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Original Article
Özgün Araştırma

Microbiological quality of some artisanal Italian cheeses retailed in the Turkish market

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Abstract: Three types of imported Italian cheeses (mozzarella, Parmigiano, and robiola) were evaluated for microbiological qualities in this study with the methods offered by ISO. The existence of *S. aureus*, *L. monocytogenes*, Coliform bacteria, *E. coli*, *C. perfringens*, *Salmonella*, Yeast, Mold, and *Staphylococcal* enterotoxins was investigated in 120 (40 each) samples. *L. monocytogenes* and *Salmonella* were not detected. The VIDAS SET2 technique searched *Staphylococcal* enterotoxins, and no toxin was spotted. The count of *S. aureus*, Coliform bacteria, *E. coli*, *C. perfringens*, Yeast, and Mold was found under the toleration limits in all 120 samples ($P<0.001$). The use of good quality raw materials, low bacterial load at the beginning of production and respect for the principles of good manufacturing practices are known as essential steps to obtain quality products in every respect. The samples we analysed in our study were found to be valuable in terms of public health.

Keywords: Food-borne Pathogens, Mozzarella, Parmigiano, Public Health, Robiola

Türkiye'de perakende olarak satışa sunulan bazı el yapımı İtalyan peynirlerinin mikrobiyolojik kalitesinin belirlenmesi

Özet: Bu çalışmada, ülkemizde satışa sunulan ve en çok tüketilen üç tip İtalyan peynirinin (mozzarella, Parmesan ve robiola) mikrobiyolojik kalitesi ISO (International Organization for Standardization) tarafından belirlenmiş standartlarla değerlendirilmiştir. Her biri 40'ar adet olmak üzere toplam 120 örnekte *S. aureus*, *L. monocytogenes*, Koliform bakterileri, *E. coli*, *C. perfringens*, *Salmonella*, Maya, Küf ve Stafilokokal enterotoksinlerin varlığı araştırılmıştır. Yapılan analizler sonucunda, numunelerde *L. monocytogenes* ve *Salmonella* tespit edilmemiştir. Stafilokokal enterotoksinler, VIDAS SET2 tekniği araştırılmış ve benzer şekilde toksin varlığı tespit edilememiştir. 120 örneğin tamamında ise *S. aureus*, Koliform bakterileri, *E. coli*, *C. perfringens*, Maya ve Küf sayıları yasal tolerans sınırlarının altında bulunmuştur ($P<0,001$). Kaliteli hammadde kullanımı, üretimin başlangıcında düşük bakteri yükü ve iyi üretim uygulamaları ilkelerine uyulması, her açıdan kaliteli ürün elde etmenin vazgeçilmez adımları olarak bilinmektedir. Çalışmamızda analiz ettiğimiz örneklerin halk sağlığı açısından risk oluşturmadığı ve hijyenik olarak değerli olduğu görülmüştür.

Anahtar kelimeler: Gıda Kaynaklı Patojenler, Halk Sağlığı, Mozarella, Parmesan, Robiola

Introduction

Cheese is defined as a dairy-based fermented food and is known as a highly nutritious dairy product with thousands of varieties according to the region of production, production technique, type of milk, ethnic and social characteristics. At the same time, another reason for this variousness has been reported as having different physical, chemical, and sensory qualities thanks to the rich bacterial flora it contains. This rich bacterial flora is a significant supporter of the scented sensory attributes of the various cheese types owing to their complicated interaction with milk proteins, carbohydrates and fats that mainly appear in an essential technological procedure in cheese production, understood as "ripening" (Forde ve Fitzgerald, 2000; Gülhan, 2023a; Khattab et al., 2019).

Ripening is known as one of the critical stages in cheese production technology in terms of providing variety and developing various savours. At this stage, cheese types with different textural properties are obtained thanks to the flavouring substances produced as a result of the biochemical and microbiological reactions carried out by the starter cultures (Akarca et al., 2015; Fox, McSweeney and Paul, 1998; Gülhan, 2023b). Briefly, it has been reported that macromolecules in milk are fragmented by the proteolytic and lipolytic enzymes contained by the

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starter bacteria, and aromatic components materialise after these enzymatic reactions (Forde and Fitzgerald, 2000). The outline of some cheese kinds and their associated microbial flora is displayed in Table 1 (Khattab et al., 2019).

Table 1. Different cheese types, their microflora, and aromatic properties (Khattab et al., 2019)

Cheese Kind	Starter Cultures	Sensory Characteristics
Camembert	*Streptococcus cremoris* *Penicillium camemberti* *Geotrichum candidum*	Mushroomy, soft
Roquefort	*Streptococcus cremoris* *Penicillium roqueforti*	Pepery
Mozzarella	*Streptococcus cremoris*	Delicate
Provolone	*Streptococcus thermophilus*	Smoky
Feta	*Lactococcus lactis* *Enterococcus faecium*	Light colour
Cheddar	Streptococcus cremoris Streptococcus thermophilus	Sharp

It has been reported that significant economic losses result from microbial contamination of foods. Similarly, milk and dairy products are known as a good breeding environment for bacteria, thanks to the macromolecules they contain. For this reason, the usage of quality raw materials, hygienic milking, production, transportation, and storage conditions are among the critical aspects affecting milk and dairy products' microbial quality and shelf life (Losito et al., 2014; Ruegg, 2003).

The European authorities license traditional dairy productions of the European countries by giving the 'Protected Designation of Origin' (PDO) status, which fixes the requisites of genuineness for products from specified geographical provinces (Giammanco et al., 2011).

The Mediterranean coast and its surroundings are known as a region rich in biodiversity. The diversity of animal and plant resources and cultural richness have also been positively reflected in the variety of foods produced in the territory. For this reason, the Mediterranean region is known as a wealthy area, especially regarding dairy product multiplicity. Italy has a significant place in terms of cheese culture and variousness in the Mediterranean region (Di Trana et al., 2022).

Mozzarella is white, unripened, soft, elastic, and smooth textured cheese in the "pasta filata" family. Thanks to its melting and elastic characteristics, it is highly preferred in pizza recipes. Both bovine and buffalo milk can be used to produce mozzarella. The physical and sensory quality of Mozzarella cheeses produced from either bovine or buffalo milk looked similar. However, it was determined that mozzarella cheeses produced from buffalo milk contain more fat, protein, and mineral essences than mozzarella derived from bovine's milk (Jana, 2001; Jana and Tagalpallewar, 2017).

Parmigiano-Reggiano is a hard and cooked cheese with a high level of proteolytic ripening, which began to be produced approximately 2000 years ago in Parma, Italy (Erbay et al., 2017). It is an additive-free and nutritious cheese in thermophilic cultures used during its production and has a long ripening period of about 12 months (Tedeschi et al., 2022).

Roccaverano is a commune in the province of Asti, located in northwest Italy. Moreover, it has been reported that many important cheese varieties, especially Robiola, emerged in this environment. This PDO-type fresh cheese has five days of the ripening period, produced from Roccaverano-originated raw goat, sheep, and cow kinds of milk. However, it has been reported that it is obligatory not to use thermalization and to use 50 percent goat's milk to adhere to traditional methods in the production process of Robiola cheese (Biolcati et al., 2019; Biolcati et al., 2021).

Since the Anatolian region is wealthy in cheese diversity, it is also known as the land where these products are consumed in elevated quantities. For this reason, it is essential to determine the microbiological quality of imported cheeses that have just joined our country's market. This study aimed to analyse the PDO-type Italian cheeses marketed in our country regarding critical foodborne pathogens.

Material and Method

Material

This study examined a total of 120 PDO-type Italian kinds of cheese (40 mozzarellas, 40 Parmigiano, and 40 Robiola) with different lot numbers between January 2022 – September 2022. The existence of *S. aureus*, *L. monocytogenes*, Coliform bacteria, *E. coli*, *C. perfringens*, *Salmonella*, Yeast, Mold, and *Staphylococcal* enterotoxins was analysed to understand the microbiological quality of samples. After the samples were purchased from different sales points, they were carried to the laboratory under the cold chain and analysed instantly.

Method

In this investigation, the identification methods suggested by ISO for determining foodborne bac-

teria were used as a reference. For this attempt, ISO 6888-1, ISO 11290-1, ISO 4832, ISO 7937, ISO 6579, ISO 21527 and VIDAS® Staphylococcal Enterotoxins (SET2) techniques were used for the detection of *S. aureus, L. monocytogenes*, Coliform bacteria, *E. coli, C. perfringens, Salmonella*, Yeast, Mold, and *Staphylococcal* enterotoxins, respectively (ISO 2004, 2006, 2008, 2017a, 2017b, 2021). For the analyses of Coliform bacteria, *E. coli, S. aureus, C. perfringens*, Yeast, and Mold, 10 grams of each sample were diluted in 90 ml Maximum Recovery Diluent (Merck 112535) under aseptic conditions then homogenized with a stomacher (Interscience Bagmixer 400) for 1 minute and the mixture was plated on to bacteria/pathogen specific media or enrichment. For *Salmonella* spp. and *L. monocytogenes* analysis, 25 grams of each sample were weighed and inoculated on specific selective media or enrichment, adhering to the methods recommended by ISO. Identification of pathogen suspected colonies were done with specific biochemical tests. Finally, all inoculated media/enrichment were incubated at the specific temperatures advised in ISO methods. Furthermore, eventually for the detection of *Staphylococcal* enterotoxins, VIDAS SET2 (rapid automated detection of *Staphylococcal* Enterotoxins) strips were used according to the manufacturer's recommendations.

Results

The present study examined 120 PDO-type Italian kinds of cheese (40 mozzarella, 40 parmigiano, and 40 robiola) with different lot numbers between January 2022 – 2023. *S. aureus*, *L. monocytogenes*, Coliform bacteria, *E. coli*, *C. perfringens*, *Salmonella*, Yeast, Mold, and Staphylococcal enterotoxins were determined in the samples with ISO and VIDAS-based methods. In our research, the analyses were repeated twice, and the average of the results was taken as the basis. The results obtained were evaluated within the limits of PDO and the Turkish Food Codex (TFC, 2011). Study findings are presented in Table 2. According to the data we obtained from the study, none of the analysed samples exceeded the legal limits. In other words, imported Italian cheeses in Turkey which we examined in our study were found to be both hygienic and safe in terms of food-borne pathogens and public health.

Table 2. Microbiological quality of Artisanal Italian Cheese Samples

Chese Type	*S. aureus* (cfu/g)	Staphylococcal Enterotoxins	L. monocytogenes	Coliform Bacteria (MPN)	*E. coli* (MPN)	C. perfringens	*Salmonella* spp.	Yeast	Mold
Mozzarella (n: 40)	$<1.0x10^1$	ND	ND	$\bar{x} < 3,6$	$\bar{x} < 3$	$<1.0x10^1$	ND	$<1.0x10^2$	$<1.0x10^2$
Parmigiano Reggiano (n: 40)	$<1.0x10^1$	ND	ND	$\bar{x} < 3,6$	$\bar{x} < 3$	$<1.0x10^1$	ND	$<1.0x10^2$	$<1.0x10^2$
Robiola di Roccaverano (n: 40)	$<1.0x10^1$	ND	ND	$\bar{x} < 3,6$	$\bar{x} < 3$	$<1.0x10^1$	ND	$<1.0x10^2$	$<1.0x10^2$

Discussion

This study displayed that the microbiological properties of imported Italian cheese in the Turkish market were quite good. The results of our study showed that the hygienic quality of the samples we examined was high and did not pose any risk to public health.

Contamination levels of mozzarella samples are widely ranged. In research conducted in Egypt, 120 soft cheese samples (Kareish and Mozzarella) were investigated in terms of the presence of *Staphylococcus aureus*, coliforms, *E. coli*, mould, and yeast. The study's data showed that the prevalence of *Staphylococcus aureus*, coliforms, *E. coli*, mould, and yeast was 23%, 40%, 16.6%, 14%, and 10%, respectively (Mohamed et al., 2019). In another study, the presence of coliforms, *Staphylococcus aureus*, *Salmonella* spp., and *Listeria monocytogenes* were investigated in mozzarella cheese samples. According to the research data, 12.5%, 10%, 2.5%, and 2.5% of samples harbour coliforms, *Staphylococcus aureus*, *Salmonella* spp., and *Listeria monocytogenes,* respectively (Marinheiro et al., 2015). Similarly, Garbaj et al. (2007) studied coliforms, *Salmonella* spp., and *Staphylococcus aureus in* traditionally made and imported mozzarella cheese in Tripoli city. As a result of the study, it was determined that the samples produced by the traditional method included higher rates of coliforms, *Salmonella* spp., and *Staphylococcus aureus* than the imported ones. In addition, it was specified that the imported samples had 13%

coliform and 26% *Staphylococcus aureus*. However, similarly to our work *Salmonella* spp. Was not detected in samples. A study conducted for the microbiological evaluation of mozzarella cheeses sold in Cameroon was aimed to determine the levels of coliform bacteria, *S. aureus*, and *E. coli*. According to the study results, different coliform levels, *S. aureus*, and *E. coli* were detected in mozzarella cheese samples. However, it has been documented that the determined values are below the permitted limitations (Belli et al., 2013). The fact that the findings acquired with the research were more elevated than the findings of our study can be attributed to the low hygienic conditions and the usage of different technologies in the dairy production process, and other inefficient conditions during storage and at sale points. Furthermore, climate conditions, transport temperature, and staying time of cheese on market shelves can be thought that they are effective in growing the bacterial load. Tirloni et al. (2014) investigated the presence of *Enterobacteriaceae*, *E. coli*, Coagulase positive *S. aureus*, yeasts, and moulds in Italian cheeses such as Crescenza, Robiola, Primo sale, Formaggella, Mozzarella, and Burrata. According to their study data, the level of *Enterobacteriaceae*, *E. coli*, Coagulase positive *S. aureus*, yeasts, and moulds in Robiola was 5.9-7.1, 2.7-7.0, 2.6-5.5, 4.3-7.4, and <2.0 cfu/g respectively. Similarly, the level of *Enterobacteriaceae*, *E. coli*, Coagulase positive *S. aureus*, yeasts, and moulds in mozzarella was detected at 3.2-6.0, 2.0-6.0, <2.0-3.0, <2.0-4.7, and <2.0 cfu/g respectively. Öksüz et al. (2004) found Coliform group bacteria and *E. coli* in white pickled cheese <4 cfu/g. The similarity of the study results can be attributed to the suppressive effect of the high acidity of cheeses on bacteria. However, in another study, *L. monocytogenes* was detected in 11% of some dairy products in Turkey (Kevenk and Koluman, 2022). Alike, *L. monocytogenes* was found in 8.1% of traditionally produced cheese types in the Black Sea Region of Turkey (Kevenk and Terzi Gulel, 2016). This situation may be attributed to *L. monocytogenes*, an acid-resistant agent that can survive in severe conditions such as acidic environments or lower temperatures. Future studies are planned to reveal the hygienic quality of different imported foods.

Conclusion

With the rapid increase in the circulation of communication, transportation, knowledge, and people in the world, the interest in different cultures is increasing at the same rate. The sense of curiosity that arises primarily in the cultural field brings an interest in various types of foods.

It is a fact that should be noted that we live in a world where favorite foodstuffs from different countries can be found even in very distant countries. While this situation has good aspects, it can also have adverse effects that may threaten public health due to low quality. Although the regulations of importing countries protect public health, food-borne infections and intoxications cannot be controlled entirely.

For this purpose, the imported and most consumed Italian cheeses in Turkey were analyzed, and their hygienic quality was tried to be determined in our investigation.

In a world that shrinks in parallel with the developing technology within the scope of safe food concept from farm to fork, studies like this need to be increased not to raise the rate of food-borne infections and intoxications and to protect public health better.

Ethical Statement: This study does not present any ethical concerns.

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