reduction in the number of lactobacillus bacteria during storage, which our results is consistent with these researchers.

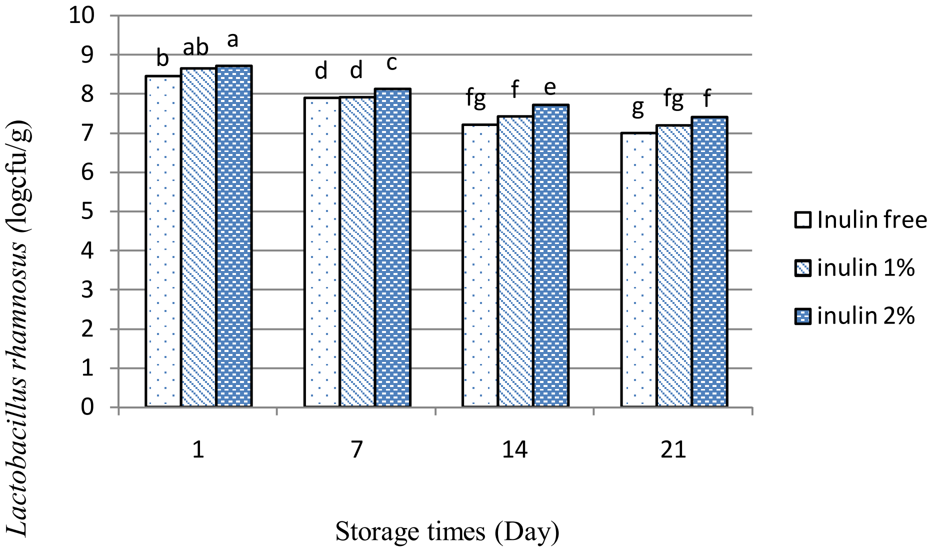


Figure 9. Mean comparison for interaction of inulin and storage time on *lactobacillus rhamnosus* of synbiotic yoghurt

Sensory properties

The results of the sensory evaluation of yogurt samples during storage time are shown in Table 2. Regarding the sensory evaluation scores, it was found that the sample without inulin in 7 and 14 day, samples with 1% inulin in day 14 and the sample containing 2% inulin in day 14 had the highest score compared to other samples during storage time.

Yogurt sample with 1% inulin compared to the inulin-free sample had a higher score in terms of sensory properties, which is indicative of improving the sensory properties of our sample by adding 1% inulin concentration.

For taste, the highest scores were allocated to the 1 and 2% insulin samples in the 14th day and the lowest score was allocated to the insulin-free sample in the first day. As shown in Table 2, the scores of yogurt samples with over time was slightly reduced, which may be due to the increased bacteria activity and the production of acidic lactate. In conclusion, it can be concluded that the addition of inulin to the yogurt has a favorable effect on the sensory properties, but desirability of yogurt is reduced by increasing the storage time up to 21st days.

Table 2. Results of average comparison of yoghurt sensory properties

Sample	Storage time (day)	Appearance	Aroma	Taste	acceptance
Control (inulin free)	1	3.6^{b}	4.1^{a}	3.1^{c}	3.6^{b}
	7	3.8^{ab}	4.2^{a}	3.7^{a}	3.9^{a}
	14	4.2^{a}	3.9^{ab}	3.7^{a}	3.9^{a}
	21	3.9^{ab}	3.7^{b}	3.2^{bc}	3.6^{b}
Inulin 1%	1	3.7^{ab}	4.1^{a}	3.3^{bc}	3.7^{ab}
	7	3.8^{ab}	4.2^{a}	3.5^{b}	3.8^{ab}
	14	4.0^{a}	4.2^{a}	3.8^{a}	4.0^{a}
	21	4.1^{a}	3.9^{ab}	3.8^{a}	3.9^{a}
Inulin 2%	1	3.6^{b}	3.9^{ab}	3.1^{c}	3.5^{b}
	7	3.9^{ab}	4.4^{a}	3.6^{a}	3.9^{a}
	14	4.2^{a}	4.3^{a}	3.8^{a}	4.1^{a}
	21	4.2^{a}	4.0^{ab}	3.5^{b}	3.8^{ab}

Means in each column, followed by similar letter(s) are not significantly different.

CONCLUSION

The results of physicochemical tests of yogurt samples showed that with over time, pH decreased, and acidity and synergies significantly increased. In all storage times, synergistic of inulin (1 and 2%) was significantly lower and viscosity and all of the sensory factors studied in these treatments were significantly higher than inulin-free treatment. The highest amount of synergy and lowest sensory score were observed in treatment without inulin and in the first day. Also, the lowest synergy and the highest sensory score in 2% inulin treatment were observed on the 14th day of storage. The number of *Lactobacillus rhamnosus* decreased with over time, As with time passed, its number decreased in all treatments, but the addition of inulin reduced this trend, so that the number of *Lactobacillus rhamnosus* bacteria in 14 and 21 days, There was no statistically significant difference between them.

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