

Determination of Aflatoxin M1 Level in Motal Cheese Produced in Azerbaijan

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Abstract

In this study, it was aimed to determine the presence and level of aflatoxin M1 (AFM1) in motal cheese. The research has shown that the mean of AFM1 values of motal cheese is quite low, and the positive sample ratio is similar to the literature. Possible reasons for the low concentration of determined AFM1 include the feeding of the animals in the area, where the samples were collected, the spread of the sheep breeding, the grazing of the animals at the pastures most of the year, and the production of motal cheese especially during the summer when there is a lot of milk. Motal cheese can be evaluated in a group of fatty, soft and/or semi-hard cheeses. It is considered that production and sales centers of the cheese do not have sufficient hygienic and technological capacity and that necessary precautions should be taken in this respect when considering the high numbers of microorganisms.

Keywords: Aflatoxins, Milk, Dairy Products, Cheese, Microbiological Analyses

Introduction

Milk and dairy products are of great importance in terms of human nutrition as they are basic foodstuffs. For this reason, it has been tried to use milk in different ways because milk cannot be stored for a long time. Since milk is not a durable product, besides its direct consumption, it can be consumed extensively by transforming it into many products with different flavors, aroma and structure. Among these products, cheese ranks first in terms of variety and consumption (Tekinşen *et al.*, 1996; 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). Considering this ratio, the industrial importance of cheese is also remarkable. Milk is an easily digestible product with high nutritional and biological value. In recent years,

effective development has been observed in cheese production in the dairy industry in most developed countries. In many countries with a developed dairy industry, there is an active development in cheese production (Kireççi *et al.*, 2007; Kaynar 2011). In parallel, the demands and production volumes for cheese are constantly increasing, the product range is expanding and developing. Today, it is believed that there are more than 2000 types of cheese. It is seen that every country and region has a habit of consuming its own type of cheese (Khodadadi *et al.*, 2014; Tekinsen 2000).

Contamination of food and feed is an important problem all over the world. In this context, mycotoxins are considered to be very important natural toxins due to the fact that molds can be found almost everywhere and develop toxins in many foods. The Food and Agriculture Organization reported in 1985 that 25% of the world food production was contaminated with mycotoxins. This situation causes negative economic consequences due to the producer's product loss, animal and milk losses (Pietri *et al.*, 1997; Govaris *et al.*, 2002; Özkaya *et al.*, 2003).

In this study, it was aimed to determine the AFM1 level of motal cheese, which is a cheese produced from sheep's milk and left to ripen for three months by traditional methods in the districts and villages of Ganja city and other regions of Azerbaijan.

Despite the fact that cheese has been used as a food since the past, it has not been revealed exactly in which region and by whom it was first made. There are many different claims on this subject. The researcher named R.W. Menges states that the first cheese was obtained after the milk started to coagulate by chance during the transportation that an Arab traveler made in a bag made from the internal organ (stomach) of the sheep. However, there are common opinions regarding the history of cheese that it was produced from horse milk raised by the Scythian Turks living in Russia between 600 and 200 BC. It is also common for Turks and Mongols to obtain fermented food using goat milk during the transition to Europe. Kuntze reported that the method of cheese making was first introduced by the Finns. (Eralp, 1974). Although there is no evidence, there are also opinions that cheese was produced by herders in the Mediterranean coasts, Mesopotamia or South-west Asia after the domestication of dairy animals between 8000-6000 BC. (Bernard *et al.*, 2002). However, there is a claim that, following the domestication of horses, the Scythian Turks discovered cheese between 600 and 200 BC. It is also emphasized that the history of cheese production goes back to the Sumerians, which have a much older history. As a result of the readings on the figures found in a relief discovered in Mesopotamia, it was understood that the Sumerians had important information about milk preservation. When the historical information of that period is examined, there are various estimates that the Akkadians and Sumerians produced many types of cheese. Likewise, it is stated that the Hittites produced various types of cheese

from milk obtained from cows. (Delamy *et al.*, 1974). It has been reported that in the Babylonian period dating back to 2000 BC, milk production was advanced compared to that period and cheese was consumed as the food of noble people. In an archaeological excavation in Switzerland, the discovery of strained milk containers revealed that the production of cheese made by the people goes back to the Sumerians. A similar study was carried out in Italy and France, and it was revealed that the ancient milk-cut strainers were found in different regions. (Ünsal, 1997).

As for cheese production, many production methods and types have been provided to ensure that people consume high quality cheese. Accordingly, new modern equipment has been invented and the standard has been achieved in the construction technique. In addition, new methods have been developed for the breakdown of protein, lactose and fat, which are the main components of cheese, and ripening methods that give flavor and aroma specific to cheeses. (Kesenkaş & Akbulut, 2006). In addition to the consumption of cheese at home from retail sales, it is also widely used in the food and food industry. An important part of the cheese produced for home consumption is the type of cheese produced by traditional methods, and it is a food that people directly consume. On the other hand, a significant portion of the cheese used in industrial cheese production is evaluated as food ingredient after various processes before turning into products.

As with any food, there are various dangers threatening human health in contamination of milk and dairy products. Aflatoxins are one source of these threats. Contamination of milk with aflatoxin M1 (AFM1), which is a carcinogenic substance, causes serious health problems. Toxins in milk, which is an important part of child nutrition, can cause harmful effects in children. Since AFM1 is resistant to heat, it does not show a decrease in pasteurization, and it is even stated that it contains a certain amount in UHT milk (Pietri *et al.*, 1997). This substance passes from raw milk to dairy products. (Brackett & Marth, 1982; Van Egmond, 1989; Calaresu *et al.*, 2006).

Aflatoxins can also be included among metabolites produced by other species, such as *Aspergillus*, *Penicillium* and *Rhizopus* molds, especially *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*, under suitable temperature and humidity (Stubblefield & Kwolek, 1986; Steyn, 1995; D'Mello & AMC, 1997; Lee *et al.*, 2009).

Tablo 1. Aflatoxins and species of fungi that synthesize them

Fungus Türleri	Aflatoksinler			
	B1	B2	G1	G2
<i>A. flavus</i>	+	+	+	+
<i>A. flavus var. Columnaris</i>		+		
<i>A. oryzae</i>	+	+		
<i>A. parasiticus</i>	+	+	+	+
<i>A. parasiticus var. globosus</i>	+	+	+	+
<i>A. niger</i>	+			
<i>A. ventii</i>	+			
<i>A. rubber</i>	+			
<i>A. ostianus</i>	+	+		
<i>A. ochraceus</i>	+			
<i>P. variable</i>	+			
<i>P. puberulum</i>	+	+	+	+
<i>P. citrinum</i>	+			
<i>P. frequentas</i>	+			

Aflatoxins

Aflatoxins consist of six basic groups as aflatoxins B1, B2, G1, G2, M1 and M2. However, it is stated that there are 18 species of aflatoxins, together with their metabolites (such as aflatoxin B2a, G2a, P1, Q1, and aflatoxicol) formed from mold fungi and animals through various stages (Pittet, 1998). *Aspergillus* molds that release aflatoxin and show toxic effects are widely found worldwide. (Dragacci *et al.*, 2001). Under conditions where the ambient temperature is about 25 ° C, aflatoxins have the ability to reproduce in all agricultural consumer goods and food-borne diseases with an aw value of 0.80 and above. Mold species belonging to *Aspergillus* strain, which is considered to be the most important of these fungal strains, can easily develop and produce aflatoxins under the appropriate conditions shown in Table 2 (Özturan, 2007; Jay, 1992; Concon, 1998).

Table 2. Some values of the growth of molds belonging to the genus *Aspergillus*

Temperature	10-45° C
Aw	0.8 and more
pH	1.6-9.3
Relative Humidity	%60 and more

The Importance and Effects of Aflatoxin Contamination on Milk and Dairy Products

An important reason for researching aflatoxins in milk and dairy products is the harm these substances cause to human health. Very important health problems arise when the AFM1 found in these foods is consumed at a high rate, especially by growing children. Aflatoxins cause acute, subacute and chronic toxicity in living creatures depending on the intake level and time. The disease caused by aflatoxin consumption is called aflatoxicosis. The organ that is directly affected in acute aflatoxicosis is the liver. Generally, symptoms such as jaundice, anorexia, hemolytic anemia and diarrhea are encountered. However, the liver parenchyma cells are damaged, and the process of serial division of the cells in the liver and bile ducts occurs. Then various bleeding occurs and the nervous system is affected. As a result, cramps, paralysis, balance disorders occur. Chronic toxicity is a result of long-term consumption of high doses of AFB1 with liver cancer and malformations in this organ, cancer formation in the colon and kidneys also observed. In addition, the liver is the organ most affected by aflatoxins, so it has hepatotoxic effects. (Frobish et al., 1986; Harvey et al., 1991; Herrman, 1995; Creppy, 2002).

Contamination of milk and dairy products with aflatoxins

As a result of feeding dairy cows aflatoxin-containing foods, their milk is contaminated. (Van Egmond, 1989). AFB1 taken with feeds is converted to AFM1 by settling in the liver of dairy cows and passes from here to milk. Consequently, AFM1 contamination may occur in various dairy products obtained from contaminated milk. (Galvano *et al.*, 2001). It is also possible to develop toxin-producing species of bacteria and contamination of the released aflatoxins with dairy products. (Marth, 1979). Aflatoxins can easily occur and reach high levels, especially in cheeses that contain a suitable environment for *Aspergillus* growing (Steyn, 1995).

Contamination of milk and dairy products with aflatoxin varies according to geographical regions, countries and seasons. Contamination rates with AFM1 also vary according to hot or cold seasons due to the abundance of AFM1 in the spring and summer season, meadow, pasture, grass, roughage varieties compared to winter.

Consequently, as a result of feeding animals with non-contaminated food, the amount of aflatoxin in milk decreases and disappears completely. Therefore, AFM1 level decreases to undetectable levels in dairy products made with this milk. (Özkaya & Temiz, 2003; Pittet, 1998).

Effects of Aflatoxin in Humans

Aflatoxins cause adverse effects on human health. (Tayfur, 2002). These can be listed as various acute diseases, primarily liver, cirrhosis (liver cirrhosis), carcinomatous formations (liver carcinoma), teratogenic and genetic disorders. (Erol, 1999). Toxic effects include hepatocellular carcinoma, acute hepatitis, Reyes syndrome, and cirrhosis; kwashiorkor has been reported to cause various diseases. (Shukla *et al.*, 2002). Aflatoxins can cause major liver cancer in humans and can lead to various changes in the human gene structure. Moreover, toxins can disrupt the hormonal balance, weaken the immune system, cause infertility and birth defects, reduce the absorption rate of food, and weaken bone structure. The European Union Food Scientific Council reported that even low rates of AFB1 can lead to liver cancer and mutations in genes. (Akpınar, 2015)

Aflatoxin detection methods

Sample preparation techniques

Stages include determination of aflatoxin in foods; sampling, sample preparation, extraction, purification, qualitative and quantitative analysis of concentration and verification stages. Sampling and sample preparation are of great importance in aflatoxin analysis. While there is no problem with homogeneous products such as milk in sampling and preparation for AFM1 analysis, aflatoxins in products such as cheese can be found in different proportions on the outer surface and inside of the cheese. It is very important to determine the amount of analysis sample that will represent the entire batch. The sample taken must be ground and homogenized in order to ensure effective distribution of aflatoxin contaminated particles. (Tiryaki, 2011).

Although thin layer chromatography and high-pressure liquid chromatography are most frequently used in the analysis of aflatoxins, the radio-immunoassay method and the enzyme-linked immunosorbent method (ELISA) are also widely used. (Tiryaki, 2011).

ELISA method

ELISA is a method developed in 1971. In this method, the antigen or antibody is marked with an enzyme, and the immunological reaction is measured as a result of an enzymatic activity (Acaröz *et al.*, 2016). Antigens are reversibly linked to antibodies by non-covalent bonds such as hydrogen bonds, electrostatic interactions, hydrophobic interactions, and Vander Wals forces. (Deligöz and Bilge, 2017). This method is used with antibodies. It consists of two steps: the reaction between the toxin and the measurement of the substrate reaction with the enzyme-bound toxin. (Atik, 2012). Free and enzyme-bound antigens compete for the vacant antibody binding sites on the antibody surface. After the reaction is complete, unbound substances are washed away. After this process, the specific substrate is added to the enzyme. This substrate is catalyzed by the enzyme in an inverse proportion to the amount of free antigen and enzyme activity is measured. (Atik, 2012; Acaröz *et al.*, 2016).

The advantages of the ELISA method are that it is inexpensive, easy to prepare and simple. Many analyses can be made in a short time, and not requiring too much solvent. However, it is a method that requires verification. In addition, the sampling should be done very thoroughly since aflatoxins do not show a homogeneous distribution in the food and the ELISA method uses a small amount of sample. In this case, the difficulty of obtaining a representative sample constitutes the most important problem encountered in the ELISA method. (Tiryaki, 2011; Deligöz & Bilge, 2017).

ELISA has two types, direct competitive and indirect competitive. In the direct competitive ELISA method, the specific antibody is coated on a solid excess on the microtiter plate, while in the indirect competitive ELISA method, the toxin is coated on the protein conjugated microtiter plate. (Aycicek *et al.*, 2005). In the direct competitive ELISA method, specific antibodies for aflatoxin are coated on the wells in microtiter strips. Cheese test samples or AFM1 standards are added to the wells. After incubation and washing, enzyme conjugate is added to the wells. Free AFM1 and AFM1 enzyme conjugate compete in the wells for AFM1 antibodies. The washing step removes any unbound enzyme conjugate. Then the base (sheet) / chromogen is added to the wells and incubated. The bound enzyme converts the conjugate into a colorless chromogen product. The stop solution is added, and this causes a blue to yellow color change. The measurement is then made photometrically with waves at 450 nm.

Motal Cheese Production

Making a bag of sheep [goat skin] for storing motal cheese

Sheep or goat skins are used to make motal cheese. Removing the skin of animals consists of several stages. After the slaughter of the animal, the body is inflated with gas. After the gas has completely penetrated between the skin and the adjacent tissue, the skin begins to tear off the neck. The front and hind legs are cut from the body at the level of the wrist and the hide is completely detached from the body. After the skin has been removed, the inside is well washed, cleaned and salted, and left to dry. After a while, the hide is washed and dried again. After making sure that the skin is dry, it turns over and the process ends with the hairy part. The open cuffs are made of finished hide and are closed with a flat cord. Air is supplied from the open part and it is checked if the bag is leaking air. A bag made of skin, which must be airtight, is considered suitable for storing cheese.

Motal cheese preparation

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

Milk from sheep and goats used to make dry cheeses is filtered and, after milking, placed in a special flat container. Further, special yeast is added, and the formation of clots is observed, after which the milk is transferred into pre-prepared bags so that the clotting process can continue for some time. The bag of coagulated milk is then placed in a flat container and a weight is placed on it so that, during coagulation, the water in the clot flows out of the bag. This clot is expected to mature and completely lose all liquid in about 24 hours. At the end of this period, the curd mass is cut into small pieces, salted in accordance with the traditional rules and kept in this state for about 10 days. Thus, after sufficient ripening and hardening, the cheese is placed in small pieces into a bag made of hide and tied tightly. Then it is transferred to a cellar or cave for two or three months. Sometimes these bags of cheese are buried in the soil. After this ripening process, the finished motal cheese can be stored for 7 or 8 months.

Materials and methods

110 samples of coiled cheese used in the study were collected in February-June 2016 from local markets and from farms in Ganja, Dashkesan, Gook-gool, Tovuz and Shamkir.

In this study, the presence and level of AFM1 in motal cheese samples were determined. The ELISA method (R-Biopharm, R 1101 Test Kit r-biofarm RIDASCREEN Aflatoxin M1 test kits) was used for the detection of AFM1 in motal cheese samples.

Preparation of samples

2 g of the finely chopped and crushed cheese sample was weighed. 40 ml of dichloromethane was added and mixed for 15 minutes by shaking. The resulting suspension was filtered and evaporated at 60 ° C under weak nitrogen gas until 10 ml of extract was obtained. The oily residue was then mixed and dissolved with 0.5 ml methanol, 0.5 ml PBS buffer and 1 ml heptane. The solution was centrifuged at 15 ° C for 15 minutes at 2700 rpm in a cooled centrifuge. Then, by removing the heptane layer on the upper part completely, 100 µL of the methanol-aqueous phase was taken and the content was diluted to 10% by adding 400 µL buffer. 100 µl of this mixture was used for each well of the plate in the test.

Test procedure

A sufficient number of microtiters for standard solutions and prepared samples were placed in the well holder. Standard solutions and 100 µl of prepared samples were taken and added to the wells. After shaking the holder by hand, it was incubated for 30 minutes at room temperature (20-25 ° C) in a dark environment. Then the liquid in the wells was drained out. The microplate holder was vigorously inverted against the adsorbent paper to ensure complete removal of liquid from the wells. Then each well was washed by adding 250 µL of wash buffer and the liquid was poured again. The washing step was repeated twice. Then, 100 µl of diluted enzyme conjugate was added to the wells, the holder plate was shaken and mixed and left to incubate again for 15 minutes in the dark at room temperature. After the incubation, the liquid in the wells was discharged and 2 times washing was done again. Then 100 ul substrate / chromogen

The holder plate was added and mixed gently by shaking again and incubated for 15 minutes at room temperature in the dark. At the end of the incubation, 100 μ l of stop solution was added to each well and mixed thoroughly and its absorbance was measured at 450 nm in a spectrophotometer. The evaluation of the results was made using the RIDAWIN computer package program. While calculating the results, the concentrations obtained on the calibration curve were multiplied by the dilution factor of 10.

Results and discussions

The study was carried out on 110 motal cheeses produced from sheep milk with the traditional method provided in the provinces of Ganja, Daşkesen, Goy-Gol, Touz, and Shemkir in Azerbaijan. In the study, while AFM1 over 5 ng / kg was detected in 49 (44.55%) of the total 110 cheese samples examined, 61 (55.46%) samples were within the measurable limits (the minimum measurable limit in the ELISA method and device was 5 ng / kg. AFM1 could not be detected. AFM1 concentration of positive samples ranged from 5.19-16.42 ng / kg and was found to be 8.00 ± 2.43 ng / kg on average.

Table 3. AFM1 concentration in motal cheese

Number of samples	Positive samples (>5 ng/kg) (%)	Negative samples (<5ng/kg) (%)	Number of samples exceeding legal limits *	Positive samples (ng / kg) Range Mean \pm SD	
110	49 (44.55)	61 (55.46)	Not determined	5.19-16.42	8.00 \pm 2,43
* European Union Standards 0.05 ppb (μ g / kg)					

Cheese is a food with a high nutritional value, obtained as a result of the formation of clot by the action of organic acids contained in cheese, fatty milk, skimmed milk, milk fat, buttermilk and the treatment of these raw materials with rennet. (Akarca *et al.*, 2013; Prazeres *et al.*, 2012). In this respect, this Since it is a dairy product that is enjoyed all over the world. Cheese is a product that is increasing its influence on the demand of people today and is an extremely important part of the food consumption chain. In the different product groups in this sector, there are a wide variety of products such as drinking milk, cheese, butter and cream, fermented milk, ice cream, condensed milk and milk powder. (Akarca *et al.*, 2013). Various raw materials and

production methods, with different maturation times, A wide variety of cheese types are produced today as a result of the demands. (Kaynar, 2011). It is stated that there are more than 1000 types of cheese production, which has a historically important history. (Akarca et al., 2013). However, approximately 40% of the milk produced in the world is used in cheese production. Therefore, the importance of cheese production and consumption stems from this. (Özturan, 20076; Akarca et al., 2013).

Thanks to the developing industry, the importance and rate of cheese types used as food additives in the ready-to-eat food 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 started to be consumed as a food additive. 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).

Since cheese has a wide range of uses, the disease risks it can carry and the presence of metabolites that may be harmful to human health in cheese have been the subject of many studies. In this context, the presence of aflatoxins, which have a high risk of being found in milk and cheese, has been an important research subject for scientists in recent years (Erkan et al., 2009). Aflatoxins have a carcinogenic effect on various organs and tissues, especially the liver, especially in human health. These metabolites also pose an important risk in animal nutrition. Scientific studies on milk and dairy products have shown that these metabolites can be found in various countries at a level that can be harmful to the body of living beings and that they are transported entirely with milk and dairy products. (Galvano et al., 2001; Kim et al., 2000; Roussi et al., 2002). It is thought that aflatoxin formation occurs in three different ways in dairy products. Toxin formation in raw milk as a result of the consumption of aflatoxin-containing feeds, the presence of molds that can produce toxins (e.g. *A. flavus* and *A.parasiticus*) that develop on the surface of cheeses, the passage of additives used in cheese and yoghurt and toxins in milk powder to dairy products and aflatoxin in milk and milk products. cause the formation. It is stated that the distribution of AFM1 in milk is not homogeneous and it is found in the skim part of the milk around 80%.

The presence of aflatoxin M1 (AFM1) in milk and dairy products is important for public health. It is reported that aflatoxin M1 level is higher in cheese types than milk. (Galvano et al., 2001). This situation is thought to be caused by the affinity of aflatoxin M1 to casein. In cheese production, casein content is much higher than milk because casein fractions coagulate and form a very important part of the cheese mass. (Galvano et al., 2001; Dinkçi et al., 2012). During cheese production, the highest aflatoxin content in dairy products is detected in cheese, as AFM1 clings to the casein fraction due to hydrophobic binding. (Trucksess & Page, 1986). It has

been reported that the concentration of aflatoxin M1 is 2.5-3.3 times higher in soft cheeses and 3.9- 5.8 times higher in hard cheeses compared to the milk it is made from (Bakirci, 2001). As a matter of fact, some experimental studies (Oruc *et al.*, 2007; Oruc *et al.*, 2006) also support this situation. In the study conducted by Oruc *et al.* (2007) on cheddar cheese, raw milk samples were contaminated with AFM1 at levels of 50, 250 and 750 ng / L, and AFM1 concentrations of cheeses produced from these milks were found to be 2.93, 3.19 and 3.37 times higher, respectively, compared to the raw milk produced. Again, Oruch *et al.* (2006) examined the distribution and stability of AFM1 in the ripening process in white pickled cheese, curd, whey and cheese samples produced according to traditional techniques. Cheese artificially with AFM1 at levels of 50, 250 and 750 ng / L. They were prepared from contaminated milk and left to mature for three months. It was determined that the cheeses produced in the study had 3.6, 3.8 and 4.0 times higher AFM1 levels, respectively, compared to the milk they were produced.

It is important to control the process steps to determine at what stage the aflatoxin is contaminated with the cheese and to prevent this situation. Therefore, HACCP applications in businesses are important in terms of reliability. (Wiseman & Marth, 1983).

Despite its widespread consumption, as a result of the literature reviews, no research has been found on motal cheese in Azerbaijan or other countries of the region. Based on the lack of any research to determine the chemical and microbiological quality of motal cheese and the content of Aflatoxin M1 (AFM1), this study was planned to eliminate this deficiency in the literature to some extent.

Motal cheese is a semi-hard and fatty cheese that is produced from sheep's milk with traditional methods in the regions and villages of Ganja province, especially in the Karabakh region of Azerbaijan and left to mature for three months. On the other hand, production from cow's milk has become widespread in recent years due to the scarcity of sheep's milk. Motal cheese production is generally maintained in rural areas are considered as another version of cheese are produced in Turkey. It is known that this cheese can be produced from time to time by mixing cow's milk and sheep's milk. Since Motal cheese does not have any industrial production in today's conditions, it is produced completely by traditional methods, so the production stages remain out of control. For this reason, many factors and metabolites that can threaten human health carry the risk of contamination of milk and cheese or the transmission of the factors in the milk to the cheese. As a matter of fact, the disease risks of this type of cheese increase even more during the ripening period and it is possible to continue these risks with preservation methods. In Azerbaijan, the risk of exposure to various factors is high, since no heat treatment is applied to the milk used in the

production of cheeses. However, it is highly possible that various microorganisms and fungi that produce aflatoxin, especially aspergillus, and the aflatoxins they produce are in these production areas due to the unhygienic environment where cheese production is made. In addition, although there is a legal regulation regarding the minimum aflatoxin value that can be accepted in cheeses offered for consumption in many countries, there is no reference value related to this issue due to the limited researches on this subject in Azerbaijan. Due to all these deficiencies, studies on these issues are extremely important.

In this study, while AFM1 over 5 ng / kg was detected in 49 (44.55%) of 110 total cheese samples, in 61 (55.46%) samples (below 5 ng / kg) AFM1 was not detected. The AFM1 concentration of positive samples ranged from 5.19 to 16.42 ng / kg, with an average of 8.00 ± 2.43 ng / kg. Looking at these values, it is seen that the average values are quite low, and the rate of positive samples is similar to the literature. Possible reasons for the very low AFM1 concentration include feeding the animals on pastures in the region where the samples were obtained, the prevalence of sheep farming and grazing on the pastures for most of the year, and the production of total cheese especially during the summer months when milk is high (therefore feeding the animals with fresh grass). and the consumption of dry feed is relatively low, the climatic conditions are not suitable for the growth of mold in the feeds, and the geography of this region is far from the tropical area where mold growth is high. It has been reported that the use of corn and silage in the diet of dairy cattle can be the main source of AFB1 contamination, (Cavallarín *et al.*, 2011; Pereyra *et al.*, 2008) and the presence of aflatoxin in milk and dairy products may differ according to geographical regions, countries and seasons. (Bakirci, 2001).

Although mycotoxins are known to pose a threat to public health, aflatoxins are the most studied toxic metabolites among mycotoxins. Aflatoxin contamination reports are still being made today in many milk and dairy products. Preserving animal feeds in unsuitable conditions may pave the way for aflatoxin formation in feeds, and consumption of contaminated feeds by animals can lead to aflatoxin presence in milk and dairy products. In addition, the presence of mold types that can produce toxins in cheeses can cause aflatoxin formation. For this reason, in order to keep the AFM1 level in milk and dairy products at very low levels, modern production techniques should be applied, the storage conditions of the feeds given to dairy animals should be made suitable and necessary controls should be made and milk producers should be made aware of this issue. Looking at the results, it is seen that the AFM1 content of the total cheese samples is not very high. However, it is thought that this toxin may pose a public health risk due to its accumulation in the body. This study confirms the information that the presence of aflatoxin in milk and dairy products varies according to geographical regions, countries and seasons. Because although

very high AFM1 contaminations were reported from different parts of the world, it was seen that this toxin was at very low levels in cheese samples obtained from Azerbaijan. Since there are not enough studies to determine the presence of aflatoxin in this region, new and more comprehensive studies are needed. It is thought that motal cheese may pose a public health risk in terms of microbiology because it contains high levels of microorganisms studied. The variation in chemical properties of motal cheese indicates the need for standardization in the production of this product.

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