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USAGE OF RICE MILK IN PROBIOTIC YOGHURT PRODUCTION

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ABSTRACT

There are previous studies focusing on the production of probiotic and fermented dairy products made using vegetable based raw materials like oats and soy, however there is a limited number of studies on the usage of rice milk in fermented dairy products. Four different types of yoghurt samples were produced and stored for 21 days at 4°C. Physical, chemical, microbiological and sensory characteristics of the samples were performed at the 1st, 7th, 14th and 21st days of the storage. It was determined that rice milk increased the viscosity values but decreased the values of the texture, whey separation and the chemical and microbiological properties of yoghurts. Acetaldehyde, acetoin, acetone and diacetyl of carbonyl compounds were detected as main flavor components of yoghurt samples. In the sensory analysis, scores decreased as the rice milk proportions in yoghurt was increased and the panelists reported that P1 sample (25% rice milk + 75% cow's milk) was the closest sample to the control sample (100% cow's milk). Generally speaking, samples containing rice milk did not give good results. However, P1 samples were the most favored products among the samples containing rice milk as they were the closest product to the control group. The consumption of such products is continuously increasing as the customers' tendency to consider them as functional products rather than traditional food products increase.

1. Introduction

The content of yoghurt, which is produced with lactic acid fermentation using *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* and has a rich content in terms of carbohydrates, protein, fat, vitamins, calcium and phosphate, show similarities with milk, however, differences occur due to fermentation (Shahani & Chandan, 1979; Caglar & Çakmakçı, 1999). The positive effects of yoghurt-like fermented dairy products on human health have been determined. Yoghurt, which is suitable for lactose intolerant individuals, is also easy to digest (Dewit, Pochart & Desjeux, 1988; Marteau et al., 1990; Rosado, Solomons & Allen, 1992). Due to its bacterial content, it stimulates the growth of other useful bacteria in the body and shows antagonistic effects with antimicrobial substances produced against various pathogens (Hayaloglu & Konar, 1998).

Depending on the changing customer preference and advancing scientific researches, yoghurt production using different starter cultures has become widely a subject of interest in the recent years. Studies also focus on the production of yoghurt using cultures with probiotic properties and hence having supporting health effects, but generally focused on the effects of using different yoghurt cultures on the common qualities of yoghurt. Increasing population of the world makes the

efficient use of natural sources in human nutrition essential. As the welfare and life standards of the countries rise, phytonutrients leave their place to more quality and rich in terms of protein foods of animal origin. Among the zoological nutrients, milk products take an important place (Tamime & Marshall, 1997; Ziemer & Gibson, 1999; Tamime & Robinson, 1999; Yerlikaya, Akpınar & Kılıç, 2013).

Among the milk products, yoghurt and similar fermented dairy products have high digestibility and contain starter cultures protecting the microflora which have inhibitor activities against harmful microorganisms and show anti-tumor, anti-carcinogenic and anti-cholesterol activities, also they can be safely consumed by individuals with lactose intolerance. Also, fermented dairy products which have important functions as protein source of animal origin, contain balanced amounts of carbohydrates, protein and fat, high amounts of calcium for the healthy development of bones and are an important group of nutrients with low calorie, high nutritional value, refreshing properties and being ready to be consumed anywhere, anytime (Granato et al., 2010; Ozer & Kirmaci, 2010; Soccol1 et al., 2010; Divya et al., 2012).

There are previous studies focusing on the production of probiotic and fermented dairy products made using vegetable based raw materials like oats and soy, however there is a limited number of studies on the usage of rice milk in fermented dairy products. In our study, we aimed to produce fermented dairy product by using rice milk or cow milk-rice milk mixture instead of milk with multi-functional properties, containing amino acids and nitrogen essential for growth and development and bioactive peptides which are recently found to have specific functional properties. In researches, knowing the functional properties of food components, enable us to produce nutritive, healthy and resistant foods with good taste, flavor and consistency (Faccin et al., 2009; Ramos et al., 2011; Coda et al., 2012).

For this purpose, in order to increase its commercial acceptance and enhance the sensory properties, rice milk, an important raw food source considered worldwide, was used in probiotic yoghurt production. In Turkey, there are limited amount of studies focused on the production of such products. Besides, food formulations and diets are needed for individuals with various health problems. With this perspective, we aimed to use rice milk, which is a fermented product added into cow milk and newly introduced in Turkey, with probiotic adjunct cultures used combined with standard yoghurt culture to enhance its physical, chemical, microbiological and sensory properties.

2. Materials and methods

2.1. Materials

Milk used in this study was obtained from Ege University Menemen Research and Application Farms. Beneo (Mannheim, Germany) Nutriz, rice bran formula was obtained from Artisan Gida San. Tic. Ltd. Sti. For the preperation of rice milk, 13.6 g of rice bran was diluted in 100 mL of water. MYE 96-98 starter culture for yoghurt production containing *S. thermophilus* and *L. bulgaricus* was obtained from Maysa Gida San. Tic. A. S. In addition to the yoghurt culture, *Lactobacillus gasseri* ATCC 4963 and *Bifidobacterium longum* DSM Lafti B22 strains were used. Filling and packaging were done with packages obtained from Ege University Faculty of Agriculture Menemen Farms and Ege University Faculty of Agriculture Department of Dairy Technology.

2.2. Methods

Yoghurt samples were encoded as C: 100 % cow milk probiotic yoghurt; P1: 25 % rice milk - 75 % cow milk probiotic yoghurt; P2: 50 % rice milk - 50 % cow milk probiotic yoghurt; P3: 75 % rice milk - 25 % cow milk probiotic yoghurt.

2.3. Probiotic yoghurt production

In probiotic yoghurt production, raw milks were treated according to Turkish Food Codex Communiqué on Fermented Milk. Production was conducted in Ege University Faculty of Agriculture Department of Dairy Science Pilot Production Plants. Mixtures containing only cow milk and three different proportions of cow milk-rice milk (C, P1, P2 and P3) were pasteurized (90°C minutes) in different containers. Following this process, milks were cooled to fermentation temperature (42-43 °C) and inoculated with preactivated lactic cultures with 2 % proportions. Inoculated milks were incubated at 42°C. pH values of yoghurt samples were measured and incubation was ended at pH 4.6. Samples were kept at room temperature for 15 minutes and taken to cold storage at +4°C. Physical, chemical, rheological, microbiologic and sensory properties of samples were measured on the 1st, 7th, 14th and 21st days of the storage.

2.4.Physical analysis

At physical analyses, syneresis rate, viscosity and some textural properties were analyzed.

2.4.1.Syneresis

Volumetric method is used for the determination of syneresis rate. Yoghurts samples were taken with a constant volume (40 mL) ice cream scoops in one go (in order to keep the coagulum as intact as possible) and put onto the filter papers placed in a cone which was fixed over a cylindrical graduate. The amount of syneresis was measured in mL at 30th, 60th and 90th minutes (Gönç, 1986).

2.4.2. Apparent viscosity

For the viscosity analysis, Brookefield DV II Pro+ Viscometer (Brookfield Engineering Lab Inc., Stoughton, Mass., U.S.A.) was used. Samples were mixed 20 times (10 times clockwise, 10 times counter clockwise) and measured at 10 rpm in +8°C in mPa (Aryana et al., 2007; Yerlikaya, Akpınar & Kılıç, 2013).

2.4.3.Texture analysis

Brookfield Texture Analyzer (TA - CT3, Brookfield Engineering Laboratories, Inc., Middlebore, MA, USA) (Probe: TA4/1000) was used for the texture analysis (Lee & Yoo, 2011). Samples were analyzed in 150 mL beakers at +8°C. Texture analyzer CT3 was calibrated before use. Parameters for the calibration; Used probe; TA 4/1000, pretest speed: 2 mm/s, test speed: 1 mm/s, spinning speed: 1 mm/s, shape: cylinder, sample length: 76 mm and sample depth: 45 mm.

2.5.Chemical analysis

Dry matter and fat contents of the samples were measured according to TS 1330. Titrable acidity values were measured according to TS 1330 in ºSH (Soxhelet-Henkel) and lactic acid % (Anonymous, 1999). Total nitrogen content of yoghurt samples were analyzed with Kjeldahl method. Protein contents were calculated by multiplying the nitrogen content by 6.38 (Anonymous, 1999; Barbano et al., 1990).

2.5.1.DL Lactic acid

Lactic acid analysis was performed with Assay Proceolurs K-DLATE 12/11 kit (Megazyme, Wicklow, Ireland) (Amatayakul et al., 2006).

2.5.2.Proteolysis

OPA solution was prepared daily and 25 mL 100 milimoles of Sodium tetraborate, 2.5 mL 20 % Sodium dodecyl sulphate, 40 mg OPA solved in 1mL methanol and 100 μm beta-mercaptoethanol were added and diluted in 50 mL water. As substrates, milk proteins (usually 5-100 μg protein in 10-50 μL) were added to 1 mL OPA. Samples were placed in quartz cuvettes of the standard spectrophotometer and kept in room temperature for 2 minutes and absorbance values were read in 340 nm (Church et al., 1983).

2.5.3.Aroma compounds

The volatile compounds of yoghurt samples were determined with a solid-phase-micro extraction (SPME) method using a fiber (57348-U, Supelco Inc., Bellefonte, PA, USA) coated with the sorbent material, divinylbenzene/Carboxen/Polydimethyl-siloxane, and GC-MS (Trace GC Ultra/ISQ, Thermo Scientific, U.S.A.) equipped with flame ionization detector. Prior to GC-MS analysis, yoghurt samples stored at -20°C were conditioned to room temperature. The yoghurt samples (10 mL) were placed into a 20 mL headspace vial containing a micro stirring bar and a PTFE silicone septum was sealed with an aluminum crimp seal. Before extraction, stabilization of the headspace in the vial was obtained by equilibration for 30 min at 60°C. Then SPME fiber was inserted into headspace of the vial and waited for 30 minutes at 60° C for the absorption of volatile compounds. After equilibration time, the fiber was inserted into the GC injector port and held for 5 minutes for desorption of absorbed molecules at 250 °C. The volatile compounds were separated by using 30 m × 0.2 μm i.d. TR-5MS column (Thermo Scientific, U.S.A.) with 0.25 μm film thicknesses. Carrier gas (He) flow rate was 1 mL/min. Oven temperature was programmed as: 40 °C for 5 min then the temperature was raised to 100 °C (4 °C/min) to a final temperature 240 °C (10°C/min) and hold that temperature for 1 min. Volatile compound fractions were expressed as percentage area. The volatile compounds were defined by using the library of GS/MS (NIST and WILEY). Two replicates of each yoghurt sample were analyzed.

2.6.Microbiologic analysis

2.6.1.Preparation of dilutions

8.5 g NaCl were diluted in 1 L pure water. 90 mL of this solution were taken to special glass bottles and 9 mL to test tubes. Bottles and tubes were sealed and sterilized at 121 ºC, 1.1 atm pressure for 15 minutes. Homogenized 10 g probiotic yoghurt samples were added to 90 mL of saline solution and stirred. 1mL of this solution was added to 9 mL saline containing test tubes. Finally, dilutions were prepared in appropriate proportions.

2.6.2.Lactobacillus delbruecki subsp. bulgaricus counts

For *L. bulgaricus* counts, MRS-Agar (Merck, Germany) fixed at pH 5.2 with 1.0 M HCL was used. 1 mL of dilutions were taken to petri dishes and approximately 15-20 mL of MRS-Agar were added and mixed. After gelation of the media, petri dishes were incubated upside down at 43ºC for 3 days in anaerobic conditions. Following the incubation, white colored colonies were counted and determined as *L. bulgaricus* count in CFU/g (Tharmaraj & Shah, 2003).

2.6.3.Streptococcus thermophilus counts

For the determination of *S. thermophilus* counts were determined with M-17 Agar (Merck, Germany) according to pour plate technique. 1 mL of dilutions was taken to petri dishes and approx. 15-20 mL of pre-liquefied M17-Agar at 40 – 45oC were added until forming a thin layer and mixed. After gelation of the media, petri dishes were incubated upside down at 370C for 3 days in aerobic conditions. After the incubation, round shaped yellowish colonies were counted as *S. thermophilus* in CFU/g (Dave & Shah, 1996; Donkor *et al.,* 2006).

2.6.4.Lactobacillus gasseri counts

For *L. gasseri* counts, MRS-Agar (Merck, Germany) fixed at pH 5.2 with 1.0 M HCL was used. After diluted in proper proportions, 1 mL of dilutions was taken to petri dishes and approx. 15-20 mL of MRS-Agar was added until forming a thin layer and mixed. After gelation of the media, petri dishes were incubated upside down at 43ºC for 3 days in anaerobic conditions. Following the incubation, white colored colonies were counted and determined as *L. gasseri* count in CFU/g (Tharmaraj & Shah, 2003).

2.6.5.Bifidobacterium longum counts

For *Bifidobacterium longum* counts, Rogosa Agar was used by adding tetramethybenzidin at 37ºC for 72 hours at microaerophilic conditions. 1 mL of appropriate dilutions was taken to petri dishes and approx. 15-20 mL of Rogosa Agar were added until forming a thin layer and mixed. After gelation of the media, petri dishes were incubated upside down at 37 ºC for 3 days in aerobic conditions. Following the incubation, round shaped yellowish colonies were counted and determined as *B. longum* count in CFU/g (Lapierre, Undeland & Cox, 1992; Vinderola & Reinheimer, 1999).

2.7. Sensory analysis

In order to evaluate the consuming quality of the probiotic yoghurts, sensory analysis were made. For this purpose grading method was used in sensory analysis (Bodyfelt F.W., Drake M.A., Rankin, 1998; Uysal, Kınık & Kavas, 2004). Grading sensory analysis was made with a trained group of panelists consisting the academic staff and graduate students of Ege University Faculty of Agriculture Department of Dairy Technology. For the grading, evaluation criteria specified in TS-1330 were considered. According to these criteria, evaluation forms graded between 1 and 5 were given to the panelists and were asked to fill for the evaluation.

2.8. Statistical analysis

In the study, 4 different yoghurt types were produced in 2 repetitions. For the statistical evaluation of results Analysis of Variance was used and for the determination of different groups Duncan Test was applied. Accordingly, statistical analysis software SPSS version 19.0 was used.

3. Results and discussions

3.1.Physical Properties of Probiotic Yoghurt Samples

3.1.1.Syneresis

The average syneresis values of probiotic yoghurt samples were given in Table 1. On the 30^{th} minute of the measurements, the highest syneresis rate was determined in P2 sample on the 1^{st} day (18.50 mL), while the lowest syneresis rate was in K sample (12 mL) on the 14^{th} and 21^{st} days. As a result of the analysis of variance, the difference between the storage days were significant ($p<0.05$). In the samples containing rice milk, the syneresis rate was lower in P3, compared to the two other samples. Among all the results, difference between P1 sample and K sample was not significant ($p>0.05$). On the 60^{th} minute of the measurements, the highest syneresis rate was in P2 sample on the 1^{st} day (17 mL) while the lowest syneresis rate was seen in K sample (13 mL) on the 21^{st} day. As a result of the analysis of variance, the difference between the samples according to days were be significant ($p<0.05$). On the sample groups P1, P2, P3 differences were associated with rice milk proportions. The difference between K and P1 on the 1^{st} day was not significant ($p>0.05$), whereas on the 7^{th}, 14^{th} and 21^{st} days, the differences between K sample and samples containing rice milk were significant ($p<0.05$).

At the end of the 90^{th} minute, syneresis values varied between 18.50 mL – 26 mL. The highest value was obtained in P2 on the 21st day and the lowest in control sample on the 14^{th} and 21^{st} days of the storage. As a result of the analysis of variance, the difference between the samples according to days were significant ($p<0.05$). The differences between P2 and P3 on the 7^{th} day, K and P1 on the 21^{st} day and, P2 and P3 on the 21^{st} day were statistically not significant ($p>0.05$). The syneresis rates usually had a decline on the 30^{th}, 60^{th} and 90^{th} minutes since the 1st day of the storage. This continued until the last day of the storage. The decline in the syneresis rates in yoghurt samples during storage period were also reported in some of the studies (Atalay, 1994). This was associated with the water holding capacity of proteins, and reported that the water holding capacity of proteins increased as the pH levels decreased down to 4.00. Atamer & Sezgin (1987) also reported a decline during storage periods as the pH dropped to 4.00.

Table 1. The avarage syneresis values of probiotic yoghurt samples (ml) (n=2).

		Storage Period (Day)			
Time (Minute)	**Sample**	**1**	**7**	**14**	**21**
30	**K**	13.25 ± 0.35^{aX}	13.00 ± 0.00^{aX}	12.00 ± 0.00^{aY}	12.00 ± 0.00^{aY}
	P1	13.00 ± 0.00^{a}	12.50 ± 0.70^{a}	11.50 ± 0.70^{a}	11.50 ± 0.70^{a}
	P2	18.50 ± 0.70^{bX}	17.25 ± 0.35^{bXY}	16.75 ± 1.06^{bXY}	16.00 ± 0.00^{bY}
	P3	15.00 ± 0.00^{c}	14.50 ± 0.70^{c}	14.00 ± 0.00^{c}	14.00 ± 0.00^{c}
60	**K**	16.25 ± 0.35^{aX}	15.50 ± 0.70^{aX}	13.50 ± 0.70^{aY}	13.00 ± 0.00^{aY}
	P1	17.00 ± 0.00^{a}	17.00 ± 0.00^{b}	16.75 ± 1.06^{b}	16.50 ± 0.70^{b}
	P2	24.00 ± 0.00^{bX}	23.50 ± 0.70^{cXY}	22.50 ± 0.70^{cYZ}	22.00 ± 0.00^{cZ}
	P3	21.50 ± 0.70^{cX}	20.00 ± 0.00^{dY}	20.00 ± 0.00^{dY}	20.00 ± 0.00^{dY}
90	**K**	19.50 ± 0.70^{a}	19.00 ± 0.00^{a}	18.50 ± 0.70^{a}	18.50 ± 0.70^{a}
	P1	21.00 ± 0.00^{b}	21.00 ± 0.00^{b}	20.75 ± 1.0^{b}	20.50 ± 0.70^{a}
	P2	26.00 ± 0.00^{cX}	25.00 ± 0.70^{cX}	25.00 ± 0.00^{cX}	24.00 ± 0.00^{bY}
	P3	24.00 ± 0.00^{d}	23.00 ± 1.06^{c}	23.00 ± 0.00^{d}	22.75 ± 1.06^{b}

Table 2. The average viscosity values of probiotic yoghurt samples (Pa.s)

	Sample	**Storage Period (day)**			
		1	**7**	**14**	**21**
Viscosity (Pa.s)	**K**	3.52 ± 0.33^{aXY}	3.29 ± 0.03^{aY}	4.32 ± 0.61^{aX}	3.79 ± 0.14^{aXY}
	P1	3.22 ± 0.13^{a}	3.22 ± 0.11^{a}	3.20 ± 0.05^{b}	3.36 ± 0.31^{a}
	P2	1.09 ± 0.08^{bX}	1.43 ± 0.24^{bXY}	1.53 ± 0.08^{cY}	1.63 ± 0.13^{bY}
	P3	0.85 ± 0.03^{b}	0.83 ± 0.09^{c}	0.82 ± 0.15^{c}	0.89 ± 0.25^{c}

3.1.2.Apparent viscosity values

The average viscosity values of probiotic yoghurt samples were given in Table 2. At the end of the 21 days of the storage period, viscosity of the samples were found between 0.89 and 3.43 Pa. The highest value was obtained in control sample (4.22) on the 14th day, and the lowest in P3 (0.87) again at the 14th day of the storage. The difference between the storage days were found to be significant ($p<0.05$).

On the first day the difference between K and P1 and the difference between P2 and P3, on the 7th day the difference between K and P1, on the 14th day the difference between P2 and P3 and on the 21st day the difference between K and P1 were found to be statistically not significant ($p>0.05$). As seen on the table, since the first day, the viscosity of probiotic yoghurt samples increased. During the storage period, the values for syneresis decreased, while the viscosity values increased. In other words, for obtaining the firm structure, syneresis decreased while the viscosity increased. The reason for this, as Akin & Konar (1999) explained, is the increase in the water holding capacity of proteins during the storage period and tightening of the gel structure during the storage period.

Table 3. Textural properites of probiotic yoghurt samples

Parameter	Sample	Storage Period (day)			
		1	7	14	21
Hardness (g)	K	233.25±76.01^{a}	241±95.45^{a}	242.5±96.87^{a}	194±125.86
	P1	124.75±34.29^{ab}	124.25±39.24^{ab}	125±41.71 ab	106.75±67.52
	P2	66.75±21.56^{b}	68.5±23.33^{b}	67.5±26.16 b	65.75±18.03
	P3	39.25±2.47^{b}	39±2.82^{b}	38.75±1.76 ^{b}	40±0.70
Consistency (mj)	K	21.08±5.72^{a}	22.20±8.32^{a}	22.53±8.00 ^{a}	18.95±12.31
	P1	12.51±4.49^{ab}	12.68±4.86^{ab}	12.89±4.99 ^{ab}	10.81±8.29
	P2	5.98±2.18^{b}	6.16±2.49^{b}	6.11±2.63 ^{b}	6.00±2.15
	P3	3.78±0.17^{b}	3.76±0.29^{b}	3.73±0.29 ^{b}	3.85±0.11
Cohesion Force (g)	K	39 ± 10.60^{a}	29.25 ± 41.36	48.75 ± 21.56	44.75 ± 23.68
	P1	35 ± 14.84^{ab}	23.25 ± 30.05	23 ± 31.11	24 ± 33.94
	P2	11 ± 14.14^{ab}	0.5 ± 0.70	10.25 ± 14.49	0.50 ± 0.70
	P3	4.0 ± 5.65^{b}	1.0 ± 0	1.25 ± 1.761	0.25 ± 0.35
Cohesiveness (mj)	K	3.38 ± 1.08	2.5 ± 3.52	3.26 ± 1.64	3.37 ± 0.96
	P1	3.56 ± 2.05	2.75 ± 3.88	2.3 ± 3.24	2.42 ± 3.42
	P2	1.32 ± 1.86	0 ± 0	1.46 ± 2.06	0 ± 0
	P3	1.02 ± 1.43	0 ± 0	0.43 ± 0.60	0 ± 0

Table 4. Dry matter, fat and protein content of probiotic yoghurt samples

	Sample	Storage Period (Day)	
		1	21
Dry matter	K	16.43 ± 0.05^{a}	16.30 ± 0.06^{a}
	P1	16.13 ± 0.15^{a}	16.14 ± 0.10^{a}
	P2	15.47 ± 0.28^{b}	15.32 ± 0.28^{b}
	P3	14.30 ± 0.16^{c}	14.34 ± 0.24^{c}
Fat	K	3.25 ± 0.06^{a}	3.14 ± 0.02^{a}
	P1	3.15 ± 0.01^{b}	3.11 ± 0.01^{ab}
	P2	3.05 ± 0.01^{c}	3.07 ± 0.01^{bc}
	P3	2.94 ± 0.01^{d}	2.90 ± 0.01^{d}
Protein	K	3.43±0.05^{a}	3.42±0.03^{a}
	P1	3.28±0.02^{b}	3.31±0.04^{b}
	P2	2.88±0.01^{cX}	2.82±0.02^{cY}
	P3	2.53±0.01^{dX}	2.57±0.02^{dY}

3.1.3. Texture

Firmness is the force to be applied to a food material in order to provide a certain deformation. In our study, the average firmness values of probiotic yoghurt samples were given in grams (g) and shown in Table 3 with the standard deviations. The firmness values of samples varied between 41.13 g and 240.88 g. The highest value was obtained in control sample on the 14th day and the lowest in P3 on the 14th day of the storage. The difference between the different storage days were not significant ($p>0.05$). In the samples containing rice milk, the firmness values decreased as the ratio of the mixture increased. During the storage period, firmness values of the samples decreased. Regarding the increase in denaturation of whey proteins and heat process applied, it was determined that, as hydrophilic properties increased up to a certain level, the firmness increased and the storage period also caused an increase in firmness. Additionally, low acidity affected the water holding capacity

of proteins and the firmness negatively. Water holding capacity of proteins decreased in high acidity as well, causing shrinkages in gel formation and syneresis. Between pH 4.6 - 4.0 water holding capacity increases and syneresis does not occur (Atamer & Sezgin, 1986). Homogenization also affects the firmness of yoghurt structure, the firmness increase as the homogenization pressure applied increase.

Consistency is the energy needed to initiate the flow. The average cohesiveness values of probiotic yoghurt samples with standard deviations were given in mj (milijoule) in Table 3. Consistency values of probiotic yoghurt samples varied between 3.77 – 24.41 mj. The highest value was obtained in control sample on the 14th day, and the lowest in P2 on the 14th day of the storage. The difference between the storage days were not significant ($p>0.05$). Herrero & Requena (2005), in their study focusing on production of yoghurts from goat milks fortified with whey concentrate in a ratio of 1 %, reported that whey concentrate has a positive effect on the structure and consistency of yoghurt products. In a similar study, using whey concentrates caused an increase in the firmness and dry matter of the yoghurt products, and consistency deformations due to over softening of yoghurt samples fortified with 15 % whey concentrate occurred and weak flavor formation was observed (Tosun, 2007).

The average cohesiveness values of probiotic yoghurt samples with standard deviations were given in Table 3These textural values varied between 1.16 and 41.82 g. The highest value was obtained in control sample on the 14th day, and the lowest in P3 on the 21st day of the storage. The cohesion force of K sample increased from the 1st day of the storage, where P1, P2 and P3 samples decreased.

Cohesiveness is the work done to break the attraction force of the surface (tongue, tooth, palate or probe) in contact. Cohesiveness values of probiotic yoghurt samples varied between 0.30 – 5.08 mj. The highest value was obtained in P1 on the 1st day, and the lowest in P2 and P3 on the 7th and 14th days of the storage The difference between the storage days were not significant ($p>0.05$). K and P1 had higher values compared to others, where P2 and P3 had changing values with descents and ascents.

3.2.Chemical Properties of Probiotic Yoghurt Samples

3.2.1.Total dry matter

Total dry matter analysis of the probiotic yoghurt samples were made at the 1st and 21st days of the storage. The results with standard deviations were given in Table 4. The difference between the dry matter contents of probiotic yoghurt samples were statistically not significant on the 1st and 21st days of the storage ($p>0.05$). Dry matter contents of samples varied between 14.30 – 16.43 %. The results obtained were similar to those of Akalin (1993) and Dave & Shah (1997a; 1997b) (%15.30-15.80). The dry matter contents of the yoghurt-like fermented dairy products vary in a wide range. The dry matter results reported by other researchers and our current results share partial similarities but some differences. The types of raw milk used in the production, dry matter, rice milk, the process applied during the production and degree of cultures to ferment lactose are the factors that affect the dry matter contents.

3.2.2.Fat

Fat content of the probiotic yoghurt samples were measured at the 1st and 21st days of the storage. The results with standard deviations were given in Table 4. Fat contents of samples varied between 3.25 – 2.90 %. The difference between the fat contents of probiotic yoghurt samples were statistically not significant at the 1st and 21st days of the storage ($p>0.05$).

3.2.3.Total protein

Total protein analysis of the probiotic yoghurt samples were made at the 1st and 21st days of the storage. The results with standard deviations were given in Table 4. At the end of the 21 days of the storage period, protein

contents of the samples found between 2.53 and 3.43 %. The difference between the protein contents of probiotic yoghurt samples on the 1st and 21st days of storage found to be significant ($p<0.05$). The protein contents of K and P1 samples were found complying with the rates (3% minimum) specified in Fermented Milks Regulations, where P2 and P3 were found lower than the rates specified. In previous studies protein contents were found between 2.66 % and 8.38 % (Yaygın, 1981; Akin & Konar, 1999; Küçüköner & Tarakçı, 2003; Ayar, Sert & Kalyoncu, 2006). Protein values found in our study had similarities with these results.

3.2.4.Titratable acidity

Titratable acidity values of probiotic yoghurt samples in lactic acid % and (°SH) were given below.

Lactic acid

The average lactic acid % values of probiotic yoghurt samples with standard deviations were given in Table 5. On the 1st, 7th, 14th and 21st days, the difference between lactic acid (%) values were found insignificant ($p>0.05$). Although the differences between storage days were not significant, lactic acid levels of the samples increased during the storage period. Lactic acid (%) values of probiotic yoghurt samples varied between 0.41 and 1.14 % during the storage. According to the Turkish Food Codex Fermented Milks Regulations, lactic acid levels shall be between 0.6 - 1.5 %. The levels of K, P1 and P2 have complied with the regulations and kept their compliance during storage period. The highest level of lactic acid was usually measured in sample K, where the lowest was P3. In all the samples, as a result of periodical increase during the storage period, total acidity values increased. This was associated with the continuous acid production by culture bacteria. The change in acidity after incubation is important in terms of determining the shelf life of the products. Also, the increase in the titratable acidity may be due to the increase in the levels of protein, phosphate, citrate, lactate and some minerals, as well as dry matter (Tamime & Robinson, 1999). In a study on yoghurts produced from soy milk, lactic acid levels in samples containing soy milk were reported lower (Lee & Yoo, 2011). In a similar study, syneresis due to the increase in lactic levels in yoghurts affected the consumability of the yoghurts significantly. It was reported that lactic acid levels in yoghurts produced exclusively from soy milk were considerably lower than those of produced from cow milk, additionally; lactic acid levels in soy milk yoghurts were increased to standard yoghurt levels by adding ingredients such as milk powder (Granta & Morr, 1996).

Soxhelet Henkel (°SH) acidity

The average °SH values of probiotic yoghurt samples were given in Table 5, with standard deviations. °SH values varied between 16.5 and 45.5. The highest value was obtained in control sample on the 21st day, while the lowest in P3 on the 1st and 7th days of the storage. Although the °SH levels of samples increased during the storage, the effect of the storage period were not significant ($p>0.05$). Among the groups, the difference between the °SH levels were significant ($p<0.05$). Only on the 7th day, the difference between K and P1 samples were not significant. Various researchers reported the °SH levels in their studies between 39.19 and 53.55 °SH (Yaygin, 1981; Akin & Konar, 1999; Akalin, 1993; Sarı, 2005; Tosun, 2007; Yalçınkaya,2002). Akın & Konar (1999), in their study, produced fruit added/flavored yoghurts from cow and goat milks and stored for 15 days and reported an increase in titratable acidity during the storage period.

3.2.5.DL Lactic acid

Lactic acid, which is formed as a result of fermentation of lactose, has three isomers, ; L(+) which rotates the light clockwise direction, D (-) which rotates the light counterclockwise direction and DL with no optical activity. This is related with the location

of the hydroxyl groups on the 2nd carbon atom. If the hydroxyl group is on the right, called D(-), and if on the right, it is called L(+). Lactic acid isomers vary depending on the starter cultures used in the production of dairy products. In the studies, it is found that *L. casei*, *S. thermophilus*, *L. lactis*, *L. cremoris*, *L. diacetylactis*, *Bifidobacterium bifidum* produce L(+), *L. bulgaricus* D(-) and *Lactobacillus helveticus* and *Lactobacillus acidophilus* DL lactic acid.

Lactic acid isomers have positive effects on human health as well as maintaining the typical formations desired in dairy products. One of the two stereo isomer forms of lactic acid, L(+) lactic acid, is formed as an intermediary product of human metabolism, and then it is partially hydrolyzed to CO_2 and H_2O, and used as an energy source, also partially used in glycogenesis for the formation glycogens. Therefore, L(+) lactic acid is defined as physiologic lactic acid. On the contrary, D(-) lactic acid is metabolized slowly and insufficiently in the organism, causing a burden for the organism.

DL lactic acid values of probiotic yoghurt samples were given in Table 5. DL values of samples varied between 0.44 mg and 1.21 mg. The highest value was obtained in P3 on the 1st day and the lower in control sample on the 21st day of the storage. As a result of the analysis of variance, the difference between the storage days were found to be significant ($p<0.05$). The difference between K and P1, P2 and P3 at the 1st day and the difference between K and P1 samples at the 14th day were statistically not significant ($p>0.05$).

Table 5. Some physic-chemical properties of probiotic yoghurt samples

Parameter	Sample	Storage Period (day)			
		1	7	14	21
Acidiy (Lactic acid%)	K	1.11 ± 0.01^{a}	1.09 ± 0.05^{a}	1.13 ± 0.10^{a}	1.14 ± 0.05^{a}
	P1	0.90 ± 0.07^{b}	0.95 ± 0.00^{a}	0.95 ± 0.03^{b}	0.93 ± 0.03^{b}
	P2	0.66 ± 0.053^{c}	0.64 ± 0.08^{b}	0.70 ± 0.03^{c}	0.70 ± 0.00^{c}
	P3	0.41 ± 0.01^{d}	0.41 ± 0.05^{c}	0.43 ± 0.00^{d}	0.43 ± 0.03^{d}
Acidity (ºSH)	K	44.50 ± 0.70^{a}	43.50 ± 2.12^{a}	45.00 ± 4.24^{a}	45.50 ± 2.12^{a}
	P1	36.00 ± 2.82^{b}	38.00 ± 0.00^{a}	38.00 ± 1.41^{b}	37.00 ± 1.41^{b}
	P2	26.50 ± 2.12^{c}	25.50 ± 3.53^{b}	28.00 ± 1.41^{c}	28.00 ± 0.00^{c}
	P3	16.50 ± 0.70^{d}	16.50 ± 2.12^{c}	17.00 ± 0.00^{d}	17.00 ± 1.41^{d}
Acetaldehyde (ppm)	K	16.81 ± 2.15^{W}	11.5 ± 0.61^{aX}	8.89 ± 0.43^{aY}	6.82 ± 0.46^{aZ}
	P1	16.71 ± 2.09^{W}	11.427 ± 0.21^{aX}	9.05 ± 0.41^{abY}	6.75 ± 0.24^{aZ}
	P2	15.5 ± 1.16^{W}	10.96 ± 0.08^{abX}	8.22 ± 0.35^{bcY}	6.44 ± 0.20^{abZ}
	P3	14.46 ± 1.00^{W}	10.625 ± 0.16^{bX}	7.72 ± 0.63^{cY}	6.11 ± 0.38^{bZ}
DL Lactic acid (mg/100g)	K	0.92 ± 0.05^{aW}	0.77 ± 0.02^{aX}	0.50 ± 0.03^{aY}	0.44 ± 0.02^{aZ}
	P1	1.03 ± 0.08^{abW}	0.84 ± 0.01^{bX}	0.59 ± 0.03^{aY}	0.51 ± 0.03^{bZ}
	P2	1.11 ± 0.09^{bcW}	0.94 ± 0.04^{cX}	0.69 ± 0.05^{bY}	0.56 ± 0.03^{cZ}
	P3	1.21 ± 0.04^{cW}	0.98 ± 0.04^{cX}	0.81 ± 0.08^{cY}	0.65 ± 0.01^{dZ}
Proteolytic activity (OPA Value)	K	0.93 ± 0.02^{bcX}	1.06 ± 0.02^{aW}	0.88 ± 0.01^{abY}	0.742 ± 0.02^{bZ}
	P1	0.99 ± 0.03^{aX}	1.02 ± 0.05^{aX}	0.85 ± 0.04^{aY}	0.66 ± 0.02^{cZ}
	P2	0.97 ± 0.03^{abX}	0.90 ± 0.07^{bX}	0.91 ± 0.01^{bX}	0.80 ± 0.04^{abY}
	P3	0.89 ± 0.04^{cX}	1.10 ± 0.02^{aY}	0.92 ± 0.03^{bX}	0.81 ± 0.05^{aZ}

3.2.6.Proteolytic activity

OPA (ortho-phthalaldehyde), (ortho-phthalaldehyde) is the chemical compound with the formula $C_6H_4(CHO)_2$. The molecule is a dialdehyde, consisting of two formyl (CHO) groups attached to adjacent carbon centers on a

benzene ring. The molecule was first described in 1887 when it was prepared from α,α,α',α'-tetrachloro-ortho-xylene.α,α,α',α'-tetrachloro-ortho-xylene. Related to ortho-phthalaldehyde are the meta- and para-isomers, which are respectively named isophthalaldehyde and terephthalaldehyde. It is sensitive to UV illumination and air oxidation. In dairy industry, proteolysis occurring during the production of milk and milk products have both positive and negative effects. The negative effects of proteolysis on milk and dairy products during storage is related to heat resistant alkaline milk proteinases, storing milk for an extended length of time and heat resistant proteinases produced by psychotropic microorganisms. Therefore OPA is essential regarding the determination of proteinase and proteolysis tracking. OPA values of probiotic yoghurt samples were given in Table 5. OPA levels of samples varied between 0.66 – 1.10. The highest value was obtained in P3 on the 7th day, and the lowest in P1 on the 21st day of the storage. As a result of the analysis of variance, the difference between the storage days were found to be significant ($p<0.05$). Also, the difference between different periods were significant ($p<0.05$). The difference between K and P2, P1 and P2, K and P3 among each other on the 1st day, K, P1 and P3 among each other on the 7th day, K and P1, K, P2 and P3 among each other on the 14th and K and P2, P2 and P3 among each other on the 21st day were not significant, whereas the difference between all the samples among each other were statistically significant ($p<0.05$).

Table 6. Microbiological properties of probiotic yoghurt samples (CFU/g)

Bacteria	Sample	Storage Period (day)			
		1	7	14	21
***Lactobacillus bulgaricus* (CFU/g)**	**K**	8.48 ± 0.12^{aX}	7.45 ± 0.09^{aY}	7.44 ± 0.04^{aY}	6.57 ± 0.05^{aZ}
	P1	5.92 ± 0.03^{bX}	5.58 ± 0.08^{bY}	5.24 ± 0.06^{bZ}	5.06 ± 0.07^{bZ}
	P2	5.65 ± 0.04^{cX}	5.47 ± 0.06^{bX}	5.23 ± 0.07^{bY}	4.92 ± 0.08^{bZ}
	P3	4.94 ± 0.04^{dX}	4.66 ± 0.06^{cY}	4.31 ± 0.05^{cZ}	4.25 ± 0.04^{cZ}
***Streptococcus thermophilus* (CFU/g)**	**K**	9.75 ± 0.09^{aX}	8.33 ± 0.12^{aY}	8.2 ± 0.02^{aY}	8.13 ± 0.04^{aY}
	P1	8.57 ± 0.07^{bX}	8.4 ± 0.07^{aXY}	8.21 ± 0.07^{aYZ}	8.06 ± 0.07^{aZ}
	P2	7.27 ± 0.07^{cX}	6.42 ± 0.02^{bY}	6.43 ± 0.02^{bY}	6.36 ± 0.05^{bY}
	P3	6.94 ± 0.05^{dW}	5.94 ± 0.02^{cX}	5.66 ± 0.06^{cY}	5.19 ± 0.02^{cZ}
***Lactobacillus gasseri* (CFU/g)**	**K**	7.34 ± 0.03^{aY}	7.60 ± 0.02^{aX}	7.58 ± 0.00^{aX}	7.67 ± 0.06^{aX}
	P1	7.48 ± 0.02^{aY}	7.31 ± 0.07^{bYZ}	7.15 ± 0.04^{bZ}	7.75 ± 0.14^{aX}
	P2	6.17 ± 0.10^{bY}	6.09 ± 0.04^{cY}	5.29 ± 0.03^{cZ}	6.41 ± 0.00^{bX}
	P3	6.9 ± 0.11^{cX}	6.67 ± 0.07^{dY}	6.62 ± 0.02^{dY}	6.96 ± 0.02^{cX}
***Bifidobacterium longum* (CFU/g)**	**K**	8.77 ± 0.07^{aX}	8.67 ± 0.06^{aX}	8.15 ± 0.01^{aY}	7.6 ± 0.02^{aZ}
	P1	8.93 ± 0.02^{bW}	8.41 ± 0.21^{aX}	7.93 ± 0.03^{bY}	7.29 ± 0.04^{bZ}
	P2	7.33 ± 0.01^{cW}	6.51 ± 0.01^{bX}	6.19 ± 0.04^{cY}	6.85 ± 0.04^{cZ}
	P3	6.40 ± 0.00^{dW}	5.91 ± 0.00^{cX}	5.23 ± 0.02^{dY}	5.07 ± 0.01^{dZ}

3.2.7.Aroma compounds

Flavor is one of the most important properties of food products and is an important factor determining its acceptability and preference. The sensory properties of dairy products depend largely on the relative balance of flavor compounds derived from fat, protein or carbohydrate in the milk types (Cheng, 2010; Routray & Mishra, 2011). During storage, the volatile constituents in yoghurt may differ depending on the culture, mix formulation, milk type and the storage conditions. Table 6 summarizes the main aroma compounds identified in yoghurts produced from cow milk and cow/rice milk mixtures. Quantitatively, the major volatile compound in

the headspace and contributing to the flavor of yoghurt samples appeared to be 2-3 butanedione, acetoin, methyl benzene, 3,4 dihydroxyphenethyl alcohol and isoamyl hexanoate. Ketones and aromatic compounds mentioned above are common constituents of yoghurt samples as volatile compounds. However, some methyl ketones were not detected in all yoghurt samples. Even though di ketone di acetyl (2,3 butanedione) significantly varied from sample to sample. Monnet & Corrieu (2007) indicated that di ketones in yoghurt come only from pyruvate, since thermophilic starter cultures are not able to metabolize citrate. S. thermophilus strains process an α-acetolactate synthase and acetohydroxy acid synthase which produce α-acetolactate and 2-hydroxyacetolactate respectively from pyruvate (Cheng, 2009; Güler & Park, 2011). These two α-acetoacids are generally metabolized into neutral compounds to protect pH homeostasis by decarboxylation. Also, they could be converted into branched-chain amino acids such as valine, leucine or isoleucine. Also, diacetyl/2-3 butanedione was negatively related to acetoin as mentioned earlier (Warsy, 1983; Güler et al., 2009; Güler & Park, 2011). This may be sourced to the reduction of diacetyl to acetoin. There were significant differences in acetoin concentration in yoghurt samples. This compound is derived from β-oxidation of saturated free acids depending on the lipolytic activity of yoghurt starters (Tsau, Guffanti & Montville, 1992; Güler & Park, 2011; Routray & Mishra, 2011).

Acetaldehyde, acetoin, acetone and diacetyl of carbonyl compounds are main flavor components of yoghurt. But, many researchers indicated the importance of acetaldehyde for a favorable flavor in yoghurt (Tamime & Deeth, 1980). Heating the milk at high temperatures, increase in dry matter content, milk or milk powder addition, type of milk and properties of yoghurt bacteria have effects on the acetaldehyde content (Yaygın, 1981). The average acetaldehyde values of probiotic yoghurt samples are given in Table 5. Acetaldehyde levels of samples varied between 6.11 and 16.81 ppm. The highest value was obtained in control sample on the 1st day, and the lowest in P3 on the 21st day of the storage. The difference between the samples according to 21 days of storage were significant ($p<0.05$). Acetaldehyde contents tended to decrease since the 1st day of storage. It is reported that the decrease in the acetaldehyde contents during the storage period is related to reduction of acetaldehyde to ethyl alcohol (Tamime & Deeth, 1980).

Robinson et al. (1977) reported that high quality yoghurt contains 27.6 ppm acetaldehyde, whereas Rasic & Kurmann (1987) reported that this value should be between 23-41 ppm. Beyatli & Tunail (1980) reported that the acetaldehyde levels of yoghurts in Turkish markets varied between 12.28 and 34.72 ppm and in yoghurts produced with selective cultures according to their acid production and proteolytic activity changed between 19.14-32.21 ppm. In another study, it was reported that acetaldehyde levels in bio-yoghurts produced with *S. thermophilu*s and *L. acidophilus*, for a favorable flavor, should be between 3-5 ppm (Sezgin, Yıldırım & Karagül, 1994). Quantitavely, the major volatile compound in the headspace and contributing to the flavor of set type yoghurt and Turkish yoghurt appeared to be acetaldehyde which was mentioned by other researchers (Kneifel et al., 1992; Ott, Germond & Chaintreau, 2000). The acetaldehyde contents of yoghurt samples have changed during storage. During storage, these non-regular changes in acetaldehyde may depend on the culture, mix formulation and storage conditions (Brauss et al., 1999; Tamime & Robinson, 1999). Also the lower concentration of acetaldehyde may be related to the nonstarter lactic acid bacteria. There were significant variations in hexanal and 2 heptanal concentrations in yoghurts. Level of hexanal concentration decreased during storage in P1 yoghurts and P3 yoghurt samples, however, hexanal concentration increased until the 7th day then reduced until 21st day of storage in control group. In comparison of all samples,

hexanoic acids initially increased in all samples after the first day, then increases were observed only in control samples during all storage days. The carbonyl compounds cover aldehydes, ketones etc. also related to the fat contents of fermented milks. Stelios et al. (2007) found that carbonyl compounds, especially ketones, increased in yoghurts depending on the increase in fat content and storage time. 2-3 Pentanedione was changed in all samples during storage. The highest 2-3 pentanedione level was observed in control compared to P2 sample. However, the lowest 2-3 pentanedione level was found in P2 sample at 1st and P3 sample at 21st day of storage.

The other main aromatic volatile compounds such as methyl benzene, methyl 2 benzoate showed significant differences between yoghurt samples. These volatile aromatic compounds may be derived from oxidation of carboxylation or naturally occurred depending on the activity of yoghurt strains. Additionally, Stelious et al. (2007) mentioned that these volatiles may be related in yoghurts depending on the composition of milk and storage time. According to our results; differences between studies may be associated with the factors including the starter culture, synergistic effects of the microflora, fermentation conditions and the composition parameters of milk used in yoghurt production. Furthermore, the applied analytical method may also be a source of divergent volatile compound concentrations.

3.3.Microbiological Properties of Probiotic Yoghurt Samples

3.3.1.Lactobacillus bulgaricus counts

The average *L.bulgaricus* counts of probiotic yoghurt samples were given in Table 7. The average *L. bulgaricus* counts of probiotic yoghurt samples varied between 4.25 – 8.48 log CFU/g. The highest value was obtained in control sample on the 1st day, and the lowest in P3 on the 21st day of the storage. The difference between the storage days were found to be significant ($p<0.05$). *S. thermophilus* and *L. bulgaricus* present in the traditional yoghurt culture have a symbiotic living and during the fermentation, first *S. thermophilus* and then *L. bulgaricus* get active. Our results were similar to those by Medina & Jordano (1994), Akalin (1993) and Donkor et al. (2006).

3.3.2.Streptococcus thermophilus counts

The average *S. thermophilus* counts of probiotic yoghurt samples were given in Table 7. The average *S. thermophilus* counts of probiotic yoghurt samples varied between 5.19 – 9.75 log CFU/g. The highest value was obtained in control sample on the 1st day and the lowest in P3 on the 21st day of the storage. The difference between the storage days were significant ($p<0.05$). The *S. thermophilus* counts obtained in our study was similar to those reported by Scmazny & Reinartz (1982), Akalin (1993), Vinderola et al. (2000), Oliveira et al. (2002), and lower than those reported by Fenderya (2002) and Mada (1981). These differences may be due to type and ratio of culture used (DVS or liquid), strains, incubation temperature and microorganisms present in culture combination, production methods, and media used in determination of microorganism counts. Considerably lower live counts determined in fermented dairy products produced with liquid cultures than those of produced with freeze dried cultures may cause different results in different stages of the researches.

3.3.3.Lactobacillus gasseri counts

The average *L. gasseri* counts of probiotic yoghurt samples were given in Table 7. The average *L. gasseri* counts of probiotic yoghurt samples varied between 5.29 – 7.75 log CFU/g. The highest value was obtained in P1 on the 1st day and the lower in P2 on the 21st day of the storage. As a result of the analysis of variance, the difference between the storage days were found to be significant ($p<0.05$).

3.3.4.Bifidobacterium longum counts

The average *B. longum* counts of probiotic yoghurt samples were given in Table 7. The

average *B. longum* counts of probiotic yoghurt samples varied between 5.07 – 8.93 CFU/g. The highest value was obtained in P1 on the 1st day and the lowest in P3 on the 21st day of the storage. The difference between the storage days were significant ($p<0.05$). *Bifidobacterium* ssp. counts in our studies were similar to those by Mada (1981) and Kim et al. (1992), lower than those reported by Sonoike et al. (1986) and Dave & Shah (1997a). These differences may be due to the different strains used in production, different production methods (cystein, ascorbic acid addition etc.), inoculation ratios, the temperature and duration of incubation and different microorganisms found in the production. Also, Dave & Shah (1997a) reported that, polysaccharide production by *S. thermophilus* during fermentation may suppress the growth of Bifidobacterium ssp.

3.4.Sensory Evaluations of Probiotic Yoghurt Samples

The sensory analysis of samples was performed using grading method according to TSE criteria. An ideal yoghurt is clean, with a bright appearance, having a milkish color (pale yellowish in none homogenized, porcelain white in homogenized), no cracks or gas bubbles, consistent, a viscose structure after stirring, low syneresis and characteristic odor and flavor (Anonymous, 1999).

3.4.1.Appearance

Our probiotic yoghurts were graded out of 5 and evaluated according to their state of being clean, bright, milk colored, having no syneresis, cracks and gas bubbles and being homogenous. The average appearance values of probiotic yoghurt samples were given in Table 8. The appearance values for sensory properties of probiotic yoghurt samples varied between 2.11-5.00. The highest value was obtained in control sample on the 21st day, and the lowest in P3 on the 1st day of the storage. The difference between the different storage days were not significant ($p>0.05$). K and P1 had close grades, where P2 and P3 had lower. In all the samples, appearance grades were the highest on the 21st day of the storage. Rasic & Kurman (1987) reported that protein hydration and gel formation which effects the appearance occured after a length of time.

3.4.2.Consistency

Consistency evaluations were graded out of 5, according to their smoothness and meaty consistency, viscose structure after stirring, having no syneresis and easy dispersion in the mouth. The average cohesiveness values of probiotic yoghurt samples were given in Table 8. The consistency values for sensory properties of probiotic yoghurt samples varied between 1.67 – 4.82. The highest value was obtained in control sample at the 21st day, and the lowest in P3 at the 1st day of the storage. The difference between the different storage days were not significant ($p>0.05$). K and P1 had close grades, where P2 and P3 had lower. Although the panelists have been trained, a slight vision of flaw in appearance might have stimulated the panelist for grading low. Therefore, our consistency values show a great range.

3.4.3.Odor

Odor evaluations were graded out of 5 according to the characteristic odor of yoghurt. The average cohesiveness values of probiotic yoghurt samples were given in Table 8. The odor values for sensory properties of probiotic yoghurt samples varied between 3.05-5.00. The highest value was obtained in control sample at the 21st day, and the lowest in P3 at the 21st day of the storage. The difference between the storage days were found to be not significant ($p>0.05$).

3.4.4.Flavor

Flavor evaluations were graded out of 5 according to the characteristic flavor of yoghurt. The average cohesiveness values of probiotic yoghurt samples were given in Table 8. The flavor values for sensory properties of probiotic yoghurt samples varied between 2.11 – 4.86. The highest value was obtained in

control sample at the 14th day, and the lowest in P3 at the 1st day of the storage.

The difference between the storage days were not significant ($p>0.05$). There were limited sources on the evaluation of sensory properties of probiotic yoghurts in the literature, usually results on sensory properties and flavor components concentration of yoghurt products were available. Akalin (1993), reported that the products that gained the highest flavor and odor scores were Bio-yoghurt and Bifi-yoghurt, where products showed no significant consistency and appearance differences, but the researcher determined a decrease in sensory evaluation grades at the 28th day of the storage, similar to our results.

Table 8. Sensorial properties of probiotic yoghurt samples

Sensory Criteria	Sample	Storage Period (day)			
		1	7	14	21
Appearance	**K**	4.72±0.21^{a}	4.78±0.04^{a}	4.86±0.01^{a}	5.00±0.00^{a}
	P1	4.33±0.58^{a}	4.55±0.27^{a}	4.57±0.20^{ab}	4.6±0.15^{ab}
	P2	3.20±1.30^{ab}	3.28±0.65^{b}	3.50±0.70^{bc}	3.96±0.45^{b}
	P3	2.11±0.36^{b}	2.48±0.15^{b}	2.64±0.30^{c}	2.78±0.50^{c}
Consistency	**K**	4.38±0.24^{a}	4.63±0.06^{a}	4.65±0.31^{a}	4.82±0.25^{a}
	P1	4.14±0.50^{a}	4.42±0.28^{a}	4.35±0.30^{a}	4.57±0.20^{a}
	P2	2.93±1.32^{ab}	2.92±0.54^{b}	2.92±0.70^{b}	3.46±0.45^{b}
	P3	1.67±0.45^{b}	2.01±0.15^{c}	2.35±0.10^{b}	2.21±0.30^{c}
Odour	**K**	4.81±0.26^{a}	5.06±0.08^{a}	4.85±0.20^{a}	5.00±0.00^{a}
	P1	4.37±0.35^{ab}	4.52±0.03^{b}	4.64±0.10^{a}	4.57±0.00^{a}
	P2	3.59±0.83^{ab}	3.81±0.00^{c}	3.26±0.02^{b}	3.92±0.30^{b}
	P3	3.05±0.27^{b}	3.41±0.31^{c}	3.21±0.10^{b}	3.03±0.25^{c}
Flavor	**K**	4.58±0.28^{a}	4.81±0.26^{a}	4.86±0.01^{a}	4.67±0.05^{a}
	P1	4.31±0.34^{ab}	4.22±0.12^{a}	4.32±0.05^{a}	4.03±0.25^{a}
	P2	3.30±0.53^{b}	3.34±0.30^{b}	2.85±0.80^{b}	3.14±0.40^{b}
	P3	2.11±0.36^{c}	2.30±0.18^{c}	2.85±0.00^{b}	2.42±0.40^{b}
General Evaluation	**K**	4.52±0.03^{a}	4.85±0.14^{a}	4.78±0.10^{a}	4.87±0.17^{a}
	P1	4.14±0.41^{a}	4.35±0.11^{a}	4.42±0.00^{a}	4.39±0.45^{ab}
	P2	3.11±0.99^{ab}	3.22±0.38^{b}	2.78±0.70^{b}	3.53±0.65^{bc}
	P3	2.06±0.26^{b}	2.33±0.31^{c}	2.20±0.11^{b}	2.57±0.40^{c}

3.4.5.General evaluation

General evaluation was obtained by calculating the average values of all sensory parameters of the samples. The average cohesiveness values of probiotic yoghurt samples with standard deviations were given in Table 7. The general evaluations for sensory properties of probiotic yoghurt samples varied between 2.06 - 4.87. The highest value was obtained in control sample at the 21st day, and the lowest in P3 at the 1st day of the storage., The difference between the different storage days were found to be not significant ($p>0.05$). Among all the groups, control group had the highest points, where rice milk added samples had a decline with inverse proportions with their rice milk content. The reasons why samples with rice milk had lower points (especially P2 and P3) are that the rice milk has a sweet flavor and it cannot provide the desired consistency and appearance in yoghurt. This sweet flavor can be sensed slightly in samples with lower rice milk content (P1 and P2), whereas it was felt intensely in sample with high rice milk content (P3).

Table 7. Aroma compounds of probiotic yoghurt samples

Compounds	RT (min)	Control				P1				P2				P3			
	Days	1	7	14	21	1	7	14	21	1	7	14	21	1	7	14	21
2-Ethyl-N-methyl-1-hexanamine	1.53	1.61	1.85	0.90	1.22	1.61	0.91	1.84	1.68	3.78	2.59	1.61	1.50	2.79	1.96	2.05	1.43
Acetaldehyde	1.61	2.39	2.11	1.57	1.63	1.52	1.89	1.49	1.58	2.92	1.69	2.15	1.59	0.75	0.93	0.91	0.65
2-Fluoropropan-1-ol	1.70	ND	ND	ND	0.94	0.71	0.94	0.36	0.57	1.69	0.80	1.19	1.09	1.39	1.32	1.70	1.30
Methyl acetoacetate	1.80	3.97	3.87	3.75	4.89	2.76	3.20	2.74	3.47	3.54	2.22	2.21	3.92	1.90	1.86	1.92	1.74
Acetic acid	2.16	4.06	3.06	2.52	3.05	2.30	3.93	3.03	4.48	1.38	2.29	3.98	2.13	1.23	1.18	ND	ND
2,3-Butanedione	2.23	7.88	6.95	7.62	9.32	4.53	6.93	5.82	8.15	8.59	5.04	5.12	6.32	4.45	5.71	5.27	3.58
Dichloromethyl ethyl sulfone	2.51	6.46	6.35	6.78	5.52	4.38	3.93	6.18	5.82	12.11	5.67	5.42	7.24	3.52	4.52	10.04	4.44
Triphenylborane–Sodium hydroxide	2.90	2.54	2.50	2.88	2.29	2.18	1.78	2.55	2.84	3.12	2.49	1.99	4.50	2.10	2.14	3.07	2.52
3,3-Difluoro-2-propen-1-ol acetate	3.33	3.42	2.75	3.03	2.03	2.99	3.51	3.74	4.15	3.13	2.73	3.67	3.72	2.71	2.36	1.10	1.57
2,3-Pentanedione	3.50	5.42	4.82	5.11	6.39	3.37	4.47	3.99	4.51	2.19	2.72	3.20	3.85	2.27	2.92	3.85	3.20
Acetoin	3.81	9.68	7.41	6.87	6.87	6.41	9.80	7.59	9.76	3.59	3.98	4.11	4.16	3.02	1.89	0.82	1.88
Methyl benzene	5.22	16.13	14.55	17.75	14.74	13.64	10.31	13.81	16.17	18.99	16.52	18.42	16.65	11.38	12.97	24.39	14.31
Ethyl butyrate	6.48	2.67	2.08	2.34	2.52	2.18	2.24	2.43	2.60	2.36	3.08	2.79	3.06	2.41	1.96	3.15	2.35
Hexanal	6.56	5.2	10.05	6.87	5.75	5.94	3.74	3.16	2.22	4.03	1.09	1.16	2.44	5.69	3.97	2.33	1.85
2-Heptanone	10.28	2.8	2.50	3.48	3.39	1.87	1.82	2.18	2.00	1.58	2.20	2.11	2.80	1.50	1.68	1.67	2.25
Heptanal	10.84	0.74	1.50	1.26	0.86	1.18	0.74	1.30	0.83	2.06	0.67	0.89	1.18	1.42	0.90	0.81	0.80
Methyl 2-[(trimethylsilyl)oxy]benzoate	13.47	4.62	4.39	3.68	4.67	7.71	7.82	7.86	7.33	4.90	4.77	5.71	5.02	12.26	8.99	6.62	13.60
Hexanoic acid	14.38	3.75	3.91	4.17	4.71	3.42	4.08	3.34	3.55	2.22	2.84	2.33	2.31	1.66	1.21	1.97	1.79
2-Nonanone	18.65	1.98	1.82	2.29	2.13	1.14	1.24	1.24	1.18	1.39	1.42	1.55	1.49	1.67	1.32	1.36	1.68
3,4-dihydroxyphenethyl alcohol	19.44	5.04	3.76	2.82	4.98	10.46	13.33	10.82	6.48	6.99	14.57	13.72	12.49	20.58	19.88	14.57	19.69
Isoamyl hexanoate	20.54	3.39	4.99	5.10	4.19	8.11	5.18	5.66	3.97	3.03	8.50	7.20	5.14	6.38	8.87	4.80	8.22
Pentyl 2-methylvalerate	20.60	4.41	6.42	6.69	5.56	9.50	6.06	6.95	4.91	3.97	10.03	8.03	6.22	7.56	10.26	5.33	9.26
Octanoic acid	21.45	1.85	2.34	2.53	2.35	2.06	2.14	1.91	1.73	2.43	2.08	1.44	1.19	1.37	1.19	2.25	1.92

4. Conclusions

Dairy industry is focusing on development of new production methods or active marketing strategies in order to meet the various flavor and health claiming expectations of consumers and to increase the dairy product consumption per capita. Production of food products from rice milk is newly developing and the effects on human health are the subject of scientific searches.

This study aimed to add a new fermented dairy product to the dairy technology in our country, inform the public regarding its dietetic and therapeutic benefits, giving the consumer a choice for a healthier diet and increase the consumption per capita. Also we considered that our study may be a guide for further studies and selections of starter culture for similar productions. Generally speaking, samples containing rice milk did not gave good results. However, P1 samples were the most favored products among the samples containing rice milk as they were the closest product to the control group. The consumption of such products is continuously increasing as the customers' tendency to consider them as functional products rather than traditional food products increase. The demand on convenience foods with single portions with no extra process required for consumption is rapidly increasing. While drinking fermented dairy products market were developing, custom labeled products are entering the market, bringing the competition to a climax. The place of functional foods is maintained by the hand of brandization causing safety and consumer loyalty.

5. References

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