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Original Article/Özgün Araştırma

Determination of microbiological, aromatic and sensory properties in different ripening period of Bayramiç cheese produced with and without bifidobacteria inoculation and comparison with similar industrial cheeses

Bifidobakteri inokule edilerek ve edilmeden üretilen Bayramiç peynirinin farklı olgunlaşma dönemlerindeki mikrobiyolojik, aromatik ve duyusal özelliklerinin belirlenmesi ve benzer endüstriyel peynirlerle karşılaştırılması

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Abstract

Objective: In recent years, interests in artisan cheeses have also increased with the interest in traditional foods. In this context, researchers are studying on revealing the properties of artisan cheeses, increasing their quality with new technologies and revealing their differences with other cheeses. This study aimed to determine the differences in microbial properties, major aromatic components and sensory properties of Bayramiç cheese, which are traditionally produced with and without inoculation of bifidobacteria and ripened in brine at different periods. In addition, these traditional cheeses were also compared with other industrially produced cheeses.

Materials and methods: In this study, Bayramiç cheeses were produced from cow milk by using traditional methods with and without bifidobacteria inoculation. Samples were taken at 1, 45, 90 and 135 days of ripening period for analysis. Three different ripened cheeses were used to compare. It is known that these cheeses were produced on an industrial scale from cow's milk, starter culture was not used in their production, standard production methods were used, they were ripened at 4ºC for 3 months and offered for sale as full-fat ripened white cheese. Bifidobacteria, lactic acid bacteria, yeast and mold counts were determined and aromatic compounds and sensory properties were also analyzed.

Results and conclusion: Bifidobacteria inoculated samples showed more regular increase in lactic acid bacteria counts. It has been observed that Bayramiç cheeses produced with 7 log cfu/mL inoculation of bifidobacteria may also provide an advantage in the marketing of probiotics. In further studies, will be done in future with much more samples, capric acid, capronate (ethyl-) and benzene, 2,4-diisocyanato-1-methyl can be used as indicator components for Bayramiç cheeses. Interreaction of bifidobacteria inoculation and ripening days were determined important factors for product quality. It is thought that producers who want to use bifidobacteria as a starter culture should also pay attention to ripening times.

Keywords: artisan cheese, starter culture, lactic acid bacteria, capric acid

Öz

Amaç: Son yıllarda geleneksel gıdalara olan ilgiye paralel olarak artizan peynirlere olan ilgi de artmıştır. Bu bağlamda araştırmacılar artizan peynirlerin özelliklerini ortaya çıkarmak, yeni teknolojilerle peynirlerin kalitesini artırmak ve diğer peynirlerle olan farklılıklarını ortaya çıkarmak için çalışmalar yapmaktadır. Bu çalışma, geleneksel olarak bifidobakteri ilavesiyle ve ilavesiz olarak üretilen, farklı zamanlarda salamurada

olgunlaştırılan Bayramiç peynir örneklerinin mikrobiyolojik, aromatik ve duyusal özelliklerindeki farklılıkları belirlemeyi amaçlamaktadır. Ayrıca bu geleneksel peynir çeşidimizin endüstriyel olarak üretilen peynirlerle karşılaştırmaları da yapılmıştır.

Materyal ve yöntem: Bu araştırmada geleneksel yöntemlerle 2 çeşit Bayramiç peyniri, inek sütünden Bifidobakteri inokülasyonlu ve inokülasyonsuz olarak üretilmiştir. Analiz için 1, 45, 90 ve 135 günlük olgunlaşma sürelerinde örnekler alınmıştır. Karşılaştırma için üç farklı olgunlaştırılmış peynir kullanılmıştır. Bu peynirlerin inek sütünden endüstriyel ölçekte üretildiği, üretiminde starter kültür kullanılmadığı, standart üretim metotlarının kullanıldığı, 4ºC'de 3 ay olgunlaştırmaya tabi tutulduğu ve tam yağlı olgunlaştırılmış beyaz peynir olarak satışa sunulduğu bilinmektedir. Bifidobakteri, laktik asit bakterileri, maya ve küf sayımının yanı sıra aromatik bileşikler ve duyusal özelliklerin belirlenmesine yönelik analizler yapılmıştır.

Bulgular ve sonuç: Bifidobakteri ilavesiyle hazırlanan örneklerde laktik asit bakteri sayılarında daha düzenli bir artış görülmüştür. Bifidobakterilerin 7 log kob/mL inokülasyonu ile üretilen Bayramiç peynirlerinin probiyotik olarak pazarlanmasında avantaj sağlayabileceği görülmüştür. Daha fazla örnekte yapılacak çalışmalarda; kaprik asit, kapronat (Etil-) ve benzen, 2,4-diizosiyanato-1-metil'in Bayramiç peynirlerinin diğer peynirlerden farklılığını ortaya koyabilecek indikatör bileşenler olarak kullanılabileceği değerlendirilmiştir. Bifidobakteri inokülasyonu ve olgunlaşma günlerinin interaksiyonu ürün kalitesi için önemli olarak belirlenmiştir. Starter kültür olarak bifidobakteri kullanmak isteyen üreticilerin olgunlaşma sürelerine de dikkat etmeleri gerektiği düşünülmektedir.

Anahtar kelimeler: artizan peynir, starter kültür, laktik asit bakterileri, kaprik asit

1. Introduction

Bifidobacteria and lactic acid bacteria are not only associated with health and nutritional benefits for the cheese consumer, but are also responsible for the aromatic components of cheese (ReyesGavilán et al., 2004; Homayouni et al., 2020; Coelho et al., 2022). In recent years, probiotic microorganisms have been considerable interest for functional food products. Fermented dairy products are at the forefront of functional foods containing probiotics. Especially ripened white cheese provides both time for the development of probiotic bacteria and a good growth environment for them (Hammam and Ahmed, 2019; Hamdy et al., 2021). Thanks to the maturation period of at least three months in accordance with the legal regulations, bacteria such as lactic acid bacteria develop in the environment and provide the desired physical, chemical and sensory properties for cheese (Castro et al., 2015; Hamdy et al., 2021).

Milk microbiota has a pivotal role in the cheese properties, flavor and aroma. Cheese-making technologies affect the dairy microbiota composition and activity. Ripening selects microorganisms responsible for the cheese flavor and aroma (Tilocca et al., 2020).

Understanding the dairy microbiota dynamics is of paramount importance for controlling the qualitative, sensorial of the cheeses (Montel et al., 2014; Tilocca et al., 2020). Raw milk microbiota is affected by the type of feed (grass, silage, hay etc.) fed to animals (Montel et al., 2014). Different raw milk microbial compositions have been reported in relation to different pasture characters and pasture's altitude. Results highlighted the presence of diverse microbial profiles between samples collected at the diverse altitudes and pastures altitude, pasture characters and weather conditions were effective on the raw milk microbiota composition (Bonizzi et al., 2009; Montel et al., 2014).

Different types of cheeses require different time to enrich their own sensory and organoleptic characteristics. Ripening temperature, time and brine and/or storage conditions (humidity, air motion/velocity or air microbiota etc.) are critical for final cheese quality (Ong and Shah, 2009; Cardarelli et al., 2009; Homayouni et al., 2020).

Ripening process of cheese has positive affect on its organoleptic and texture properties. Some studies reported the positive effect of probiotics some sensory characteristics of cheese during ripening. However, some of the studies indicates statistically insignificant effects of probiotics. The added probiotics should be beneficial to the health and should not adversely affect the flavors of the cheese product to be successfully marketed (Urala and Lähteenmäki, 2004; Burns et al., 2008; Hammam and Ahmed, 2019). Cheese is reported as one of the most effective carrier food for probiotic bacteria (da Cruz et al., 2009). During digestion cheese has advantages on protecting probiotic bacteria because of its buffer capacity in the acidic conditions of the gastrointestinal tract and in stomach (Castro et al., 2015).

This study aimed to determine the differences in bifidobacteria and lactic acid bacteria counts, major aromatic components and sensory properties of Bayramiç cheese samples, which are traditionally produced with and without inoculating starter culture and ripened in brine at different periods. In addition, this traditional cheese was compared with industrially produced some cheeses.

2. Material and methods

Fresh cow milk was obtained from a local farmer in Bayramiç (Çanakkale). Traditional methods were used for Bayramiç cheese production. Raw milk was pasteurized (70±1 °C for 20 min.) and cooled to 33 °C. As starter culture, *Bifidobacterium animalis* subsp. *lactis* Bb 12 (7 log cfu/mL) (Hørsholm, Denmark) was added to milk (Ozdemir ve Erigdeci, 2022). Rennet (0.026 g/L) was added to milk for coagulation and incubated for 1.5-2 hours at 32 °C. Curd was cut and whey was drained. Cheese curd was Pressed and moulded. Cheese was portioned (~500 g) and rested in brine (contained 14 % salt) at 28 °C for 24 hours.

Cheeses were put in cube-shaped sealed plastic packs with 4% brine. Cheese:brine ratio was adjusted as 10:1. After packaging, cheeses were stored at 4 °C. Samples were taken 0, 45, 90 and 135 days of storage for analysis. Three different industrial processed cheeses were purchased by market. Information on those cheeses was obtained from the producer companies. They were produced on an industrial scale from cow's milk, no starter culture is used in their production, standard ripened white cheese production methods are used, they are aged at 4°C for 3 months and offered for sale as full-fat ripened white cheese. They were named as industrial sample A, B and C.

Bifidobacteria counts were done by using MRS (Man Rogosa Sharpe) broth, which was conventionally prepared and supplemented with NNLP. NNLP solution was prepared with lithium

chloride (3 g), nalidixic acid (15 mg), neomycin sulfate (100 mg), paromomycin sulfate (200 mg) and L-cysteine hydrochloride (0.5 g), diluted in distilled water (100 mL), filter sterilized (0.22 mm), and added to MRS broth at 10% (incubation at 37 ℃ for 48 hours) (Miranda et al. 2011). Lactic acid bacteria count was done with 3M Petrifilm™. Lactic Acid Bacteria Count Plate (incubation at 30 °C for 48 hours) (Nero et al., 2020).

For mold and yeast counting, samples were homogenized with Maximum Recovery Diluent in accordance with food microbiology laboratory rules. Dilutions were prepared according to the expected number in the sample, as serial dilutions of 1/10. Petri dishes containing 1 ml of DRBC (Dichloran Rose Bengal Chloramphenicol) agar medium were inoculated from each dilution. It was incubated at 25 ℃ for 5 days (FDA/BAM, 2001).

Aromatic components were extracted according to the method of Delgado et al. (2011). The external part of the cheese was removed (1.5 cm). 10 g of cheese taken as a sample and placed in a 50 mL vial and then 10 ml NaH_2PO_4 (25%, w/v) were added. The sample was stirred for 30 min at 50 ℃ to accelerate the equilibrium of headspace volatile compounds between the cheese matrix and the headspace. Chromatographic conditions were used same as Delgado et al. (2010).

Flavor, texture, taste, color and overall acceptance characteristics of each cheese samples were determined. Sensory panel test was done by 10 trained and experienced panelists. The panelists had at least food or agricultural engineering degree. Panelists used a nine-point hedonic scale; the higher score means the higher quality (Afkhami et al., 2019; Zonoubi and Goli, 2021).

In this study two factorial (ripening day and inoculation status) experimental design was used for statistical analysis. Analysis of variance was applied to determine the presence of significant differences ($p<0.05$) between the bifidobacteria inoculated and spontaneous samples. Statistical analysis was performed by using the JMP 7 statistical package program. Different letters indicate significant difference in same colon of tables.

3. Results and discussion

Bifidobacteria are not member of the lactic acid bacteria. Bifidobacteria are also not closely related to any of the traditional lactic acid bacteria used in the production of cheese or other fermented foods (Mokoena et al., 2016). In this study, the effect of bifidobacteria inoculation and microbiota of Bayramiç cheese during ripening were determined. Results were also compared with some industrially processed ripened cheeses. Bifidobacteria could not be detected in spontaneous fermented cheese and industrial processed cheese samples. These results were given in Table 1. It is clearly seen from Table 1 that, bifidobacteria inoculation supported the growing of lactic acid bacteria significantly. On the other hand, statistically least yeast and mold counts were detected in bifidobacteria inoculated samples.

Table 1. Microbiologic counts of Bayramiç and industrially processed cheeses (log cfu/g).

Inoculation status	Day	Bifidobacteria	Lactic acid bacteria	Yeast	Mold
Bifidobacteria inoculated	0	6.81±0.12b	8.7±0.14c	2.1±0.02c	4.1±0.02c
	45	6.83±0.04b	9.4±0.12b	2.1±0.07c	3.5±0.07d
	90	7.02±0.11a	9.7±0.07a	2.2±0.07c	3.7±0.06d
	135	7.15±0.04a	9.6±0.21ab	2.4±0.14b	4.2±0.12c
Spontaneous	0	ND	7.3±0.21f	3.2±0.14a	4.2±0.14c
	45	ND	8.1±0.07d	2.5±0.20b	4.6±0.21ab
	90	ND	7.8±0.01e	2.6±0.08b	4.8±0.07a
	135	ND	7.6±0.07ef	3.1±0.12a	4.5±0.12b
Industrial sample A		ND	7.2±0.10	3.7±0.08	5.8±0.20
Industrial sample B		ND	7.7±0.10	3.5±0.06	4.5±0.20
Industrial sample C		ND	7.4±0.16	3.1±0.06	4.1±0.10

Different letters in column represent statistical difference. ND: not detected.

Hamdy et al. (2021) aimed to produce low-fat Feta cheese by using yogurt cultures, bifidobacterium cultures (*Bifidobacterium bifidum* and *Bifidobacterium longum*) and mixed of them. They reported the bifidobacteria and total yeast and mold counts of cheeses between 5.1-7.3 and 2.1-2.8 log cfu/g respectively. In this study similar results were found for bifidobacteria, but higher results were

found for yeast and mold counts than those of Hamdy et al. (2021). Our results showed that bifidobacteria inoculated samples had the highest bifidobacteria and lactic acid bacteria counts after 90 and 135 day of ripening. Interactions of inoculation status and storage days were detected as statistically significant for all microbial counts. In particularly, no linear or regular change was observed for all samples for mold and yeast counts. Bifidobacteria inoculated samples had more regular increase in lactic acid bacteria counts.

In addition to its beneficial effects on health as a probiotic microorganism, it has been reported that bifidobacteria can contribute positively to industrial cheese production and help cheese stability during storage (Miranda et al., 2011). Many strains of bifidobacteria can be used as starter culture which is well adapted to survive in large numbers during the manufacturing process and storage up to consumption for fermented milks (Gueimonde et al., 2004). This study is in agreement with the literature which expressed increase in the number of bifidobacteria during storage. For marketing the food as a probiotic, food must have probiotic bacteria at least 6 log cfu/g. This study also showed that 7 log cfu/mL inoculation of bifidobacteria can ensure the probiotic effects and probiotic marketing of cheese.

Microorganisms especially, lactic acid bacteria, bifidobacteria, yeast and molds contribute to the organoleptic and textural profile of cheeses (Fernández et al., 2015; Coelho et al., 2022). Lactic acid bacteria and bifidobacteria are also responsible to produce lactic acid and they contribute to the organoleptic and textural profile of cheeses (Mattu and Chauhan, 2013; Tajic Ahmadabadi et al., 2020).

Main aromatic components of Bayramiç cheese and industrially processed cheese were given in Table 2. Bifidobacteria inoculated samples were found to have higher aromatic components than spontaneous samples and industrially processed samples. The results also showed that bifidobacteria inoculation promoted the increase in aromatic components during the ripening process. Both bifidobacteria inoculated and spontaneous samples had higher benzene, 2,4- diisocyanato-1-methyl and capric acid content than industrially processed cheeses. Bayramiç cheeses have been produced traditionally by spontaneous fermentation. In this study, spontaneous fermented cheese has similar ethanol and capronate (ethyl-) content with industrial cheese samples. On the other hand, they had higher Benzene diisocyanato-1-methyl and capric acid content than industrial samples. Bifidobacteria inoculated samples showed more regular ripeness. This is thought as important advantage for controlled production of standard quality cheese.

Table 2. Relative concentration of the main aromatic components of Bayramiç cheese and their comparation with industrially processed cheese samples (%)

Inoculation status	Day	Ethanol	Capronate (ethyl-)	Benzene, 2,4-diisocyanato-1-methyl	Capric acid	Hexanoic acid	Acetic acid
Bifidobacteria inoculated	0	8.45±0.35d	8.48±0.40c	6.39±0.24d	8.28±0.26e	10.42±0.21e	8.29±0.13cd
	45	13.95±1.06b	9.98±0.30b	8.39±0.24c	10.28±0.26c	12.15±0.24c	8.38±0.17c
	90	18.05±0.92a	11.90±0.85a	9.73±0.16b	11.67±0.26b	13.33±0.20b	8.22±0.11d
	135	18.40±1.27a	12.75±0.78a	11.45±0.42a	12.44±0.16a	16.52±0.22a	9.89±0.12a
Spontaneous	0	8.41±0.20d	8.75±0.21c	8.53±0.22c	7.55±0.28f	10.49±0.26e	8.47±0.21c
	45	9.20±0.14cd	8.79±0.28c	8.40±0.13c	9.04±0.03d	10.16±0.31de	8.55±0.21c
	90	9.25±0.06cd	9.00±0.21bc	8.53±0.20c	8.77±0.04de	11.33±0.22d	8.70±0.18b
	135	10.18±0.19c	9.25±0.08bc	8.75±0.21c	9.10±0.28d	12.03±0.20c	9.14±0.21ab
Industrial sample A		9.62±1.10c	1.35±0.44d	1.38±0.29d	ND	8.62±0.23f	8.42±0.21c
Industrial sample B		11.17±1.04bc	1.07±0.32d	ND	ND	6.51±0.11g	6.59±0.33e
Industrial sample C		3.29±0.18e	ND	ND	ND	6.88±0.14g	13.96±0.46

Different letters in column represent statistical difference. ND: not detected.

In the inoculated samples, a more regular increase was observed in the aromatic components. The increase in ethanol and capronate (ethyl-) contents during the ripening of cheeses produced by the spontaneous method was remarkable. In order to be able to sell under the ripened cheese label, the cheeses must be matured for at least 90 days legally. In this study a significant increase in

aromatic substances was observed after 90 days of maturation in both bifidobacteria inoculated and spontaneously produced cheeses. Increase in ripening period more than 90 days also has caused an increase in the contents of aromatic components. Bifidobacteria inoculated samples had higher aromatic content in almost all ripening periods

Ethanol content of sample was reported as 10.26% of total aromatic components but other major aromatic compounds capronate (ethyl-), benzene, 2,4 diisocyanato-1-methy and capric acid were not detected in ripened white cheese (Suzuki-Iwashima et al., 2020). Total lactic acid bacteria counts (as log. values) were determined at 7.4 and 7.5 after 90 and 180 days ripening periods for white cheese (Tzora et al., 2021).

Non-starter lactic acid bacteria contribute to ripening along with the starter lactic acid bacteria of cheese. Non-starter lactic acid bacteria can increase the content of small peptides and free amino acids by increasing proteolysis and peptide hydrolysis and produce aroma compounds to enhance the quality of cheese (Beresford and Williams, 2004; Settanni and Moschetti, 2010; Li et al., 2021).

Li et al (2021) isolated some lactic acid bacteria from traditional cheeses. They inoculated these lactic acid bacteria to contribute to ripening of cheese. Lactic acid bacteria inoculation showed an enhancing effect on several acids, such as capric acid which add flavor to cheese. After 40 days of ripening, the researchers reported that octanoic acid or capric acid was not detected in the control samples, but it was detected in cheese inoculated with lactic acid bacteria. (Li et al., 2021). Similarly in this study, capric acid was not detected in industrial samples. Capronate (ethyl-) and benzene, 2,4-diisocyanato-1-methyl were not detected or detected very low relative concentration but they can be detected in Bayramiç cheese sample bifidobacteria inoculated or not. It is thought that this difference is due to the fact that the milk used in Bayramiç cheese is obtained from cows grazing in the Kaz Mountains region. It is reported that different aromatic components are more common in milk obtained from cows fed with fresh and rich grass diversity (Coppa et al., 2011; Kilcawley et al., 2018; Formaggioni et al., 2020)

Sensorial test scores of Bayramiç and industrially processed cheese samples were given in Table 3. When the sensory analysis results were evaluated, starter inoculated and spontaneous processed samples were determined in different statistical groups for overall acceptance scores. Statistical differences were not determined for color scores. Statistical analysis results showed that, starter inoculation and ripening period were effective on these sensory scores.

Table 3. Sensorial test scores of Bayramiç and industrially processed cheese samples.

Inoculation status	Day	Flavor	Texture	Taste	Color	Overall Acceptance
Bifidobacteria inoculated	0	7.03±0.14d	6.35±0.35d	6.22±0.21f	8.10±0.14	6.46±0.21f
	45	8.61±0.15a	6.90±0.28c	7.03±0.17cd	7.90±0.10	8.55±0.13a
	90	8.25±0.28b	6.85±0.07c	7.50±0.13b	7.85±0.21	8.16±0.14b
	135	8.60±0.28a	7.40±0.13a	7.88±0.15a	7.70±0.13	8.07±0.20b
Spontaneous	0	6.52±0.16e	6.30±0.42d	6.09±0.27f	7.95±0.20	6.20±0.22g
	45	7.86±0.14c	6.85±0.35c	6.94±0.24d	7.80±0.28	7.15±0.07e
	90	7.90±0.14c	6.92±0.16c	7.55±0.16c	7.65±0.35	7.80±0.12c
	135	8.00±0.22bc	7.00±0.55b	7.40±0.22c	7.45±0.09	7.73±0.10c
Industrial sample A		7.15±0.28d	6.91±0.35b	6.65±0.40de	8.17±0.47	7.12±0.20e
Industrial sample B		7.86±0.44c	6.72±0.42c	6.48±0.44e	8.08±0.35	7.35±0.26d
Industrial sample C		7.94±0.75c	6.37±0.46d	6.69±0.68de	8.02±0.26	7.06±0.38e
Scoring range		1-9	1-9	1-9	1-9	1-9

Different letters in column represent statistical difference.

The color and appearance scores of low-fat feta cheese were reported between 9-12 (Perfect score: 15 point). Flavor scores and overall acceptance of this cheese was also determined between 20-46 (Perfect score: 50 point) and 58-91(Perfect score: 100 point) (Hamdy et al., 2021). Similar to the results of Hamdy et al. (2021), high color and appearance scores were determined for both starter inoculated and spontaneous processed samples in this study. In Bayramiç region, Bayramiç cheeses

are produced with spontaneous methods. In this study, ripened spontaneous Bayramiç cheese had higher taste and overall acceptance scores than all industrial samples at 90 days ripening period. Bifidobacteria inoculated cheeses had higher overall acceptance scores than all samples. Higher sensory scores of bifidobacteria inoculated cheese possible related to higher content of aromatic components which were showed in Table 2. Bifidobacteria inoculation allowed more aromatic cheese production. Color values of industrial samples were higher than all ripened cheese samples.

4. Conclusion

Study result showed that bifidobacteria inoculation as a starter culture can enhance regular improvement of ripeness and more standard cheeses production. Interaction of bifidobacteria inoculation and ripening days were determined as important for product quality. So that producers who are willing to use starter culture should also give attention for ripening durations. Some aromatic components of Bayramiç cheese were remarkably higher than industrial processed cheeses. More detailed studies are necessary on this aromatic components, as an indicator to reveal the differences between Bayramiç cheese and other cheeses. The possibility that it may originate from the milk of cows fed with vegetation belonging to the Kaz Mountains may provide an idea for future researches.

One of the most significant challenges for probiotic products is the survival of probiotic bacteria during processing and shelf life. Bifidobacteria inoculation can be used as a method for production of probiotic cheese without any sensorial disadvantages or aromatic component losses.

It was seen that, bifidobacteria inoculation provided an advantage in terms of sensory properties, aromatic substance content and microbiological counts. It has been also observed in regular maturation for cheese ripening. On the other hand, it has been determined that Bayramiç cheese contains more aromatic substances that can differentiate it from other industrial cheeses. It is thought that the results obtained in this research may provide an advantage in marketing to cheese producers in the Bayramiç region. The other important issue is that, more studies are necessary for providing a competitive advantage by differentiating/diversifying these traditional foods.

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