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RESEARCH ARTICLE

Investigation of Some Physicochemical and Microbiological Quality Parameters of Toast Cheese Sold Retail in Afyonkarahisar Province

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ABSTRACT

In this study, the physicochemical and microbiological quality of toast cheese offered for sale in markets in the center of Afyonkarahisar province was examined. The acidity (pH) and water activity (a_w) values of the samples were determined to be 4.89 and 0.932 on average, respectively. As a result of microbiological analyses, the total aerobic bacteria, total aerobic psychrophilic bacteria, yeast and mold, total coliform group bacteria, lactic acid bacteria counts, *Lactococcus/Streptococcus* spp., *Pseudomonas* spp., *Staphylococcus aureus, Bacillus cereus* average counts were determined to be 5.81, 3.30, 4.14, 2.89, 4.69, 4.93, 4.74, 2.42 and <2.00 log CFU/g, respectively. Furthermore, the samples were contaminated in percentages as follows: *Escherichia coli* 24%, *Clostridium perfringens* 8%, *Salmonella* spp. 18%, and *Listeria* spp. 12%, while *Brucella* spp. growth did not occur in the samples.

Keywords: Toast cheese, Afyonkarahisar, microbiological quality.

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Afyonkarahisar İlinde Satışa Sunulan Tost Peynirlerinin Bazı Fizikokimyasal ve Mikrobiyolojik Kalite Kriterlerinin İncelenmesi

ÖZ

Bu çalışmada Afyonkarahisar il merkezinde marketlerde satışa sunulan tost peynirlerinin fizikokimyasal ve mikrobiyolojik kaliteleri incelemiştir. Örneklerin asitlik (pH) ve su aktivitesi (a_w) değerleri ortalama olarak, sırasıyla 4.89 ve 0.932 olarak belirlenmiştir. Yapılan mikrobiyolojik analizler sonucunda; toplam aerobik bakteri, toplam aerobik psikrofilik bakteri, Maya Küf, toplam koliform grubu bakteri, laktik asit bakteri sayıları, *Lactococcus/Streptococcus* spp., *Pseudomonas* spp., *Staphylococcus aureus, Bacillus cereus* ortalama sayıları ise sırasıyla; 5.81, 3.30, 4.14, 2.89, 4.69, 4.93, 4.74, 2.42 ve <2.00 log kob/g olarak tespit edilmiştir. Ayrıca örneklerin %24'ünde *Escherichia coli*, %8'inde *Clostridium perfringens*, %18'inde *Salmonella* spp. cinsi, %12'sinde *Listeria* spp.cinsi bakteri varlığı belirlemesine karşın örneklerin hiçbirisinde *Brucella* spp. cinsi bakteri gelişimi tespit edilmemiştir.

Anahtar Kelimeler: Tost peyniri, Afyonkarahisar; mikrobiyolojik kalite.

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INTRODUCTION

It is stated that cheese with the highest number of types among dairy products has now more than 4000 types in the world (Akin 2010), whereas this accounts for 200 in Turkey (Çetinkaya 2008). Kashar cheese, which is one of the three most produced types of cheese in Turkey and is the most consumed type of cheese following white cheese, can be produced using different processes (Tekinsen 2000). Traditional or ripened (aged) kashar cheese is usually produced artisan ally without machine and is subjected to the ripening process for at least four months at 18 ± 2 °C, before being offered for sale. Nevertheless, the type, which is known as fresh kashar or kashar cheese, is produced with the help of machines and offered for sale without being subjected to the ripening process (Aran 1998).

A kind of processed cheese, which has been called fresh kashar in recent years but has no similarity in the appearance to original fresh kashar, has been offered for sale under the name of fresh kashar. A serious price difference between the two products and the same name of both products have led to confusion among consumers and the emergence of an unfair competition. This cheese has been named "Toast Cheese" by the cheese communique which was issued in the Turkish Codex (2015), so this confusion has been eliminated (Anonymous 2015).

However, there are no standards for the production of toast cheese in the communiqué. Ingredients which can be added and/or cannot participate in the cheese are not mentioned. This may lead to unfair competition in terms of the producer and health risks (foodborne infections, food poisoning, etc.) for the consumer.

Toast cheese is eventually a kind of processed cheese. And any processed cheese is defined as obtained by heat treatment and deicing salt (such as citrates and phosphates) with the use of hard and semi-hard, or sometimes soft types of cheese (Uçuncu 2004).
In this study, it was aimed to examine some physicochemical and microbiological quality parameters of toast cheeses, offered for sale in markets across Afyonkarahisar province.

MATERIAL and METHOD

2.1. Material

In the study, 50 toast cheese samples were purchased from markets across Afyonkarahisar province, and some physicochemical and microbiological quality parameters were examined. The samples were taken in the containers in which they were offered for sale, and they were transferred to the laboratory under cold conditions (4 ±0.1ºC). The samples were stored at 4±0.1ºC until further analyses.

2.2. Physicochemical Properties

The water activity of the samples was measured using a Novasina (Lab Touch aw-Switzerland) device (AOAC, 2005a). The pH value of the samples was measured using the Ohaus (ST 5000) device (AOAC, 2005b).

2.3. Microbiological Analyses

Total aerobic mesophilic bacteria (TAMB), total aerobic psychrophilic bacteria (TAPB), yeast/mold, total coliform group bacteria (TCGB), Lactic acid bacteria (LAB), *Lactococcus/Streptococcus* spp., Pseudomonas spp., *Staphylococcus aureus, Bacillus cereus* counts, and the presence of *Escherichia coli, Clostridium perfringens*, *Salmonella* spp., *Listeria* spp. and *Brucella* spp. were investigated using the spread plate technique on appropriate media (Halkman and Sagdas 2011).

Ten grams of the samples were taken into Stomacher bags (Lp Italiana Spa-174538) under aseptic conditions, and decimal serial dilutions were prepared until 10-7 (Anonymous 2001, Sekin and Karagozlu 2004).

The total aerobic mesophilic bacteria (TAMB) and total aerobic psychrophilic bacteria (TAPB) count analysis were performed by the spread plate technique using Plate Count Agar (PCA) (Merck 1.05463). Spread petri plates were incubated at 30°C for 48-72 hours for TAMB count under aerobic conditions (ISO 2013a, ISO 2013b), where TAPB plates were incubated at 4°C for 5-7 days (Halkman and Sagdas 2011).

Rose Bengal Chloramphenicol Agar (Merck 1.00467) (RBC) was used for yeast/mold analysis, and the petri plates were incubated at 22°C for 5-7 days under aerobic conditions (ISO 2008).

Violet Red Bile Agar (Merck 1.01406), de Man Rogasa and Sharpe Agar (MRS) (Merck 1.10661), M-17 Agar (Merck 1.15108) and *Pseudomonas* Selective Agar Base (PSA) (Merck 1.07620) were used for the following analysis: Total coliform group bacteria (TCGB), Lactic acid bacteria (LAB), *Lactococcus/Streptococcus* spp., *Pseudomonas* spp. All petri plates were incubated at 30°C for 24-48 hours under aerobic conditions (ISO 1991, Kneifel and Berger 1994, Corroler et al. 1998, ISO 2010).

The analysis of *Staphylococcus aureus* was performed by the spread plate method using Baird Parker Agar (Merck 1.05406). Plates were incubated at 30-35°C for 24-48 hours under aerobic conditions (ISO 1999).
The analysis of *Bacillus cereus* and *Escherichia coli* were performed by the spread plate method using Mannitol Egg Yolk Polymyxin Agar (MYP) (Merck 1.05267) and Chromocult TBX Agar (Merck 1.16122), respectively. The cultivated petri plates were incubated at 37 °C for 24 hours under aerobic

conditions. The colonies growing in the petri dishes were checked under a UV (366 nm) lamp (ISO 2004a, ISO 2001, ISO 2015).

The analysis of *Clostridium perfringens* was performed by the spread plate method using Tryptose Sulfite Cycloserine (TSC) Agar (Merck 1.11972). The petri plates were incubated at 35-37°C for 20-24 hours under anaerobic conditions in the anaerobic jar (Merck 1.16387) (ISO 2004b).

The analysis of *Salmonella* spp. was performed by the spread plate method using Nutrient Broth (NB) (Merck 1.05443), Rappaport Vassiliadis *Salmonella* Enrichment Broth (RVS) (Merck 1.07700), Brilliant Green Phenol Red Lactose Sucrose Agar (BPLS) (Merck 1.10747) and Xylose Lysine Deoxycholate Agar (XLD) (Merck 1.105287). The plates were incubated at 37°C for 24-48 hours under aerobic conditions (Greenwood et al. 1984, Flowers et al. 1992, ISO 2017a).

The analysis of *Listeria* spp. was performed by the spread plate method using Fraser broth (Merck 1.10398) and Oxford Agar (Merck 1.07004). The plates were incubated at 37°C for 24-48 hours under aerobic conditions (ISO 2017b, ISO 2017c).

The analysis of *Brucella* spp. was performed by the spread plate method using Brucella selective supplement (Oxoid SR 83) and Farrell's Agar (Oxoid CM 169). To prevent drying of the medium, the petri dishes were wrapped around with parafilm (3M). The plates were incubated at 37°C for 21 days under conditions with 6% CO2 (Kara 2011).

RESULTS

3.1. Physicochemical Properties

The aw and pH values of the toast cheese samples are presented in Table 1.

3.2. Microbiological Analyses

The microbial load of the samples is shown in Table 2. The presence of some pathogenic bacteria in toast cheese samples is shown in Table 3.

DISCUSSION

4.1. Physicochemical Properties

The aw values of the samples (n=50) were presented in Table 1. It was determined that the aw values of the samples varied between 0.899 and 0.962 and were 0.932 on average (Table 1).

Topal (1987) determined that the aw values of ripened kashar cheese varied between 0.850 and 0.970. Hence, our samples' aw values were lower compared to Topal's (1987) findings. It is thought that low aw values were caused by various ingredients, which bound water molecules in cheese production.

The pH values of the samples varied between 4.64 and 5.11 (Table 1). Oksuztepe et al. (2009) reported that the pH values of fifty fresh kashar cheese samples, offered for sale in Elazığ, were found to be from 5.01 to 5.92, and the average was 5.49 ± 0.32. The pH values in our study were lower than those results (Oksuztepe et al., 2009). The difference is caused by the production methods and the selection of additives (e.g. emulsifying salts).

It has been indicated that the properties of raw materials used in toast cheese production, the amount of emulsifying salts used, type, pH value, buffering capacity, calcium chelating properties, etc. are effective on the pH value in the last product (Shirashoji et al. 2006). The results obtained in the present study revealed that the pH of the samples varied between 5.4-5.9 as a result of the hydrolysis, dissolution and some interactions of emulsifying salts used in the production of toast cheese, and the best pH value in the end product is 5.7 (Guinee et al. 2004, Lu et al. 2007).

4.2. Microbiological Analyses

Aerobic-mesophilic bacteria constitute the great majority of microorganisms found in foodstuffs. These bacteria do not need specific nutrients, and they can easily grow under neutral and mild acidic conditions (Dogan and Tukel 2000).

It was determined that the TAMB counts of the samples varied between 3.59 and 7.36 log CFU/g, and their average was 5.81 log CFU/g, while TAPB counts varied between 2.30 and 3.90 log CFU/g, and their average was 3.30 log CFU/g (Table 2).

According to a survey held in Elazığ, the microbiological load (TAMB) of fresh kashar cheese samples was as follows: 6.59 log CFU/g (min) and 7.43 log CFU/g (max), and the average was 7.021 log CFU/g (Oksuztepe et al. 2009). At the same time, Cetinkaya and Soyutemiz (2006) investigated the microbiological quality of traditional kashar cheese during the ripening process and reported that the TAPB count varied between 5.04-5.59 log CFU/g.

The results obtained by the researchers were higher than the results obtained in our study. The main reason for the difference may be variation in pH and aw values.

As a result of the yeast-mold count analysis, it was determined that the yeast-mold counts of the samples varied between 2.58 log CFU/g and 7.78 log CFU/g and the average was 4.14 log CFU/g (Table 2). Gulmez et al. (2004) reported that they determined the yeast-mold count to be 8.59 log CFU/g on average in fifty Kars kashar cheese samples marketed

for consumption in Kars province. They found quite higher values when compared to our data. The difference is thought to be due to the processing methods, the additives used in the production and the physicochemical values (aw, pH, etc.) of the end product.

Table 1. aw and pH values of the samples.

Parameter	Samples (n: 50)		
	Minimum	**Maximum**	**Average**
aw	0.899	0.962	0.932±0.04
pH	4.64	5.11	4.89±0.29

Table 2. Microbial load of the samples (Log CFU/g).

Parameters	Samples (n: 50)		
	Minimum	**Maximum**	**Average**
TAMB	3.59	7.36	5.81±0.18
TABP	2.30	3.90	3.30±0.62
Yeast/Mold	2.58	7.78	4.14±0.42
TCGB	NG	3.64	2.89±0.06
LAB	4.06	6.08	4.69±0.36
Lactococcus/Streptococcus spp.	3.88	5.75	4.93±0.14
Pseudomonas spp.	2.65	5.48	4.74±0.71
Staphylococcus aureus	< 0.1	5.07	2.42±0.12
Bacillus cereus	< 0.1	3.86	1.08±0.02

NG: No Growth, TAMB: Total aerobic bacteria count, TABP: Total aerobic psychrophilic bacteria count, TCGB: Total coliform group bacteria count, LAB: Lactic acid bacteria count.

Table 3. The Presence of Some Bacteria in the Samples

Bacteria	Samples (n: 50)	
	Number	**%**
Escherichia coli	12	24
Clostridium perfringens	4	8
Salmonella spp.	9	18
Listeria spp.	6	12
Brucella spp.	0	0

It was determined that the TCGB counts of toast cheese samples offered for sale across Afyonkarahisar province varied between NG (No Growth) and 3.64 log CFU/g and that the average was 2.89 log CFU/g. Ozdemir and Demirci (2006) investigated the microbiological properties of kashar cheese stored with the addition of potassium sorbate and reported that they determined the TCGB counts of kashar cheese samples to be 2.54 log CFU/g on average. The results obtained by the researchers are similar to the data obtained in our study.

It was determined that the LAB counts of 50 toast cheese samples, the microbiological quality of which

we examined, varied between 4.06 and 6.08 log CFU/g and that *Lactococcus/Streptococcus* bacteria counts varied between 3.88 and 5.75 log CFU/g. Yilmaz and Dagdemir (2012) indicated that the LAB counts of kashar cheese, ripened for 120 days by being covered with beeswax, on the 5th day of ripening varied between 6.77 and 7.20 log CFU/g and that *Lactococcus/Streptococcus* bacteria counts varied between 6.73 and 7.09 log CFU/g.

The researchers obtained higher data compared to the results of our study. It is thought that this difference is due to the production methods, the presence and number of starter bacteria used in production and the ripening process applied to cheese after production.
Pseudomonas bacteria are among the most common microorganisms that cause degradation in raw and pasteurized milk and dairy products (Mc Guiggan et al. 1994) and constitute the most important and the most diverse group of bacteria in the world ecologically.

Furthermore, they have the ability to physiologically and genetically adapt to different environmental conditions. Most of these bacteria (58-91%) also have the ability to exhibit enzymatic extracellular proteolytic, lipolytic and phospholipolytic activity (Wijman et al. 2007). The duration of development of Pseudomonas is reported to be quite short compared to other psychrotrophic bacteria, and a surviving cell in milk at 4°C would reach 106 CFU/mL within 8 days (Larsen and Jorgensen 1997).

As a result of microbiological analyses, it was determined that Pseudomonas spp. counts of toast cheese samples varied between 2.65 and 5.48 log CFU/g and that their average was 4.74 log CFU/g.
As a result of *Staphylococcus aureus* analysis performed in 50 toast cheese samples offered for sale in Afyonkarahisar province, it was determined that counts varied between <0.1 and 5.07 CFU/g.

Kocak (2014) investigated the microbiological quality of kashar cheese produced and offered for sale in the dairies in Aydın province and determined the *Staphylococcus aureus* bacteria count to be 4.14±0.36 log CFU/g on average. The values obtained in the study are higher than the values obtained in our study. It is thought that this difference is caused by the differences in the production process steps of cheese, the additives used in production, the differences in physicochemical values of the end product, and the failure to adequately comply with the hygiene and sanitation rules.

Bacillus cereus is a Gram (+) spore-forming and enterotoxin (spores are resistant to heat treatment at 63 °C for 30 min) producing bacterium that causes two types of food poisoning (diarrheal and emetic) in humans (Ray and Bhunia 2013, Karagozlu 2017, Aytac and Taban 2017). It was determined that *Bacillus cereus* bacteria count in toast cheese samples varied between <0.1 and 3.86 log CFU/g and that the average was 1.08 log CFU/g. Piatkietwicz and Fabijanska (1992) reported that they determined *Bacillus cereus* average count to be 2.75 log CFU/g, as a result of the analysis performed in Fromage Fin type cheese.

The analysis results of the presence of *Escherichia coli* in the samples are presented in Table 3. Accordingly, *Escherichia coli* bacterial growth was detected in 12 (24%) of 50 toast cheese samples offered for sale in Afyonkarahisar province. Kocak (2014) reported the presence of *Escherichia coli* in 4 (13.3%) of kashar cheese samples offered for sale in Aydın province. Similarly, Oksuztepe et al. (2009) determined the presence of *Escherichia coli* in 4 (8%) of fresh kashar cheese offered for sale in Elazığ.

The presence of *Clostridium perfringens, Salmonella* spp. and *Listeria* spp. was found in 4 (8%), 9 (18%) and 6 (12%), respectively, of 50 toast cheese samples examined in terms of their microbiological qualities. Nevertheless, *Brucella* spp. growth was not detected in the samples. (Table 3). Turantas et al. (1989) reported that they did not observe *Clostridium perfringens* growth in 38 white cheese samples offered for sale in Izmir province. Similarly, Cetin et al. (2015) reported that they did not find the presence of *Salmonella* spp. in 17 kashar cheese samples offered for sale in Kırklareli province, and *Listeria monocytogenes* grown was reported in one sample.

It is thought that the differences among the relevant studies are various heat treatment norms applied in cheese production, processing differences, and the failure to adequately comply with the hygiene and sanitation rules in the production and packaging stages.

CONCLUSION

The reasons such as the lack of a certain standard in the production of toast cheese, inadequate or no heat treatment applied to the milk used in the production of cheese, the use of too many additives, relatively low temperature applied to curding, the failure to pay adequate attention to hygiene and sanitation rules in the process stages, and the microbial contaminations resulting from misapplications in storage, transportation and sales conditions may negatively affect the microbiological quality of cheese.
Many numbers and types of pathogenic bacteria, such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella, Listeria*, and *Brucella*, could be present in the milk to be processed into cheese. These bacteria can change the characteristic features of cheese and may also cause poisoning and diseases as a result of their consumption. Therefore, since the consumption of cheese made with raw milk or insufficient heat

treatment may be risky, the milk to be processed into cheese should be pasteurized at the appropriate temperature and time.

As a result of the microbiological analyses performed, it was determined that especially the microbiological quality of toast cheese samples offered for sale in Afyonkarahisar province was generally low and was not in conformity with the criteria specified in the Turkish food codex communique on microbiological criteria and that the consumption of these cheeses could pose a risk for public health.

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