

A COMPARATIVE STUDY ON THE PRODUCTION METHODS OF AYRAN

AYRAN ÜRETİM METOTLARI ÜZERİNE KARŞILAŞTIRMALI BİR ÇALIŞMA

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ABSTRACT: Ayrân is a traditional Turkish yoghurt beverage containing plain yoghurt, water and salt. It is produced commercially by two methods. The main difference between these two methods is the dilution of milk with water before incubation or the dilution of yoghurt with water after incubation of milk. The aim of this research was to reveal any differences between Ayrân properties resulting from the different methods. The results indicated no significant differences between Ayrân samples in terms of gross composition and consumer acceptance ($P>0.05$). However, dilution of yoghurt reduced the acetaldehyde content on day 7 and the number of *Streptococcus thermophilus* on day 1 significantly ($P<0.05$) compared to those produced from diluted milk.

Key words: Ayrân, yoghurt beverage, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, acetaldehyde.

ÖZET: Ayrân, yoğurt, tuz ve sudan oluşan, geleneksel bir Türk içeceği. Ticari olarak Ayrân iki şekilde üretilmektedir. Bu iki üretim şekli arasındaki başlıca fark, inkübasyondan önce Ayrân sütüne su ilave edilmesi veya inkübasyondan sonra yoğurda suyun ilave edilmesidir. Bu nedenle bu araştırma, bu iki üretim metodunun Ayrânın kimyasal, mikrobiyolojik ve duyu özellikleri üzerindeki etkilerini araştırmayı amaçlamaktadır. Elde edilen sonuçlar, üretim metodlarının Ayrânın genel bileşimi ve tüketici beğenisi üzerinde herhangi bir etkilerinin bulunmadığını göstermiştir ($P > 0.05$). Ancak, yoğurda su katılarak üretilen ayrânların asetaldehit içeriklerinde 7. gün depolamada, *S. thermophilus* içeriğinde ise 1. gün depolamada diğer metoda göre daha düşük değerler elde edilmiştir ($P < 0.05$).

Anahtar kelimeler: Ayrân, yoğurt içeceği, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, asetaldehit.

INTRODUCTION

In Turkey, one form of yoghurt consumption is, as a traditional yoghurt beverage, Ayrân which is a mixture of plain yoghurt, water and salt. It is estimated that approximately 20-30% of yoghurt produced in Turkey is used for Ayrân production (1). It is consumed throughout year; but, the consumption as a refreshment considerably increases during summer.

According to Turkish Food Codex (2), Ayrân is produced by two methods, which are also used in commercial production. Either milk is diluted with water (ca. 1/3) before being processed into yoghurt, or milk is processed into yoghurt first and diluted with water afterwards (ca 1/3). The main difference is therefore the initial gross composition of milk to be processed into yoghurt. It has already been shown that the total solids content of milk affects the microstructure (3), viscosity (4), bacterial count (5) and aroma compounds content of yoghurt (6). However, it is not clear that, whether differences in yoghurt properties caused by the different

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total solids content during makeup are reflected in the properties of the final Ayran product. Also, it is unknown whether any potential differences in the physical, chemical and microbiological properties of Ayran samples arising from the yoghurt properties produced by two different methods will affect consumer preferences.

To our knowledge, there is no research revealing the effects of different production methods on the properties of Ayran. The aim of the present study was therefore to reveal any chemical, microbiological and sensory differences resulting from the different production methods of Ayran during a storage period of 7 days at 5°C.

MATERIALS AND METHODS

Materials: For Ayran production, whole milk powder was used in order to standardize the milk base throughout the study. The powder was supplied from ENKA Co. (Konya, Turkey) and stored at 2°C until use. Some properties of milk powders were given in Table 1. A direct-vat-set, freeze-dried starter culture (YC-380) containing a blend of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* was obtained from Peyma-Hansen Ltd. Co (Istanbul, Turkey). This culture contains ropy strains of yoghurt bacteria and has been commonly used by Ayran producers to increase the viscosity of the final product. Table salt was purchased from the local market.

Table 1. Some chemical properties of whole milk powder, reconstituted milk A and B (n=3).

	Whole milk powder	Reconstituted milk (A)	Reconstituted milk (B)
Total solids (g/100g)	93.4±0.12	7.9±0.04	12.1±0.17
Fat (g/ 100g)	18.0±0.05	1.6±0.07	2.5±0.07
Acidity (g lactic acid/100g)	0.17±0.02	0.12±0.12	0.19±0.4
pH	-	6.73±0.01	6.64±0.01
Density, g/mL	-	1.0221±0.000	1.0344±0.000
Solubility (mL)	99.8±0.01	-	-

Preparation of starter culture: As the starter culture used was designed to be used in a direct-vat-system, in order to obtain the exact amount needed for inoculation, a sachet of starter culture (~6-7g) was added into 500 mL of previously reconstituted and heat treated (90°C/5 min) skim-milk with total solids of 110g/kg at 43±2°C. The mixture was stirred continuously for 30 min to dissolve the culture powder completely. From this mixture, the amount needed for inoculation was taken with a sterile pipette. The required amount of starter culture for Ayran production was determined with preliminary experiments so that the acidity of milk after incubation should reach pH 4.4-4.6 within 4-4.5 hours as recommended by the supplier.

Production methods: According to Turkish Food Codex (2) three types of Ayran can be produced, non-fat (<0.5%), half fat (0.5-1.5%) and full fat (≥1.5%) with a minimum non-fat dry matter content of 6.0%. In this study, full fat Ayran (8% total solids and 1.5%fat) containing 0.5% salt was produced. In Method A, 320 g of whole milk powder was reconstituted in 3680 mL of deionized water in a stainless steel container (5 L) by a high-speed homogenizer (Ultraturrax, Type T45, Janke and Kunkel, IKA-Werk Labortechnik, Germany) to achieve the composition of full fat Ayran (reconstituted milk A, see Table 1 for composition). Reconstituted milk A was heated at 85°C for 15 min in a boiling water bath with continuous stirring, followed by immediately cooling to 43°C using tap water (7). Heat treated reconstituted milk was then inoculated with starter culture mix (16 mL) and incubated at 43±2°C for about 4.5 hours until a gel formed and pH value of 4.40±0.05 was reached. The gel was cooled down to 20°C in an ice bath by stirring, and mixed with a high speed homogenizer

(90 s) to produce Ayran. During mixing, salt (20 g) was added. Ayran was filled into sterile glass bottles (200 mL) and capped under aseptic conditions. Samples produced by this method were referred to sample A. In method B, 320 g of whole milk powder was reconstituted in 2330 mL of deionized water to give a composition of 11.5% total solids and 2% fat (reconstituted milk B, see Table 1 for gross composition). After heat treatment, inoculation and incubation were carried out same as above. The yoghurt obtained was added to previously boiled and cooled water (1350 mL) and salt (20 g), and mixed with a high-speed homogenizer (90 s) in order to achieve the composition of full fat Ayran. The mixture was filled into glass bottles and capped under aseptic condition as above. Ayran samples produced by method B were referred to sample B. All samples were stored at 5°C for 7 days. Analyses were done on days 1 and 7. The experiment was carried out three times.

Methods of analysis: The powder was analyzed for total solids, fat, acidity and solubility by Turkish Standards (TS) (8) and the results were revealed in Table 1. Ayran samples were analyzed for total solids, fat and density as described in TS (9). Protein content was determined as described by Rowland (10). As major taste and flavour components, salt, acetaldehyde and lactic acid contents were determined by TS (11), Lees and Jago (12) and Steinshold and Calbert (13), respectively. pH values were measured using a combined electrode connected to a pH-metre (Orion 420, Model 250, Orion Research Inc., USA). For the determination of whey separation, Ayran samples were filled into a measuring flask of 200 mL and closed tightly and kept under quiescent condition at $5 \pm 1^\circ\text{C}$ throughout the storage period as described by Atamer et al (14). The amount of whey separated was measured volumetrically and expressed as whey mL/100 mL Ayran. Viscosity was determined by using a falling ball viscometer (Haake, Model B, Germany; ball weight is 4.892 g) at 20°C.

Enumeration of yoghurt starter bacteria: Microbiological enumeration of yoghurt bacteria was done as described by Bracquart (15). Fresh medium was prepared before each trial and distributed into sterile petri dishes. The petri dishes were dried at 36°C for 18 h before use. For dilution of yoghurt and Ayran samples, serial dilutions in sterile quarter-strength Ringer's solution were prepared. 0.1 mL of aliquots of dilutions were pipetted into petri dishes and spread on duplicate plates. The petri dishes were incubated at 36°C for 48 hours within candle jars under anaerobic conditions. The differentiation of the yoghurt bacteria was made based on the morphology of their colonies. The colonies of *S. thermophilus* were opaque and spherical while the colonies of *L. delbrueckii* subsp. *bulgaricus* appeared as larger and irregular in shape. In order to check any post-contamination, coliform bacteria and yeast-moulds were also counted as described by Harrigan and McCane (16).

Sensory analysis: A consumer preference test was carried out as described by Bodyfelt et al. (17), using a 9-point hedonic scale (1=dislike extremely, 9=like extremely). Seventy-five students of the Agricultural Faculty of Ankara University participated in the sensory evaluation.

Statistical analysis: Data were analyzed using SAS® Version 7 (18). Two-way analysis of variance (ANOVA) and the Duncan's multiple comparison test were used to determine significant differences in response means at the significant level of 0.05. Log₁₀ transformations were performed on microbial data.

RESULTS

Gross composition of Ayran samples are presented in Table 2. Production methods affected neither the total solids, fat and protein, lactic acid contents, pH values nor the whey separation ($P > 0.05$). During storage, however, significant changes were observed in pH values, lactic acid contents and whey separation ($P < 0.05$). The pH values dropped about 0.1 unit and lactic acid contents increased about 10% in both samples. At the end of the storage, more than 20% of Ayran was separated as whey in both samples. Surprisingly, no significant difference was observed between the viscosity values of the samples due to the production methods ($P > 0.05$), nor due to the effect of storage ($P > 0.05$). In contrast with other comparisons, an interaction effect of production methods and storage time were observed on the acetaldehyde contents of the samples ($P < 0.05$). When compared, the acetaldehyde content of sample B was found to be approximately 30% lower than that of sample A on day 1 ($P < 0.05$), coinciding with the amount of water added. During storage, the

acetaldehyde content in sample B increased ($P < 0.05$) while that of sample A remained almost unchanged ($P > 0.05$). On day 7, however, the acetaldehyde content of sample B was still significantly lower than that of sample A ($P < 0.05$).

When the behaviour of yoghurt bacteria was monitored (Table 3), it was found that the production methods and storage time affected the growth of yoghurt bacteria differently. Incidentally, for the sake of better explanation, an additional microbiological enumeration of samples was also done immediately after the incubation before the addition of water and these results were included in the table. In case of *S. thermophilus*, an interaction effect of production methods and storage time was observed ($P < 0.05$). In sample A, a small but significant increase was observed in the number of *S. thermophilus* over one day storage ($P < 0.05$), which remained unchanged thereafter ($P > 0.05$). In sample B, however, addition of water after incubation resulted in small (0.2 log) but significant reduction in the number of *S. thermophilus* after one day storage ($P < 0.05$). By the end of storage, *S. thermophilus* in sample B appeared to recover ($P < 0.05$), and no differences between the sample A and B were observed ($P > 0.05$).

In the case of *L. delbrueckii subsp. bulgaricus*, it appeared that neither differences in the initial composition of milk nor the addition of water caused significant changes in the number of *L. delbrueckii subsp. bulgaricus* ($P > 0.05$), unlike *S. thermophilus*. However, the number of *L. delbrueckii subsp. bulgaricus* in sample B tended to be lower at all times. *L. delbrueckii subsp. bulgaricus* continued to grow significantly in both samples on the first day of storage ($P < 0.05$) and then showed insignificant fluctuations ($P > 0.05$). Finally, the result of sensory analysis is presented in Table 4, with no significant changes in any of the sensory properties ($P > 0.05$).

DISCUSSION

The data in Table 2 confirmed that similar gross composition in Ayran samples were achieved at the end of the production, as designed. Changes in lactic acid contents and pH values of Ayran samples during storage indicated an ongoing bacterial activity (19,20,21). Amongst the other quality parameters, whey separation appeared to be the major defect in the Ayran samples in agreement with the others' findings (14). Whey separation is caused by the differences between the density of whey and acid curd, or low viscosity (17). In our study, it could be speculated that either the differences in the water content of reconstituted milks prior to curd formation did not alter the protein matrix significantly, or that mixing with a high-speed homogenizer destroyed the structure of the curd to such extent that no difference that might occur in viscosity and whey separation was observed. It is also possible that the use ofropy bacteria may have concealed the any effect arising from the differences in the production methods.

It is well known that yoghurt bacteria, particularly *L. delbrueckii subsp. bulgaricus*, are mainly responsible for acetaldehyde production (8, 22). Therefore, the changes in the acetaldehyde content of Ayran samples can be attributed to microbial activity. It is evident from the Table 3 that changes in the acetaldehyde content of the samples closely followed the changing pattern of yoghurt bacteria. The fate of acetaldehyde during storage is controversial. Some authors observed a decrease (20, 22) while the others reported an increase (23). In any case, the acetaldehyde contents of Ayran samples were found to be within the range of those reported for yoghurt and yoghurt-related products (2 to 40 mg g⁻¹) (8, 17, 24).

It is another well established fact that *S. thermophilus* is less acid tolerant than *L. delbrueckii subsp. bulgaricus* (8). When incubated together, *S. thermophilus* grows first until the pH drops to 5.0 where its growth slows down due to the developed acidity, followed by the growth of *L. delbrueckii subsp. bulgaricus* (8, 25, 26). Therefore, it is most likely that the growth of *S. thermophilus* in sample B had ceased before the addition of water, resulting in a decrease. Due to its acid-tolerant nature (8, 25, 26), *L. delbrueckii subsp. bulgaricus* continued to growth, even after the addition of water ($P < 0.05$), eliminating the effect of dilution. At the end of the storage period, numbers of both bacteria tended to increase slightly. Possibly, addition of water made yoghurt bacteria late to reach their lag phase. The growth of yoghurt bacteria during yoghurt production and

Table 2. Changes in some physico-chemical properties of Ayran samples produced by two different methods during storage at 5 °C for 7 days along with statistical evaluation (n=3)^{a, b}

	Day 1		Day 7	
	Method A	Method B	Method A	Method B
Total solids (g/100g)	8.3±0.14 ^a	8.3±0.07 ^a	-	-
Fat (g/100g)	1.6±0.03 ^a	1.6±0.03 ^a	-	-
Protein (g/100g)	2.26±0.02 ^a	2.28±0.03 ^a	-	-
Salt (g/100g)	0.65±0.05 ^a	0.66±0.02 ^a	-	-
Density (g/mL)	1.0250±0.001 ^a	1.0255±0.001 ^a	-	-
Lactic acid (g/100g)	0.56±0.07 ^b	0.56±0.05 ^b	0.61±0.04 ^a	0.60±0.02 ^a
pH	4.15±0.02 ^a	4.17±0.10 ^a	4.05±0.05 ^b	4.06±0.02 ^b
Acetaldehyde (mg/kg)	10.9±0.83 ^a	7.6±0.22 ^c	10.8±0.70 ^a	9.1±0.51 ^b
Whey separation (mL/100mL)	3.2±0.63 ^b	2.9±0.85 ^b	24.8±3.18 ^a	22.0±1.74 ^a
Viscosity (mPa.s)	1.7±0.05 ^a	1.8±0.09 ^a	1.7±0.07 ^a	1.9±0.15 ^a

^aMean values± standard deviation^bMeans in the same row without a common superscript differ (P < 0.05).**Table 3.** Effect of different production methods on the numbers of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in Ayran samples stored at 5°C for 7 days (log₁₀ cfu/mL) (n=3).^a

	<i>S. thermophilus</i>		<i>L. delbrueckii</i> subsp. <i>bulgaricus</i>	
	Method A	Method B	Method A	Method B
After Incubation	8.41±0.06 ^{a, b}	8.57±0.21 ^a	7.97±0.24 ^b	7.78±0.44 ^b
Day 1	8.47±0.02 ^a	8.23±0.03 ^b	8.40±0.02 ^a	8.31±0.06 ^a
Day 7	8.53±0.10 ^a	8.44±0.02 ^a	8.45±0.12 ^a	8.36±0.19 ^a

^aMean values± standard deviation^bMeans in the same column, row and species without a common superscript differ (P<0.05)

early days of storage have been reported by others (20). However, in contrast to our results, a reduction in the number of yoghurt bacteria after 7 days (19), 15 days (20) and 21 days (21) of storage at 4°C were also reported. In this study, the storage time was not long enough to observe any decrease in the number of yoghurt bacteria. Incidentally, no coliform, or yeast-mould was counted in the samples during the storage, indicating that there was no post-production contamination (data not shown).

Finally, the result of sensory analyses showed that the test panel were not conscious of the differences between the acetaldehyde content of the samples (P>0.05) (Table 4). Similar results were observed when Labneh was produced with different techniques (26). The taste panel were not able to differentiate Labneh samples although the acetaldehyde content of the products varied from 9.9 to 22.8 mg g⁻¹. In an earlier study,

however, Robinson et al. (27) reported that a test panel of students from Mediterranean countries found the sensory properties of yoghurt samples containing $37.5 \pm 2.3 \text{ mg g}^{-1}$ of acetaldehyde superior to that containing $10.4 \pm 0.3 \text{ mg g}^{-1}$ acetaldehyde. Although the acetaldehyde values were found to be well above threshold value (28), recent studies (29) showed that pH is the main factor responsible for the intensity of flavour perception of yoghurt, not the aroma compounds like acetaldehyde. Therefore, these results were not surprising since the pH values of the samples were same. In terms of texture and overall acceptability, the consumer detected no differences (Table 4).

Table 4. Flavour (a), body/texture (b) and overall acceptance (c) scores of Ayran samples produced by two different methods. (n=75)^a,

	Flavor		Body/texture		Overall acceptance	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
Method A	6.7 $\pm$ 1.62	6.4 $\pm$ 1.94	6.0 $\pm$ 1.82	6.2 $\pm$ 1.72	6.6 $\pm$ 1.47	6.6 $\pm$ 1.62
Method B	6.6 $\pm$ 1.66	6.3 $\pm$ 1.82	6.4 $\pm$ 1.43	6.0 $\pm$ 1.92	7.2 $\pm$ 4.16	6.4 $\pm$ 1.84

^a Mean values $\pm$ standard deviation

It can be concluded that either processing method could be employed since the differences caused by the production methods were not detectable by the consumer. However, dilution of milk before heat treatment, as in method A, can be recommended in terms of Good Manufacturing Practice as addition of water to yoghurt may increase the risk of post-contamination.

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