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Araştırma Makalesi / Research Article

A Preliminary Study on the Physicochemical, Microbiological and Sensory Characteristics of Purslane and/or Cayenne Pepper Added Drinking Yogurt, A Traditional Anatolian Beverage

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Abstract

Drinking yogurt (ayran) is one of the valuable traditional Anatolian dairy products. Ayran is prepared traditionally through adding water and salt to yogurt or manufactured industrially by fermentation of yogurt culture added diluted milk. Nowadays, interest and consumption of traditional different fermented dairy products have spread rapidly around the world. Although cucumber-yogurt (cacik), soda ayran, fruitful ayran have a commercial face, there is still locally consumed drinking yogurt ayran types. One of the locally consumed ayran types is herby-hot ayran from Puturge-Malatya. In the preparation of herby-hot ayran, the flavoring herbs/spices are added to the ayran in earthenware pots then buried under the soil and left to ferment spontaneously for at least 15 days. The objectives of this study were to determine physicochemical, microbiological, and sensory properties of traditional purslane and/or Cayenne pepper added drinking yogurt from Puturge-Malatya, Turkey. The analysis results were found total dry matter 5.80-7.03 %, pH 3.77-4.30, titratable acidity 0.61-1.17 % lactic acid, serum separation 11.17-21.5 mL (50 mL)$^{-1}$. Levels of lactic acid bacteria (LAB) ranged from 7.00 to 8.04 log cfu mL^{-1} and the mold and yeast (MY) levels varied from 5.25 to 6.98 log cfu mL^{-1} for studied samples. Properties of traditional herby-hot ayran are found different from plain ayran. The results demonstrated that this product may have the potential for consumer preference. To reveal the changes occurring in product characteristics during the fermentation period would be thus of interest.

Keywords: Fermented products, Drinkable yogurt, Herby hot ayran, Purslane, Cayenne pepper

Geleneksel Bir Anadolu İçeceği olan Semizotu ve/veya Acı Biber İlaveli Ayranın Fizikokimyasal, Mikrobiyolojik ve Duyusal Özellikleri Üzerine Bir Ön Çalışma

Öz

İçilebilir yoğurt (ayran), Anadolu'nun değerli geleneksel süt ürünlerinden biridir. Ayran geleneksel olarak yoğurda su ve tuz ilave edilerek hazırlanır veya yoğurt kültürü ilave edilen seyreltilmiş sütün fermantasyonu ile endüstriyel olarak üretilir. Günümüzde, geleneksel farklı fermente süt ürünlerinin tüketimi ve onlara olan ilgi dünya çapında hızla yayılmaktadır. Cacık, sodalı ayran, meyveli ayran ticari bir yüze sahip olsa da, hala yerel olarak tüketilen ayran çeşitleri mevcut olup bunlardan biri de Puturge-Malatya'nın otlu-acılı ayranıdır. Otlu-acılı ayran üretiminde, çömleklerdeki ayrana aroma verici otlar/baharatlar eklenir ve en az 15 gün toprak altındaki çömleklerde spontan fermantasyona bırakılır. Bu çalışmanın amacı, Puturge-Malatya'nın geleneksel semizotu ve/veya acı biber ilaveli ayranının fizikokimyasal, mikrobiyolojik ve duyusal özelliklerini belirlemektir. Örneklerin toplam kuru madde % 5.80-7.03, pH 3.77-4.30, titre edilebilir asitlik % 0.61-1.17 laktik asit ve serum ayrılması 11.17-21.5 mL (50 mL) $^{-1}$ aralığında belirlenmiştir. Laktik asit bakteri (LAB) seviyeleri 7.00 ile 8.04 log cfu mL^{-1} ve küf ve maya (MY) değerleri 5.25 ile 6.98 log cfu mL^{-1} arasında değişmiştir. Geleneksel otlu-acılı ayranın özellikleri sade ayrandan farklı bulunmuştur. Çalışmanın sonuçları, bu ürünün tüketicilerin tercih edebileceği bir potansiyele sahip olabileceğini göstermiştir. Fermantasyon sırasında ürün karakteristiğinde meydana gelen değişiklikleri ortaya çıkarmak bundan sonraki çalışmamızda ilgi alanımız olacaktır.

Anahtar Kelimeler: Fermente ürünler, İçilebilir yoğurt, Otlu acılı ayran, Semiz otu, Acı biber.

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1. Introduction

Dairy products have been widely consumed since the early history of mankind. Milk-based products especially fermented ones make a considerable contribution to human health depend on rich sources of beneficial metabolic compounds. Nowadays, a wide range of yogurt forms is consumed all over the world. Fermented milk beverages are called drinking yogurt in Europe, kefir and kumis in the Middle East, lassi in India and ayran in Turkey with different names according to cultures (Karim et al., 2017). Ayran, drinkable yogurt, is a fermented dairy product made by the addition of water to yogurt (homemade) or addition of yogurt cultures; *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* to industrially produced standardized milk (TPC, 2009). Additively plain ayran, different types of ayran are generally consumed such as cucumber-yogurt (tsatsiki), herby ayran, soda ayran, fruitful ayran. The dairy product marketplace continues to go forward as the popularity of the diet-health relationships is on the rise among consumers. This increase is also due to radical cultural, social and economic changes worldwide.

A certain product variety is currently available for the dairy industry. However, a number of traditional dairy products are still waiting for commercial interest. One of these is Puturge ayran which may meet functional alternatives' demand of consumers. Herby-hot ayran called “Puturge ayran” in Turkish, is mainly consumed in Puturge-Malatya, Turkey. Puturge County is located in the most eastern area of Malatya City, Turkey and is surrounded by the southeastern Taurus Mountains (Tuna et al., 2020). The ingredients of this traditional fermented beverage are yogurt, purslane, Cayenne pepper, water, and salt. By those functional ingredients, a fortified, flavored, health-promoting form of plain ayran has been conventionally obtained. One of the aims of the present study was to examine the physicochemical, microbiological and sensorial properties of Puturge ayran. The other outstanding point was to compare characteristics of plain ayran with Puturge ayran. There are studies mentioning the physicohemical properties of fruit-added yogurt. Only one other study has analysed powdered and sliced Cayenne pepper addition on the properties of ayrans (Akcay et al., 2020). To the best of our knowledge, this is the first paper to investigate physicohemical and microbiological characteristics of Cayenne pepper and/or purslane added ayran as a traditional Anatolian beverage: Malatya’s Puturge ayran.

2. Material and Methods

Puturge ayran samples manufactured in August 2017 were supplied from Puturge Food, Agriculture and Livestock District Direction, Malatya, Turkey. Samples were identified and named as A (ayran with purslane and Cayenne pepper), B (ayran with purslane), C (ayran with Cayenne

pepper), D (plain ayran). Traditional ayran samples were manufactured by mixing of 0.5 kg yogurt, 3.5 L water, 160 mL soda, and 50 g salt. Produced drinking yogurts contain different amounts of purslane and/or Cayenne pepper. The formulation of samples along with details is shown in Table 1. Spontane fermentation in earthenware pots buried under the soil was performed for 15 days. Ayrans were passed through by using classic kitchen utensils to remove solid parts of samples in accordance with the traditional consumption. Drinkable yogurt samples were refrigerated at +4 °C for two days until measurements.

Table 1. Formulation of drinking yogurts

Sample	Ingredients					
	Yogurt (g)	Water (L)	Salt (g)	Soda (mL)	Cayenne pepper (g)	Purslane (g)*
A	500	3.5	50	160	240	400
B	500	3.5	50	160	-	400
C	500	3.5	50	160	240	-
D	500	3.5	50	-	-	-

* Purslane was cutting into small pieces, boiled for 20 minutes and drained.

2.1. Physicochemical Analysis

Total dry matter, water-soluble dry matter (°Brix), pH, % titratable acidity (lactic acid based) contents of samples were determined according to AOAC (2005) standardized methods. Serum separation analysis was determined based on Koksoy and Kilic (2003). Results were given as mL of serum separation per 50 mL ayran.

2.2. Color Analysis

The color values of the samples were determined using the A Hunterlab Colorflex CFLX 45-2, VA instrument and measured CIE L*, a* and b* values (Estrada et al., 2011).

2.3. Microbiological Analysis

10 ml of sample and 90 ml of sterile 0.85% NaCl solution were homogenized for 3 minutes and inoculated into petri dishes from the 10^{-9} dilution series. This process was repeated for each ayran sample. Total mesophilic aerobic bacteria (TMAB) were determined using Plate Count Agar (PCA-Merck) at 37 ºC for 48h and mold-yeast count were determined by incubating in Potato Dextrose Agar (PDA-Merck) at 25 ºC for 3-5 days. Also, Lactobacilli and Lactococci numbers were counted in MRS agar at 44 ºC and in M17 agar at 37 ºC for 48h, respectively. Additionally, possible

Escherichia coli and coliform bacteria colony numbers in ayran were defined with Violet Red Bile Agar (Downes and Ito, 2001). Results were indicated as colony-forming units (log cfu) per mL of ayran.

2.4. Sensory Evalutaion

Coded samples were kept at room temperature for 10-15 minutes before sensory evaluation and served with water. The sensorial characteristics of studied ayran samples were evaluated by a jury of 12 panelists with 10-point scale according to five parameters: appearance, body-texture, taste-flavor, odor and general acceptance (Altug Onogur and Elmaci, 2011).

2.5. Statistical Analysis

Obtained results were shown as means ± standard deviations, and processed by ANOVA followed by Duncan's test (p-value 0.05) using the software SPSS 20.

3. Results and Discussion

The results of physicochemical properties of ayran samples are shown in Table 2. Control ayran differed statistically from other ayran samples. A study on some physicochemical properties of 20 different plain ayran samples were reported that the pH values range between 3.60-4.70 (Cetin et al., 2014). The pH range were given as 4.10-4.17 by Erkaya et al. (2015). Our results for the samples were similar to those in the stated reports. Ayran samples were formulated by different ingredients i.e. purslane and Cayenne pepper that may affect the physicochemical characteristics of samples. The effects of purslane and Cayenne pepper addition on pH and titratable acidity were found to be significant. However, no significant differences were found between pH, %titratable acidity, and °Brix of Puturge ayran samples. This result was actually expected since the purslane and Cayenne pepper in their content were sieved before the storage stage in accordance with the traditional consumption. Control sample has the highest pH (pH: 4.30) and the lowest titratable acidity values. The acidity and the pH level of a fermented product are associated with the microbial load and activity conditions (Erkaya et al., 2015). This may be because of the bacterial growth and the production of lactic acid in samples. The presence of the prebiotic polysaccharides that might sustain the metabolism of lactic acid bacteria and lactic acid bacteria decrease pH by producing organic acids. As it is well known that the proteolytic activity of Lactobacilli is higher than that of Lactococci. Cayenne pepper and/or purslane added samples were dominated by Lactobacilli. Therefore, it is

thought that the increase in Lactobacilli counts improve proteolysis and may contribute to titratable acidity of samples.

Table 2. pH, titratable acidity (%), total soluble dry matter content (°Brix) of samples

Sample	pH	Titratable acidity (%lactic acid)	Total soluble dry matter (°Brix)	Serum separation mL (50 mL) $^{-1}$
A	3.87±0.01^{a}	1.12±0.01^{a}	4.10±0.01^{a}	21.50±0.36^{d}
B	3.77±0.02^{a}	1.15±0.01^{a}	4.00±0.01^{a}	16.17±0.17^{b}
C	3.89±0.01^{a}	1.17±0.07^{a}	4.00±0.10^{a}	17.33±0.19^{c}
D	4.30±0.31^{b}	0.61±0.03^{b}	5.00±0.20^{b}	11.17±0.10^{a}

* Different superscripts letters (a–d) in the same column represent significant differences between means ($p<0.05$).

Serum separation in acidic fermented dairy products is the formation of the serum phase at the top of the product, which is due to the loss of water from a continuous protein matrix (Gursoy et al., 2016). It is one of the main textural defects in fermented milk drinks (Shirkhani et al., 2015). Serum separation occurs due to sedimentation of large particles and casein proteins at the bottom due to aggregation (Kiani et al., 2008). Fermentation temperature and final pH are important factors influencing serum separation as they would affect the structure of the protein network (Ozdemir and Kilic, 2004). Low dry matter content, high incubation temperature, rapid acidification, low acid production, and storage conditions have a profound impact on serum separation. The serum separation is unavoidable for traditionally produced ayran because of the lack of homogenization and standardization process. The serum separation results of the analyzed ayran samples were statistically different ($P<0.05$). Obtained results are in complete accordance with the values of 21.5, 16.17, 17.33, and 11.17 mL (50 mL)$^{-1}$ serum separation for A, B, C, and D samples, respectively (Table 2). The water holding capacity of protein molecules is limited under low acidity conditions. Since the highest pH and the lowest serum separation values belong to the plain ayran, it can be said that the acidity does not cause this type of change. It seems that, in this case, serum separation may be associated with total soluble solid content of samples. The total soluble solid content in plain ayran was higher than that in purslane and/or Cayenne pepper added samples. The use of total soluble solids by higher number of bacteria for their metabolism in purslane and/or Cayenne pepper added samples may cause lower soluble solid content and higher serum separation phenomenon. Many studies have been performed considering stabilizing agents (Laurent and Boulenguer, 2003; Janhoj et al., 2008; Anli et al., 2013; Gharibzahedi and Chronakis, 2018), emerging technologies such as ultrasound (Ertugay et al., 2012) and thermosonication (Riener et al., 2009) to prevent serum separation for fermented dairy products.

The color parameters L*, a*, and b* of all studied ayran samples (Table 3) presented significant differences (P <0.05). Samples formulated with the purslane (B), purslane and Cayenne pepper (A) had darker color values (lower L* and higher a*) compared with plain ayran. The decrease in luminosity was increased with the addition of the Cayenne pepper and purslane. Those values may be attributed to the leakage of natural pigments to the drinking yogurt samples. The purslane and Cayenne pepper added sample made higher contribution of the red color than purslane added ayran. However, the same changes were not detected in Cayenne pepper added samples. The anthocyanins can be easily degradable and affected by the microbial flora and pH, and can exhibit colourless or brown-coloured compounds (Karaaslan et al., 2011). From this point of view, it is thought that the differences in the color values of the Cayenne pepper added samples (C) are not only due to the formulations, but also to the complex reactions including anthocyanin degradation and microbial activity.

Table 3. Color values of samples

Sample	L*	a*	b*
A	4.81 ± 0.23^{a}	7.08 ± 0.38^{d}	7.99 ± 0.23^{a}
B	7.00 ± 0.07^{b}	5.97 ± 0.10^{c}	11.60 ± 0.14^{b}
C	16.74 ± 0.23^{d}	0.08 ± 0.03^{a}	24.01 ± 0.40^{d}
D	10.45 ± 0.14^{c}	0.56 ± 0.04^{b}	16.76 ± 0.18^{c}

* Different superscripts letters (a–d) in the same column represent significant differences between means ($p<0.05$).

Table 4. Sensory evaluation results for ayran samples

Sample	Appearance	Texture	Taste	Odor
A	7.05 ± 0.76^{a}	8.76 ± 1.17^{b}	7.20 ± 1.59^{ab}	8.44 ± 0.38^{bc}
B	7.05 ± 1.18^{a}	8.36 ± 1.07^{ab}	7.61 ± 0.54^{ab}	7.78 ± 1.41^{b}
C	$7,30\pm1.52^{a}$	7.36 ± 0.82^{a}	6.19 ± 1.43^{a}	6.54 ± 0.43^{a}
D	9.80 ± 0.27^{b}	8.88 ± 0.83^{b}	8.30 ± 0.65^{b}	9.00 ± 0.61^{c}

*Different superscripts letters (a–c) in the same column represent significant differences between means ($p<0.05$).

Plain ayran had the highest score, while the others did not show a significant difference for in the case of appearance (Table 4). The darkening effect of purslane and Cayenne pepper in formulation did not reflect the appearance scores of Puturge ayrans. Texture descriptors were used as homogeneity, smoothness, and thickness Ayran with Cayenne pepper (C) were rated lower (P <0.05) than the others in terms of texture. Although the color measurement results do not completely overlap with the appearance scores, the low texture score of the sample with Cayenne pepper (C) indicates that there is a problem in terms of homogeneity and smoothness in the sample. This problem may be the other reason for the difference in L*, a*, and b values of Cayenne pepper added samples. There is no statistical difference between texture scores of A (ayran with purslane and Cayenne pepper) and

D (plain ayran). Taste values of samples containing purslane were found similar according to statistical analysis. Plain ayran received the highest score while other ayran types received different scores by panelists when the general acceptability was evaluated (Figure 1).

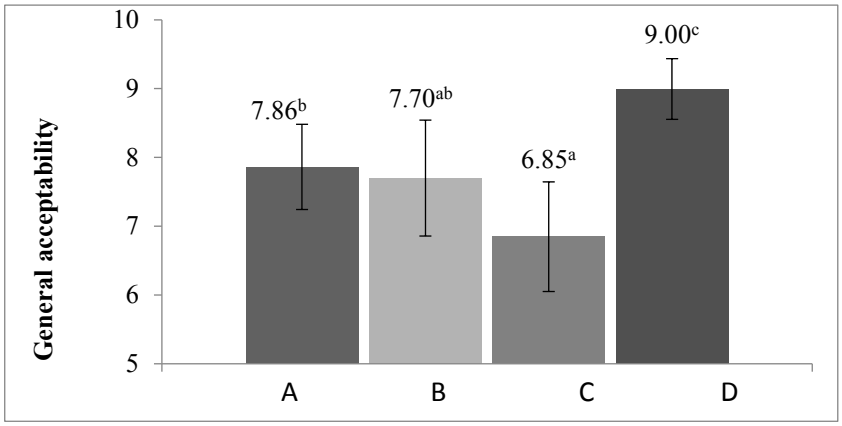

Figure 1. General acceptability results of ayran samples (Values with different superscripts letters on the bars indicate significant differences ($p<0.05$)).

Quality characteristics including flavor and textural properties are also directly associated with the microbial load. The content of viable microflora and its development is determining factor for the quality and shelf life of dairy products. Types of microorganisms may be increased either by contamination or by the growth of the microorganisms already present (Cardak and Yılmaz, 2011). Lactic acid bacteria are the most common group of microorganisms in those products. LAB fabricates lactic acid using lactose and can grow at extremely high acid concentrations (Kucukoner et al., 2006). Levels of LAB ranged from 7.00 to 8.04 log cfu mL^{-1} for studied samples (Table 5). Significant differences were found between microbial analysis results of herby ayrans and control sample. The LAB counts in herby ayrans were significantly higher than for plain ayran ($P<0.05$). There are studies that specifically addressed the plants as a rich source of functional nutrients and prebiotic substances (Joung et al., 2016). Since those substances enhanced the metabolic activities and counts of yogurt bacteria, the pH was decreased by the production of organic acids in fermentation process (Amirdivani and Baba, 2011). Thus, this result may be evaluated as an evidence of the positive effect of purslane and/or Cayenne pepper towards the counts of traditional yogurt microflora. As it was also expected, traditional drinking yogurts have a higher microbial load than plain ayran. The mold and yeast (MY) levels in the samples were high and varied from 5.25 to 6.98 log cfu mL^{-1}. *E. coli* and coliform bacteria were detected in any of the four drinking yogurt. Same maximum range of microbiological values for plain ayran collected from Aydin, Turkey were reported by Cardak and Yilmaz (2011). Kucukoner et al. (2006) found lower values for LAB, TMAB, and yeast and mold in herby cacik. Those authors have also reported that the higher numbers of the mold and yeast may originate from the added herbs. This statement can be accepted for plain ayran (D) and purslane and

Cayenne pepper added samples (A). However, it was found that plain ayran (D) have the higher mold and yeast loads than ayran with purslane (B) and Cayenne pepper (C). Therefore, there is need for the detailed knowledge on the competition and adaptation of LAB, yeast and molds in Puturge ayran fermentation.

Table 5. Lactic acid bacterial counts, total mesophilic aerobic bacterial counts and mold and yeast counts of ayran samples

Sample	Microbial counts (Log cfu mL^{-1})			
	Lactobacilli	Lactococci	TMAB	MY
A	7.70±0.14^{b}	7.55±0.21^{b}	8.39±0.14^{bc}	6.98±0.19^{c}
B	8.04±0.28^{b}	8.00±0.04^{c}	8.10±0.29^{ab}	5.28±0.22^{a}
C	8.03±0.36^{b}	7.94±0.17^{c}	8.58±0.14^{c}	5.25±0.04^{a}
D	7.00±0.02^{a}	7.38±0.08^{a}	8.00±0.05^{a}	6.00±0.02^{b}

* Different superscripts letters (a–c) in the same column represent significant differences between means ($p<0.05$).

4. Conclusion

Lately, food products at remained regional level are well-perceived and demanded. One of the traditional products, Puturge ayran, was researched in terms of some physicochemical, microbiological and sensory characteristics. The results are demonstrated that Puturge ayran has potential in terms of consumer attitude. It is thought that rheological characteristics and shelf life of this product should also be studied. Detailed investigations, further studies and advertising are vital for awakening food producers' interest and providing added value to Malatya's Puturge ayran. To reveal the changes occurring in product characteristics during the fermentation period would be thus of interest.

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Contribution of the Authors

The 1st, 2nd and 3rd authors contributed 75% equal in total and the 4th author 10% and the 5th author 15% contributed.

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Research and Publication Ethics Statement

Research and publication ethics were followed in the study.

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