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DETERMINATION OF TOTAL AEROBIC MESOPHILIC BACTERIA (TAMB), TOTAL AEROBIC PSYCHROPHILIC BACTERIA (TAB), *Lactococcus spp., Lactobacillus spp., Staphylococcus-Micrococcus*, COLIFORM, YEAST AND MOLD IN MOTAL CHEESE SAMPLES, AZERBAIJAN

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Abstract. This research was aimed for determination of the microbiological properties of cheese in the Western region of Azerbaijan by traditional methods. 110 samples of cheese used in the study were collected in markets and farms in the regions of Ganja, Dashkesan, Goy-Gol, Touz and Shamkir. The microbiological properties of the samples were determined. Motal cheese can be included in the group of fatty, soft and /or semi-hard cheeses. Centres for the production and sale of cheese do not have sufficient hygienic and technological conditions, and in this regard, it is envisaged to take the necessary precautions when considering a large number of microorganisms.

Keywords: *sheep milk, motal cheese, microbiological quality, Lactococcus spp., Lactobacillus spp., Staphylococcus-Micrococcus.*

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1. Introduction

Since milk is a frequently consumed product, and especially given its role in the diet of children, it is necessary to comply with the above indicators in order to avoid mass poisoning. It should be noted that in recent years, no serious violations in this direction have been revealed.

The quality of dairy products is determined by a complex of organoleptic, physico-chemical and microbiological indicators in accordance with the requirements of the current regulatory documentation.

The most important characteristics of dairy products is their safety and microbiological resistance. Quantitative and qualitative microbiological indicators are used to assess the quality of dairy products. Quantitative indicators indicate the total number of certain microorganisms in 1 g or 1 cm^3 of the product. Qualitative indicators indicate the absence (presence) of microbes of specific species or groups in a certain mass or volume of the product.

The sanitary evaluation of dairy products, indirect methods are used to determine the level of contamination of the product by human secretions (the level of fecal contamination). The higher this level, the more likely it is that pathogens - pathogens of intestinal infections-will enter the object under study. Such methods include a quantitative method for determining the number of mesophilic aerobic and facultative anaerobic microorganisms (Cmafanm) and a qualitative method for determining

sanitary-indicative microorganisms - bacteria of the Escherichia coli group (BGCP).In the sanitary evaluation of dairy products, indirect methods are used to determine the level of contamination of the product by human secretions (the level of fecal contamination). The higher this level, the more likely it is that pathogens - pathogens of intestinal infections-will enter the object under study. Such methods include a quantitative method for determining the number of mesophilic aerobic and facultative anaerobic microorganisms and a qualitative method for determining sanitary-indicative microorganisms - bacteria of the Escherichia coli group (BGCP).

Milk and dairy products are of great importance for human nutrition as they are the main foodstuffs. For this reason, milk has been tried to be used in many different ways. Milk; since it is not a durable product, it can be consumed widely by converting it into many products with different flavour, aroma and structure that have longer preservation period as well as direct consumption. Cheese in terms of diversity and consumption are among the first in these products (Tekinşen *et al*., 2006; Fallah *et al*., 2009; Khodadadi *et al.*, 2014). Today, approximately 40% of the milk produced in the world is used in cheese production (Fox, 2011). When this ratio is taken into consideration, the importance of cheese in industrial terms draws attention. Milk is an easily digestible product with high nutritional and biological value. In recent years, efficient development of cheese production has been observed in most of the developed countries of the dairy industry. In many countries with an advanced dairy industry, an active development in cheese production is seen (Kireçci *et al*., 2007; Kaynar *et al*., 2011). In parallel, demand for cheese and production volumes are constantly increasing, and the product range is expanding and developing. Today, cheese is thought to be more than 2000 varieties. It is seen that each country and region has the habit of consuming its own kind of cheese. (Khodadadi *et al.*, 2014; Tekinsen , 2000).

Contamination of food and feed is a major problem worldwide. When it comes to cheese production, many types of production have been provided to ensure high quality cheese consumption. As a result, new modern equipment was invented and the standard in construction technique was tried to be achieved. In addition, new methods have been developed for the ripening of the main components of cheese, protein, lactose and fat as well as ripening methods that give flavour to specific cheeses (Hussein & Brasel, 2001) was initiated. An important part of the cheese produced for home consumption is the cheese produced by traditional methods and is a direct food consumed by people. On the other hand, a significant portion of the cheeses used in the production of industrial cheese are processed as food ingredients before they are converted into products. Today, the prevalence of cheese-containing foods, which are prepared in parallel with the developing modern food consumption habits, is increasing. Approximately 25% of the cheeses produced in Europe are consumed by adding to food, while this ratio is thought to be around 35-45% in the world (Fox & McSweeney, 2017).

Preparation of a bag from sheep [goat] skin for the storage of motal cheese

Sheep or goat skins are used to make cheese coils. Removal of animal skins consists of several stages. After slaughtering the animal, the body is inflated with gas. Once the gas has completely penetrated between the skin and the adjacent tissue, the skin begins to break away from the neck. The front and back legs are cut off from the body at wrist level, and then the skin is completely separated from the body. After the skin is removed, the inner surface is well washed, cleaned and salted, it is left to dry. After a while the skin is washed and dried again. After making sure the skin is dry, it flips over and the process is completed with the scalp. The open cuffs of the finished

skin are fastened with a flat rope. The air is supplied from the open part and checked whether the bag does not pass air. A skin bag, which must be airtight, is considered suitable for storing cheese.

Preparation of motal cheese

Motal cheese is historically known as a type of cheese produced by nomadic communities living in the Karabakh region of Azerbaijan. In the following years, it was expanded and began to be produced in neighboring regions and villages of Azerbaijan. In recent years, more technological methods of production have been developed for the production of motal cheese, and various commercial firms have begun to produce motal cheese on a commercial basis, while maintaining the old style of production. This cheese is produced both by people living in villages and by companies established for commercial purposes.

Milk obtained from sheep and goats used for the production of dry cheeses is filtered and after milking it is placed in a special flat container. Next, special yeast is added and the formation of clots is observed. After the formation of clots, the milk is transferred to pre-prepared bags so that the milk can continue the coagulation process for some time. The bag of coagulated milk is then placed in a flat container and a load is placed on it. Thus, while the cheese continues the coagulation process, on the one hand, it is ensured that the water in the clot comes out. This clot is expected to mature and completely lose all fluid in about 24 hours. At the end of this period, the cheese mass is cut into small pieces, salted in accordance with the rules and kept in this state for about 10 days. Thus, after sufficient maturation and hardening, the cheese is placedin small pieces in a bag made from skin and the bag is tied hermetically. It is then placed in a cellar or cave for two or three months. Sometimes these bags of cheese are buried in the soil. After this maturation process, the finished motal cheese can be stored for 7 or 8 months.

2. Materials and methods

Material

110 samples of cheese cheese used in the study were obtained in February-June 2016 from markets, local markets and family businesses in the provinces of Ganja, Dashkesen, Goy-Gol, Touz and Shamkir of Azerbaijan.

Method

Total aerobic mesophilic bacteria (TAMB), total aerobic psychrophilic bacteria (TAB), *Lactococcus spp*., *Lactobacillus spp*., *Staphylococcus-Micrococcus*, coliform and yeast mold were determined in motal cheese samples.

Microbiological analysis

Dilution Preparation: 10 g of motal cheese samples which were homogenized by mixing thoroughly were weighed and homogenized with 90 ml sterile buffered water.1 ml of this liquid was taken and transferred to a tube containing 9 ml sterile buffered water. In this way up to 106 decimal dilutions were prepared. Samples were made by smear method. The plates were poured into pre-prepared media, solidified, then diluted samples on -0.1 ml of media were transferred to the media was drigalski and incubated. Colonies that developed after incubation were counted.

Determination of **Total Aerobic Mesophilic Bacteria** Number: Plate Count Agar (Merck, 1.05463) medium was used for TAMB count.0.1 ml of smears from the appropriate dilutions of the medium were cultured for 48 hours at 37°C and then counted (Harrigan & McCance, 1979).

Determination of **Total Number of Aerobic Psychrophilic Bacteria** Number: Plate Count Agar medium was used for the samples for TAPB count.0.1 ml of smears from the appropriate dilutions of the medium were planted by incubation at 5°C for 10 days and then counted.

Lactococcus spp. M17 (Oxoid, CM0785) Agar was used for *Lactococcus* count. Petri dishes were incubated at 37 ° C for 48 hours and counted (Tekinşen et al., 2002). *Lactobacillus spp.* Determination of *Lactobacillus spp.* Man Rogosa and Sharpe Agar (Merck, 1.10660) were plated by smear plate technique. Anaerobic medium was formed and incubated at 37 ± 1 ° C for 48 hours. Colonies formed after incubation were counted (Harrigan & McCance, 1979).

Staphylococcus-Micrococcus count: Mannitol Salt Agar (Merck, 105404) was used to count these microorganisms. After the plates were incubated at 37 ± 1°C for 36-48 hours, the resulting colonies were counted (Harrigan & McCance, 1979).

Determination of *Coliform Bacteria* Count: Violet Red Bile Agar (Oxoid, CM107) was used in the counting of coliform microorganisms. The plates were incubated at 30ºC for 24 hours. After incubation, purplish red colonies with a diameter greater than 0.5-1 mm in diameter were counted.

Determination of Yeast and Mold Count: Rose Bengal Chloramphenicol Agar for yeast and mold count (Merck, 1.00467).medium was used. Microorganisms were counted after incubation at 25ºC for 5-7 days by sowing with 0.1 ml smear plate method from the appropriate dilutions to the medium (Harrigan & McCance, 1979).

3. Results and discussions

Thanks to the developing industry, the importance and proportion of cheese types used as food additives especially in the catering sector is increasing day by day. It has been stated in various sources that more than 25% of the total cheese produced in Europe is consumed as food additives. It is known that this level is significantly high in the USA and that cheese is widely used, especially in the fast food industry (Kaynar, 2011; Güley *et al.*, 2013; Akarca et al., 2013).

Because of the wide usage area of cheese, the risks of disease and the presence of metabolites in the cheese that may be harmful to human health have been the subject of many studies.In this context, the presence of microbes and bacteria at high risk in milk and cheese has been an important research topic for scientists in recent years (Erkan *et al.*, 2009).

Although the consumption is widespread, as a result of the literature surveys, no research has been found on motal cheese either in Azerbaijan or in other countries of the region.The aim of this study, which was planned based on the absence of any research to determine the microbiological quality of motal cheese, was to eliminate this deficiency in the literature to a certain extent.

In Azerbaijan, there is a high risk of exposure to various factors as no heat treatment is applied to the milk used in the production stages of cheeses. However, it is highly probable that various microorganisms originate from the non-hygienic environment in which cheese production is made. In addition, although there is a legal

regulation on the minimum acceptable microorganism value in the cheeses offered for consumption in many countries, there is no reference value on this subject due to limited research in Azerbaijan. Because of all these deficiencies, the studies to be carried out on these issues are of great importance.

In this study, the total number of mesophilic aerobic microorganisms was determined as a minimum of 4.34 log kob / g, a maximum of 7.45 log kob / g and an average of 6.51 ± 1.09 log kob / g. When these values are examined, it is seen that the number of mesophilic aerobic microorganisms in the samples is high. High level of this factor; It is closely related to the low microbial quality of the raw material from which the product is obtained, to produce raw milk and to be a fermented product. The number of microorganisms in fermented foods is generally higher than the raw material from which they are obtained. Therefore, microbiological quality of fermented foods; the microbial load of the raw material depends on the processes applied before fermentation, the processing conditions and the conditions under which the food is maintained after fermentation (Patır & Ateş, 2002).

In addition, high levels of other bacteria and coliforms determined in parallel with the high number of mesophilic aerobic viable bacteria seen in the cheese samples examined may be caused by deficiencies and errors in the processing process. These deficiencies and errors are thought to be caused by insufficient heat treatment and lack of hygienic production and storage conditions. The presence of high levels of bacteria and microbial enzyme production negatively affect the organoleptic quality of the product, but may threaten consumer health.

If the total number of aerobic mesophilic microorganisms used as indicators in determining the general hygienic quality and microbial load of foods is high, the number of other microorganisms is expected to be high. Many pathogenic microorganisms that are of human and animal origin and spread to food have mesophilic character and reproduce under aerobic or facultative anaerobic conditions. The presence of a high number of aerobic mesophilic microorganisms in a product indicates that the product is produced and stored under conditions that allow the development of pathogens and the likelihood of such pathogens is high.[20]In microbiologically degraded foods, this group of microorganisms is generally expected to be high. The deterioration in odor, taste and structural properties of foods has a microorganism load higher than 10 kob /g in many foods.

In this study, the total number of mesophilic aerobic microorganisms determined in motal cheese samples showed similarity with the findings of some previous studies Morul and İşleyen (2006), Erceyes et al. (2006), and in some studies [13] is higher than the determined level.It is thought that this observed difference may be related to the initial microorganism load of the raw material, production technique and whether or not heat treatment is applied in some products.

In this study, the number of yeast and molds of cheese samples ranged between 2.00 log kob/ g and 2.90 log kob/ g and the average was found to be 2.61 ± 0.26 logkob/ g. This difference may be due to differences in raw material, production and storage conditions.

Yeasts and molds develop at wide pH, a_w and temperature levels and cause appearance, odor and flavor disorders in foodstuffs. These microorganisms adversely affect both the nutritional value and preservation of foodstuff. In the dairy industry, yeasts that ferment lactose are important. Since they causeodor and taste disorders in cheese, they are not required to be present in the product. Oxidative yeasts forming the

membrane on the cheese surface reduce the acidity of the milk and reduce the acidity of the medium. It contributes to the ripening in part when it is low, and when it is high it can cause the cheese to disperse due to the CO_2 formed (Pekel, 2008).

The coliform level determined in this study was minimum 2.00 log kob/g, maximum 3.05 log kob/g, and the average level of 2.76 ± 0.28 log kob/g was higher than the data of Pekel (2008), similar to previous studies (Keles & Atasever, 1996; Erceyes *et al*., 2006; Morul & İşleyici, 2006). The reason for this is thought to be due to differences in production methods and storage conditions. Bacterial *coliform* bacteria break up lactose in the milk heterofermentatively, producing lactic acid, acetic acid and alcohol as well as CO_2 gas, causing premature swelling in cheeses. The presence of coliform microorganisms in cheeses is an indication that poor sanitation conditions, inadequate or incorrect pasteurization applications, re-contamination after cooking and pasteurization. *Lactococcus spp*. The minimum number of 2.00 log kob/g, maximum 6.56 log kob / g and average 4.75 ± 1.34 logcfu /g level is expected in terms of cheese being fermented product.

The bacteria of the genus *Lactococcus* are immobile, non-spore forming, facultative anaerobic, mandatory homofermentative lactic acid bacteria, present in pairs and short chains or groups. They can produce lactic acid in a suitable liquid medium and lower the pH to about 4.5. Some lactococci are used as starter cultures in the production of fermented dairy products (Kaynar e al., 2005).

In this study, Lactobacillus spp. The level was determined as at least 4.95 logkbo / g, maximum 6.32 log kob / g and average 5.69 ± 0.40 log kob / g. These findings are consistent with the data of Morul and Processor. *Lactobacillus spp*. level is a result of cheese being fermented product.

Lactobacillus species are commonly found in foods (eg, milk and dairy products, meat and meat products, fruit and juice, pickles, olives, beer, wine and cereals). The most important feature of these species is to produce lactic acid by breaking down carbohydrates.

Psychophilic bacteria can cause significant problems in milk and dairy products stored at 2-7 ºC. Therefore, it is stated that the number of psychrophilic bacteria should be low. These microorganisms break down carbohydrates, fats and proteins as fermentative during their growth and this fragmentation changes the taste and odor of protein containing food products. Psychophilic microorganisms are commonly found in foods. The source of these organisms is water, soil and, in some cases, food itself (for example, milk, meat products, marine fish and vegetables).

In this study, the minimum number of 3.00 log kob / g, maximum 5.09 log cfu/g and the average psychophilic microorganism number of 3.94 ± 0.64 logkob/g can be considered as normal values for fermented products. While these values are compatible with Morul et al (2012), it is lower than the findings of Pekel (2008).

In this study, *Staphylococcus-Micrococcus spp*. load was determined as a minimum of 2.90 log kob / g and a maximum of 5.60 log kob / g on the average of 4.40 ± 0.85 log kob / g. Erceyes et al. (2006) reported that they did not encounter Staphylococcus species in their samples. High values may be due to the inadequacy of the milk used in production, processing equipment and storage conditions.

The importance of *Staphylococcus-Micrococcus* species in foodstuffs is that some strains cause food poisoning. Strains that cause food poisoning are defined as *enterethoxigenic Staphylococcus.*

Useful properties of cheese are largely explained by its nutritional value. The composition of cheese includes vital and valuable human proteins, milk fat, minerals, vitamins and extractives. Their concentration is almost 10 times higher than in milk, from which cheese is made. According to nutritionists, 50 grams of cheese is equivalent to the use of 0.5 liters of milk. Cheese is one of the most nutritious and high-calorie foods. Its nutritional value is due to the high concentration of protein and fat, the presence of essential amino acids, vitamins, calcium and phosphorus salts necessary for the normal development of the human body. Currently, cheese consumption is increasing almost everywhere: in countries with developed markets, and in countries with developing market relations. According to the results of the chemical examination of the cheese, it was determined that all samples meet the established requirements.

References

Akarca, G., Çağlar, A., Tomar, O. (2013). Mozzarella Cheese: Definition, Production Techniques and Quality Parameters. *Academic Food Journal*, 11 (in Turkish).

Erkan, M.E., Vural, A., Güran, H.Ş. (2009). Searching the presence of Aflatoxin M_1 and verotoxin 1 and 2 in Diyarbakir örgü cheese. *Dicle Üniv. Vet. Fak. Derg*, 1, 19-25.

Erkmen, O. (2010). *Gida Mikrobiyolojisi*, Baskl, Ankara, Efil Yayınevi.

Fallah, A. A., Jafari, T., Fallah, A., & Rahnama, M. (2009). Determination of aflatoxin M1 levels in Iranian white and cream cheese. *Food and Chemical Toxicology*, *47*(8), 1872-1875.

Fox, P. (2011). Introduction. History of Dairy Products and Processes.

Fox, P. F., McSweeney, P. L., Cogan, T. M., & Guinee, T. P. (Eds.). (2004*). Cheese: Chemistry, Physics and Microbiology*, Volume 2: Major Cheese Groups. Elsevier.

Güley, Z., Uysal, H.R., Kılıç, S. (2013). Investigation of the presence of aflatoxin M_1, aflatoxin B_1 and aflatoxigenic moulds in some naturally mould-ripened traditional cheeses. *Ege Üniversitesi Ziraat Fakültesi Dergisi*, 50 (in Turkish).

Harrigan, W.F., & McCance, M. E. (1976). *Laboratory methods in food and dairy microbiology*. Academic Press Inc.(London) Ltd.

Hussein, H.S., & Brasel, J.M. (2001). Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology*, *167*(2), 101-134.

Kaynar, P. (2011). Microbiological Research on Turkish Cheese. *Türk Mikrobiyol. Cemiy. Derg*, 41, 1-8.

Kaynar, Z., Kaynar, P., Koçak, C. (2005). A Research on Hygienic Qualıty of Pıckled White Cheeses in Ankara Market. *Türk Hijyen ve Deneysel Biyoloji Dergisi*, 62, 1-10.

Keles, A., Atasever, M. (1996). Chemical, microbiological and sensory quality characteristics of Divle Tulum cheese. *Sut Teknolojisi*, 1, 47-53 (in Turkish).

Kesenkaş, H., Akbulut, N. (2006). Yeasts as Adjunct Starter Cultures Used in Cheese Production. *Ege Üniversitesi Ziraat Fakültesi Dergisi*, 43 (in Turkish).

Khodadadi, M., Khosravi, R., Allahresani, A., Khaksar, Y., Rafati, L., & Barikbin, B. (2014). Occurrence of aflatoxin M1 in pasteurized and traditional cheese marketed in southern Khorasan, *Iran. Journal of food quality and hazards control*, *1*(3), 77-80.

Kireçci, E., Savaşçı, M., Ayyıldız, A. (2007). Detectıon of aflatoxın M_1 ın mılk and cheese products consumed ın Sarıkamış, Turkey. *Turkish Journal of Infection*, 21, 93-96 (in Turkish).

Little, C. L., Rhoades, J. R., Sagoo, S. K., Harris, J., Greenwood, M., Mithani, V., ... & McLauchlin, J. (2008). Microbiological quality of retail cheeses made from raw, thermized or pasteurized milk in the UK. *Food microbiology*, *25*(2), 304-312.

Morul, F., İşleyici, Ö. (2012). Chemical and Microbiological Properties of Divle Tulum Cheese. *Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi*, 23, 71-76 (in Turkish).

Patır, B., Ateş, G. (2002). Research on some microbiological and chemical properties of dry matter. *Turk J. Vet. Anim. Sci.*, 26, 785-792 (in Turkish).

Pekel, M. (2008). Determination of Microbiological Properties and Mold Flora of Sivas Cube Cheese. Graduate School of Natural and Applied Sciences. Uludag University (in Turkish).

Tekinsen, O. (2000). *Sut urunleri teknolojisi*. Selcuk University, Turkey, 1, 86.

Tekinşen, O.C., Atasever, M., Keleş, A. (2006). Chemical and Organoleptical Properties of Civil Cheese. *Vet. Bil. Derg.*, 12, 65-71 (in Turkish).

Ünlütürk, A., Turantaş, F., (2003). Food Microbiology. İzmir, Basıç Matbaaçılık.