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Microbial Assessment of Dairy Byproducts in Albania as a Basis for Consumer Safety

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Abstract**—**Dairy by-products are a fairly good environment for microorganisms due to their composition for their growth. Microbial populations have a significant impact in the production of cheese, butter, yogurt, etc. in terms of their organoleptic quality and at the same time some also cause their breakdown. In this paper, the microbiological contamination of soft cheese, butter and yogurt produced in the country (domestic) and imported is assessed, as an indicator of hygiene with impact on public health. The study was extended during September 2018-June 2019 and was divided into three periods, September-December, January-March, and April-June. During this study, a total of 120 samples were analyzed, of which 60 samples of cheese and butter locally produced, and 60 samples of imported soft cheese and butter productions. The microbial indicators analyzed are *Staphylococcus aureus and E. coli*. Analyzes have been conducted at the Food Safety Laboratory (FSIV) in Tirana in accordance with EU Regulation 2073/2005. Sampling was performed according to the specific international standards for these products (ISO 6887 and ISO 8261). Sampling and transport of samples were done under sterile conditions. Also, coding of samples was done to preserve the anonymity of subjects. After the analysis, the country's soft cheese products compared to imports were more contaminated with *S. aureus* and *E. coli*. Meanwhile, the imported butter samples that were analyzed, resulted within norms compared to domestic ones. Based on the results, it was concluded that the microbial quality of samples of cheese, butter and yogurt analyzed remains a real problem for hygiene in Albania. The study will also serve business operators in Albania to improve their work to ensure good hygiene on the basis of the HACCP plan and to provide a guarantee of consumer health.

Keywords**—**Consumer, health, dairy, by-products, microbial.

I. Introduction

MILK and its by-products are a great source of protein, vitamins, calcium and phosphorus. They also have health benefits due to the components they contain: functional proteins, bioactive peptides, fatty acids, calcium and vitamin D that have an effect on the immune system, cardiovascular system, gastrointestinal tract, etc. Dairy components have antimicrobial effects.

Even in Albania, milk and its by-products occupy a considerable amount of consumption in the population. In 2018, the amount of milk collected was about 139,000 tons, marking an increase of 5, 87% compared to 2017, referred Institute of Statistics, Albania 2018.

Full processed milk is the most consumed, by 79% of the population. The amount of cheese produced in Albania in 2017, increased by 2.87% compared to 2016. The butter production increased by 6.55% compared to 2016, referred Institute of Statistics, Albania 2018.

In addition to the benefits and the necessity of the consumption of milk, butter, cheese etc., there is also the risk of the diseases that these products cause to the consumer [3], [7].

Soft cheeses have higher moisture content, around 45-75% compared to strong cheeses. Excessive moisture in it may cause the appearance and reproduction of unwanted microorganisms.

Their consumption when they are contaminated with pathogens can lead to some cases of severe illness [7]. The presence of pathogens such as *S. aureus, E. coli, Salmonella* spp. etc., is the cause of milk and by-products being a source of food poisoning [1], [2].

Hence, maintaining a high standard of hygiene is one of the most important objectives of milk production and its by-products today. The level of hygiene affects directly the economy and public health [4]. For this reason, every day it is required continual increase in the quality of milk and its by-products [8]. Recently, consumers in Albania are concerned about the safety of milk-based products and the conditions under which they are produced. Therefore, it is very important to ensure good hygienic conditions in the production and marketing of these products. Also, it is necessary to apply control measures to protect human health.

II. Materials and Methods

A. Microbiological Analyzes: Indicators S. aureus and E. coli

For performing this study, in order to evaluate the hygiene of domestically produced dairy products compared to those imported, as a basis for consumer safety, a series of microbiological analyzes were conducted based on the standard methods applied by the Food and Veterinary Institute, Tirana, Albania, the Microbiological Food Division of Animal Origin. Research was conducted over the period from September 2018 to June 2019 in the Laboratory of Microbiology of Food (FSNI) and in the Laboratory of Microbiology and Food Biotechnology (FNS).

For comparison of microbiological indicators, *E. coli* and *S. aureus*, hygiene determinants in soft cheese and butter, a total of 120 samples were taken and analyzed. 60 of these samples are soft cheese samples, where 30 are domestic products and 30 imports, while 60 other samples are butter, with 30 being domestically produced butter and 30 being imported butter. Sampling and analysis are divided into three periods:

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September-December 2018, January-March 2019, and April.

TABLE I
SOFT CHEESE SAMPLES OF COUNTRY AND IMPORTED

Period	Soft Cheese Country	Soft Cheese Imported
	E. coli	*S. aureus*
September - December 2018	10	10
January - March 2019	10	10
Aprile – June 2019	10	10

TABLE II
BUTTER SAMPLES OF COUNTRY AND IMPORTED

Period	Butter Country	Butter Imported
	E. coli	*S. aureus*
September - December 2018	10	10
January - March 2019	10	10
Aprile – June 2019	10	10

B. Sampling and Packaging

The sampling and packaging of samples is carried out under sterile conditions in order to avoid the possibility of cross-contamination. Samples come into the lab with 5 units per sample. The quantity of each sample unit should not be less than 250 g for milk-based products. Each sample is coded before it comes to the lab for analysis. Sampling is done according to specific international standards (ISO 6887 and ISO 8261) [4].

Fig. 1 Taking samples randomly under sterile conditions

Fig. 2 Sample codification with minimum 250

C. Transport of Samples

Selected samples are placed in a thermo box (2-8 °C) in order to ensure the best and most accurate storage during transport to laboratory.

Their transport to the laboratory is done according to the rules established by the international organism ISO 6887 [4].

D. The Confirmation of Staphylococci aureus

Initially was done the homogenization of the samples in the laboratory from which the gradient dilutions 1:10 were prepared.

The confirmation of *staphylococci aureus* is *based* on ISO 6888-1: 1999. [5]

The plates with Baird Parker medium are initially incubated at 37 °C for 24 hours, checked and then are incubated again for another 24 hours at 37 °C.

The typical 24-hour colonies are 1-1.5 mm, while the 48-hour ones are 1.5-2.5 mm surrounded by a clear, bright area.

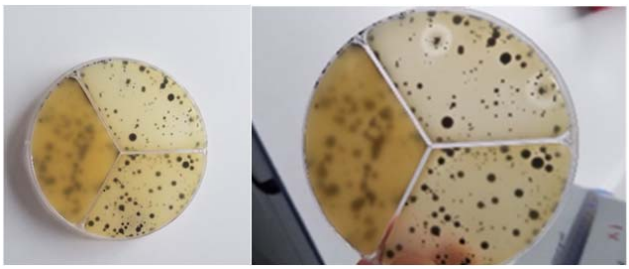

Fig. 3 Presence of *Staphylococcus aureus* colonies

For confirmation, the Coagulase Test was performed: in BHI (Brain-Heart Infusion) tubes and incubated at 37°C for 24 hours.

The test is considered positive when the volume of coagulase accounts for more than half of the initial volume of fluid.

E. The Confirmation of Escherichia coli

For the identification of E. coli was used the horizontal method of counting Escherichia coli β-glucuronidase positive in products intended for human consumption based on ISO/TS 16649-2 [6], [9].

Mix inoculum with TBX is incubated at 44 °C for 18-24 hours. The colonies are counted.

III. RESULTS AND DISCUSSIONS

A. Comparison of the Presence of E. coli and S. aureus in the Country's Soft Cheese and Imported

Following the results obtained, the interpretation of these microorganisms was done in accordance with EU Regulation 2073/2005 and 645/2016 by the Ministry of Agriculture. [3]

To evaluate the presence of *E. coli* and *S. aureus* in domestic and imported cheese and butter, a total of 120 samples were taken. These samples were taken from subjects such as: big factories, dairies and various sales points. For each of the products, domestic and imported cheese and butter, an equal number of 30 samples were analyzed during the three study periods. The samples were taken at the same time and conditions, in order to make comparisons.

To compare the presence of *E. coli* and *S. aureus* in the domestic cheese and imported, we analyzed 60 samples, 30 of which were domestic soft cheese, and 30 others imported soft cheese. Also, a comparison of 30 country butter samples was made with 30 import butter samples that are consumed in our country.

From 30 soft cheese samples analyzed for *E. coli* throughout the study period from September 2018 to June 2019, 5 of them or 16.6% have loads smaller than 100

colonies/g. Nine samples or 30% of them contain 100-1000 colonies/g. More than half, 16 or 53% of the specimens do not meet the criteria of the regulation by exceeding the microbial load limit of 1000 colonies/g for *E. coli*.

During the period September-December are analyzed 10 samples of imported soft cheese from which six resulted in lower load than 100 colonies/g. 3 of them had loads within the 100-1000 colon/g rates. Only one specimen passed the rate of 1000 colonies/g of *E. coli*.

In the second quarter, January-March, 10 other samples of soft cheese were analyzed, out of which five had less than 100 colonies/g of *E. coli*. Four samples were within the rate of 100-1000 colonies/g. Only one sample resulted out of the norms from the regulations.

During the April-June period, another 10 samples of soft cheese were analyzed again. Six of them did not exceed the 100 colon/g load. Three samples were within the 100-1000 colon/g norms. Only one sample resulted out of the norm, i.e. with a content higher than 1000 colonies/g (Fig. 4).

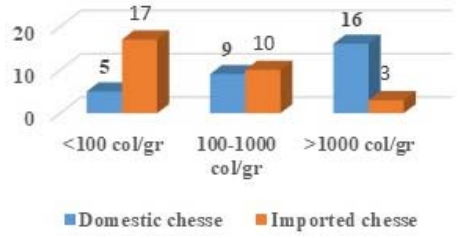

Fig. 4 Comparison of the presence of *E. coli* in the country's soft cheese and imported

Unlike domestic cheese where more than 50% of the samples did not meet the criteria for *E. coli*, only 10% of the analyzed samples exceeded the limits in import cheese, and were not suitable for consumption (Fig. 6).

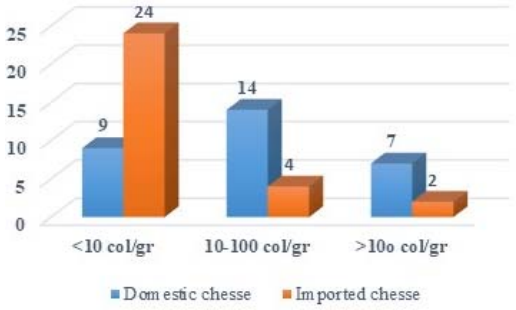

Fig. 5 Comparison of the presence of *S. aureus* in domestic and imported soft cheese

During September to December, from 10 samples analyzed for *S. aureus*, two contained less than 10 colonies per gram of bacteria, five resulted within the norm with 10-100 colonies/g, while three were out of the norm with a content higher than 100 colonies/g. In the months from January to March, three samples contained less than 10 colonies/g, and five samples did not exceed 100 colonies per gram of bacteria. Only two samples were out of control norms with more than 100 colonies/g. During April-June, four samples out of 10 contained less than 10 colonies/g. Four samples containing 10-100 colonies/g, while two samples were out of the norm with a bacterium content higher than 100 colonies/g.

The same samples analyzed for *E. coli* were also analyzed for *S. aureus*. During the first three months, seven samples contained less than 10 colonies/g. Two samples were within the 10-100 colon/g norms. Only one sample exceeded the specified rate with more than 100 colonies per gram of *S. aureus* bacteria. In the second quarter, eight samples contained less than 10 colonies/g, one sample was within rates of 10-100 colonies/g. Only one sample resulted outside the norms, which contained more than 100 colonies/g *S. aureus* bacteria. In the last quarter, nine samples contained less than 10 colonies/g. Only one import cheese sample resulted within the rate of 10-100 colonies/g and no sample was out of the norm.

The *Staphylococcus aureus* indicator showed that only 6.6% of the import cheese samples exceeded 100 colonies per gram, much different from domestic cheese with 23% of the samples beyond the norms of the regulation (Figs. 5, 6).

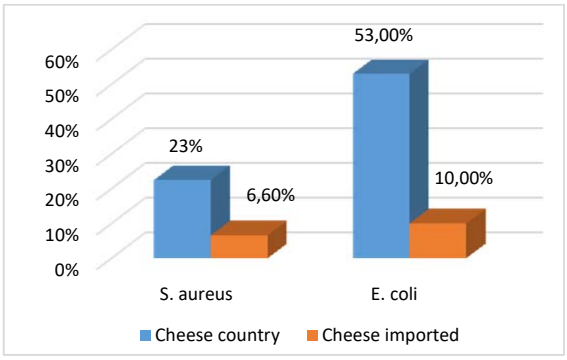

Fig. 6 The presence in % of *S. aureus* and *E. coli* in domestic and imported soft cheese

B. Comparison of the Presence of S. aureus in Domestic and Imported Butter

During the study period, 30 country butter samples were analyzed for *E. coli* and *S. aureus* indicators. In the first three months, from September to December, from 10 butter samples analyzed for the *S. aureus* indicator 2 resulted in a microbial load of less than 10 colonies/g. Three samples containing 10-100 colonies/g. Meanwhile, five samples passed the rate of 100 colonies/g of *S. aureus* referring to the regulation for this indicator. In the second three months, four samples resulted in a load of less than 10 colonies/g. Four of the samples resulted within the norm with 10-100 colonies/g. Only two samples crossed the 100 colony/g limit of *S. aureus* bacteria. In the third quarter of April-June, three samples showed a microbial load of less than 10 colonies/g. Six samples showed a load of 10-100 colonies/g. Only one sample was out of norms for the *S. aureus* index with more than 100 colonies/g.

The same samples of domestic butter that were analyzed for *S. aureus* were analyzed also for *E. coli*. During the months from September to December, three samples contained less than 100 colonies/g of *E. coli*. Four samples were within the

rate of 100-1000 colonies/g, while three samples crossed the limit of 1000 colonies/g of bacteria. In the months from January to March, five samples had less than 100 colonies/g. Three samples did not exceed the rate of 1000 colonies/g. Meanwhile two domestic butter samples crossed the standard prescribed by the regulation for *E. coli* of more than 1000 colonies/g. In April-June, three samples showed less than 100 colonies/g. Four samples contain 100-1000 colonies/g, so they were within the prescribed rate. Meanwhile, three samples exceeded the limit of 1000 colonies/g.

Domestic butter resulted in 26% of the samples above the permissible rate for the *E. coli* indicator, unlike imported butter in the analysis of which 100% of the samples were within the norm allowed by the regulation (Fig. 7). Also for the *Staphylococcus aureus* indicator, the imported butter sample was 100% within the allowed norms unlike domestic butter with 26% of the samples above 100 colonies/gram.

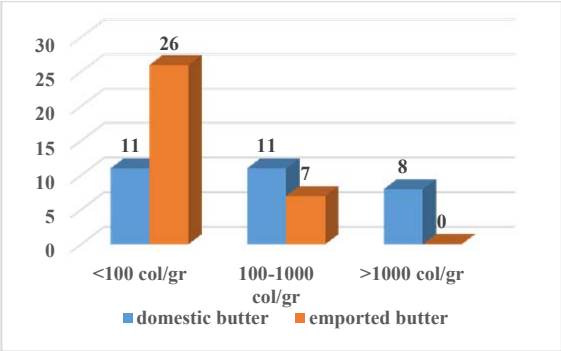

Fig. 7 Comparison of the presence of *E. coli* in the domestic butter and imported

The study on milk-based products showed that the microbiological quality of hygiene indicators remains a current problem in Albania.

The results of this paper will serve business operators to improve their work on processing and production lines to ensure hygiene on the basis of the HACCP plan, and will therefore guarantee the health of the consumer.

IV. Conclusions

- From 30 domestic soft cheese samples analyzed for the S. aureus indicator, 22.5% resulted above the norm set by the EU regulation.
- From 30 domestic soft cheese samples analyzed for the *E. coli,* 53% resulted outside the allowed norms.
- From 30 samples of imported cheese analyzed for the *S. aureus*, 6.6% resulted above the norm.
- From the same samples analyzed for *E. coli*, 10% of them were above the allowed rate.
- From 30 domestic butter samples analyzed for the S. aureus, 26.6% resulted outside the allowed norms.
- From the same samples analyzed for *E. coli* 26.6% were out of stock.
- Out of 30 imported butter samples analyzed for S. aureus and *E. coli*, 100% of samples were within the norm for both indicators.
- Unlike domestic cheese with 22.5% of positive samples for *S. aureus*, imported cheese has only 6.6% of positive samples for this bacterium. Also, for the *E. coli* in domestic cheese, 53% of the samples were positive, i.e. outside the norms, while in the imported cheese only 10%.
- Analysis of 30 imported butter samples for S. aureus and *E. coli* showed in both cases 100% of samples were negative for the presence of these bacteria. Very unlike domestic butter, where 26.6% of the samples were positive for *S. aureus* and 26.6% of the samples were positive for *E. coli.*

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