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The effect of using pro and prebiotics on the aromatic compounds, textural and sensorial properties of symbiotic goat cheese

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Abstract

The aim of this study was to evaluate the effects of probiotics as an adjunct culture, and the use of inulin and oligofructose as a prebiotic product, on symbiotic goat cheeses during their ripening period. The control group had the lowest value in terms of aromatic compounds, and the probiotics used in the production of cheese increased the aromatic substances. The control group was found to have the highest hardness values and that the use of prebiotics and probiotic cultures in cheese production significantly changed the textural profile depending on the probiotic and prebiotic type. The most favoured cheeses were found to contain *E. faecium* and oligofructose.

Key words: symbiotic goat cheese, aroma, texture profile

Introduction

Goat milk and its products are highly preferred for their valuable nutritional value (Mukdsi et al., 2013). Goat milk has been described as having higher digestability and lower allergenic characteristics than cow milk. Goat milk is considered to have a therapeutic value in human nutrition (Alferez et al., 2001; Diaz-Castro et al., 2012; Mukdsi et al., 2013). The possibility of improving the nutritional benefits of these dairy products by enriching them with probiotic and prebiotics strains has been investigated. The probiotics are live microorganisms that exert a beneficial effect on the health of the host when they are consumed in adequate quantities (FAO/WHO, 2002). The prebiotics are nondigestible food ingredients, whose purpose is to serve as food for the probiotic microorganisms and thus increase their survival chances and subsequent implantation in the host's digestive system (Zamora-Vega et al., 2013). Inulin and fructooligosaccharides are more important ingredients of prebiotics contained in foods (Ziemer and Gibson, 1998; Zamora-Ve-

ga et al., 2013). Current research tends to use prebiotics and probiotics in the development of functional foods called "symbiotic food", that is, those foods that contain probiotic cells and prebiotic ingredients (Araujo et al., 2009; Zamora-Vega et al., 2013).

Functional dairy foods, such as fermented milk and yoghurts, have a limited shelf life compared to cheese species that have a longer temporary storage capacity (Staton et al., 1998; Mukdsi et al., 2013). Cheese is one of the suitable dairy products for carrying probiotic bacteria due to its higher pH value, high fat content and solid matrix that protect bacteria more efficiently than fermented milks, such as yoghurt or kefir, during its passage through the gastrointestinal tract (Alves et al., 2013; Mukdsi et al., 2013). A developing number of probiotic and prebiotic cheese types have been reported, such as fresh Minas type cheese, fresh Argentina cheese, Pategras cheese, Whey cheese, Ras cheese, Turkish White Pickled cheese, Fior di latte cheese, Iranian Ultrafiltrated Feta cheese, Akami cheese, Panela cheese, Petit suisse cheese and Lighvan cheese etc.

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All have shown satisfactory results for the viability of probiotic bacteria during ripening or storage times (ShababLavasini et al., 2012). While only a few studies look at its potential as a functional food, its use as a food matrix carrier for probiotic bacteria and prebiotic ingredients characterize it as a symbiotic food. In many studies, it has been reported that a food matrix is a suitable carrier for probiotic bacteria, and the combination of probiotics and prebiotics have an important potential in functional food production (Buriti et al., 2007; Araujo et al., 2010; Effat et al., 2012; Alves et al., 2013).

The aim of this research is to evaluate the effect of using some probiotics and prebiotics on aromatic compounds, and on the textural and sensory properties of symbiotic goat cheese during its ripening period.

Material and methods

Table 1. The average microorganism counts and pH of cultures used in symbiotic goat cheese production

Culture	pH	cfu/g
Cheese culture	4.65	2.00x10 ¹¹
<i>E. faecium</i>	4.67	3.98x10 ⁹
<i>Lb. paracasei</i>	4.80	1.72x10 ¹⁰
<i>B. longum</i>	4.88	4.00x10 ¹⁰

Material

The Şemsi Ege Balkan Dairy Company, Izmir, Turkey supplied the whole goat milk. Probiotic cultures of *Enterococcus faecium* NRRL B-2354, *Bifidobacterium longum* NRRL B-41409 and *Lactobacillus paracasei* subsp. *paracasei* NRRL B-4560 were obtained from the United States Department of Agriculture Research Service (USDA-ARS). Freeze-dried cheese culture (*Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*) was obtained from Maysa (Kozyatağı, Istanbul, Turkey) (Table 1). Calcium chloride from Merck (Darmstadt, Germany) was used in the cheese production. The brine solution was prepared from rock salt.

Methods

Cheese production

Whole goat milk was heated at 25 °C and then 3 % inulin, 3 % oligofructose or 1.5 % inulin and 1.5 % oligofructose was added. Afterwards the milk samples were pasteurized at 67±1 °C for 10 minutes. Calcium chloride (CaCl₂) was also added to the cheese milk at a ratio of 0.02 %. The probiotic culture addition and the experimental design of the study can be seen in Table 2. Liquid rennet was added to the milk at 32±1 °C and coagulation occurred at about 90 minutes. After coagulation, the curd was cut into cubic centimetres and allowed to

Table 2. Experimental design of symbiotic goat cheese production

Cheese	Culture				Prebiotics	
	Cheese culture	<i>E. faecium</i>	<i>Lb. paracasei</i>	<i>B. longum</i>	Inulin %	Oligofructose %
C	X					
EF-C	X	X				
EF-I	X	X			3	
EF-O	X	X				3
EF-IO	X	X			1.5	1.5
LP-C	X		X			
LP-I	X		X		3	
LP-O	X		X			3
LP-IO	X		X		1.5	1.5
BL-C	X			X		
BL-I	X			X	3	
BL-O	X			X		3
BL-IO	X			X	1.5	1.5

rest in whey for 15 to 20 min. Then, the curds were covered with cheese cloth for syneresis, drained for 25 to 30 minutes without pressing. After draining, the curd was molded and pressed. Then, the cheese blocks were cut into cubes, placed in vats and brine-salted (16 g/100 g NaCl at 12 to 14 °C) for 3 to 4 hours. After the cheeses were removed from the brine solution, they were kept at room temperature for 12 hours and then packaged with 11 % brine solution to cover the surface of the cheese blocks. The cheeses were ripened at 4 °C for 90 days. Samples were selected randomly at intervals of 15 days for analysis and the analyses were carried out in triplicate.

Sampling for analyses

Cheese sampling for analyses was carried out according to International Dairy Federation (Anonymous, 1980) procedures.

Aromatic compounds

Aromatic compounds were analyzed by gas chromatography and mass spectroscopy, and a solid-phase micro extraction method was used for volatile compound extraction (PerkinElmer Fision Instrument GC 8000 series GC and Perkin Elmer Fisions Instrument MD 800 MS, USA). An SPME 75 µm fiber assembly (CAR/PDMS) was used to extract aromatic compounds. Samples were defrosted at 4 °C before the day of analyses. The outer surfaces of the samples were removed and the samples were grated. Then the cheese samples were sliced into thin shapes weighing 100 g and then distilled water was added. Steam distillation head space was collected and hexane was added to the extract obtained during boiling. The oven temperature was programmed at a steady 40 °C for 6 minutes then the temperature was raised to 100 °C (within 5 °C for a minimum of 2 minutes) and then to a final temperature of 220 °C (within 10 °C for a minimum of 5 minutes). The inlet temperature was 220 °C. This analysis was duplicated. Identification was completed by comparing GC/MS mass spectral data, retention time and aroma with standards and the Mass Spectral Library.

Textural analyses

Textural analyses of the goat cheese were carried out according to Awad et al., (2002), by using TA.XT Plus Texture Analyser (Vienna Court, Surrey Gu7 YL, England). A flat probe of 35 mm width was attached to a moving crosshead. The dimensions of the cheese specimens were 20 mm in diameter and 25 mm in height. The operating conditions were a crosshead speed of 1 mm/second, chart speed of 10 seconds and 80 % compression ratio. Samples were compressed between two stainless steel plates using a texture analyser with a 50 kg force load cell and a 75 mm compression plate. A double bite compression cycle was carried out with a rest period of 3 hours between bites.

Sensory evaluation

A sensory evaluation of the cheese samples was carried out by scoring a test taken by eight trained panelists from the Department of Dairy Technology, Faculty of Agriculture, Ege University, according to Hayaloğlu (2003). The cheeses were evaluated for color, texture, taste and overall acceptability. Samples were coded with randomly chosen three digit numbers and served at room temperature. Water and bread were also provided to the panelists to clean their palate between samples.

Statistical analysis

Results were assessed using analysis of variance (ANOVA) and the SPSS 15.0 for Windows software package.

Results and discussion

Aromatic compounds of symbiotic cheeses

The characteristic flavor of cheeses is one of the quality components that are of particular importance to cheese producers because, among them any organoleptic quality components such as color or other rheologic properties, flavor takes priority, i.e., the odor and taste sensations received when eating. Thus, the presence, contents and composition of volatile compounds in food have a substantial influence on its quality. The unique flavor of a cheese variety is the result of a complex balance between

volatile and non-volatile chemical compounds, originating during the ripening process of milk fat, protein and carbohydrates (Delgado et al., 2011).

The volatile components can be divided into several groups based on the chemical analysis and sensory evaluation of the cheese. These components are fatty acids, esters, aldehydes, alcohols, ketones, sulfur compounds and various other components.

Thirty-five major volatile compounds were identified in the present study. Delgado et al., (2011), detected 64 volatile compounds in goat cheese, and Sabio and Arogön (1996) detected 29 volatile compounds in Ibores cheese. Aromatic compounds in the cheese samples are given in Table 3. During the ripening of cheeses, carboxylic acids (acetic, butanoic, deconoic, hexanoic, heptanoic, octanoic, isovaleric, pentanoic etc.) can originate from three main biochemical pathways: lipolysis, proteolysis and lactose fermentation. Lactose is metabolised to lactate in all cheese varieties, and lactate may also be further metabolised to cheese flavor compounds such as acetic and propanoic acids by microbial metabolism (McSweeney and Sousa, 2000). It was found that among all cheese types, the maximum number of aromatic compounds was found in the LP-IO sample with 19, and the minimum number of aromatic compounds was found in the LP-O, BL-C and BL-O samples. The main aromatic substances determined in all samples were 2 butanone-3 hydroxy, decanoic acid, hexanoic acid, heptanoic acid and octanoic acid. Initially the most abundant compound was 2-butanone. The amount of methyl ketones were significantly reduced at the end of ripening. This reduction was probably due to the decrease of the microbial activity during this storage time, because free fatty acids arising from lipolysis are generally catabolised to methyl ketones by the microorganisms (Delgado et al., 2011). Generally, decanoic and octanoic acid increased at the end of the storage period compared to the first day of storage. Carboxylic acids are not only aromatic compounds but they are also precursors of other compounds, such as methyl ketones, alcohols, lactones, aldehydes and esters (Collins et al., 2003). Hexanoic acid had the highest flavor rate of all samples. This compound, originating through lipolysis, contributes significantly to goat cheese odour and has been identified as the main odorant in different cheese types such as aged Cheddar (Christensen and Reineccius,

1995). For this reason, hexanoic acid, a short-chain carboxylic acid, could contribute importantly to the typical aroma of goat cheese. Octanoic and decanoic acid are also listed among the major odorants of cheese. Hexanoic, octanoic and decanoic fatty acids have been widely recognised as being responsible for the characteristic aroma of goat cheeses, giving rise to the popular terms caproic, caprylic and capric acids, respectively (Poveda and Cabezas, 2006; Delgado et al., 2011). Hexanoic acid also contributed to the aroma of cheese in this study. Branched-chain alcohols with propanol and butanol were determined on 90th day of the ripening period in cheeses which contained *L. paracasei*. It can be said that amino acid compounds (especially leucine) are formed by the degradation of aldehydes resulting from Strecker degradation (Larsen, 1998; Bintsis and Robinson, 2004). When the aromatic compounds formed during the ripening period in the BL-IO sample were evaluated, containing *B. longum* as a probiotic adjunct culture and oligofructose-inulin as a prebiotic, sixteen different aromatic compounds were determined. On the first day and the last day of the storage period, 9 and 13 different aromatic compounds were determined, respectively. The maximum aromatic compound rate of 43.6 % at the beginning of the storage period was due to 2-butanone; the maximum rate on day ninety, with approximately 40 %, was hexanoic acid. Decanoic acid, pentanoic acid, octanoic acid and pyridine storage were determined on day 1 and day ninety; 2-hydroxy, 3-pentanol on only the first day, and butanoic acid, propionic acid and trichloromethane were determined only on the last day of the 90-day storage period. The compound 2,3-butanediol, which is one of the compounds that is found in fermented dairy products in low concentrations is highly effective in aromatic formation (Margalith, 1981). In this study, in all cheese samples, 2-3 butanediol was determined at various concentrations at different stages of the storage period. At different stages of ripening, in probiotic cheeses that contain *E. faecium* as an adjunct culture, low concentrations of hexanol, methanol and phenylethanol, as well as alcohols mentioned above, were found. These alcohols were also determined in groups containing different prebiotics in cheeses containing *L. paracasei* and *B. longum*. Hexanol, an aliphatic alcohol, was determined only in the EF-I sample containing

E. faecium as an adjunct culture on day ninety of the storage period with an 0.67 % rate. In general, the strong reducing conditions in cheese favour the formation of alcohols from aldehydes and ketones, following reaction pathways that involve alcohol dehydrogenases (Molimard and Spinnler, 1996). The levels of alcohol were significantly affected by the maturation process. Chloroform, having a quite low rate among aromatic compounds, was not detected in any samples containing the *B. longum* adjunct culture, but it was detected in the control, EF-O and LP-IO samples only on 90th day of the storage. In the EF-C sample, the main aromatic compound on the first day of the storage was 2-butanone-3 hydroxy, while octanoic acid reached its highest value by the end of the storage period. Ethyl acetate and acetic acid were detected only on the first day, while butanoic acid and methanol was only detected on day ninety of the storage period. In the EF-I, EF-IO and EF-O samples, 19 different aromatic compounds were detected. This shows that the prebiotics that were used had an effect on the variety of aromatic compounds. Decanoic acid, hexanoic acid, heptanoic acid and octanoic acid were detected in all *E. faecium* group samples (EF-C, EF-I, EF-O and EF-IO) on the first and ninetieth days of storage. Depending on the storage, the highest increase in aromatic substances was determined in *L. paracasei* samples. In cheeses containing *L. paracasei*, 2-butanone had its maximum rates in the LP-I, LP-O and LP-IO samples on the first day of storage, whereas octanoic acid had its maximum rate in LP-C. At the end of the storage, in all cheese groups except LP-I, octanoic acid reached its maximum value, while hexanoic acid had its maximum rate in LP-I. The highest numbers of flavor components determined in this study were due to organic acids and free fatty acids. Generally, using a probiotic adjunct culture in cheese production caused an increase in the number and amount of volatile fatty acids in cheese. Free fatty acids commonly determined in all cheese types were butanoic acid with short-chain fatty acids (C4, butyric acid), hexanoic acid (C6, caproic acid) and octanoic acid (C8, caprylic acid). As well as these fatty acids in the cheeses; other fatty acids were determined in the cheeses in different concentrations as potential aroma-active compounds, including: decanoic acid (C10, capric

acid), decanoic acid (C14, myristic acid), hexadecanoic acid (C16, palmitic acid), octadecanoic acid (C18, stearic acid), 9-octadecanoic acid (C18: 1, oleic acid), pentanoic acid (valeric acid), 3-methyl butanoic acid (isovaleric acid) and acetic acid. Butanoic acid has a rancid cheese-like odor and plays an important role in the flavor of many cheese types such as Camembert, Cheddar, Grana Padano, Gruyère, Pecorino, Ragusano and Roncal. Among the fatty acids mentioned, acetic acid was determined in all cheese types.

Acetic acid production may be due to the metabolism of lactose by lactic acid bacteria, or the metabolism of citric and lactic acid, or the catabolism of amino acids. When all the cheese groups had been evaluated in this study, the aromatic compounds in samples containing inulin-oligofructose in all groups were higher than the other cheeses produced. This showed an increase in the aromatic compound values in cheeses when inulin-oligofructose is used. Also, the number of aromatic compounds increased in all cheeses during storage. In recent years, studies on aromatic chemistry have focused on cheese. Thus, the components responsible for the characteristic taste and flavor of many cheeses have been determined. For example, Avşar et al., (2010), found that the desired nut-like flavor in cheddar cheese is caused by aldehydes, 2-methyl propanal and 2/3 methyl butanol. In another study, it was found that the raw potato flavor, which was seen as an aromatic defect, was caused by 2,3-dimethyl-5-methylpyrazine. Novikova and Ciprova (2009), produced Krievijas cheese and divided the cheeses into two groups. One group of cheese was stored at 6 °C, and the other group of cheese was stored at 12 °C, both for 60 days. During ripening, 2-pentanone, diacetyl and 2-heptanone from fruity flavoring ketones were found. It was also found that phenyl acetaldehyde, a benzene derivative, causes an increase in aroma. The main components responsible for fruity flavors and sweetness are esters. In all examples except unripened cheese and cheese ripened at 12 °C, ethyl butyrate and ethyl acetate were found. Sulfur compounds caused a garlic-like and excessively ripened cheese aroma. Their increase caused an undesired taste. Also, methional and dimethyl-trisulfide were found in all samples. Alcohol levels, as an aromatic substance, have been related to using *Lactococcus*

strains in combination with *B. bifidum* in cheese production (Starrenburg and Hugenholtz, 1991). The volatile composition of the analyzed Gouda type cheeses using *Lactobacillus paracasei* strains as adjunct cultures was mainly characterised by aromatic compounds derived from lipolysis, proteolysis and metabolism of residual lactose, lactate and citrate. The main volatile compounds in cheese were reported as methylketones, volatile acids, lactones, alcohols and sulphur compounds. The same type of aromatic compounds were also previously identified in Gouda type cheeses (Van Hoorde et al., 2010; Van Leuven et al., 2008). Lynch et al., (1999), also observed an increase in flavor intensity and bitterness, whereas the creaminess and milky flavor decreased in the ripening of Cheddar cheese using *L. paracasei* subsp. *paracasei* as an adjunct culture. Probiotic cheeses made with *L. paracasei* were perceived as significantly different from the non-probiotic cheeses with higher levels of bitter compounds (Ong et al., 2007). Iličić et al., (2012), investigated the volatile compounds of traditional and probiotic functional cheeses. In that study, volatile compounds affecting the flavour of traditional and probiotic fresh cheeses were investigated. The traditional starter culture, Flora Danica, and a combination of the probiotic starter ABT-1 and FD (ABT-1:FD-1:1) were applied as starters. Nineteen compounds were identified with gas chromatography and mass spectrometry as well as eight hydrocarbons (decane, undecane, tridecane, tetradecane, pentadecane, hexadecane, octadecane and 2,6,10,14 tetramethyl hexadecane); 6 ketones (2 heptanone, 2 nonanone, 2 undecanone, 2 pentadecane, 2 heptadecanone, 2 tridecanone); 3 aldehydes (nonanal, tetradecanol, hexadecanol); 1 fatty acid (decanoic acid) and disulfide (bis-1-methylethyl). The researcher emphasized that the highest levels were associated with hexadecanol, 2 pentadecanone, 2 tridecanone and 2 undecanone in all cheeses examined, regardless of the starter culture and type of milk used. According to the results of this study, it is possible that these aromatic compounds changes are dependent on cheese type, the starter culture type and the cheese curd matrix used as the vehicle for prebiotics. So both similar and different results were found in various other studies when compared to this study.

Textural profile analysis of symbiotic cheeses

Texture is one of the most important parameters in determining the quality of cheese and its acceptability by consumers. Texture involves quality characteristics that are closely related to the structural and mechanical features of food.

Among the experimental cheeses, the maximum hardness values were produced by the BL-C sample with 3.35, followed by EF-C with 2.63, LP-C with 2.01 and C with 2.15, respectively. The softest cheeses were determined to be the ones with inulin and oligofructose together. When the cheeses were evaluated within their own groups, it was found that using prebiotics had no significant effect on the hardness values of the cheeses ($p > 0.05$). In all cheeses, except the samples containing inulin-oligofructose, the hardness of the cheeses were affected by the different cultures used ($p < 0.05$). In the first 30 days of storage, the control, all EF type cheeses and all cheeses in the LP group, except LP-C and only BL-C of the BL group cheeses, saw a decrease in the hardness values. The hardness value of LP-C stayed stable. In BL-I, BL-O and BL-IO type cheeses a decrease in the first thirty days was followed by a slight increase over the next sixteen days. The hardness value of EF-C type cheese started to rise after the day 60 and this rise continued until the end of the storage period. The hardness value of LP-C decreased during storage, which was 2.15 at the beginning and 2.05 at the end. The hardness of BL-C, which was the hardest cheese on the first day of storage, decreased to one third of that rate within the first thirty days. Generally, among the cheeses, it can be said that the hardness of all samples showed a decrease during storage. The highest hardness value of cheeses at the beginning of storage was expected, as low fat cheeses have a rubbery and hard texture due to the predominant role of milk proteins (Mistry, 2001). This is because proteolysis had not yet started, which otherwise would have resulted in a softer sample texture. Generally, the hardness of the samples were observed to decrease significantly with increasing storage time. Proteolytic enzymes produced by lactic acid bacteria (LAB) which initially cleave proteins to peptides and further peptides to small peptides and amino acids, implies that intact proteins decrease during storage resulting in cheese softening (Mushtaq et al., 2015).

Cohesiveness can be defined as the degree of deformation of a food sample before its disintegration in the mouth (Altuğ, 1993). In this study, cohesiveness values of the control samples were found to be higher than the samples containing prebiotics. The maximum internal cohesiveness value was in EF-IO with 2.07, whereas the lowest was found in the control group with 0.60. The cohesiveness values of the two other group cheeses, except EF group cheeses, were found to be affected by the prebiotics used ($p < 0.05$). Using starter cultures in samples containing inulin-oligofructose (EF-IO, LP-IO and BL-IO) and in control samples (C, EF-C, LP-C and BL-C) had no effect on the cohesiveness values ($p > 0.05$), but samples containing only inulin and only oligofructose were affected by the starter culture types ($p < 0.05$). Cohesiveness values of the cheese samples during storage generally decreased (Table 4), but there wasn't any linear correlation between cohesiveness and storage time. Awad et al., (2002), determined that cohesiveness decreased with storage of cheese samples. The maximum decrease in the samples was found in BL-I. The value of 0.88 on the first day dropped to 0.08 by the end of the storage period. The lowest values among the cheeses were in the control sample with 0.60 and the LP-C sample with 0.61. At the end of the storage, a very limited difference between the values of cohesiveness was observed. There wasn't any relationship between the hardness and cohesiveness of samples.

Adhesiveness in the mouth during chewing is one of the methods used for evaluation (Altuğ, 1993). It was observed that using inulin and oligofructose increased the adhesiveness values. On the first day of storage, the lowest adhesiveness values were observed only in the control group and those containing probiotic bacteria. Adhesiveness values of the samples using inulin were found to be higher. Initially the maximum adhesiveness value was found in LP-I at 46.67, whereas the lowest was found in the LP-C group at 6.96. The adhesiveness values of the BL and EF group samples were not affected by the use of prebiotics ($p > 0.05$), whereas LP group cheeses were affected ($p < 0.05$). The starter cultures used were found to have no effect on the adhesiveness values of cheeses ($p > 0.05$). The BL-C sample showed a steady increase from a value of 10.02 on the first day of storage to 44.88 by the

90th day. Generally, adhesiveness of the samples increased during storage. This is because the water-holding capacity of proteins increased during storage due to proteolysis. The highest increase in textural parameters was determined in non-stickiness. At the end of the storage, the minimum adhesiveness value was found in LP-IO with 13.90, whereas the highest was found in EF-C with 94.92.

In symbiotic goat cheeses, the gumminess values of the samples were determined to range from 0.34 to 2.51. Initially, the gumminess values of samples containing inulin-oligofructose (EF-IO, LP-IO, BL-IO) were found to be much lower than the other samples. Using prebiotics had a significant effect on the gumminess values of all samples ($p < 0.05$). The starter cultures used did not affect the gumminess value ($p > 0.05$). The gumminess value of the control sample on the first day of storage was found to be 1.21. It dropped to 0.13 by the 90th day of storage. The gumminess values of EF-C type cheese decreased to one third of its initial value during storage. As a general evaluation, although the gumminess values of all samples showed irregular changes during storage, by the end of the storage period a decreasing trend in the values was noted. Gumminess of the samples depends on the hardness of cheeses, so as previously mentioned, the hardness decreased with storage. The greatest decrease among the cheese samples was observed in the EF-I sample. The gumminess value of 2.51 at the beginning of storage decreased to 0.07 by the ninetieth day of storage. At the end of the storage period, the lowest gumminess value was found in BL-I with 0.01 and the highest value was found in BL-C with 0.65.

Springiness is the degree of restoration of the original shape of foodstuffs during chewing. As seen in Table 4, springiness was found to be the least differing textural characteristics among samples. Together with the prebiotics used in production, starter cultures had no effect on the springiness results of samples ($p > 0.05$). The lowest springiness value on the first day of the storage was 9.96 for BL-IO and the highest value was 10.00 for the BL-C sample. If a comparison is made of springiness values between the first and ninetieth days of storage, all samples showed a slight decrease. This decrease was statistically insignificant ($p > 0.05$).

Table 4. Textural parameters of cheese samples during the storage

Parameter	Day	Samples												
		C	EF-C	EF-I	EF-O	EF-IO	LP-C	LP-I	LP-O	LP-IO	BLC	BL-I	BLO	BL-IO
Hardness (kg)	1	2.01±0.23	2.63±0.41	1.38±0.56	1.70±0.89	0.45±0.02	2.15±0.48	1.92±0.19	1.76±0.25	0.36±0.09	3.35±1.10	1.55±0.08	1.40±0.09	0.62±0.06
	30	1.20±0.33	1.87±0.26	0.36±0.14	1.32±0.15	0.46±0.05	2.03±0.13	0.88±0.009	1.21±0.12	0.31±0.06	2.07±0.08	0.38±0.07	0.42±0.06	0.49±0.05
	60	1.09±0.21	1.97±0.31	0.54±0.25	1.60±0.41	0.32±0.08	1.89±0.48	0.70±0.37	0.30±0.04	0.25±0.11	2.00±0.21	0.22±0.15	0.41±0.10	0.42±0.21
	90	1.72±0.98	1.96±1.05	0.52±0.08	1.48±0.23	0.45±0.06	2.05±0.28	0.38±0.13	0.43±0.09	0.20±0.008	2.14±1.08	0.14±0.03	0.32±0.15	0.21±0.08
Cohesiveness (cm ²)	1	0.60±0.06	0.70±0.04	1.83±0.08	0.82±0.07	2.07±0.11	0.61±0.04	0.72±0.03	0.71±0.02	0.94±0.24	0.75±0.16	0.88±0.19	1.70±0.32	1.19±0.41
	30	0.78±0.07	0.88±0.24	1.45±0.33	0.93±0.22	1.63±0.16	0.96±0.27	0.75±0.08	1.66±0.12	1.13±0.19	1.37±0.08	1.41±0.09	1.26±0.24	0.89±0.08
	60	0.62±0.18	0.48±0.40	0.39±0.11	0.40±0.04	0.66±0.06	0.53±0.04	0.54±0.29	0.40±0.21	3.13±1.05	1.32±1.24	1.14±1.04	0.32±0.03	0.55±0.21
	90	0.10±0.08	0.20±0.09	0.13±0.04	0.27±0.05	0.21±0.02	0.30±0.09	0.17±0.02	0.19±0.11	0.52±0.26	0.30±0.15	0.08±0.01	0.27±0.09	0.13±0.07
Adhesiveness (kg.s)	1	10.97±2.2	12.45±3.0	32.20±6.47	18.33±2.96	18.90±2.4	6.96±1.52	46.67±9.17	33.31±4.8	28.39±4.0	10.02±1.8	30.06±5.2	23.78±3.4	21.99±4.1
	30	9.03±1.95	18.28±2.55	41.70±8.61	49.10±5.84	34.86±4.61	27.69±4.25	41.87±3.09	49.87±5.31	25.34±4.89	7.16±1.69	43.77±9.85	55.84±10.08	41.83±8.82
	60	46.30±8.79	79.86±9.66	42.78±4.37	72.25±4.15	56.67±7.26	44.17±8.05	47.10±9.14	54.80±10.26	16.20±7.02	24.83±3.86	55.89±4.26	50.17±10.22	56.33±7.29
	90	51.70±10.06	94.92±12.33	65.03±5.26	84.65±15.91	60.38±11.02	54.80±8.29	57.07±7.62	70.50±9.47	13.90±1.1	44.88±2.36	10.10±0.87	46.17±6.11	30.15±4.42
Gumminess (kg)	1	1.21±0.12	1.82±0.09	2.51±0.22	1.38±0.14	0.94±0.08	1.32±0.19	1.38±0.31	1.26±0.22	0.34±0.08	2.48±0.14	1.35±0.07	2.38±0.20	0.77±0.15
	30	0.93±0.07	1.64±0.06	0.52±0.08	1.22±0.11	0.75±0.01	1.94±0.05	0.66±0.04	2.00±0.04	0.35±0.02	2.83±0.07	0.53±0.06	0.53±0.08	0.43±0.07
	60	0.67±0.31	0.94±0.02	0.21±0.09	0.64±0.01	0.21±0.07	1.00±0.05	0.37±0.04	0.12±0.03	0.78±0.08	2.64±0.11	0.25±0.24	0.13±0.06	0.23±0.10
	90	0.13±0.08	0.41±0.07	0.07±0.008	0.38±0.11	0.31±0.07	0.59±0.08	0.06±0.00	0.08±0.00	0.11±0.00	0.65±0.05	0.01±0.03	0.08±0.00	0.02±0.00
Springiness (s)	1	9.99±0.01	9.99±0.02	9.97±0.01	9.99±0.02	9.98±0.02	9.99±0.03	9.99±0.01	9.99±0.03	9.99±0.04	10.00±0.12	9.97±0.22	9.97±0.02	9.96±0.03
	30	9.93±0.11	9.93±0.24	9.96±0.16	9.94±0.17	9.94±0.18	9.96±0.15	9.93±0.55	9.97±0.06	9.95±0.08	9.94±0.09	10.00±0.21	9.93±0.07	9.92±0.04
	60	9.93±0.02	9.93±0.03	9.93±0.06	9.94±0.07	9.93±0.09	9.97±0.11	9.93±0.16	9.94±0.09	9.95±0.08	9.93±0.14	9.95±0.03	9.93±0.05	9.93±0.06
	90	9.94±0.06	9.96±0.04	9.96±0.12	9.99±0.11	9.95±0.15	9.97±0.06	9.97±0.24	9.96±0.08	9.97±0.07	9.99±0.03	9.97±0.05	9.97±0.04	9.98±0.02
Chewiness (kg)	1	12.15±1.21	18.29±1.15	25.02±1.63	13.84±2.15	9.38±1.42	13.20±1.20	13.78±0.85	12.55±1.23	3.39±0.69	24.89±2.14	13.45±1.11	23.72±1.24	7.72±0.63
	30	9.29±0.05	16.28±1.23	5.20±0.08	12.12±1.22	7.45±0.06	19.41±1.42	6.55±0.08	20.02±2.22	3.48±0.97	28.17±2.58	5.35±0.07	5.25±0.08	4.32±0.07
	60	6.71±0.04	9.43±0.03	2.09±0.05	6.35±0.07	2.09±0.08	1.98±0.01	3.75±0.11	1.19±0.08	7.76±0.09	26.21±1.26	2.49±0.07	1.30±0.09	2.29±0.07
	90	1.34±0.55	4.10±1.11	0.71±0.06	3.84±0.68	0.94±0.42	5.95±1.02	0.65±0.08	0.87±0.07	1.17±0.34	6.58±1.06	0.12±0.08	0.85±0.09	0.28±0.07

The number of chews of food necessary to allow swallowing is defined as chewiness. The chewiness values on the first day of storage changed from 3.39 to 25.02. The lowest chewiness values were found in the samples containing inulin-oligofructose mixtures. As a result of the statistical analysis, when all cheese groups were evaluated within their groups, it was found that using prebiotics had a significant effect on the chewiness values of the samples ($p < 0.05$), while using different probiotics had no significant effect on the chewiness of the samples ($p > 0.05$). As a result of the statistical analysis, storage had a significant effect on the chewiness of the samples ($p > 0.05$). The greatest decrease in chewiness value was observed in the EF-I sample on the first day of storage. The 25.02 value on the first day dropped to 0.71 by the end of the storage period. The chewiness values show an increasing trend as the hardness values increase. The factors that had an effect on the hardness of the cheeses also had an effect on the chewiness values. Chewiness values of cheeses had a positive relationship with their hardness and gumminess values. When the textural analysis results of the cheeses were examined, it can be noted that production technology, milk composition, moisture content in cheese, pH, salt content, lipolysis, proteolysis during ripening, culture type, probiotic bacteria and prebiotic used, all have significant effects on the cheese texture profile (Lawrence et al., 1987; Fox et al., 2000; Ercan et al., 2011). On the other hand, cheeses containing salt affect the textural profile due to protein solubility and protein conformation, and also have a hard cheese texture (Prasad and Alvarez, 1999; Fox et al., 2000; Ercan et al., 2011). Furthermore, significant correlations can be observed between textural parameters and the lipolysis or proteolysis occurring during ripening. Springiness especially occurs during ripening because of the effects of proteolytic breakdown of the protein matrix. Likewise, the firmness of cheeses decreased during cheese aging (Gunasekaran and Ak, 2003; Brown et al., 2003; Ercan et al., 2011). Moreover, Buriti et al., (2007), mentioned that probiotic Minas cheese behaved similarly to their control cheeses in terms of textural and physicochemical parameters during cold storage.

Sensory properties of symbiotic cheeses

Sensory evaluation is defined as a multidisciplinary science formed by the magnitude, analysis and explanation of the reactions of characteristic sight, smell, taste, touch or hearing senses of a variety of foods. Today, sensory analysis in the food industry has become a standard tool used for the development of new products, for improving product quality and increasing quality control, sales potential and marketing. In this study, color, texture, odor, taste and general acceptance properties were investigated during the sensory analysis. The sensory analysis results are shown in Table 5.

The lowest color scores of the samples were found in the control sample on the first day of storage, while the highest acceptable color value was detected in the BL-O sample. Prebiotics used in the production have affected the color scores of all cheeses ($p < 0.05$). While using different starter cultures were effective in samples containing inulin ($p < 0.05$), no significant effect was determined on samples containing oligofructose and inulin-oligofructose ($p > 0.05$).

Initially the lowest texture values were recorded in the LP-C sample, while the highest were observed in the BL-O sample. The structure of LP-C, BL-C and LP-IO samples were found to be similar to cream cheese. Using different prebiotics in production affected the texture scores for all cheeses ($p < 0.05$). Also, it was found that using different probiotics had a significant effect on the texture scores of samples containing inulin-oligofructose ($p < 0.05$). When cheeses were considered within their own group, generally, the control sample of each group had higher scores than others. It was seen that the lowest texture score among the cheese groups at the end of the storage were those products containing inulin and oligofructose. Symbiotic cheeses containing *E. faecium* were the ones with the highest texture scores.

While the least favored smell of cheese samples was in LP-I and K, the most favored cheese was BL-O. At the beginning of ripening, it was found that using different prebiotics and probiotics had no significant effect on the odor scores of the cheeses ($p > 0.05$).

Table 5. Values of sensory attributes (scale from 0 to 10 points) of cheese samples during the storage

Attribute	Day	Samples												
		C	EF-C	EF-I	EF-O	EF-IO	LPC	LP-I	LPO	LP-IO	BL-C	BL-I	BL-O	BL-IO
Color	1	3.43±0.58	4.43±0.61	5.14±0.41	5.86±0.36	4.14±0.41	3.86±0.66	3.71±0.51	5.57±0.56	4.43±0.62	4.86±0.63	3.71±0.43	6.43±0.42	5.86±0.92
	30	5.50±1.10	6.50±0.78	4.33±0.38	6.00±0.89	5.17±0.87	6.67±0.78	5.17±1.23	5.00±1.40	5.83±0.74	5.67±0.84	5.67±1.20	4.50±1.02	5.17±1.15
	60	6.13±0.56	6.75±0.48	5.50±0.64	6.50±0.62	6.00±0.15	6.38±0.26	5.25±0.76	5.75±0.51	5.50±0.39	6.75±0.73	4.50±0.38	4.75±0.49	5.50±0.52
	90	6.75±0.75	6.00±1.15	4.75±0.69	6.50±1.14	5.00±0.63	6.75±0.89	5.25±1.17	4.75±0.65	4.75±0.84	6.25±2.23	4.75±1.51	5.50±1.47	5.50±1.36
Texture	1	3.29±0.44	3.43±0.52	5.29±1.26	5.79±0.78	3.00±0.45	2.43±0.44	2.71±0.33	5.79±1.31	3.57±0.97	2.50±0.69	4.64±0.55	6.14±0.87	6.00±0.69
	30	6.00±1.14	6.50±0.85	5.83±0.66	6.17±1.09	4.50±0.66	6.17±1.54	4.33±0.59	4.17±1.57	3.67±0.89	5.67±0.99	3.50±0.67	3.67±0.62	4.50±0.74
	60	5.50±0.48	5.75±1.97	4.13±0.87	6.50±1.59	3.50±0.06	6.00±1.22	3.00±0.07	1.88±0.49	4.38±0.97	6.38±1.24	1.25±0.08	3.38±0.46	1.25±0.09
	90	6.25±1.97	6.00±1.88	4.50±1.69	6.00±0.79	4.00±0.87	6.13±1.99	2.63±0.68	2.63±0.79	2.38±0.69	6.00±1.21	2.00±0.68	3.63±0.52	1.75±0.81
Odour	1	4.00±1.26	4.43±1.36	5.14±0.97	5.14±1.85	4.14±1.36	4.14±0.88	4.00±0.97	5.00±1.89	4.29±0.96	4.71±1.26	4.57±2.08	5.29±2.18	4.86±0.88
	30	5.33±1.05	6.17±1.08	5.50±0.89	5.83±0.99	5.50±1.95	5.67±1.86	3.83±0.87	5.00±1.35	4.17±0.97	5.50±1.64	4.50±0.61	5.17±0.88	5.17±1.25
	60	5.75±1.23	5.75±1.17	6.00±2.04	6.25±2.03	5.75±0.97	5.75±1.25	5.50±1.66	5.00±1.87	5.25±1.52	6.25±1.36	4.00±0.96	4.50±1.61	4.25±0.69
	90	6.50±0.97	6.75±0.85	5.75±2.10	7.00±2.23	5.75±0.68	6.50±0.89	5.50±1.87	5.50±1.09	5.75±0.97	6.50±1.87	5.50±0.88	5.50±2.31	5.75±1.65
Taste	1	3.64±0.65	2.93±0.28	5.00±1.21	4.86±0.68	3.71±0.98	3.71±1.15	3.64±1.21	6.64±2.05	4.67±1.25	3.79±0.98	4.50±1.62	2.64±0.98	3.14±0.09
	30	5.67±1.11	5.83±0.98	5.00±1.12	5.50±0.89	4.50±0.99	5.67±1.16	3.67±0.98	5.67±1.20	5.00±1.99	5.33±1.19	4.83±0.98	5.33±1.22	5.33±1.10
	60	5.50±0.51	5.00±0.06	5.38±0.08	6.00±1.23	3.38±0.49	5.38±0.63	5.13±0.36	3.75±0.78	5.13±0.87	5.37±1.11	3.38±0.09	4.13±1.21	3.88±0.99
	90	6.13±1.28	5.38±0.98	3.88±0.09	6.13±1.21	3.88±0.99	5.50±1.11	4.13±0.69	4.25±0.99	4.38±0.79	5.38±1.22	3.13±0.08	4.50±1.12	5.00±1.09
Overall acceptance	1	3.86±0.68	3.29±0.59	5.14±1.21	5.43±1.32	3.43±0.99	3.43±1.08	3.14±0.57	5.14±0.74	4.43±0.49	3.86±0.08	5.29±1.24	5.14±1.28	5.29±1.78
	30	5.83±1.26	6.00±1.47	5.00±1.06	6.17±1.89	4.50±0.98	5.00±1.15	3.50±0.89	5.33±1.27	4.50±0.99	4.83±1.15	4.50±1.24	4.50±1.20	4.67±0.68
	60	6.25±1.24	6.00±1.09	5.75±0.98	7.50±2.38	4.00±1.08	5.50±0.46	5.00±0.29	3.13±0.88	5.00±1.15	6.75±2.48	2.75±0.09	4.13±1.20	3.50±0.71
	90	7.25±2.25	6.00±1.81	3.63±0.53	6.50±0.76	3.50±0.91	7.25±2.18	4.50±1.79	4.50±0.86	4.75±0.84	6.50±1.24	2.75±0.07	5.00±0.91	4.50±0.57

Panelists reported a typical goat cheese taste in all samples, while taste scores varied between 2.64 and 6.64. It was found that the prebiotics used had a significant effect on the taste scores of the BL group samples ($p < 0.05$), but no significant effect on the taste scores of the EF and LP group cheeses. Using different probiotics had no effect on the taste scores of cheeses ($p > 0.05$). Considering each cheese within its own group, it was found that cheeses with inulin added received the lowest taste scores. Some panelists indicated a slight metallic taste in samples with inulin added.

In a general evaluation of cheeses by panelists, the most favored cheese was found to be EF-O, while the least favored cheese was LP-I. Prebiotics used in the production had no effect on any of the cheese groups in the overall evaluation scores ($p > 0.05$). Using different prebiotics only had a significant effect on the general evaluation scores in samples containing oligofructose ($p < 0.05$).

It was found that the overall acceptance scores of the experimental cheeses did not change significantly, a slight increase was observed on the sixtieth day, except for LP-O and BL-I. On the ninetieth day of ripening, significant increases were observed especially in the control, LP-C and LP-IO samples. At the end of the storage, when the Symbiotic cheeses were evaluated in general, the highest score was produced by the control and LP-C groups with 7.25, while the BL-I group produced the lowest scores with 2.75.

Queiroga et al., (2013), detected high rates of short-chain fatty acids (caproic, caprylic and capric) in the goat milk cheeses. The goat-like flavor observed in cheeses intensifies as the product's pH increases to six or higher (Ceballos et al., 2009). In this study, the increase in the bitter flavor in cheeses was caused by the increasing amounts of octanoic and decanoic acids during the storage period. Poveda et al., (2008), found that these fatty acids give an undesirable bitter taste to the product. In their study, Pereira et al., (2011), produced sample cheese milk from cows, goats and sheep. In the first days of production, the best cheese was the one produced from goat milk, followed by cow and then sheep milk, respectively. However, this order was reversed after ripening with sheep milk receiving the highest score in terms of textural structure ($p < 0.05$). No significant differences were detected

between the textural structures of cow and goat milk; however, after 60 days' of storage, cow milk had the lowest scores in terms of textural structure. Moreover, Buriti et al., (2007), studied three types of Minas cheese and found that differences in sensorial characteristics might be attributed to the presence or otherwise of the type O starter culture, rather than using ABT probiotic culture as a starter adjunct. Oliveira et al., (2012), observed that Coalho cheese may be a good carrier for the delivery of probiotic lactic acid bacteria, and also that Coalho goat cheeses, with the added probiotic lactic acid strains alone and in co-cultures, were better accepted in the sensory evaluations than cheeses without the probiotic strains. In another study, the addition of the symbiont *S. boulardii* and inulin in the cheeses impacted positively on the quality sensory attributes evaluated (Zamora-Vega et al., 2013). Meanwhile Araujo et al., (2009), did not observe statistically significant differences in taste, texture and total acceptability between the symbiont type cottage cheese made with *L. delbrueckii* and inulin and the control cheese, both stored from three to fifteen days at 4 °C.

Conclusion

The characteristic compounds most involved in the cheese aroma were butanoic, hexanoic, decanoic and octanoic acids, some alcohols (1-hexanol and benzenetanol) and some methyl ketones (2-butanone and 2-heptanone). Volatile acids were the most abundant compounds isolated from the cheese. Those acids with origins in lipolysis had the highest significance in the aromatic profile of the samples. The pattern of volatile acid formation, according to their most probable origin, could be associated to the differential and typical characteristics in each type of cheese, and could be an interesting pathway to investigate the characterisation of the aromatic volatile profile of cheeses. The hardness of the samples was observed to decrease with increasing storage time. A decreasing trend was observed in these parameters along with the chewiness values, which is due to the relationship between cohesiveness and springiness. The derived parameters (gumminess and chewiness) were also influenced by the storage period due to decreased hardness.

General sensory evaluations of cheeses by panellists revealed that the most favored cheese was found to be EF-O and the least favored cheese was LP-I. There are no significant effects caused by the prebiotics used in the production, or in the overall evaluation scores ($p > 0.05$). Using different prebiotics only had a significant effect in the general evaluation scores on samples containing oligofructose ($p < 0.05$).

Significant differences were found between the characteristics of symbiotic goat cheese samples. The variability of aroma, textural and sensorial attributes was mainly based on variations in some physicochemical parameters, the probiotic or prebiotic types used, the addition rates and the proteolysis/lipolysis indices during ripening.

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Utjecaj korištenja probiotika i prebiotika na aromatske spojeve i teksturalna i senzorska svojstva simbiotskog kozjeg sira

Sažetak

U ovom radu istražen je utjecaj probiotika, te inulina i oligofruktoze, na simbiotski kozji sir tijekom zrenja. U kontrolnoj grupi su utvrđene najniže koncentracije aromatskih spojeva, dok je dodatak probiotika u proizvodnji sira povećao koncentraciju aromatskih spojeva. Također, u sirevima iz kontrolne grupe su utvrđene najveće vrijednosti čvrstoće. Dodatak probiotika i prebiotika je signifikantno utjecao na teksturalne karakteristike sira. Sirevi koji su ocjenjeni kao najbolji, proizvedeni su uz dodatak *E. facium* i oligofruktoze.

Ključne riječi: simbiotski kozji sir, aroma, teksturalna svojstva

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