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Research Article/Araştırma Makalesi

Impact of pH on the salty taste perception of the yogurt drink, ayran

Ayranda tuzluluk algısı üzerine pH'nın etkisi

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ABSTRACT

Reducing the salt consumption has gained much interest in recent years. Enhancing the perceived saltiness of the food could help reducing the salt to a certain level. Other components in the food could play a role on changing the perceived saltiness during consumption. This study investigated the impact of acidity on the salty taste perception of ayran. Ayran is a fermented milk drink widely consumed in Turkey and is basically produced by diluting the yogurt with water and adding salt. Ayran samples were produced at three pH levels; pH 4.2, pH 4.4 and pH 4.6. Same amount of salt (%0.5 w/w) was added to each group. Composition, pH, sensory properties, viscosity, whey separation and microbiological properties (starter culture, yeast and mould counts) were determined. Sensory analysis was done only once at the beginning of the storage since no difference was observed between the pH of the samples after day 7. pH influenced the sensory saltiness scores significantly ($p<0.05$). Saltiness scores of the pH 4.2 samples were higher than that of the sample at pH 4.6. Ayran samples at pH 4.2 and 4.4 had higher viscosity than pH 4.6 during 20-day storage and had no whey separation at day 1. At day 20, whey separation of pH 4.2 sample was below 10%, while it was higher for pH 4.4 and 4.6 samples. We did not find any yeast and mould in samples during 20 days of storage. *Streptococcus thermophilus* counts were higher at pH 4.2 and 4.4 samples, and no difference was observed during storage while *Lactobacillus bulgaricus* counts decreased about 1 log at the end of 20 days.

Key Words: Saltiness perception, Ayran, pH, Salt reduction

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ÖZ

Fazla tuz tüketimi uzun bir zamandır kardiyovasküler hastalıklarla ilişkilendirilmektedir. Dünya Sağlık Örgütüne göre yüksek tansiyonu önlemek için günlük tuz tüketimi 5 gramı geçmemelidir. Ancak, gıdalarda tuzun azaltılması tüketici beğenisini etkilemektedir. Tuzun azaltılmasından sonra istenilen tuzlu tadı sağlamanın bir yolu, ağızda tuzluluk algısının değiştirilmesidir. Gıdanın tüketimi sırasında hissedilen tuzluluk seviyesinde gıdadaki diğer bileşenlerin de rolü vardır. Ayran ülkemizde yaygın tüketilen ve temelde yoğurda su ve tuz ilavesiyle elde edilen bir üründür. Bu çalışmada pH değerinin ayranın tuzluluk algısı üzerine etkisi araştırılmıştır. Ayran örnekleri 3 farklı pH değerinde üretilmiştir; pH 4.2, 4.4 ve 4.6. Her gruba aynı oranda tuz ilave edilmiştir (%0.5 w/w). Örneklere ait pH, bileşim, duyusal özellikler, viskozite, serum ayrılması ve mikrobiyolojik özellikler (starter kültür, maya ve küf sayısı) belirlenmiştir. Örneklere ait pH değerleri 7. günden itibaren farksız bulunduğundan, duyusal analiz sadece depolamanın başında yapılmıştır. Farklı pH değerleri tuzluluk puanlarını önemli derecede etkilemiştir. Tuzluluk puanları pH 4.2 örneklerinde, pH 4.6 örneklerinden önemli düzeyde yüksek bulunmuştur. Depolama süresince pH 4.4 ve 4.2'deki ayran örneklerinde pH 4.6'daki örneklerden daha yüksek viskozite değerleri bulunmuş, depolamanın 1. gününde serum ayrılması gözlenmemiştir. Depolamanın 20. gününde pH 4.2 örneğinin serum ayrılması %10'un altındayken, pH 4.4 ve 4.6 örnekleri daha yüksek serum ayrılması göstermiştir. Örneklerde 20 günlük depolama süresince herhangi maya küf gelişimi tespit edilmemiştir. *Streptococcus thermophilus* sayısı pH 4.2 ve 4.4 örneklerinde daha yüksek bulunmuş ve depolama süresince değişmemiş, *Lactobacillus bulgaricus* sayıları ise 20 günlük depolama sonunda 1 log düşüş göstermiştir.

Anahtar Kelimeler: Tuzluluk algısı, Ayran, pH, Tuzu azaltma

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Introduction

Salt (NaCl) is one of the most commonly used food ingredient that significantly influences the acceptability and liking of the food. Together with its vital role in many technological aspects such as structure, preservation and shelf life of food products, its overconsumption is almost inevitable. On the other hand, excess sodium intake is linked with high blood pressure and related health problems such as cardiovascular diseases and hypertension (Doyle and Glass 2010). Growing concerns on the health risks of excessive salt intake led the World Health Organization and food legislators around the world to take action for reducing the salt in food products (WHO 2007). A great number of researches have been conducted regarding salt reduction and several approaches developed such as use of salt replacers, salt enhancers, modulation of salt spatial distribution and particle size (Wilson *et al.* 2012). However, these approaches may have several limitations that restrict their application to different types of food products. Perception of the salty taste depends not only on the sodium amount in a food, but also on the presence of other components in the food (Lauverjat *et al.* 2009). The level of the salty taste sensation is proportional to the amount of sodium ions that can reach ion channels on the tongue. Since it usually takes quite a short time before swallowing the food, how fast the sodium ions release from the food matrix within that brief oral processing period is crucial for perceiving a higher level of saltiness. Food components that bind to sodium ions or limit their mobility could suppress saltiness dramatically (Kuo and Lee 2014). Therefore, expressing the amount of salt as salt-in-moisture or salt-in-dry matter is a better way of representing salt level in the food. An alternative approach to increase the mobility of sodium ions and thus to increase the saltiness sensation is, controlling the intermolecular interactions in the food matrix. Yücel and Peterson (2015) reported that, ionic protein interactions influence the salt perception significantly in Na-caseinate lipid solutions. Rosett *et al.* (1997) showed that negatively charged biopolymers such as calcium caseinate, xanthan, and κ-carrageenan gums can bind sodium ions to limit the salty taste perception while nonionic gums do not have any effect in tomato soups. They suggested that the charge on the biopolymer had more impact on the perception of saltiness than the viscosity. Therefore, pH could play an important role in manipulating the delivery properties of sodium through intermolecular ionic interactions in order to enhance the salt perception. Texture and rheological properties of most dairy products strongly depend on the pH (Lucey *et al.* 2003). As a result, changing the pH could also bring about changes in saltiness perception related to physical properties of the product as well. Our objective in this study is to determine the impact of pH on saltiness sensation of ayran. For this purpose Ayran samples produced at 3 different pH levels that are common for Ayran (pH 4.2, 4.4 and 4.6).

Material and Methods

Samples

Ayran samples were produced at Harran University Pilot Dairy Plant. Milk for ayran production was prepared by combining skim milk powder (Pinar A.S., İzmir), cream (35% milk fat) (Pinar A.S., İzmir) and water to have a minimum of 2% protein, 6% NFS and 1.8% fat. The reason for the use of recombine milk from skim milk powder instead of raw milk was to eliminate any seasonal differences in milk composition in case further replication of the trial is needed. Milk was then homogenized at 144 bar in two stages (Hommak F-HM20, İzmir, Turkey) after heating to 60 ºC, and heated at 90 ˚C for 5 min using heating tank. After cooling to 45 ˚C, inoculated with direct vat starter culture containing *Lactobacillus delbruckii bulgaricus* and *Streptococcus thermophillus* (1:1) at the prescribed amount (YF-L903, Chr Hansen, İstanbul). Inoculated milk was divided into 3 separate containers and kept in incubation room at 45˚C until reaching to pH 4.9, pH 4.6 and pH 4.4 respectively for each container. At the end of the incubation 0.5% salt was added, stirred and filled in 250 ml cups and sealed with foil using filling machine (Sezmak, İstanbul, Turkey). Samples were then cold stored at 4˚C. Trial was replicated twice.

Composition and pH

Total solids was determined by gravimetric method according to IDF (1982). Fat content was analyzed by Gerber method (IDF 1991). pH was measured throughout the storage (at d1, 3, 7, 14, 20, 28) directly by inserting the probe of the pH meter (Thermo Scientific Orion, Fort Collins, USA) into samples.

Apparent viscosity

Apparent Viscosity was measured at day 1, 10 and 20 using Brookfield Viscometer (Brookfield Model RVDV-II+, Brookfield engineering Laboratories. Inc., Middlesbrough, UK) with spindle no 3 at 100 rpm. Sample cups were shaken well before measurement and the value obtained at 15s was recorded (Ozer *et al.* 1997). Sample temperature was 4˚C during measurements.

Whey separation

100 ml sample was placed in graduated cylinder and kept at 4˚C until measurement. Once placing the samples, measurements were taken after 1 day, 3, 7, 14 and 28 days from the same cylinders. Volume of the separated whey on top was measured (Ozunlu 2005).

Sensory evaluation

Sensory evaluation was done by a group of 50 people including students, faculty, staff and experienced sensory panelists. Samples were presented at 4°C±2 with their sealed cups and ensured to be shaken well before the test. Ayran samples were evaluated for overall liking, flavour and consistency using a 9-point hedonic scale (with 1 = extremely dislike, 5 = neither like nor dislike, and 9 = extremely like), and for saltiness a just-about-right (JAR) 9-point intensity scale was used with 5 being just about right, 1= not enough salt and 9 = too much salt. Panelists were also asked to rank the samples in order from the most preferred to the least. Samples were evaluated using ranking test model according to Drake (2008).

Microbiological analysis

Ayran samples were diluted using 0.1% sterile peptone water and 1 mL aliquot dilutions were poured onto plates of the selective agars in triplicate. M17 agar was used for the enumeration of *S. thermophilus* and *L. bulgaricus* was incubated at MRS agar anaerobically (Rybka and Kailasaphaty 1996). All plates were incubated at 37˚C for 48 h. Anaerobic conditions were obtained using Anaerocult A sachets (Merck). Enumeration of the yeast and mould was done after incubating sample aliquots (1mL) dispersed on Potato Dextrose Agar at 25 ºC for 4-5 days (Taniwaki *et al.* 2001).

Statistical analysis

Statistical analysis was performed by SPSS version 16 (SPSS Inc., Chicago, IL) using One-way ANOVA. Differences between means were evaluated by Tukey multiple comparisons test.

Results and discussion

Chemical properties

Chemical properties of the milk base used for ayran production are given in Table 1. All ayran samples had 1.8% fat, 6.5% NFS and a minimum protein content of 2%. Changes in pH values during 28 days of storage are given in Fig. 1. pH values of the ayran samples were 4.6, 4.4 and 4.2 respectively at day 1 and did not change significantly in the first 3 days after manufacture. During the storage pH decreased gradually for all samples due to the growth and presence of starter culture bacteria ($p<0.05$). Since a proportionally higher amount of decrease in pH was observed at higher pH samples, the difference between the pH values of the ayran samples diminished by time and after 7 days the difference was mostly insignificant. This could be due to the slowing down in starter growth and acid development after a certain acidity level.

Physical properties

Phase separation is a well-known phenomenon for ayran that is also called as "whey separation" and caused by the sedimentation of destabilized proteins. Although it is unavoidable at some point during storage, a fast whey separation is unwanted, and the length of the time required for the phase separation to occur is a quality criteria of ayran. Stability of casein micelles controlled mainly by pH in a milk system, therefore pH is one of the key factors play role in whey separation (Lucey *et al.*, 2003). pH 4.4 and 4.2 samples did not exhibit any whey separation a day after manufacture while pH 4.6 sample had a slight whey separation (Fig. 2). During the first 3 days pH 4.2 sample did not have any whey separation and it showed significantly lower whey separation than the other samples at the rest of the storage period. Previous studies showed that higher acidity reduce the whey separation in ayran and yogurt (Akın and Akın, 2016; Tamucay-Özünlü and Koçak, 2010; Özdemir and Kılıç, 2004). Tamucay-Özünlü and Koçak (2010) reported that ayran samples having the lowest incubation end pH (4.0) did not show any whey separation at day 1, and exhibited less whey separation during 14 days of storage than those with higher pH (4.3 and 4.6). They observed the highest whey separation at pH 4.6. Similarly, Özdemir and Kılıç (2004) reported a higher serum separation in ayran samples at higher pH (4.6). It is suggested to end the fermentation of stirred-type fermented products at pH 4.2-4.4 rather than pH 4.7-4.8 in order to have higher viscosity (Özer 2006). Schkoda et al. (1999) had also observed reduced sedimentation below pH 4.6 and they claimed that this was due to the increase in the net positive charge on casein micelles below the isoelectric pH, which cause more electrostatic repulsion and less aggregation between the particles resulting in less sedimentation of those particles. Ayran is a dilute form of the yogurt and gravitational forces act upon the particles causing them to sink. According to the Stokes law, phase separation increases with particle size and decreases with the viscosity of continuous phase. Ayran samples having low whey separation had also higher viscosity (Fig. 3). Previous studies had also observed an increase in viscosity of the acidified milk gels with the decrease of the pH below 4.6 (Özdemir and Kılıç 2004, Tamucay-Özünlü and Koçak 2010).

Table 1. pH, titratable acidity and composition of recombine milk used for ayran production
Çizelge 1. Ayran üretiminde kullanılan hazırlanan rekombine sütlerin pH, titrasyon asitliği ve kompozisyonu

Parameter *Parametre*	Milk *Süt*
pH *pH*	6.64 ± 0.03
Titratable acidity (% lactic acid) *Titrasyon asitliği (% laktik asit)*	0.178 ± 0.020
NFS (%)* *YKM (%)**	6.1 ± 0.3
Fat (%) *Yağ (%)*	1.8 ± 0.2
Protein (%) *Protein (%)*	2.16 ± 0.04
Ash (%) *Kül (%)*	0.68 ± 0.05

*Non-fat solids
Yağsız kurumadde

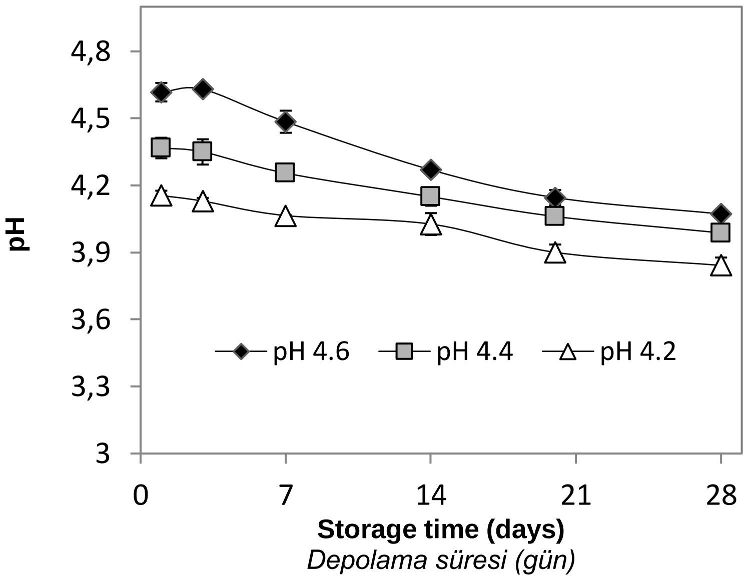

Figure 1. Change in the mean pH values of ayran samples at pH 4.6, pH 4.4 and pH 4.2 during storage

Şekil 1. pH 4.6, pH 4.4 ve pH 4.2 değerindeki ayran örneklerinin depolama süresince ortalama pH değerlerindeki değişim

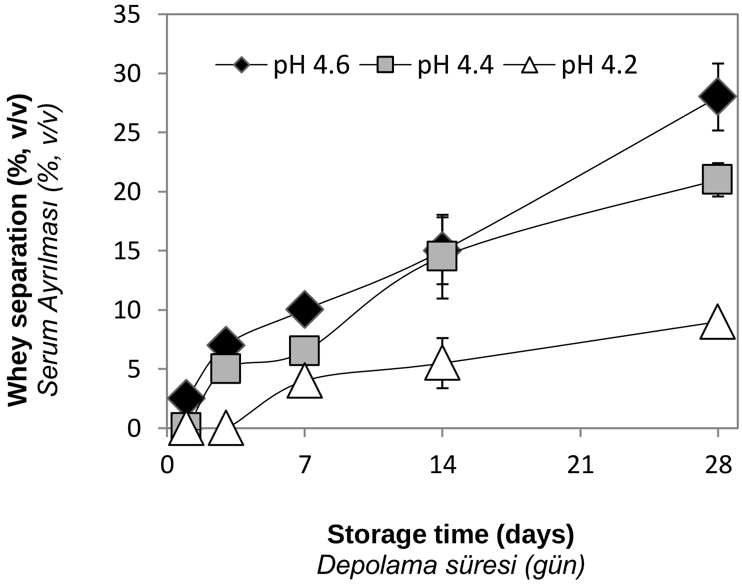

Figure 2. Change in the mean whey separation values of ayran samples at pH 4.6, pH 4.4 and pH 4.2 during storage

Şekil 2. pH 4.6, pH 4.4 ve pH 4.2 değerindeki ayran örneklerinin depolama süresince ortalama serum ayrılması değerlerindeki değişim

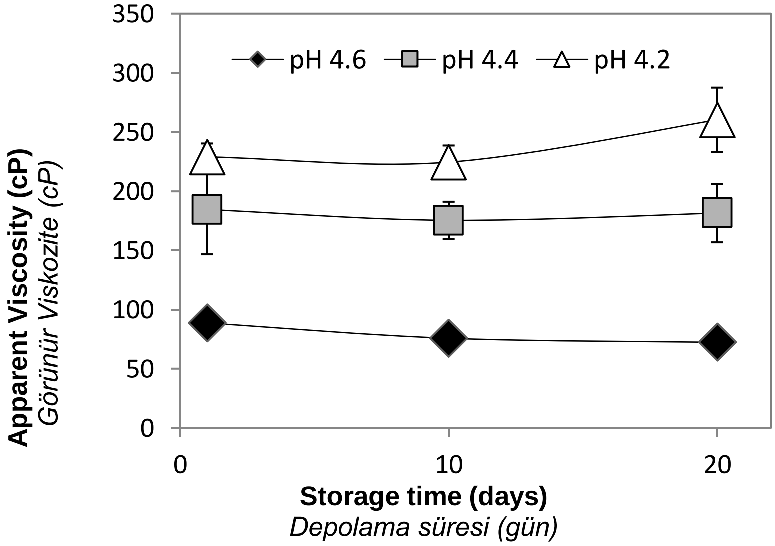

Figure 3. Change in the mean apparent viscosity of ayran samples at pH 4.6, pH 4.4 and pH 4.2 during storage

Şekil 3. pH 4.6, pH 4.4 ve pH 4.2 değerindeki ayran örneklerinin depolama süresince ortalama görünür viskozite değerlerindeki değişim

Sensory properties

We conducted sensory evaluation only at one time point in first 3 days of the production since the difference between the pH of the samples diminished by time and were insignificant after 1 week.

Ayran samples did not show any significant differences in terms of flavor, consistency and overall liking as seen in Table 3. However when we asked panelists to rank the samples from 1 to 3 according to their preference, pH 4.2 was the one most preferred with 55% of the panelists placing it to number 1. The increase in the pH of the ayran samples had a negative impact on their acceptability and preferences.

Saltiness was evaluated using JAR method. Although all samples contained the same amount of salt, panelists found the saltiness significantly different for ayran samples produced at different pH values. Saltiness level was found just-about-right (scored 5) in average for ayran sample with the mid pH, pH 4.4. Table 3 shows that the higher acidity in sample pH 4.2 was perceived as saltiness, resulting in significantly higher JAR scores than pH 4.6 samples ($p<0.05$). 50% of the panelists found the saltiness of the pH 4.6 low, labeling it not enough salt, while ayran samples at pH 4.2 received JAR, scoring 5, from half of the panelists (Fig. 4).

It has been reported in several studies that, acids enhance the saltiness at low concentrations, while at high concentrations either no sensory interaction was observed between salt and acid or the interaction was suppressive (Keast and Breslin 2002). According to Sakurai *et al.* (2009) weak acids can penetrate taste cell membranes and dissociate leading to cytoplasmic acidification, which then could affect membrane permeability and the taste response profiles. They studied the interaction of 0.5 mM HCl and 10 other tastes including solution of NaCl (0.005 to 0.05 M) by measuring the responses of the rat chorda tympani nerve; and found that interaction of HCl with NaCl was suppressive. On the contrary, most studies observed an intensifying effect at low concentrations of salt and acid (Hamajima, 1976; Hellemann, 1992; Stevens, 1997; Keast and Breslin 2002). Hamajima (1976) reported that saltiness sensation of 1% to 2% salt solution was enhanced at the presence of 0.01% acetic acid. Stevens (1997) found that salt and acid tastes were hyperadditive to each other at subthreshold levels. Hatae *et al.* (2009) observed that addition of vinegar to salt intensified the salty taste. Hellemann (1992) used lactic and acetic acid mixtures in water and in bread and measured the perceived saltiness that was increased with added acid at low salt concentrations. We observed an intensifying effect of acidity on saltiness similar to those studies. Most previous studies worked with salt and acid or other basic taste sources that are added to water, while ayran could be considered a much more complex system with the presence of other food components; proteins, carbohydrate, fat and minerals. In order to taste the saltiness, sodium ions should be absorbed on the tongue first. Food components can influence the release of sodium ions during mastication of food which would affect the amount of sodium absorbed by the tongue to initiate the electrochemical signaling that produce the sensation of saltiness (Lawrence *et al.* 2012). Yucel and Peterson (2015) showed that, ionic interactions between cationic sodium ions and anionic Na-caseinate molecules may influence the transportation and absorption of sodium ions to the tongue. They found that, in solution at high protein content, samples had significantly lower initial saltiness while having a higher salty aftertaste. It has been shown that negatively charged biopolymers, such as calcium caseinate, xanthan, and κ-carrageenan gums, can bind sodium ions to limit the salty taste perception, whereas nonionic gums do not have such an effect in tomato soups (Rosett *et al.* 1997). Reducing the pH below 4.6 could increase the mobility and release of sodium ions in ayran samples due to repulsive forces provided by positively charged proteins. Viscosity of the ayran samples was higher below pH 4.6. When the viscosity of the liquid foods increases, retention time in the oral cavity increases allowing a higher taste sensation (De Jongh and Janssen, 2007). The higher viscosity of pH 4.4 and 4.2 ayran samples could have helped sensing a higher saltiness by lengthening its duration on the tongue.

Table 2. Mean 9-point hedonic liking scores for sensory attributes and ranking for preference results of ayran samples at pH 4.6, pH 4.4 and pH 4.2

Çizelge 2. pH 4.6, pH 4.4 ve pH 4.2 değerlerindeki ayran örneklerine ait ortalama 9-point hedonik beğeni skorları

Sample *Örnek*	Attribute *Özellik*			
	Overall liking *Genel beğeni*	Flavour *Tat*	Consistency *Kıvam*	Ranking order *Beğeni sırası*
pH 4.6	6 ± 2[a]	6 ± 2[a]	6 ± 2[a]	3 (45%)
pH 4.4	6 ± 2[a]	6 ± 1[a]	6 ± 2[a]	2 (50%)
pH 4.2	7 ± 1[a]	7 ± 2[a]	6 ± 2[a]	1 (55%)

[a] Values with different superscript letter in the same column are significantly different (p<0.05)

[a] Aynı sütun içinde farklı harfle ifade edilen değerler önemli olarak farklıdır (p<0.05)

Table 3. Mean JAR scores for saltiness of ayran samples at pH 4.6, pH 4.4 and pH 4.2

Çizelge 3. pH 4.6, pH 4.4 ve pH 4.2 değerlerindeki ayran örneklerine ait ortalama JAR skorları

	Sample *Örnek*		
Attribute *Özellik*	pH 4.6	pH 4.4	pH 4.2
Saltiness *Tuzluluk*	4 ± 1[a]	5 ± 1[ab]	6 ± 1[b]

[a-b] Values with different superscript letter in the same row are significantly different (p<0.05)

[a] Aynı satır içinde farklı harfle ifade edilen değerler önemli olarak farklıdır (p<0.05)

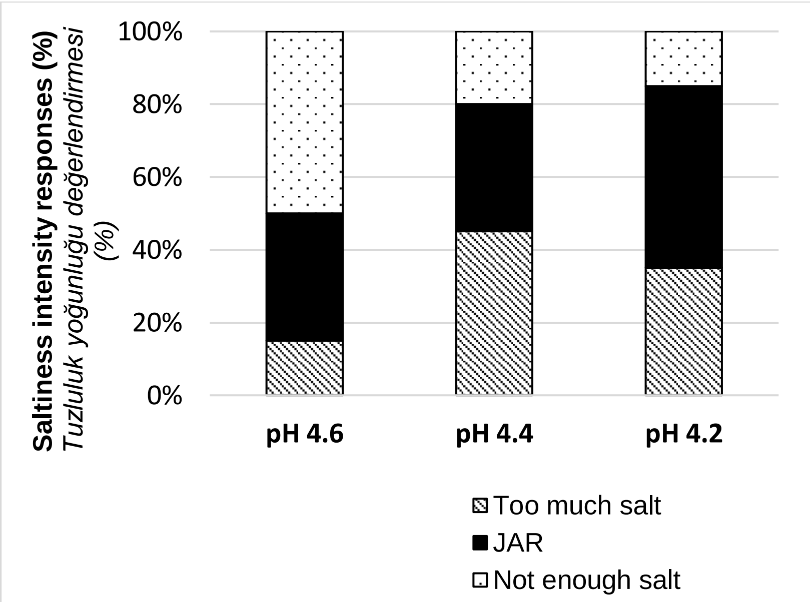

Figure 4. Saltiness intensity responses of ayran samples at pH 4.6, pH 4.4 and pH 4.2

Şekil 4. pH 4.6, pH 4.4 ve pH 4.2 değerindeki ayran örneklerine ait tuzluluk yoğunluğu değerlendirmesi

Microbiological properties

We did not find any yeast and mould growth in samples during 20d. *S. thermophilus* counts of pH 4.2 samples were significantly higher than pH 4.6 during first 10 days of the storage (p<0.05); however no significant difference was observed between the samples at d20. The slight increase in *S. thermophilus* counts at each pH level during the storage was also statistically insignificant. On the other hand *L. bulgaricus* counts decreased about 1 log at the end of 20d (p<0.05). We observed significantly higher *L. bulgaricus* counts at pH 4.2 and 4.4 samples than pH 4.6 only at day 1, and at day 10 and day 20 no differences were found.

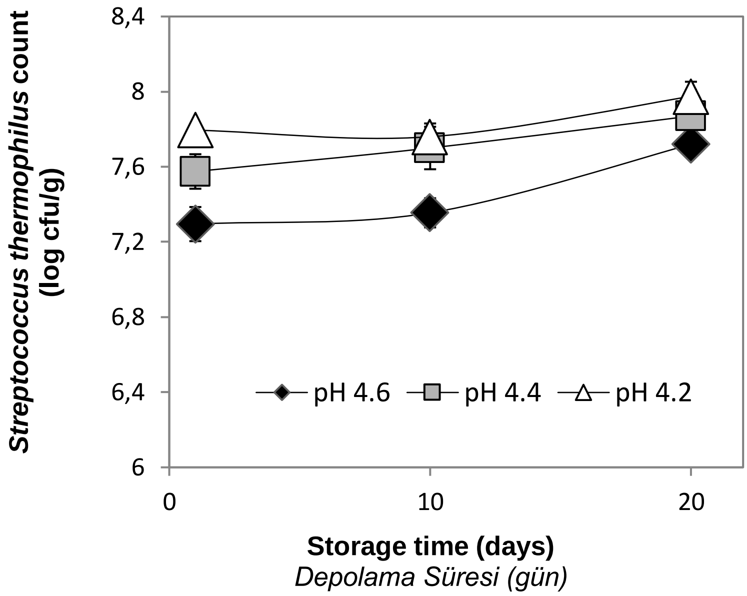

Figure 5. Change in the mean S. thermophilus count of ayran samples at pH 4.6, pH 4.4 and pH 4.2 during storage
Şekil 5. pH 4.6, pH 4.4 ve pH 4.2 değerindeki ayran örneklerinde depolama süresince ortalama S. thermophilus sayısı değişimi

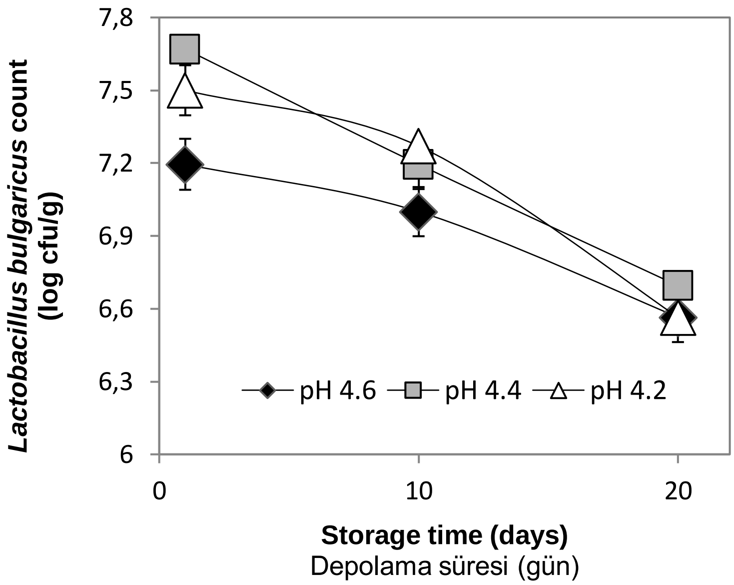

Figure 6. Change in the mean L. bulgaricus count of ayran samples at pH 4.6, pH 4.4 and pH 4.2 during storage
Şekil 6. pH 4.6, pH 4.4 ve pH 4.2 değerindeki ayran örneklerinde depolama süresince ortalama L. bulgaricus sayısı değişimi

Conclusion

In this study our results showed that pH significantly influenced the salty taste perception in ayran samples. Although all samples contained the same amount of salt (%0.5), half of the panelists found the saltiness of the ayran samples at pH 4.6 low while half of the panelists evaluated the pH 4.2 samples as just about right, scoring 5. According to our results it could be suggested to drop the pH to 4.4 and below for reducing the salt. This study demonstrated that reducing the salt content of the ayran to a certain extent could be unnoticeable when the pH is reduced. Therefore it is noteworthy to take into account the pH of the dairy products when formulating reduced salt products.

Conflict of Interest: The authors declare that they have no conflict of interest.

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