



Research Article

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Assessment of Microbiological Results of Some Albanian Food Products Compared with Microbiological Criteria

Rozeta Hasalliu^{1*} and Malgorzata Ziarno²

¹Agricultural University of Tirana, Albania

²University of Life Sciences, Poland

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*Corresponding author: Rozeta Hasalliu, Agricultural University of Tirana, Albania

Abstract

The aim of this study is to assess the microbiological results of some Albanian Food Products like milk, soft and hard cheese, meat, minced meat, eggs also products made by them, compared with microbiological criteria. These food products are most perishable products owing to their biological and chemical composition. A high level of protection of public health is one of the fundamental objectives of food law, as laid down in Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Microbiological hazards in foodstuffs form a major source of food-borne diseases in humans. Foodstuffs should not contain micro-organisms or their toxins or metabolites in quantities that present an unacceptable risk for human health. Regulation (EC) No 178/2002 lays down general food safety requirements, according to which food must not be placed on the market if it is unsafe (Commission Regulation (EC) No. 2073/2005 of 15 November 2005). Food business operators have an obligation to withdraw unsafe food from the market. In order to contribute to the protection of public health and to prevent differing interpretations, it is appropriate to establish harmonized safety criteria on the acceptability of food, in particular as regards the presence of certain pathogenic micro-organisms. Microbiological criteria also give guidance on the acceptability of foodstuffs and their manufacturing, handling and distribution processes. The use of microbiological criteria should form an integral part of the implementation of HACCP-based procedures and other hygiene control measures. The safety of foodstuffs is mainly ensured by a preventive approach, such as implementation of good hygiene practice and application of procedures based on hazard analysis and critical control point (HACCP) principles (Commission Regulation (EC) No. 2073/2005 of 15 November 2005).

Microbiological criteria can be used in validation and verification of HACCP procedures and other hygiene control measures. It is therefore appropriate to set microbiological criteria defining the acceptability of the processes, and food safety microbiological criteria setting a limit above which a foodstuff should be considered unacceptably contaminated with the microorganisms for which the criteria are set. According to Article 4 of Regulation (EC) No 852/2004, food business operators are to comply with microbiological criteria. This should include testing against the values set for the criteria through the taking of samples, the conduct of analyses and the implementation of corrective actions, in accordance with food law and the instructions given by the competent authority. This evaluation in this study is done in accordance and based on Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. These products are analyzed for the presence of different microorganisms like Aerobic colony count, *Enterobacteriaceae*, *E. coli*, *Coagulase-positive staphylococci*. Samples are taken from different places like market, retail, from raw materials and final products which were going to be exported from Albania like Meat, Milk and Dairy Products Factory in the period of 2016-2018. Some of samples from raw materials are over limit microbiological criteria. The National Food Authority of Albania must increase the microbiological controls of Albanian food products in order of Food Safety and Quality.

Keywords: Microbiological standards; Food products; Food safety; Albania

Introduction

A high level of protection of public health is one of the fundamental objectives of food law, as laid down in Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements

of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Microbiological hazards in foodstuffs form a major source of food-borne diseases in humans. Foodstuffs should not contain micro-organisms or their toxins or metabolites in quantities that present an unacceptable

risk for human health. Regulation (EC) No 178/2002 lays down general food safety requirements, according to which food must not be placed on the market if it is unsafe (Commission Regulation (EC) No. 2073/2005 of 15 November 2005). Food business operators have an obligation to withdraw unsafe food from the market. In order to contribute to the protection of public health and to prevent differing interpretations, it is appropriate to establish harmonized safety criteria on the acceptability of food, as regards the presence of certain pathogenic micro-organisms. Microbiological criteria also give guidance on the acceptability of foodstuffs and their manufacturing, handling and distribution processes. The use of microbiological criteria should form an integral part of the implementation of HACCP-based procedures and other hygiene control measures. The safety of foodstuffs is mainly ensured by a preventive approach, such as implementation of good hygiene practice and application of procedures based on hazard analysis and critical control point (HACCP) principles (Commission Regulation (EC) No. 2073/2005 of 15 November 2005).

Microbiological criteria can be used in validation and verification of HACCP procedures and other hygiene control measures. It is therefore appropriate to set microbiological criteria defining the acceptability of the processes, and food safety microbiological criteria setting a limit above which a foodstuff should be considered unacceptably contaminated with the microorganisms for which the criteria are set. According to Article 4 of Regulation (EC) No 852/2004, food business operators are to comply with microbiological criteria. This should include testing against the values set for the criteria through the taking of samples, the conduct of analyses and the implementation of corrective actions, in accordance with food law and the instructions given by the competent authority. The aim of this study is to assess the microbiological criteria of some Albanian Food Products like milk, soft and hard cheese, meat, minced meat, also products made by them. These food products are most perishable products owing to their biological and chemical composition.

This evaluation in this study is done in accordance and based on Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. These products are analyzed for the presence of different microorganisms like Aerobic colony count, *Enterobacteriaceae*, *E. coli*, *Coagulase-positive staphylococci*. Samples are taken from different places like market, retail, from raw materials and final products which were going to be exported from Albania like Meat, Milk and Dairy Products Factory in the period of 2014-2016. Some of samples from raw materials are over limit microbiological standards. The National Food Authority of Albania must increase the microbiological controls of Albanian food products in order of Food Safety and Quality. Actually in Albania several kind of cheese are vended in retail, in raw condition or mature, produced from pasteurized or raw milk, under different sanitary hygienic condition. The present investigation was compile and undertaken to determine the contamination rate of different kind of cheese considering the

high level of cheese consumption in Albania. Meantime in Albania there is no epidemiological data and the whole chain that lead to the cause of the disease and implication of cheese [1-5].

The prevalence of pathogenic microorganisms in cheese depend upon the quality of milk used, heat treatment of milk, general sanitation in the cheese plant, quality starters, occurrence of phages, cheese handling procedures, temperatures of holding at the plant, during transport and through marketing channels [6-8] Because of the relatively higher moisture levels in soft cheeses, they provide a favorable environment for the growth of pathogens. Recently there have been more than a few reports of cheese borne food infections, and food poisoning cases reported [3,9,10]. Staphylococcal food poisoning is due to a heat-stable toxin produced by the cells during growth, and a general rule is that 105 *staphylococci* are required to produce sufficient toxin to cause problems. A number of physical parameters are involved in controlling the growth of microorganisms in cheese. These include moisture, salt, ripening temperature, pH. The aim of this study is to evaluate the favorable conditions and potential sources of contamination with Aerobic mesophilic bacteria, *Enterobacteriaceae*, *Staphylococci spp.* of raw and pasteurization milk cheeses and meat, minced meat and sausage made in Albania or products that are imported in Albania. Another aim of this study is to evaluate the effect of storage conditions on the growth of total aerobic mesophilic bacteria in some stores in Tirana. The minced meat has the higher probability than the red meat for contamination with microorganisms, due to the higher contact surface. If these microorganisms find the appropriate conditions, then their number grow and over limit the microbiological standards.

Materials and Methods

A total of 176 cheese samples, 116 cheese samples produced from pasteurized milk, 60 produced from raw milk, 112 soft cheese samples, 64 hard cheese samples, were taken and submitted for bacteriological analyses for the presence of *Staphylococci spp. coagulase positive*. The samples were randomly selected. They were packed in sterile plastic bags, placed in an isotherm container, and transported under cooling conditions (4-8°C) to the Microbiological laboratory of the Food Safety Department at the Food Safety and Veterinary Institute, Tirana. Each product consists in 5 units of 150g. The same day of their arrival at laboratory, microbiological analysis of the samples, was carried out. To carry out the laboratory test were used International standard method as ISO method 6888. Each analytical sample, consisted of 25g cheese, was homogenized with 225ml Buffered Peptone Water, in a laboratory blender type "Stomacher 400" for 2 minutes. After are done the following dilutions with same diluent. A loopful from each dilution was streaked on Baird -Parker agar) and incubated for 48 h at 37°C respectively. After 48-hours incubation at 37°C the suspected colonies were submitted for the gram test and coagulase test. Typical and untypical colonies were transferred to Brain Heart Infusion broth at 37°C for 24 hours. From BHI were

passed 0.1ml of BHI to 0.3 of rabbit plasma (ISO 6888-1 2003, ISO/FDIS (2003). The coagulase positive colonies were submitted to API-staph to see the biochemical profile.

Meantime is also used the phosphatases test in cheese produced from pasteurized milk. Determination of phosphatases enzyme using the phosphatases test is realized by homogenizing 20g of cheese in 30ml of distilled water. On purpose were taken two tubes (A and C) with 10ml of distilled water where it was dropped one tablet of lactagnost I and II (tablets were let till there were complete dissolved). In tubes A was added 1ml from the homogenate obtained in tube C and 1ml of milk without phosphatase and heated in 85°C. After that the tubes were placed in a water bath at 37°C for 1 hour. At the end of process was added lactagnost III and after were compare the colour showed up. Below on table x is showed the changes of colour and valuation of result. The minced meat samples were randomly selected. They were packed in sterile tubes, placed in an isothermic container, and transported under cooling conditions (4-8°C) to the Department of Food Control at the Food Safety and Veterinary Institute, Tirana. Each product consists in 5 unit of 150g. The same day of their arrival at laboratory, microbiological analysis of the samples, was carried out. For detection of total aerobic mesophilic bacteria Plate Count Agar is used. The process of the dilutions is done according to the methods of Plate Count Agar. 25gram of minced meat is mixed in a sterile blender with 225ml of buffered

Table 1: The percentages of *Staphylococcus coagulase* (+), coagulase (-).

Microorganisms	Results
Staphylococcus spp.	72/176 (41%)
Staphylococci coagulase (+)	12/72 (17 %)
Staphylococci coagulase (-)	60/72 (83%)

The presence of *Staphylococci* spp. in raw and pasteurised milk cheese.

Table 2: The percentages of *Staphylococcus* spp. in cheeses produce from pasteurized and raw milk.

Cheese Produced from Pasteurized Milk	Cheese Produced from Raw Milk
28/116(24%)	44/60(73%)

Table 3: Accepted and unaccepted level frequency of *Staphylococci* of cheese produced from pasteurized milk.

Cfu/g	10 ¹	10 ²	10 ³	10 ⁴	10 ⁵	10 ⁶
Number of cases	0	8	12	6	2	0

The prevalence of pathogenic microorganisms in cheese is depended upon the temperatures of holding at the plant, during transport and through marketing channels. Accepted and unaccepted level frequency of *Staphylococci* for cheese produced from raw milk is shown in Table 4. A total number of cheese samples produced from pasteurized and raw milk which positive result with *Staphylococci* spp., level 10³cfu/g has the higher frequency and less the level 10⁴, 10⁵cfu/g. The staphylococcal enterotoxin can be produced at higher level than 10⁵cfu/g, based on analyses we didn't have this level. Microbiological test confirms the presence of *Staphylococci* in low percentage in cheese

Table 4: Accepted and unaccepted level frequency of *Staphylococci* for cheese produced from raw milk.

Cfu/g	10 ¹	10 ²	10 ³	10 ⁴	10 ⁵	10 ⁶
Number of cases	0	16	20	8	0	0

peptone water. The food is blended at a speed 15000-25000rpm. Petri dishes are incubated for 48h-72h at 30°C. Microorganisms failing to form colonies, of course, are not counted.

Results and Discussion

Seventy-two (72) of 176 examined cheese samples were found to contain *Staphylococci* spp. Twelve (12) from 72 was *Staphylococcus coagulase* (+) and 60 from 72 was *Staphylococcus coagulase* (-) (Table 1). From analysis of 176 samples of cheese in the markets of Tirana, from each 116 cheese samples produced from pasteurized milk, 60 produced from raw milk). Twenty-eight (28) of 116 examined cheese samples produced from pasteurized milk were found to contain *Staphylococci coagulase* (+) and forty-four (44) of 60 examined cheese samples produced from raw milk were found to contain *Staphylococci coagulase* (+) (Table 2). Presence of *Staphylococci* spp. in cheese produced from raw milk could be as consequence of transmission from animals with mastitis to milk used to produce cheese, carrier persons of *Staphylococci* spp. can be a route of transmission in cheese; equipment used to produce cheese, that are not clean enough also can be a route of transmission of *Staphylococci* spp. in cheese, not good condition of storage can affect the increasing of *Staphylococci* spp. in unacceptable level due to consumption [11-15] Accepted and unaccepted level frequency of *Staphylococci* of cheese produced from pasteurized and raw milk is shown in Table 3.

produced from pasteurized milk. Presence of *Staphylococci* in cheese produced from pasteurized milk could be as consequence of a not complete pasteurization process transmission route like a cross contamination during processing, handling or packing. Other sources of contamination include the equipment of pasteurization, not good hygienic conditions of equipment and personnel. (50% of people are carriers in skin, nose, throat of *Staphylococci*) can contaminate the surface of product. Pasteurization process can kill the vegetative forms but cannot kill the enterotoxins which are heat stable [16,17,18] (Table 5).

Table 5: Presence of *Staphylococci spp.* in soft and hard cheese.

Soft Cheese	Hard Cheese
36/112 (32%)	36/64 (56%)

Presence of *Staphylococci spp.* is in higher percentage in hard cheese than in soft one. Regarding to the higher pH in hard cheese than in soft cheese and *Staphylococci spp.* after contamination can grow and develop in pH higher than 5. Therefore, good sanitation among plant workers, general sanitation in cheese plant, effective refrigeration, the use of active starters and good packaging are important in preventing *S.aureus* related food poisoning [18,8]. Several researchers have described meat as the most perishable of all-important foods with its nutrient rich and moist surface being particularly conducive to the colonization and rapid growth of a wide range of spoilage bacteria [19-21]. The colonization and the growth on the meat surface have been widely researched and documented over several decades in some detail, with the initial being the attachment of bacterial cells to the meat surface [22,4,12]. The minced meat has the higher probability than the red meat for contamination with microorganisms, due to the higher contact surface. If these microorganisms find the appropriate conditions then the number of them grows and overpasses the

microbiological standards. In this study we analyzed 20 stores of minced meat (5 supermarkets, 5 stores in the center of the city, 5 stores in peripheries of the city and 5 in the machinery that minces the meat) total aerobic mesophilic bacteria. 5 samples from each place were analyzed for total bacterial count. Based on Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs, total aerobic mesophilic bacteria in minced meat should be 10^6 cfu/gr (Table 6). These results show that the microbiological standard for aerobic mesophilic bacteria 10^6 cfu/gr; is not overpass. The best results are in the supermarkets where the conditions of sales and storage of the minced meat are the best (Table 7). In this figure we compare the results of total aerobic mesophilic bacteria in minced meat for four categories of stores. The category 2 that means stores in periphery has the higher microbiological level. And the stores of category 3 that means minced meat in supermarkets has the lower microbiological level of total aerobic mesophilic bacteria [23].

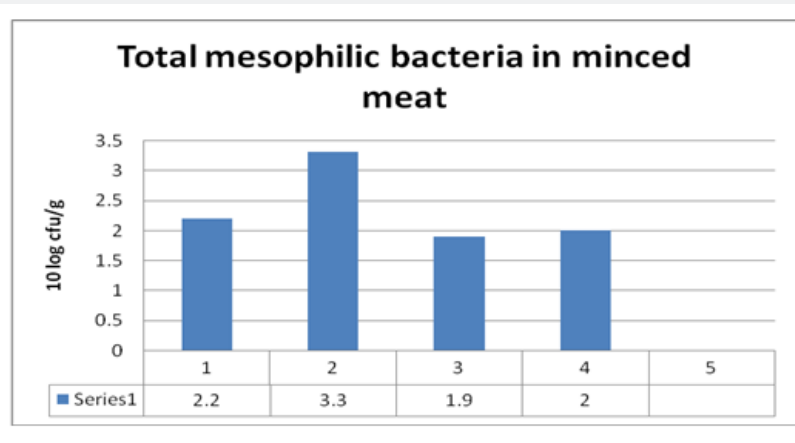


Figure 1: Total aerobic mesophilic bacteria in minced meat for 4 categories of stores (Stores in the center (cfu/g)1; Stores in peripheries (cfu/g)2; Supermarkets (cfu/g)3; In the machinery (cfu/g)4).

Table 6: The results for *Staphylococci spp.* of the sausages sold in the market of Albania.

Name of Sausages	Microbiological Level cfu/gr
Albanian sausage 1	2×10^2 cfu/gr
Imported sausage 1	6×10^3 cfu/gr
Imported sausage 2	5×10^3 cfu/gr
Albanian sausage 2	3×10^3 cfu/gr
Imported sausage 3	2×10^3 cfu/gr
Albanian sausage 3	2×10^4 cfu/gr

Table 7: The results for total aerobic mesophilic bacteria in minced meat.

Samples	Stores in the Center (cfu/g) ¹	Stores in Peripheries (cfu/g) ²	Supermarkets (cfu/g) ³	In the Machinery (cfu/g) ⁴
1	3×10^2	2×10^3	1×10^2	2×10^2
2	2×10^2	2×10^3	2×10^3	3×10^2

3	2x10 ³	3x10 ⁵	2x10 ¹	5x10 ¹
4	3x10 ²	4x10 ³	3x10 ²	5x10 ²
5	3x10 ²	5x10 ³	2x10 ¹	6x10 ²

Conclusion

The occurrence of *Staphylococci* has a higher percentage in raw milk cheese than in pasteurized milk cheese, in some markets in Albania, because the favorable conditions are more in raw milk cheese than in pasteurized milk cheese. The problem of the pasteurized milk cheese is the contamination post pasteurization. In this study we have had many cases of pasteurized milk cheeses with *Staphylococci*. After a contamination, *Staphylococci* grows better in pH higher than 5. Cheeses, with pH higher than 6 are with higher frequency of *Staphylococci*. This pH is more favorable for the growth of *Staphylococci*. Because *Staphylococci* are salt-tolerant, positive samples do not depend on the salt of the cheese. Also, *Staphylococci* grow both in high and low humidity with water activity ($A_w \sim 0.86$), and there is no dependence from the humidity. Because of their ability to grow at relatively low A_w and at high NaCl concentration, the potential for toxin production in low acid cheeses exist.

These indicate the necessity of high-quality hygienic conditions during manufacturing, storage and marketing. *Staphylococci spp.* are an indicator of post process contamination by food handlers, which most likely occurred in our case. Therefore, good sanitation among plant workers, general sanitation in cheese plant, effective refrigeration, the use of active starters and good packaging are important in preventing *Staphylococci* related food poisoning and in cheeses with pH higher than 5 the storage and sold conditions will be in a high level to decrease the possibility to grow *Staphylococci*. Although our analyses don't overcome more than microbiological standards, due to the public safety health, it should be consider more attention to those conditions and the good hygienic practices will be forever in these stores. Complete avoidance of microbial contamination of meat is practically impossible especially in our case country of living. However, control of contamination during slaughter and further meat processing and handling operations is possible. Such control is the most critical item of good manufacturing and sanitation practice (GMP, GSP) guidelines and Hazard Analysis Critical Control Point (HACCP) programs in order to produce meat products of high quality and safety. From our analyses of the minced meat for total aerobic mesophilic bacteria compare with the Directives 94/65 EC, the microbiological standard for total aerobic mesophilic bacteria that is $<5 \times 10^4$ cfu/gr, we saw that this standards are overcome or are in the limits of these parameters more in the stores that are in the periphery, where sometimes there has the lack of electricity. This is a good condition for the growth of these microorganisms. Although our analyses don't overcome more than microbiological criteria, due to the public safety health, it should be considering more attention to those conditions and the good hygienic practices will be forever in these stores.

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