Investigation of quality characteristics of industrially produced halloumi cheese

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ABSTRACT

Χαλλούμι (Halloumi)/Hellim is the traditional cheese of Cyprus and belongs to the whole Island with Turkish and Greek names. Especially with the spotlights more on the product nowadays. In the current study, cheese samples were collected as fresh and mature halloumi/hellim (sheep/goat, bovine, or both milk) and were analyzed to evaluate the physicochemical and microbiological status. As a physicochemical analysis, the potential of hydrogen (pH), titratable acidity (TA), salt-sodium chloride (NaCl), and dry-matter (DM) ratios were analyzed. For examining the quality and safety indicators; total mesophilic aerobic bacteria (TMAB), yeast and molds, lactic acid bacteria (LAB), coliform bacteria, coagulase-positive staphylococci, *Listeria monocytogenes*, *Salmonella* spp. analyzes were performed. This study will help to establish the quality profile of halloumi/hellim cheese and identify potential hazards and sources of contamination.

Introduction

Χαλλούμι (Halloumi)/ Hellim is the traditional cheese of Cyprus and belongs to the whole of the divided Island with Turkish and Greek names. Historical documents show that this cheese has been produced in Cyprus since 1554. Knowledge of the production process has been carried out from one generation to the next (1). Χαλλούμι (Halloumi)/Hellim cheese has a tight and elastic structure without holes and can be sliced easily. Χαλλούμι (Halloumi)/Hellim, which gives an elastic feeling in the mouth, does not melt like kashar cheese when fried, and due to this feature, it can be consumed by frying in a pan or on the grill. Therefore, Χαλλούμι (Halloumi)/Hellim cheese, which has a wide consumption area, can be consumed for breakfast, as well as fried or grated and added to omelets, pasta, pastries, wraps, pita bread, soups, and salads (11). The first references to halloumi were seen in 1554 when Florio Bustron referred to a cheese (calumi in Italian) made from the milk of the sheep and goats of Cyprus. Kiprianos Church of Cyprus in 1778, mentioned the taste of halloumi and that it was unique (31). Halloumi is an important part of the Cyprus dairy sector and even the economic resources of the island in general (1, 15).

The shelf-life and quality of Χαλλούμι (Halloumi)/ Hellim are reported to be affected by several factors such as the milk quality and the hygienic practices during processing (3). Although Χαλλούμι (Halloumi)/Hellim is by boiling the curds in whey, several studies have reported

the presence of contaminated microorganisms in the end product due to the low quality of raw milk (29) or due to the poor hygiene during the production process (7). On the other hand, thermoduric microorganisms may survive boiling and LAB has also been reported to be found in fresh and mature halloumi/hellim cheese (34). The microbiological load and profile in the end product may be originated from different sources (milk, starters, and contaminating microorganisms) and the growth of the microorganisms may be affected by factors such as the applications of raw milk and the maturating conditions.

The sensory qualities of halloumi/hellim may also be affected by *Lactobacillus cypricasei*, named special for halloumi/hellim. It provides a wide range of enzymatic activities in cheese and may influence the trait of the end product (9). Several research studies (4, 8, 20, 27, 35) have been performed on industrially and traditionally produced cheese varieties to promote sustained quality and safety standards for halloumi manufacturers as well as to define the unique attributes of the cheese to preserve it.

The main feature of halloumi/hellim cheese production technology is to be produced from raw milk in traditional scale production without using a starter culture. On the other hand; with the recommendations of Commission Implementing Regulation (EU) 2021/591 of 12 April 2021, the cheese should be produced by pasteurized milk. However, halloumi/hellim is made using varying proportions and types of milk, and according to PDO, the most crucial issue for the product is that it should be made with local sheep and goats (the Chios sheep and the Damascus goat) milk (1). Since no starter culture is used, the milk is coagulated by rennet at 33±1 °C and the clot is cut into 1 cm^3 pieces. In this case, after resting for 10 minutes, it is heated at 40 °C for 15 minutes, transferred to cheese vats, and applied pressure. It is then cut into dimensions of 8±1 cm x 10±1 cm x 4±1 cm. Then the whey is mixed and heated until it is brought to 80 °C. At this stage, albumin and globulin, which are serum proteins, coagulate and rise to the surface. This clot is carefully collected from the surface, pressed, and another local cheese type called "nor" is produced, which can be consumed fresh or dried (11).

It is known that different practices are followed in some process steps in the traditional production of halloumi cheese, which is common among the public of the Island. This situation causes differences in the standard features of the product. For this reason, The Regulation of PDO brought a unique and single standard for the whole island to provide exemplary quality production without changing its unique character and to maintain and increase its market share in the globalized competition. Depending on our scientific paper evaluation on halloumi/hellim quality investigation, there is not much paper regarding the situation on Northern Cyprus. That's why the current study aimed to characterize the some of the physicochemical and microbiological indices of halloumi/hellim produced in Northern Cyprus create a scientific data for the literature.

Materials and Methods

Samples collection: Totally, of 85 halloumi/hellim samples were collected from retail outlets of Nicosia. The samples were brought to the laboratory under a cold chain in their original vacuum packaging which are nearly 200-250 g in weight. The samples were collected under standard retail conditions and from the markets that have high capacity of halloumi/hellim retail.

Microbiological Analysis Of The Halloumi/Hellim Cheeses: 10 g of sample was homoginazated in 90 mL of Maximum Recovery Diluent (MRD). Then, serial dilutions were prepared and inoculated on sterile media. Total mesophile bacteria (TMAB) were enumerated on Plate Count Agar (PCA; LAB149) after incubation at 35 °C for 48h. Total coliform bacteria were enumerated on Violet-Red Bile Glucose Agar (VRBA; LAB031) at 35°C for 24h. Staphylococcal-micrococcal bacteria, Lactic Acid Bacteria (LAB), and mold/yeast were enumerated on Baird Parker Medium Agar (BPA; LAB085), MRS Agar, and Yeast Glucose Chloramphenicol Agar (YGC; LAB122), respectively. Thermophilic anaerobes, producing hydrogen sulphite were detected by Sulfite Polymyxin Sulfadiazine Agar (SPS; MERCK 110235). The media and incubation conditions are shown in Table 1. At the end of incubation, Petri dishes containing 30-300 colonies were counted and the result was reported as $\log_{10}$ cfu/g.

Physicochemical Analysis Of The Halloumi/Hellim Cheeses: After the packages of the halloumi/hellim cheeses were opened and used for microbiological analyses, the samples were prepared for physicochemical analyses. Hanna HI 98230 penetration pH meter (Hanna Instruments, Italy) calibrated with pH 4 and 7 buffers were used to determine the pH of the samples at 24°C. % lactic acid value was obtained by titration method. Humidity and dry matter (DM) analyses were performed by a humidity meter device (Shimadzu Corporation, MOC63u), and sodium chloride (NaCl) was analyzed by the Mohr method.

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Table 1. Media used incubation conditions, and references of methods.

Micro-organisms	Analytical reference method	Media name	Incubation conditions		
			Incubation temp.	Incubation period	O_2 requirement
Total Mezophile Aerobe Bacteria (TMAB)	ISO 4833	Plate Count Agar (LAB 149)	30°C ± 1 ºC	72 h ±3 h	Aerobic
Staphylococci *Staphylococcus aureus*	ISO 6888-1: 1999 + A1:2003	Baird Parker Medium Agar (LAB 085) + Egg Yolk Tellurite Emulsion (X 085)	35 º- 37 ºC	24 h ± 2 h	Aerobic
		Brain Heart Infusion Broth (LAB 049)	Confirmation for *Staphylococcus aureus*		
		Rabbit Plasma (X086)			
Salmonella spp.	ISO 6579:2002 + A1:2007	Buffered Peptone Water (LAB 204)	37 ºC ± 1 ºC	18 h ± 2 h	Aerobic
		Rappaport Vassiliadis Medium (R.V.S) single component (LAB 086)	41.5 ºC ± 1 ºC	24 h ± 3 h	
		X.L.D. Agar (LAB 032)	37 ºC ± 1 ºC	24 h ± 3 h	
		Triple Sugar Iron Agar (LAB 053)	Confirmation		
		Urea Broth Base (LAB 131)			
Coliform bacteria	ISO 4832:2006	Violet Red Bile Glucose Agar (LAB 031)	44 ºC (for *E.coli*) 30 ºC - 37 ºC	24 ± 2 h	Microaerophilic
		Brilliant Green Bile Broth (LAB051)	Confirmation		
Listeria monocytogenes	ISO 11290-1: 1996 + A1:2004	Half Fraser Broth Base (LAB 164)	30 ºC	24 h ± 2 h	Aerobic
		Fraser Broth Base (LAB 164)	37 ºC	24 h	
		Palcam Agar (LAB 148)	37 ºC	24 h ± 3 h	
		Tryptone Soya Yeast Extract Broth (LAB004)	Confirmation for *Listeria* spp.		
		Sheep Blood Agar (LAB028)	Confirmation for *L. monocytogenes*		
Lactic Acid Bacteria (LAB)	ISO 15214:1998	MRS Agar (LAB223)	30 ºC	2-3 days	Anaerobic
Yeast and mold	ISO 6611: 2004	Yeast Glucose Chloramphenicol Agar (LAB 122)	25 ºC	5 days	Aerobic
Thermophilic anaerob bacteria		Sulfite Polymyxin Sulfadiazine Agar	44 ºC	48 h	Anaerobic

Results

Results Of Physicochemical Analysis: The physicochemical analysis results of halloumi cheese are presented in Table 2. According to the physicochemical results, the minimum and maximum DM were 20-50 %, pH levels were between 4.94-6.87, salt ratios were 1-7; the titratable acidity was calculated as 0.07-0.28. With the results within the current study, it was observed that the pH of the samples is mostly in the range which stops undesired microorganism growth. The amount of salt in the analyzed halloumi cheeses was found to be min 1.00%, max 7%, and the mean value was 3% (Table 2). DM content of our samples was between 20-50% and at the mean of 40%.

Table 2. Physicochemical analysis results of halloumi cheese.

	N	Mean	Min-Max.
Dry matter (DM) (%)	85	40	20-50
pH	85	6.2	4.94-6.87
NaCl (%)	85	3	1-7
Lactic acid (LA) (%)	85	0.18	0.07-0.28

Results Of Microbiological Analysis: The microbiological analysis results of our study were presented in Table 3. Because the products are from different enterprises, very different results were obtained in TMAB and Staphylococci counts. The results were given as

Staphylococci, since all of the colonies counted on BPA agar had negative *S. aureus* confirmation test. *Listeria monocytogenes* and *Salmonella* spp. were not detected in any samples because of that, they were not included in Table 3. According to this study on the samples of halloumi/hellim cheese in the Cyprus market, *Salmonella* spp. and thermophilic anaerobe bacteria, producing hydrogen sulphite were not isolated in any of the samples. The TMAB was found to be min 3 $\log_{10}$ cfu/g, max 6.70 $\log_{10}$ cfu/g, and on average 4.54 $\log_{10}$ cfu/g in our study (Table 3). In this study, a max 2.41 $\log_{10}$ cfu/g coliform was obtained (Table 3). Considering the microbiological distribution in the samples, it was observed that only 3 (3.52%) samples were detected to contain 1.30-2.41 $\log_{10}$ cfu/g of coliform. One of the bacteria that is an indicator of hygienic quality and causes food poisoning is *E. coli*. This bacterium was not found in any of the analyzed halloumi cheeses. *Staphylococcus* spp. in halloumi cheeses was determined as a maximum of 6.70 $\log_{10}$ cfu/g and an average of 3.15 $\log_{10}$ cfu/g (Table 3). No coagulase-positive *Staphylococcus aureus* was detected in any of the samples. Yeast and molds generally affect the shelf life, quality, and flavor of foodstuffs. Yeast and molds were found to max 4.36 $\log_{10}$ cfu/g and an average of 2.50 $\log_{10}$ cfu/g in the examined halloumi cheese samples (Table 3). In the study, it was obtained a maximum LAB count of 6.70 log10 cfu/g and an average of 3 log10 cfu/g in halloumi cheeses (Table 3).

Table 3. Microbiological analysis results ($\log_{10}$ cfu/g).

	N	Mean	Min-Max.
TMAB	85	4.54	3-6.70
Staphylococci	85	3.15	1-6.70
Yeast/Mould	85	2.50	1-4.36
LAB	85	3	1-6.70
Coliform	85	1	1-2.41

Discussion and Conclusion

As reported in the scientific reports, the factors for controlling the growth of microorganisms in cheese include water activity, salt concentration, oxidation–reduction potential, pH, NO_3, temperature, and maybe the production of bacteriocins that are secreted by some of the microorganisms (19). The pH value is one of the important criteria that affect the growth of microorganisms and the shelf life of foods. Most of the bacteria require neutral pH for optimum growth. On the other hand, LAB, especially *Lactobacilli* spp., generally grows at a pH below 7, such as 4.0; most yeasts and molds can grow at a pH of 5–7 but can grow at pH values <3.0 (19). This can be accepted as a positive condition that the natural pH levels allow LAB to grow and unique properties are promoted by these LAB. In the results of various studies on halloumi cheese, the pH values were detected between 4.50–4.90 (33), and 4.79-6.12 (18) with an average of 5.38 (6), and an average of 5.97 (17).

The acidity value is also one of the important parameters in determining the chemical durability of the products. As presented in Table 2, LA% values were min 0.07, max 0.28 and mean value was 0.18. Atasever et al. (2) and Gün and Şimşek (18) determined the acidity values in halloumi as 0.53% and 1.68%, respectively. On the other hand, in the study performed by İncili et al. (21) 0.15% LA mean value was obtained which is similar to the results of our study. The main reason for the difference between the % lactic acid values is thought to be because of the microbial load of the raw milk processed into cheese, the different initial acidity, or different pressure applications.

The amount of salt added to foods affects the flavor, aroma, and also the shelf life of the products. The salt ratio is also a pivotal factor in microbiological growth. These values were found to be very low compared to the findings observed by Atasever et al. (2); Demirci and Arıcı (6); İncili et al. (21) with the results of 5.09%; 6.14% and 6.84% respectively. On the other hand, we obtained that the salt ratio diversity in commercial samples is high. This is because there is no uniformity in the production process. In the industrial process, the way the cheese is salted differs from traditional production. The cooked cheese blocks are not dry salted but left to cool in whey brine (12% NaCl) at 4ºC for ~18 h then sprinkled with dry, sterilized mint (24). As Guniee (16) mentioned the salt content of cheese varies according to the method of salting, the cheese type, cheese geometry, cheese size, etc. This affects not only its flavor but also most of its physicochemical characteristics. In addition, it is possible that the salt ratios can be different in the samples collected at different stages of their shelf lives. Within the study performed by Gün and Şimşek (18), a similar average salt ratio (4.76 ± 0.74%) was obtained. Similarly, the salt and pH values of halloumi samples were determined by Papademas and Robinson (26); Keles et al. (29); Milci et al. (33) show similarities with the results he determined. In the traditional process, the fresh cheese is dry salted and sprinkled with dry, crushed leaves of mint (*Mentha viridis*) and then kept in salted whey (12% NaCl). Storage in brine and the ratio of salt may change cheese properties such as the organic acids profile, volatile aroma compounds, and sensory features (23, 24). EU requirement for salt is maxed at 3% and 6% for fresh and mature halloumi/hellim respectively in a Single Document of the European Commission (CY-PDO-0005-01243-

17.7.2014). In this document two types of halloumi/hellim are described: fresh and mature (13).

DM of the cheese is strictly related to the DM of the raw milk that the cheese is produced from, and this also affects the yield of the cheese. Different types of milk used for halloumi/hellim production have different ratios of dry matter. This reflects the yield and the DM ratio in cheese samples The milk required to produce a kg of halloumi/hellim cheese was 5.44, 8.85, 11.30, and 6.70 kg for sheep, goat, cow, and mixed milk (sheep and goat), respectively (9). On the other hand, seasonal factors influence the DM of halloumi/hellim. As Esendağlı (12) concluded, DM ratios of halloumi/hellim samples that were produced in North Cyprus were lower in the months between April and October (min 48.8 % and max 50.14). The researcher obtained 54.1% of DM in December. The max DM that we obtained is 50% which is also below the criteria published in the Single Document of the European Commission (CY-PDO-0005-01243-17.7.2014). DM should be min 54% and 63% for fresh and matura halloumi/hellim, respectively (13). All our samples were produced with cow milk. This may be the reason for lower DM in samples. EU requires halloumi/hellim to be produced in sheep milk. Different results were concluded by researchers and those differences may depend on the origin of milk, the season of milking, the physiological and pathological condition of the animal, and the processing steps of halloumi/hellim. The obtained results were 55.02±1.78% on average as concluded by Gün and Şimşek (18), 58.89% as concluded by Atasever et al. (2) and 60.21% as concluded by Demirci and Arıcı, (6). İncili et al. (21) examined 30 halloumi samples in their study and determined that the dry matter ratio was min 37.21%, max 54.88%, and an average of 48.77±5.25 (21). In our study the avarage results are lower.

According to "Ready-to-eat foods able to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes" in Commission Regulation (EC) No 2073/2005 of 15 November 2005 (Chapter 1/1.2) on microbiological criteria for foodstuffs, *L. monocytogenes* should be absent as we obtained in our samples (5). On the other hand, the absence of other pathogens such as *Salmonella* spp. and sulfite producing anaerobes is a satisfactory result for these samples in terms of food safety and public health. The same result was reported by Özçil (30). The halloumi/hellim microbiological profile can be considered the fingerprint that creates the unique traits of the product Halloumi/ hellim is characterized by unsurpassed organoleptic characteristics and a rising contribution of the indigenous microflora in the milk (32). That's why, other than checking the pathogens in terms of food safety and the spoilage microorganisms, the presence of desired natural microorganisms is also indisputable. As Kamilari et al. (22) mentioned halloumi/hellim was a globally produced and consumed cheese up to date but the autochthonous microbial communities that affect its unique organoleptic properties and safety of the product, have not been fully evaluated yet. Moreover, these days when halloumi PDO registration is on the agenda, defining the microbial profile specific to this product will be a part of its regional uniqueness. Accordingly, Kamilari et al. (22) also mentioned that the microbiome may be used as an additional tool to define the typicity of Cyprus Halloumi/Hellim. Lawson et al. (28), characterized a novel, salt-tolerant species in halloumi/hellim cheese and named as *Lactobacillus cypricasei* sp. nov., which was later found to be a heterotypic synonym of *Lactobacillus acidipiscis* (22). *Lactobacillus cypricasei* is now mentioned and described in Regulation (EU) 2021/591 (12 April 2021) which is about PDO registration of the cheese (1). The microbiological profile of this cheese is affected by several factors, including; the heat process and curd cooking procedures, the microbiological load of rennet, as well as salt and Mentha Viridis (mint) leaves, microbial contamination from the production area environment, and the microbiological contamination from brined whey (22).

Usca and Erol (1998) reported 4.51 $\log_{10}$ cfu/g TMAB (38). Demirci and Arıcı (1989) and İncili et al. (2019) obtained TMAB counts of 8.60 and 6.39 $\log_{10}$ cfu/g, respectively (6, 21). As halloumi/hellim is produced without using a starter culture, TMAB may be used as a tool to have an idea of the microbiological quality and shelf life of the product. As Kamleh et al. (25) concluded in their study which is focused on the shelf life of halloumi/hellim, at the end of storage at 5°C, packaged halloumi cheese is expected to have TMAB of 3.8–4.0 $\log_{10}$ cfu/g.

Coliform bacteria are accepted as hygiene indicators and cause flavor, structure, and aroma disorders in cheeses. This group of bacteria can also cause technological errors during the ripening of cheeses. The number of coliform bacteria may vary depending on the technological processes applied, the type of cheese made, and whether the cheese is fresh or maturated. It is seen that the levels of coliform bacteria detected in the halloumi cheese samples examined were at levels (26% and 31.5%) lower than the levels found (6, 38). As İncili et al. (21) reported the mean number of coliforms was 2.29±0.73 $\log_{10}$ cfu/g.

Although halloumi cheese is a cheese whose curd is heat-processed due to its production technology, it is thought that the presence of *S. aureus* is probably due to the lack of personnel hygiene after heat treatment, the lack of hygiene in the use of tools and equipment that contacted with the cheese. It is known that some *Staphylococcus* strains play an essential role in foodborne poisoning. For this reason, especially *S. aureus* should not be present in

foods. *Staphylococcus* spp. in halloumi cheeses was determined as a maximum of 6.70 $\log_{10}$ cfu/g and an average of 3.15 $\log_{10}$ cfu/g (Table 3). Since Staphylococci are of human or animal origin, their myriad in foods indicates insufficient sanitation or heat treatment. İncili et al. (21) obtained the mean number for *S. aureus* as 2.81±1.54 $\log_{10}$ cfu/g. Özçil (30) reported that *S. aureus* was detected in two of them, but the researcher concluded the amounts found did not pose a public health hazard according to the Turkish Food Codex Microbiological Criteria.

It was observed that the results in this study were quite lower than the results of some researchers (2, 38). As İncili et al. (21) reported the mean numbers of yeast mold were found as 3.16±1.14 $\log_{10}$ cfu/g. This is probably due to the possible contamination from food handlers' hands during folding, the air of the production area especially where the fresh cheeses are kept together one day in brine, and the quality of the packaging materials. Brine itself can be the contamination source. As Ulusoy et al. (37) concluded in their study which focused on the contamination sources of halloumi/hellim throughout the production steps, brine may have a high microbiological load including yeast and mold. In another investigation, Atasever et al. (2), studied the effect of production technology on the vacuum-packed and brined halloumi on the microbiological quality of halloumi/hellim. They found that the coliform, total mesophilic aerobic bacteria, yeast, and mold counts were higher in vacuum-packaged products than in brine. Halloumi/hellim from different types of milk may include different species of yeast. While *Debaryomyces hansenii*, *Candida parapsilosis*, *C. boidinii*, *C. versatilis*, and *Pichia membranifaciet* were isolated from the matured halloumi cheese from sheep milk, *Cryptococcus albidus* and *Pichia membranifaciet* were isolated from halloumi cheese made from cow's milk (3). On the other hand, mold growth should also be considered in terms of the level of aflatoxins and other mycotoxins important for public health. Elkak et al. (10) reported that Aflatoxin M1 was detected in 21 samples in their analysis of 31 halloumi samples collected for sale in Lebanon and 8 of these samples exceeded the 250 ng/kg limit set by the European Union Commission.

LAB is effective on the flavor, structure, aroma, and shelf life of the products. It is a group of bacteria that can dominate in native microbial profiles and provide forming unique sensorial properties. *Lactobacilli* constitute the dominant microflora in halloumi as in cheeses such as Cheddar and Domiati, where starter culture is not used. It has been reported that lactic acid bacteria are a part of the microflora in the final product, although the milk is pasteurized in the production of halloumi and the curd is boiled during the production phase (3, 28, 33). As we previously mentioned *Lactobacillus cypricasei*, is one of the LAB that was obtained to be unique for halloumi and facultative anaerobe, homofermentative, and resistant to high salt concentrations and low pH values (28). *Enterococcus faecium*, which is one of the thermoduric microorganisms found in the natural flora of milk, can also be frequently isolated from halloumi/hellim. It has been reported that some Enterococcus strains are proteolytic and may contribute to the taste of halloumi made from sheep's milk (14).

With the results obtained by other researchers, the spore-forming bacteria, such as *Bacillus*, as well as thermophilic species, such as members of the genus *Lactobacillus* and *Enterococcus*, in addition to yeasts were identified in the cheese samples by culture-based techniques (3, 36). Kamilari et al. (22) used metagenomic analysis to characterize halloumi cheese's bacterial communities. According to the results of this study, the predominant bacteria were LAB genera, such as *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Weissella*, and *Marinilactibacillus*. Additionally, spore-forming bacteria, including the genus *Bacillus*, psychrophilic or psychrotolerant bacterial genera such as *Psychrobacter*, the halophilic genus *Halomonas*, as well as the genera *Pseudomonas*, *Staphylococcus*, *Acinetobacter*, *Macrococcus* and *Vibrio*, member of which may cause food spoilage were also commonly detected (22).

Halloumi is of great importance culturally and economically for the Turkish and Greek communities in Cyprus. Production by the registration standards prepared jointly by the two communities of Cyprus and submitted to the European Commission as a result of the application will cause halloumi to gain an important place in the world market. Both traditional small-scale enterprises and high-capacity industrial production enterprises must be subjected to inspections according to the standard set by the European Commission for registration to preserve the original character of the product. Hygienic, pathogen-free, and food-safe production is as important as quality and standard productivity.

As the conclusion of the microbiological and physicochemical analysis performed in this study, it was determined that the product did not pose a serious public health threat. However, whether small-scale or high-capacity facilities make production primarily in line with good hygiene practices (GHP) and good manufacturing practices (GMP), the product will be of higher microbiological quality.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

BHU, FKY, and HDK conceived and planned the experiments. BHU and FKY carried out the experiments. BHU, ŞF and HIK planned and carried out the simulations. BHU, FKY, HDK and CH contributed to sample preparation. BHU, FKY, HDK, ŞF, HIK and CH contributed to the interpretation of the results. BHU and CH took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

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

Ethical Statement

This study does not present any ethical concerns.

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