Presence of *Salmonella* spp., *Listeria monocytogenes* and *Staphylococcus aureus* in halloumi sold in Northern Cyprus

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ABSTRACT

Halloumi is an important part of the Cyprus dairy sector and consumed by a large volume of public. The microbiologic safety of the cheese is important in terms of public health. In this study, the presence of *Salmonella* spp., *Listeria monocytogenes* and *Staphylococcus aureus* on 1072 samples that collected for two years, their distribution according to the seasons and the effect of the seasons on the microbial load were investigated. As the result of the study, *Salmonella* spp. and *L. monocytogenes* could not be detected in any of the halloumi samples, while *S. aureus* was detected. It was determined that 39 (3.64%) of the halloumi samples contained *S. aureus* above $1x10^3$ cfu/g, 43 of them between $1x10^1$ and $1x10^3$ cfu/g, and 990 of them below $1x10^1$ cfu/g. It has been observed that the most intense contamination above $1x10^3$ cfu/g is formed in the spring season. In order to eliminate the food safety problem caused by *S. aureus*, first of all, the development of good manufacturing practices in farms, making the cold milk application cover all farms should be provided. On the other hand, within the framework of food safety from farm to fork, the end-product should be delivered to the consumer without breaking the cold chain.

Introduction

Halloumi is an important part of the Cyprus dairy sector. Historical documents showed us that this cheese has been produced in Cyprus since 1554 (24). The best-known characteristic of this cheese is to be produced the from raw milk without using starter culture. Halloumi is a type of cheese that can be consumed fresh or matured in brine. While industrial halloumi sold in cities is marketed in plastic vacuum packaging, it is preserved in brine in rural areas (1, 14, 15, 18, 19).

In the Northern Cyprus, approximately 164.250 tons of milk were produced in 2018, and a total of 144.345 tons in 2019, and 308.595 tons of milk was produced in a two-year period (9). Nowadays, implementation of Commission Regulation (EU) 2021/591of 12 April 2021 entering a name in the register of protected designations of origin (PDO) and protected geographical indications (PDI) with both Greek and Turkish names as Χαλλούμι-Halloumi/Hellim and Commission Decision (EU) 2021/586 of 12 April 2021 amending Decision 2007/330/EC lifting prohibitions on the movement of certain animal products on the island of Cyprus under Council Regulation (EC) No 866/2004 and laying down conditions for the movement of those products with regard to halloumi PDO have big importance so optimizing the food safety/quality properties (4).

It is reported that the shelf-life and quality of halloumi is affected by several factors such as the milk quality and the hygienic practices during manufacturing. Although halloumi is produced by boiling the curds in whey, several studies have reported the presence of contaminated microorganisms in the end product due to

poor hygiene during the production process and the survival of thermoduric microorganisms (22). The presence of *L. monocytogenes*, *Salmonella* spp. and *S. aureus* in ready-to-eat products is taken into account in the Turkish Food Codex Microbiological Criteria within the scope of food safety criteria in commercially available cheeses (3) and also with Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs (2). In the Turkish Republic of Northern Cyprus (TRNC) legislation, it is stated that "there will be no pathogen harmful to health" (1). It has been observed that no study has been found for cheese produced in the north of Cyprus, especially considering the incidence of *Salmonella* spp., *L. monocytogenes*, and *S. aureus*, which are also included in the food safety criteria for halloumi. In addition, considering that the Cyprus exhibits a hot and dry climate starting from the spring months due to the climate zone in which it is located seasonal temperature differences can be effective on *S. aureus*, *Salmonella* spp., and *L. monocytogenes* presence in halloumi produced in Northern Cyprus.

In addition to being a commercial product of Cyprus, halloumi also plays an important role in paving the way for the export of animal foods to the EU market in accordance with Regulation (EC) No 866/2004, also known as the green line regulation and the commission implementing decision (EU) 2021/586. Under the Commission Implementing Regulation (EU) 2021/591, Halloumi is included in the scope of protected designations of origin. In this scope, studies are carried out in the north and south of the island for export to the EU. Following the completion of both compliances with EU food safety criteria and compliance with PDO criteria both in the north and south of the island, halloumi will be able to export to the EU within the scope of PDO.

The aim of this study is to evaluate the microbiological quality of halloumi sold in markets in Northern Cyprus, using a large sample size to ensure robust results. Given the hot climate of the region, the study also examines the impact of seasonal variations on microbial contamination, with a focus on ensuring food safety.

Materials and Methods

Sample Collection: In order to investigate the presence of *Salmonella* spp., *L. monocytogenes* and *S. aureus* in halloumi marketed in Northern Cyprus, halloumi samples were collected for 2 years from market shelves. 1072 halloumi samples were delivered to the Near East University Veterinary Medicine Food Hygiene and Technology Food Laboratory in their original packaging and by maintaining the cold chain.

Microbiological Analysis: AOAC 2013.01 bioMerueux Vidas UP SPT kit protocol was used for the isolation and identification of *Salmonella* spp. (6) and AOAC 2013.11 bioMerueux LMX kit protocol was used for the isolation and identification of *L. monocytogenes* (7). The details of both protocols are as follows: 25g of halloumi sample is mixed with 225 mL of Buffered Peptone Water in a blender for 2 minutes, then 1 mL of Salmonella supplement is added to the mixture and incubated at 42±1°C or 41.5±1°C for 18-24 hours. 2-3 mL of sample is boiled (5 minutes at 95-100°C) and VIDAS SPT results can be monitored in 48 minutes. 25g of halloumi sample is mixed with 225 mL of LMX Broth (with 0.5 mL of LMX supplement) in a blender for 2 minutes, then incubated at 37±1°C for 26-30 hours. 2-3 mL of sample is boiled (5 minutes at 95-100°C), and then VIDAS LMX results are determined (6, 7).

Isolation and identification of coagulase positive *S. aureus* was performed according to TS 6582-1 EN ISO 6888-1 standard by observing coagulase positive staphylococcal colonies after aerobic incubation at 34°C to 38°C in Baird-Parker solid medium (23).

Statistical Analysis: Statistically, the SPSS package program was used, and the existence of a significant relationship between seasons and values was determined by the chi-square independence test. Percentages were calculated with a cross tabulation table.

Results

As a result of this study, *Salmonella* spp. and *L. monocytogenes* could not be detected in any of the halloumi samples, while *S. aureus* was detected in different amounts: 39 (3.64%). The seasonal distribution of *S. aureus* presence in halloumi and its microbial loads and the percentages were shown in Table 1.

In this study, it was observed that the contamination of halloumi samples with *S. aureus* over $1x10^3$ cfu/g was most common in spring months (15 samples), followed by summer (12 samples). It was determined that the season where halloumi was least contaminated with *S. aureus* above $1x10^3$ cfu/g (3 samples) was winter (P=0.15). Totally, 92.35% of all positive samples were detected to contain *S. aureus* below $1x10^1$ cfu/g.

Discussion and Conclusion

In our study, 1072 halloumi samples were analyzed, and no samples were found to contain *Salmonella* spp. or *L. monocytogenes*, and this result complies with both the Turkish Food Codex Communiqué on Microbiological Criteria for food safety (3) and the EU microbiological criteria regulation for food safety criteria (2).

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Table 1. Microbial load and seasonal distribution of *S. aureus* in halloumi (P=0.15).

Season	<1x10^1 cfu/g n (%)	1x10^1-1x10^3 cfu/g n (%)	>1x10^3 cfu/g n (%)	Total
Winter (December January February)	249 (93.96%)	13 (4.91%)	3 (1.13%)	265
Spring (March April May)	309 (92.80%)	9 (2.70%)	15 (4.50%)	333
Summer (June July August)	205 (90.71%)	9 (3.98%)	12 (5.31%)	226
Autumn (September October November)	227 (91.53%)	12 (4.84%)	9 (3.63%)	248
Total	990 (92.35%)	43 (4.01%)	39 (3.64%)	1072

The survey studies for halloumi within the borders of island are limited. However, similar results were obtained in the past out of Cyprus. Regarding *S. aureus* incidence, Değirmencioğlu (10) found *S. aureus* in 2 of 34 halloumi samples (6%) and reported that only one of them (3%) contained more than ≥1x10^3 *S. aureus*, similar to our study. The researchers concluded that microbiological load and profile in the end product may originate from different sources (milk, starters, and contaminating microorganisms), and the growth of the microorganisms may be affected by factors such as raw milk usage and the maturating conditions (24). Usca and Erol (25) reported that they detected coagulase-positive staphylococci at the level of 10^3 cfu/g in 26% (13 samples) of 50 halloumi samples. With the study carried out by Eleftheriadou et al. (12), 21% of the dairy samples (Hellim, Flavuna, and Anari) obtained to contain *Salmonella* spp. and *L. monocytogenes*. In the same study, 12.415 cheese samples were analyzed for *S. aureus* and 132 of samples (1.1%) contain between 10^3 and 10^4 cfu/g, 90 (0.7%) of the samples contain 10^4 cfu/g, a total of 222 (1.8%) of the samples contain *S. aureus*. The possibility that the halloumi produced by boiling the curd may have been contaminated with *S. aureus* in the last step from food handlers and equipment before and/or during packaging.

A variety of raw milk cheeses purchased over the internet was investigated and 108 purchases from seven European countries were examined for the prevalence of *Salmonella* spp., *L. monocytogenes*, *Escherichia coli*, and coagulase positive staphylococci. In this study, *L. monocytogenes* was detected in 1.9% of all samples, one of which had counts of 9.5 x10^3 cfu/g. *Salmonella* spp. could not be detected in any of the samples. *E. coli* and *S. aureus* could be detected in a total of 29.6% (≥10 cfu/g; 32x10^8) and 8.3% (≥100 cfu/g; 9x10^8) of samples, respectively, indicating poor conditions of hygiene (20). Unlike many cheeses generally produced from raw milk, halloumi is a curd-cooked cheese. The cooking phase of the curd is a practice that allows the elimination of pathogens originating from raw milk.

Önganer et al. (17) reported that 30 pieces of cottage cheese sold unpackaged in Diyarbakir were contaminated with 7.80±0.64 log cfu/g *Salmonella* spp. and an average of 7.53 ±1.12 log cfu/g *S. aureus* and that this might be due to non-compliance with hygienic rules in the process from production to consumption expressed. *L. monocytogenes* was detected in 2 of 85 white cheese samples produced and/or sold in Antakya region, *Listeria* spp. in 7, *L. ivanovii* in 3, *L. innocua* in 3, and *L. seeligeri* in 2 (5). Cokal et al. (8) reported that 100 Mihaliç cheeses did not contain *Salmonella* spp., 5 samples contained *L. monocytogenes* and all of them were contaminated with *S. aureus* and contained an average of 2.69 log cfu/g *S. aureus*. As a result of a study on the microbiological quality of soft, ripened soft, and semi-hard cheeses obtained from raw, terminated or pasteurized milk and sold on the market in England, it was reported that *Salmonella* was not found in any of the cheeses. In the same study, out of a total of 1819 cheeses produced from raw and thermized milk, 1 of them was ≥ 10^2 cfu/g, 16 of them <10^2 cfu/g *L. monocytogenes*, 13 of them more than 10^5 cfu/g, 13 of them between 10^3<10^4 cfu/g and backwards. While the rest contained *S. aureus* less than 10^3 cfu/g, 4 of 2618 cheese samples obtained using pasteurized milk contained *L. monocytogenes* less than 10^2 cfu/g, while the remaining 2614 samples did not contain *L. monocytogenes* and 2 of them contained 1x10^4 cfu/g (1.9x10^4 cfu/g, 4x10^4 cfu/g) and 1 of them was found to be between 10^2 <10^3 cfu/g and the remaining samples were found to contain less than 10^2 cfu/g *S. aureus* (16). In a study on the presence of *S. aureus* and other staphylococci in cheese samples sold in the Bologna region that were examined, *S. aureus* was found mostly during the hot months, while the other common species were found mostly in the period October–March (11). Teymori et al. (21) reported that *S. aureus* was found in 2

($4.75x10^2$ and $2.8x10^2$ cfu/g) of 30 cheese samples in a study conducted in West Azerbaijan region.

In the studies on the bacterial contamination of raw milk according to the seasons, Fadaei (13) stated that coliform, *E. coli*, and *S. aureus* contamination is obtained mostly in summer in 29 (96.66%) of the samples. Vahedi et al. (26), in their study, found that the highest rate of contamination of raw milk with *E. coli*, coliform, and *S. aureus* in different seasons was observed in summer and that the samples were 24 (57.1%) *E. coli*, 19 (52.8%) coliform, and 10 (45.4%) reported that it was contaminated with *S. aureus*. In current study we performed; totally 39 samples were obtained to contain *S. aureus* above $1x10^3$ cfu/g and 15 of these results were obtained in spring season which is a rainy and warm period for Cyprus.

As a conclusion of this study, absence of *Salmonella* spp. and *L. monocytogenes* hazard in halloumi make us think of a positive result in terms of food safety. On the other hand, further studies should be performed on the staphylococcal enterotoxin incidence. The boiling stage of curd makes decontamination of *S. aureus*, but the toxin which leads to food poisoning may still be presence. *S. aureus* can also contaminate to halloumi at the folding stage, at which the food handlers are in direct contact with cheese. We believe the fact that dairy product manufacturers in Northern Cyprus have largely adopted the principle of "Good Production Practices" and that they have knowledge of the principles of the Hazard Analysis and Critical Control Point (HACCP) system which based on monitoring and catching hazards from beginning to the end of manufacturing process. The most intense detection of *S. aureus* was at levels above $1x10^3$ cfu/g. This may also be because of the high temperatures in the summer months of Cyprus and the abuse of cold-chain.

The development of hygiene conditions, the application of cold milk application on the basis of all farms, the adoption of good production practices at every stage of the product, the systematic training of the food handlers on food hygiene and safety, the well-determined and monitoring of critical control points during the process flow and the selection of raw materials are accepted as important parameters to obtain a safe product. We are of the opinion that more serious implementation of the storage conditions until the end of the shelf life of the final product, especially taking all the necessary measures to prevent the cold chain and ensuring traceability, will contribute significantly to the competitiveness of the product in the foreign market by increasing the safety of the product.

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Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Author Contributions

FIM was responsible for the planning and execution of the study. FKY edited the article. BHU performed the analysis, drafted the article, and conducted a critical revision. CH was responsible for the final revision.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

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