

Effect of Some Prebiotics Usage on Quality Properties of Concentrated Yogurt

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Abstract: Physical, chemical, textural and sensory properties of concentrated yogurts made from milks including some prebiotics (inulin and oligofructose) added at different ratios were analyzed at the 1st, 7th 14th and 21st day of storage. All samples were examined for pH, titratable acidity, total solids, cholesterol, fat, ash, lactose, energy, yield, organic acids, sensory properties and textural parameters. While using prebiotic type affected texture and organic acid values of prebiotic concentrated yogurt while they didn't affect product composition. Generally, sensory scores of control samples were higher than that of other yogurts.

Key words: Prebiotic, inulin, oligofructose, concentrated yogurt, textural parameters, cholesterol

INTRODUCTION

Lactic acid fermentation is one of the oldest methods used for preservation of milk (Tamime and Robinson, 1978). By using this method a number of acidic milk products are produced. One of them is yogurt. In spite of its acidic property, it is still prone to spoilage during storage. Because water content ratio of yogurt is about 85%. For keeping its quality, separation of yogurt whey is one of the most important factors (Tamime *et al.*, 1991). Concentrated yogurt is produced by removing yogurt whey and so water content decreases to about 70%. This product is similar to Labneh in the Middle East, Skry in Ireland, Chakka and Shirkhanda in India and Ymer in Denmark (Tamime and Robinson, 1988). Concentrated yogurt is made by removing yogurt whey traditionally in a cloth bag or centrifugation.

Prebiotics are carbohydrates that increase the activity of colon bacteria, facilitate colonisation of colon bacteria which is not entropatojen, can be fermented and are non digestible. Inulin, oligofructose, carboxymethylcellulose, microcrystalline cellulose, carragenan, cellulose and oat fiber are used as prebiotic in dairy products. The most preferred prebiotics are inulin and oligofructose in many countries. They are used to reduce the calorie value of dairy products. Inulin and oligofructose have less calories value than carbohydrates because of the typical β (1-2) bond. These bonds can not be metabolized by human digestive enzymes. By this way, inulin and oligofructose passes through mouth, stomach and small intestine without being metabolized (Nilsson *et al.*, 1988). Inulin and oligofructose assure patients relaxation who has constipation by increasing amount of gaita in patient due to its high water holding

capacity and not being metabolized (Ebihara and Schneeman, 1989). Also, they prevent growing of saprophyte bacteria by reducing column pH due to formation short chained fatty acids such as acetate, propionate and butyrate at the end of fermentation and help healing of intestinal mucosa. They reduce amount of serum triglyceride and blood cholesterol degree in patients with high cholesterol (Gibson *et al.*, 1995; Fiordaliso *et al.*, 1995). The objective of this study was to determine the effect of using inulin and oligofructose on physical, chemical, textural and sensory properties of concentrated yogurts during storage.

MATERIALS AND METHODS

In this study, cow's milk was obtained from Bagyolu Village in Manisa, Turkey. Inulin and oligofructose used as prebiotic were obtained Artisan Gida (Istanbul, Turkey). Experimental and strained yogurts were made in Celal Bayar University, Food Engineering Department Laboratory.

Production of concentrated yogurts: In the production of samples, 3% Inulin (I), 3% Oligofructose (O), 1.5% Inulin and 1.5% Oligofructose (IO) mixtures were added to the milk at 25°C. Then the mixture was pasteurized at 90°C for 10 min and cooled to 43°C and inoculated with yogurt culture. Cultures were concentrated freeze-dried type starter culture (coded Bio-Industries F.Y. Sanofi. Ltd. Paris) a blend of *Streptococcus salivarius* ssp. *termophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* which was activated and then used for the yogurt production. Inoculated milks were incubated at 42±0.5°C until pH decreased to 4.7. The yogurts were

transferred into cotton cloth bags (22.5×31 cm and 23×20 threads cm⁻²) to drain the serum for 12 h. Samples were analyzed at the 1st, 7th, 14th and 21st day of storage at 4°C in refrigerator. Three replicates of yogurts preparation were made.

Analytical methods: The pH values of the samples were measured with WTW (Portuguese) pH meter. Dry matter and ash according to Oysun, fat content, density of milk and titratable acidity according to Metin, total nitrogen according to AOAC (1995), energy values according to Watt and Merrill (1995) and cholesterol according to Fletouris *et al.* (1998) was determined. Lactose values were estimated by subtraction moisture, ash, protein and fat values of yogurts from dry mater value. The yield was determined with rate of yogurt amount had to milk amount used.

Texture analyses of prebiotic strained yogurt were carried out according to modified method from Awad *et al.* (2002). The Stable Micro Systems texture analyzer (TA-XT plus, Vienna count, surrey GU7 1 YL, UK) was used to measure textural properties of strained yogurts. The four different parameters that were firmness, consistency, cohesiveness, index of viscosity/consistency were examined. These parameters were estimated from texture analysis graphs as follows.

- Firmness: maximum positive force value
- Consistency: positive area (A1)
- Cohesiveness: maximum negative force value
- Index of viscosity/consistency: negative area (A2)

Estimation of parameters can be seen from Fig. 1. The probe penetrated into the samples to a depth of 15 mm at a speed of 1.0 mm sec⁻¹ and the force exerted on the probe was automatically recorded. The diameter of cylindrical probe was 35 mm. Post test speed was 10 mm sec⁻¹. Trigger force was 10 g. About 3 yogurt samples were analyzed at 6±2°C for each trial and average readings were taken.

Organic acids contents of samples were determined according to modification of the method of Bevilacqua and Califano (1989). About 7 g of yogurt sample was taken and than 40 mL mobile phase (0.1% H₃PO₄) was added and mixed by ultraturrax for 1 min. Mixture was held in water bath (40°C) for 1 h than centrifuged at 6000 rpm for 5 min. The supernatant was filtered once through filter paper and than though 0.45 µm membrane filter. About 20 µL aliquots of individuals standards were injected to column and their retention times were determined. To obtain the calibration curves a mixture of standards of certain concentrations were also injected into HPLC and their chromatograms were

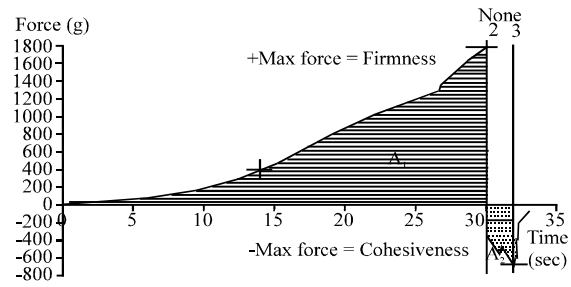


Fig. 1: A textural graph sample of concentrated yogurt

obtained. After injection of the samples (20 µL) chromatographic peaks were identified by comparing retention times of samples to known standards. A Perkin Elmer Series 200 Model HPLC (Series 200 LC pump Norwalk CT, USA) apparatus equipped with a UV absorbance detector set at 214 nm was used. Chromatographic separation was performed on a Shodex RSpak KC-118 (BIO-RAD California, USA) model ion-exchange organic acid column (8×300 mm i.d.). The mobile phases was 0.1% (w/v) of phosphoric acid in distilled water (HPLC grade) with a flow rate of 0.8 mL min⁻¹.

Six trained panellists selected from University staff members who had previous taste panel experience rated sensory properties of strained yogurts. Yogurt samples were organoleptically examined according to the method modified from Turkish Yogurt Standard with maximum scores of all of for appearance, consistency (with spoon), consistency (with mouth), odour and flavour. The results of the researches as statistical were examined using Completely Random Design and GLM Procedure of SAS Statistic Analysis Program (SAS, 2001).

RESULTS AND DISCUSSION

Chemical composition of milk used in the production of samples: The milk used for prebiotic strained yogurt production had 6.55 pH, 0.238% titratable acidity, 11.83% total solid, 3.76% fat, 13.49 mg/100 g cholesterol and 1.033 g mL⁻¹ density.

Chemical composition of prebiotic strained yogurt samples: Dry matter affects nutritional value and sensory properties of the product. Dry matter rates of yogurts were observed to be close to each other. Statistical analysis result also verifies this similarities (p>0.05). Dry matter contents of yogurts changed between 29.54-32.49% (Table 1).

Total solid contents were reported to be 19.41% in concentrated yogurt by Ozer *et al.* (1997), 23.32% in Labneh (Mehaia and El-Khadragy, 1999), 25.26% in traditional Labneh (Tamime *et al.*, 1991). Dry matter contents of yogurts are similar to the results that found

Table 1: Chemical composition of prebiotic strained yogurt * #

Analysis	I	O	IO	S
	Avr+SD	Avr+SD	Avr+SD	Avr+SD
Dry matter (%)	29.81±4.360	30.93±1.840	32.49±5.120	29.54±4.300
Fat (%)	12.42±0.760	12.14±1.500	12.40±0.720	10.91±2.410
Ash (%)	0.815±0.19	0.902±0.18	0.843±0.25	1.015±0.29
Total nitrogen (%)	1.49±0.050 ^a	1.67±0.040 ^b	1.61±0.020 ^c	1.46±0.020 ^d
Protein (%)	9.49±0.310 ^a	10.69±0.260 ^b	10.27±0.160 ^c	9.31±0.150 ^d
Cholesterol (mg/100 g)	14.71±1.320	12.14±0.530	13.13±1.060	12.90±1.160
Energy (kcal/100 g)	150.79±9.850	151.72±6.330	152.85±13.19	167.56±21.19
Lactose (%)	7.17±4.970	7.28±2.710	8.98±5.670	8.28±6.690
Yield (%)	31.72±0.028	33.00±0.003	32.85±0.019	34.12±0.002

I:3% Inulin added strained yogurt O:3% Oligofructose added strained yogurt, IO:1.5% Inulin+1.5% oligofructose added strained yogurt S: Control sample, SD: Standard Deviation Avr: Average, *Analyses are the averages of production which had three replications and made as parallel # **It presents that data in the same row are meaningful difference

32.26% Caglar and 29.44% Parlak. Fat is very valuable nutrients of products respect to sensorial attributes. Fat contents of samples changed between 10.91-12.42%. The differences in fat values of prebiotic concentrated yogurts weren't important as statistical ($p>0.05$). It was seen that the amount of fat in samples higher than that reported in strained yogurts 2.58% Atamer, 7.90% Uysal, 7.59% Ugur, 7.58% Caglar. The fat contents of strained yoghurt depend on many factors such as fat rate of the raw material used, the tissue density of bag used during the straining, time and temperature of straining. Ash is an indicator of mineral content in foods. Ash values are shown in Table 1. The prebiotics used in the production were not effective on. The ash content of samples in the research is similar to other researches.

Total nitrogen amounts of prebiotic concentrated yogurts have changed between 1.46-1.67%. It is seen that O type yogurt had the highest (10.69%) and S had the lowest protein value (9.31%). It was determined that prebiotic type had significant affect on total nitrogen and protein values ($p<0.05$). The protein values were determined as 13.31% by Atay, 14.97% by Toral, 12.01% by Atamer, 19.02% by Caglar, 10.67% by Seckin and Nergiz, 4.46-9.22% Kirdar and Gun, 8.45% by Akin, 9.63% by Sahan and Kacar, 12.55% by Kirdar and Gun protein values of samples are similar to the results that found Sahan and Kacar, Seckin and Nergiz.

Cholesterol is an organic substance that is found in human tissue, animal tissue and cells and it has an important role in metabolism,. The average amounts of cholesterol of prebiotic yogurt samples are shown in Table 1. The amounts of cholesterol in yogurt samples changed between 12.14-14.71 mg/100 g. It was

Table 2: Acidity and pH values of prebiotic concentrated yogurts during storage **

Prebiotic concentration	Day	I	O	IO	S
		Avr+SD	Avr+SD	Avr+SD	Avr+SD
Acidity	1	1.16±0.4	1.19±0.4	1.10±0.4	1.23±0.3
	7	1.21±0.4	1.20±0.4	1.18±0.3	1.24±0.3
	14	1.22±0.4	1.21±0.4	1.19±0.3	1.24±0.3
	21	1.30±0.3	1.39±0.4	1.27±0.2	1.28±0.3
pH	1	3.84±0.0	3.88±0.0	3.86±0.0	3.93±0.1
	7	3.87±0.0	3.89±0.0	3.87±0.0	3.90±0.0
	14	3.90±0.0	3.92±0.0	3.88±0.0	3.95±0.0
	21	3.93±0.0	3.95±0.0	3.92±0.0	3.95±0.0

I = 3% Inulin added strained yogurt, O = 3% Oligofructose added strained yogurt IO = 1.5% Inulin+1.5% oligofructose added strained yogurt, S = control sample SD = Standard Deviation Avr. = Average *: Analyses are the averages of production which had three replications and made as parallel

determined as a result of statistical analysis that prebiotics used in production hadn't important effect on cholesterol amounts of samples ($p>0.05$). Lactose contents of samples I, O, IO and S were 7.17, 7.28, 8.98 and 8.28%, respectively. Prebiotics used in production had not important effect on lactose amounts of samples ($p>0.05$). Energy is the ability to do business. Energy which are had via consuming of foods was obtained from carbohydrate, protein and fat. Energy values for IO, O, I and S was 152.85, 151.72, 150.79 and 135.86 kcal/100 g, respectively. Prebiotic type hadn't affect on energy values ($p>0.05$). Researchers stated that concentrated yogurt had 81-161 kcal/100 g energy value (Martensson *et al.*, 2001). Energy results of samples are similar to this result.

The yield values of samples for I, O, IO and S were 31.72, 33.00, 32.85 and 34.12%. Moreover, it wasn't determined that there weren't significant differences between yield values of samples in terms of statistical ($p>0.05$) (Table 1). The titratable acidity values of samples changed between 1.10-1.39% (Table 2). The acidity values of prebiotic strained yogurt were lower than that found in the strained yogurts (O'Neil *et al.*, 1979). The titratable acidity values of all samples increased throughout storage. The effect of storage hadn't on the titratable acidity values during storage of prebiotic strained yogurt samples ($p>0.05$). At the same time, prebiotics used in productions hadn't significant effect on the titratable acidity of samples ($p>0.05$). The pH values of strained yogurts changed between 3.84-3.95. pH values of prebiotic strained yogurt are similar to that found in strained yogurts O'Neil *et al.* (1979) but lower than that found in the strained yogurt (Musaiger *et al.*, 1998). These diversities can be come from fermentation and storage conditions of products.

Textural characteristics: About 4 different texture parameters impending firmness, consistency, cohesiveness and index of viscosity/consistency

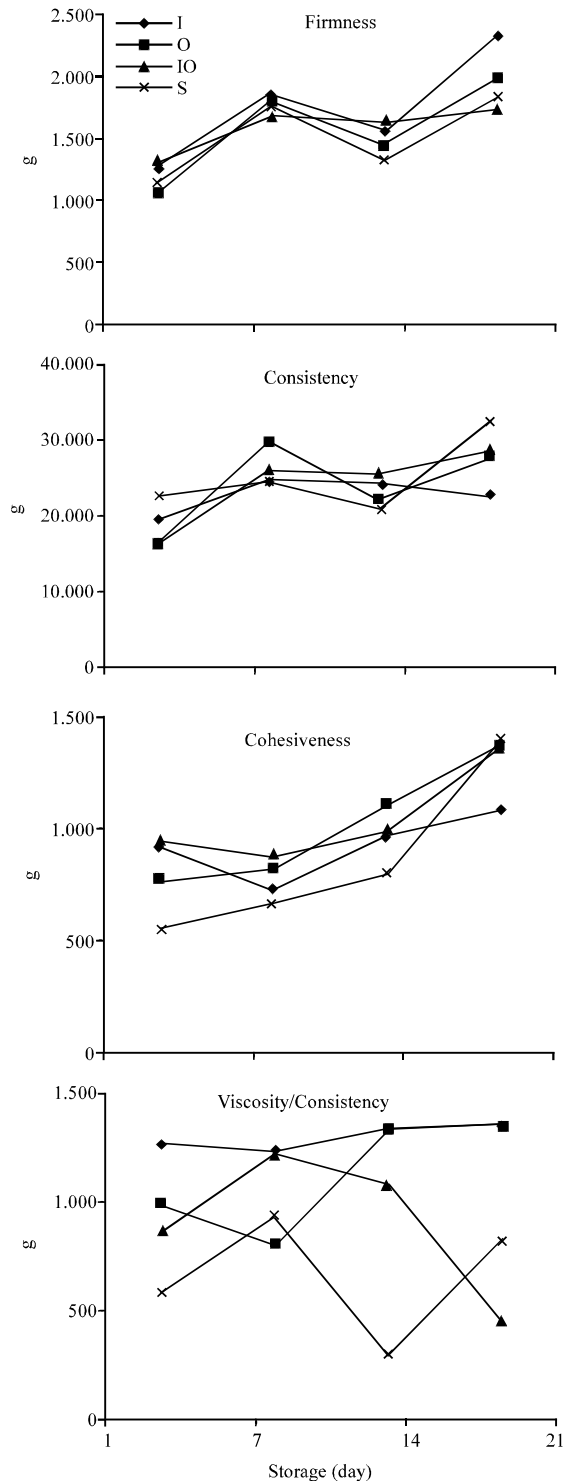


Fig. 2: Textural parameters of yogurt samples throughout the storage

were determined (Fig. 2). Storage period and used prebiotics found to be effective on the firmness values of samples ($p < 0.05$). Firmness values in all yoghurt showed

a decrease on 14th day and increased again on 21st day. This situation can be come from increasing water holding capacity of yogurt proteins with storage. Tamime *et al.* (1991) stated that firmness of concentrated yogurt made with ultrafiltration method was 62-90 g and firmness of concentrated yogurt made with traditional method was 180-200 g Akin stated that firmness values concentrated yogurt made cow milk via traditional method was 68 and 64 g with ultrafiltration method. Ekinci and Gurel (2008) informed that firmness value of yogurts varied between 0.22-0.26 N.

The other parameter measured in the textural analysis was consistency. Storage process affected consistency of O, IO and S samples. Varieties of prebiotic have a significant impact on the values of the consistency of yogurts ($p < 0.05$). Generally, consistency values have increased during storage for all samples. It has been stated that the difference between cohesiveness values of prebiotic strained yogurts were significant on 1st, 7th and 14th days of storage ($p < 0.05$). As it is shown in Fig. 2 that the storage period had important effect on the cohesiveness values of O and IO type prebiotic strained yogurts and hadn't in I type sample. The cohesiveness values of control samples increased during storage but the cohesiveness values of I, O and IO type prebiotic strained yogurts decreased on 7th day of storage and increased on other storage days. During the storage, it has been observed that cohesiveness was the most variable parameter. This changing can be come from result of chemical and biochemical changing of samples during storage.

Storage period was observed to be ineffective on index of viscosity/consistency of I type strained yogurt ($p > 0.05$) and to be effective on other yogurts ($p < 0.05$). During the storage, it has been observed that index of viscosity/consistency was less variable parameter. On storage days, it has been seen that differences between index of viscosity/consistency values of yogurt was important (Fig. 2) ($p < 0.05$).

Organic acids compositions: Organic acids appear in dairy products as a result of hydrolysis of milk fat (free fatty acids such as acetic or butyric), direct addition as acidulates (citric and lactic) normal bovine biochemical metabolism (citric, orotic and uric) or bacterial growth (lactic, acetic, pyruvic, propionic and formic). Organic acid values of prebiotic concentrated yogurts are shown in Fig. 3. It is seen that storage period is effective on oxalic acid values of IO type strained yoghurt ($p < 0.05$) and hadn't effect in the other yogurts ($p > 0.05$). The

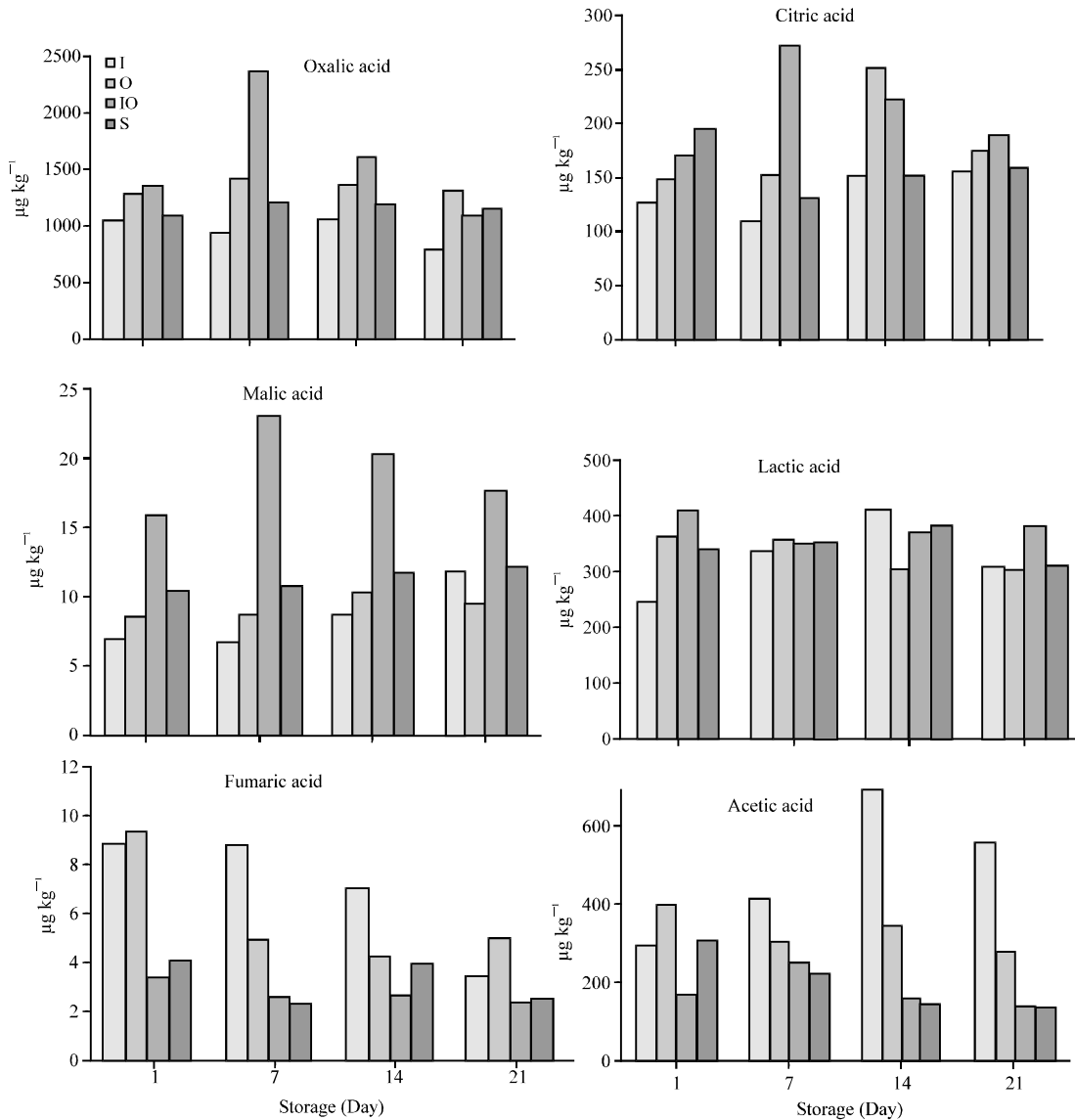


Fig. 3: Organic acid contents of prebiotic yogurt samples throughout the storage

difference between oxalic acid values of yogurts were seen to be insignificant on 14th day of storage and to be important in the other days ($p > 0.05$). It was seen that the effect of storage period on citric acid values of prebiotic strained yogurts was important in other yogurts except I type yoghurt ($p < 0.05$). The difference between citric acid values of yogurts were seen to be unimportant on 21st day of storage but to be important in the other storage days. During storage, the changes in the amount of citric acid were determined to be irregular. This situation can come from that during the storage some organic acids broken down. The effect of storage period on malic acid values of O and S samples were founded to be negligible ($p > 0.05$) but to be considerable in I and IO samples

($p < 0.05$). Prebiotic type had significant effect on malic acid values of yogurts (Fig. 3). During storage, the highest malic acid values were observed in IO type yogurt. Malic acid levels in all samples had a bumpy appearance during storage but increased the end of storage. During storage, all samples of yoghurt reached the highest levels of malic acid on different days.

The most important organic acid of yogurts is lactic acid. Lactic acid values at first day of storage were for I, O, IO and S in order 244.85, 360.74, 409.35 and 339.85 ($\mu\text{g kg}^{-1}$) and on 21st day of storage 306.72, 301.66, 377.83 and 311.80 ($\mu\text{g kg}^{-1}$). During storage, the amount of lactic acid in yogurts didn't follow a regular course. These changes are thought to originate from

Table 3: Sensory evaluation scores of probiotic concentrated yogurts during storage **

Parameters	Day	I	O	IO	S
		Avr+SD	Avr+SD	Avr+SD	Avr+SD
Appearance	1	4.39±0.5	3.83±0.0	3.94±0.20	4.17±0.3
	7	4.00±0.6	3.89±0.2	3.94±0.40	4.28±0.2
	14	3.94±0.4	4.00±0.2	4.11 ±0.2	4.17±0.2
	21	3.61±0.1 ^b	4.00±0.2 ^a	3.94±0.10 ^{ab}	3.78±0.1 ^{ab}
Consistency (with spoon)	1	4.44±0.5	4.67±0.3	4.56±0.10	4.28±0.4
	7	4.61±0.4	4.67±0.3	4.56±0.10	4.72±0.3
	14	4.11±0.3	4.17±0.4	4.39±0.20	4.22±0.4
	21	3.89±0.6	4.28±0.4	4.11±0.50	4.11±0.5
Consistency (with mouth)	1	4.00±0.5	4.22±0.4	4.05±0.20	4.06±0.2
	7	4.16±0.3	4.11±0.3	4.11±0.20	4.17±0.3
	14	4.17±0.0	4.17±0.2	4.22±0.30	4.22±0.1
	21	3.83±0.4	4.17±0.2	4.00±0.40	3.78±0.7
Odor	1	4.11±0.6	4.28±0.2	4.11±0.40	4.06±0.4
	7	3.89±0.3	3.94±0.1	3.72±0.40	3.95±0.2
	14	3.83±0.2	3.94±0.2	3.66±0.30	3.94±0.1
	21	3.56±0.3	3.83±0.0	3.72± 0.2	3.78±0.2
Flavor	1	3.94±0.7	3.61±0.4	3.78±0.50	4.00±0.5
	7	3.50±0.6	3.50±0.4	3.67±0.30	3.72±0.2
	14	3.56±0.4	3.39±0.5	3.44±0.30	3.56±0.1
	21	3.22±0.2	3.33±0.6	3.11±0.20	3.22±0.2

I = 3% Inulin added strained yogurt O = 3% Oligofructose added strained yogurt IO = 1.5% Inulin+1.5% oligofructose added strained yogurt S = control sample SD =Standard Deviation Avr = Average * :Analyses are the averages of production which had three replications and made as parallel, #^{a-d}: It presents that data in the same row are meaningful difference

fragmentation of lactic acid to other products as a result of chemical and biochemical reactions during storage (Fig. 3). The lowest organic acid amount of samples was fumaric acid. Difference between amounts of fumaric acid of yogurt samples is important ($p>0.05$). In all type yogurts, the highest amount of fumaric acid has been reached on 1st day of storage. Acetic acid identified in yogurt is another important organic acid. The effect of storage period on the amounts of acetic acid of yogurts is trivial ($p>0.05$). Probiotic type was determined to be unimportant at first and end of storage.

Marsili *et al.* (1981) stated that lactic acid, citric acid and acetic acid values in yogurt were in order of 14550, 710 and 120 $\mu\text{g g}^{-1}$. Bevilacqua and Califano (1989) stated that lactic acid, citric acid and acetic acid values in yogurt were in order of 19.60 0.60 and 0.49 $\mu\text{g g}^{-1}$. Ancos *et al.* (2000) informed that lactic acid values varied between 6961-11101 $\mu\text{g g}^{-1}$ and citric acid values varied between 2394-3806 $\mu\text{g g}^{-1}$. Tormo and Izco (2004) stated that oxalic acid, citric acid, lactic acid, acetic acid values were in order of 73.0, 1938.1, 14509.8 and 469.0 mg/100 g Ekinçi and Gurel (2008) informed that lactic acid values varied between 15.58-22.47 mg g^{-1} and citric acid values varied between 2.09-2.75 mg g^{-1} .

Sensory characteristics: The appearance scores of I, O, IO and S samples were 4.39, 3.83, 3.94, 4.17, respectively

on 1st day of storage and these scores changed to 3.61, 4.00, 3.94, 3.78 on 21st day of storage. During storage, appearance points of sample have changed as irregular (Table 3). On 1st day of storage, I had the highest appearance score (4.39) and O had the lowest with 3.83 at the end of storage, I had the lowest appearance score and O had the highest with 4.00. Generally, consistency with the spoon scores decreased during storage. The scores of I and S increased on 7th day of storage but no changes did not observed in O and IO samples. It has been seen that storage period wasn't caused a significant change in samples ($p>0.05$). It was determined that used prebiotics hadn't effect on consistency with the spoon scores of samples.

Consistency with mouth scores of yogurts had with ups and downs change during storage. But these changes had a significant impact has been identified ($p>0.05$). Consistency with mouth scores of all yogurts increased on 7th and 14th days of storage and decreased on 21st day of storage. Odour scores of all yogurts decreased, odour score of O sample did not change on 14th day of storage and increased on 21st day of storage for IO sample. Changes in scores of yogurts are seen to be unimportant ($p>0.05$). O sample had the highest odour score at the first day and last day of storage.

When looking at flavour values of yogurt samples, it has been observed at 1st day of storage that the highest score was in S and the lowest score was in O sample. By the last day of storage, O sample had the highest value and IO had the lowest value. Flavour scores of all samples decrease during storage. Flavour scores during storage hadn't significant difference ($p>0.05$).

Yazici and Akgun (2004) stated that flavour scores, structure and texture, colour and appearance scores of probiotic yogurts changed in order of 4.63-8.14, 3.13-4.42 and 3.25-4.50. Sarioglu stated that appearance scores, odour scores, consistency (spoon) and consistency (mouth) scores of probiotic yogurts changed in order of 1-5, 1-5, 2-5 and 1.5-5. Sensory results of probiotic concentrated yogurts are higher this results.

CONCLUSION

According to results, yield of the samples were between 31.72-34.12% and energy values of yogurts changed between 135.86-152.85 kcal/100 g. When inulin and oligofructose were used in concentrated yogurt production, it wasn't observed in chemical results that there are significant changes as compared with other yogurts but it was determined that there are important differences in textural parameters and organic acid values.

Sensory scores of control samples were higher than that of other yogurts. But looking sensorial scores of prebiotic added samples, it was decided that inulin and oligofructose were acceptable for consumption and can use in prebiotic concentrated yogurt in result of sensory analyses. More healthy products will be had with use of prebiotics in concentrated yogurts which is one of traditional products. It has been thought that prebiotic yogurt production will play a significant role in nutrition of public and in development of milk industry.

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