

# **Aflatoxins M1 and M2 in Dairy Products**

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## **1. Abstract:**

The term Mycotoxins is derived from the Greek word ‘mycos’ meaning mould, and the Latin word ‘toxicum’, which means poison. Mycotoxins are relatively low-molecular weight secondary metabolites of fungal origin that are harmful to animals and humans. Mycotoxins such as Aflatoxins B1,B2,G1,G2,M1and M2, Ochratoxins and Zearalenone are toxic secondary metabolites produced by various fungi such as *Aspergillus*, *Penicillium* and *Fusarium* which affect a wide range of agricultural products meant for human consumption and animal feed. Mycotoxins present in food products and animal feeds are an important problem concerning food and feed safety and significant economic losses are associated with their impact on human and animal health. Mycotoxins contamination of food and feeds remains a worldwide problem, the United Nation Food and Agriculture Organization (FAO) has estimated that up to 25% of the world’s food crops are significantly contaminated with mycotoxins. Aflatoxin B1 (AFB1) is the most potent hepatotoxin with a large variety of biological effects, such as carcinogenicity, teratogenicity and mutagenicity in humans and farm animals and it is included in the group 1B by International Agency for Research on Cancer. Aflatoxin M1 (AFM1) and Aflatoxin M2 (AFM2) are the hepatic hydroxylated metabolites of AFB1 and AFB2, respectively. AFM1 is found in milk and milk products obtained from livestock that have ingested AFB1contaminated feed. The carcinogenicity of AFM1 is about ten times less than that of AFB1, and for these reason it has been included in the class 2B by International Agency for Research on Cancer. In lactating animals the conversion rate of AFB1 to AFM1 ranges between 0.5 and 6%. Variability is due to different factors such as individual response, AFB1 intake level, stage and order of lactation. Several research workers reported that there is a linear relationship between the amount of AFM1 in milk and AFB1 in feed which is consumed by dairy cattle. Aflatoxin M1 in milk and milk products is considered to pose certain hygienic risks for human health. These metabolites are not destroyed during the pasteurization and heating process.

Aflatoxin contamination in milk and its products is produced in two ways. Either toxins pass to milk with ingestion of feeds contaminated with Aflatoxin, or it results as subsequent contamination of milk and milk products with fungi. Like other mycotoxins, Aflatoxins M1 and M2 can be detected by using chromatography (HPLC) or ELISA. Many countries' standards limits of Aflatoxins M1 and M2 ranged between 0 to 0.5 ppb, in milk and dairy products. Some European Community and Codex Alimentarius prescribe that the maximum level of AFM1 in liquid milk and dried or processed milk products should not exceed 50 ng/kg. So, in this review article, we want to highlight on this dangerous mycotoxins in our dairy products by reporting all the information which is available in the literature.

**Key words:** Mycotoxins, Aflatoxins M1 and M2, Dairy Products.

## **2. Introduction:**

Good health starts with good nutrition and good nutrition can protect against diseases later in life. Liquid milk and other dairy products are common health consumed by people of all age groups especially children. A large population in our countries depends on milk from local suppliers. Milk is a product of biological evolution, its role in human nutrition is well known and its biochemical complex which appears to be the only material to function solely as a source of food. The complements of proteins in milk are ideal in quality and balance to satisfy human amino acid requirements. Confirmation of this nutritive image is the widespread use of milk and milk products as a part of the daily diet of peoples in the highly developed countries. As a consequence, such societies enjoy almost complete freedom from nutritional disease among infants, children, young and adults. In contrast, the underdeveloped areas of the world have a primitive or nonexistent milk supply and have numerous inhabitants suffering from nutrient deficiencies, especially infants and children (**Hoppe *et al.*, 2006**). Human health is highly attractive world, so food safety remains a major challenge to food producers and to legislators endeavoring to adequate consumer protection. Both man and animals live under a certain degree of “biological hazard” from natural toxicants that occur in food and foodstuffs (**Abdelhamid *et al.*, 2002**). Naturally occurring toxins such as mycotoxins pose intense challenges to food safety widespread in many countries, especially in tropical and subtropical regions where temperature and humidity conditions are optimum for growth of moulds and toxins production, so they are found in a wide variety of

agricultural products (such as corn, wheat, soybean, barley and rice), and animal foods as well as meat products, milk products including ultra-high treated (UHT) milk and as a result of carry-over from contaminated animal feed (**Trucksess *et al.*, 2006**). Mycotoxins contamination of food and feeds remains a worldwide problem, the United Nation Food and Agriculture Organization (FAO) has estimated that up to 25% of the world's food crops are significantly contaminated with mycotoxins. Mycotoxins are unavoidable food contaminants even when good agricultural practices are applied. Crop transfers through international trade have made Aflatoxins contaminated food a worldwide problem.

### **3. Mycotoxins:**

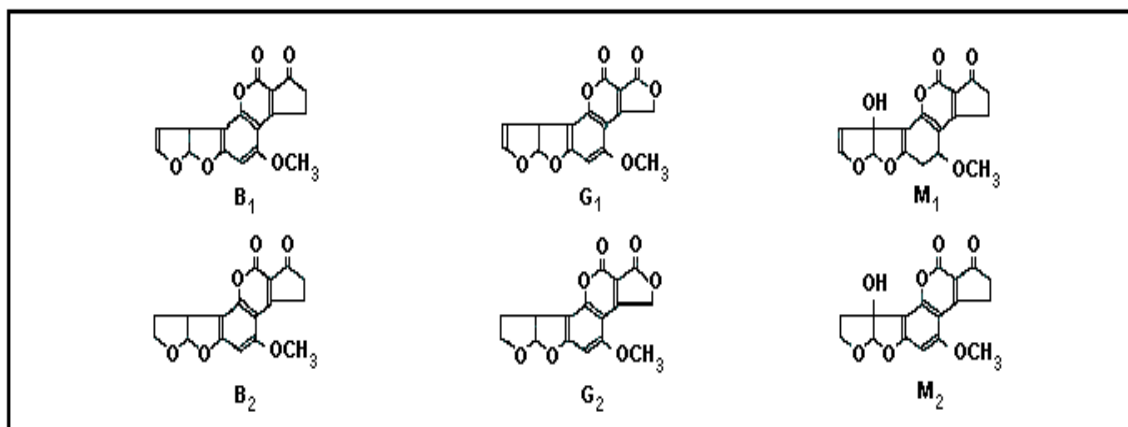
Mycotoxins are those secondary metabolites of fungi which are associated with certain disorders in animals and humans. The manifestation of toxicity in animals is as diverse as the fungal species which produce these compounds. In addition to being acutely toxic, some mycotoxins are now linked with the incidence of certain types of cancer and it is this aspect which has evoked global concern over feed and food safety, especially for milk and milk products. The term Mycotoxins is derived from the Greek word 'mycos' meaning mould, and the Latin word 'toxicum', which means poison. Mycotoxins are relatively low-molecular weight secondary metabolites of fungi that are harmful to animals and humans, and produced by various fungi which affect a wide range of agricultural products meant for human consumption and animal feed. Mycotoxins present in food products and animal feeds are an important problem concerning food and feed safety and significant economic losses are associated with their impact on human and animal health (**Shundo *et al.*, 2009b**).

#### **4. 1. Aflatoxins:**

Aflatoxins are fungal metabolites generally produced by *Aspergillus* species, namely *A. flavus*, *A. parasiticus*, *A. ochraceoroseus*, *A. bombycis*, *A. nomius*, *A. fumigatus* and *A. pseudotamari* (**Cheraghali *et al.*, 2007**). Aflatoxins are potent toxins and carcinogens which can be excreted in the milk of exposed lactating mothers mainly in the form of aflatoxinM1 (AFM1). Aflatoxin M1 (AFM1) and Aflatoxin M2 (AFM2) are the hepatic hydroxylated metabolites of AFB1 and Aflatoxin B2 (AFB2), respectively. AFM1 is found in milk and milk products obtained from livestock that have ingested AFB1-contaminated feed.

## **4. 2. Aflatoxins chemical structures:**

Aflatoxin (AF) is the strongest known naturally occurring carcinogen. Animal feed and food products are strictly inspected for AF contamination. Figure 1 shows the chemical structures of B1, B2, G1, G2, M1, and M2 Aflatoxins.



**Figure 1 – Aflatoxin chemical structures.**

## **4. 3. Analysis:**

Methods for determining Aflatoxins in agricultural commodities and food products have been verified by Method of the Association of Official Analytical Chemists (AOAC, 2005). The methods have greatly improved in recent years. A number of approaches have been used to analyze Aflatoxins and their metabolites in human tissues and body fluids. Such as high-performance liquid chromatography (HPLC), or using the competitive enzyme-linked immunosorbent assay (ELISA).

## **4. 4. Aflatoxins M1 and M2:**

AFM1 is a metabolite of Aflatoxin B1 (AFB1) and originally discovered in milk of humans and animals fed on moldy grains containing AFB1. In mammals, after 12–24 h of AFB1 ingestion, AFM1 can be detected in the milk and usually disappears within 24–72 h after stopping the consumption of contaminated feed. The carcinogenicity of AFM1 is about ten times less than that of AFB1, and for this reason has been included in the class 2B by International Agency for Research on Cancer. The quantity of AFM1 in the milk depends on the concentration of AFB1 in the contaminated feed. It has been reported that milk is one of the main risk factors of human exposure to AFM1. Infants are the foremost milk consumers, which make them more susceptible to the adverse effects of mycotoxins (Shundo *et al.*, 2009a).

Human milk is the best source of nutrition for infants providing a range of benefits for growth, immunity, and development (WHO, 2003). WHO defined food-borne diseases as illnesses of an infectious or toxic nature due to consumption of food or water, if the diseases transmittable via farm animals or their products to humans, it is considered as food-borne zoonoses. Also, it was suggested that exposure to toxicants via food from animal origin may be considered as food-borne zoonoses. There are some assumptions declare that, AFM<sub>1</sub> may be considered as communicable due to its possible transmission from food producing animals to humans and from mother to child. The lactating animal could be regarded as intermediate host also due to the biological transformation of AFB<sub>1</sub> to AFM<sub>1</sub> inside the animal body. Consequently, the farm animals may be considered as a reservoir for AFM<sub>1</sub>. The milk could be established as a major carrier of AFM<sub>1</sub> which affects the human health. Further studies are needed to detect the exact symptoms and incubation period in both animals and humans regarding the bioaccumulation of AFB<sub>1</sub> and AFM<sub>1</sub> in their bodies. Although it was suggested that the main source of AFB<sub>1</sub> is the plants, AFB<sub>1</sub> plays a major role in the epidemiology of AFM<sub>1</sub>. Generally, presence of Aflatoxins in animal or human bodies cause a disease named Aflatoxicosis, so the presence of AFM<sub>1</sub> may be specified as Aflatoxicosis M<sub>1</sub>. We can presume, thereafter, a novel concept to consider AFM<sub>1</sub> as an etiological factor for a food-borne zoonosis terming Aflatoxicosis M<sub>1</sub>. The main target organ in mammals is the liver so Aflatoxicosis is primarily a hepatic disease. Aflatoxins also cause decreased milk and egg production. AFM<sub>1</sub> from dairy products arises from several studies. Moreover, as milk is the main nutrient for growing young, who are potentially more sensitive and have less variety in their diets, the occurrence of AFM<sub>1</sub> in milk and milk products is a serious problem of food hygiene. In the lactating cow, AFM<sub>1</sub> is produced via hydroxylation of the fourth carbon in the AFB<sub>1</sub> molecular. AFM<sub>2</sub> results from hydroxylation of the fourth carbon in the AFB<sub>2</sub> molecule. The concentration of AFM<sub>1</sub> in milk increases proportionally with the amount of AFB<sub>1</sub> in the diet of the lactating cow. When ingestion is continuous, milk concentrations will increase until an equilibrium with intake is established. Recent studies indicate that a greater percentage of AFB<sub>1</sub> is secreted in milk as AFM<sub>1</sub> (58:1 to 75:1). The ratio of dietary AFB<sub>1</sub> to milk AFM<sub>1</sub> in such cows approached the range of 66:1 to 75:1. The present actionable FDA guide lines for AFM<sub>1</sub> in milk is 0.5 ppb and for AFB<sub>1</sub> in feed of lactating cows is 20 ppb.

Aflatoxin contamination in milk and products is produced in two ways. Either toxins pass to milk with ingestion of feeds contaminated with Aflatoxin, or it results as subsequent contamination of milk and milk products with fungi. AFM1 has been reported to cause certain hygiene difficulties in milk and milk products used for food. During the obtaining of cream, AFM1 disperses heterogeneously in milk. AFM1 is not destroyed during the pasteurization process or in yoghurt and cheese making. European Communities and Codex Alimentarius have fixed the limit to a maximum of 50 ng AFM1/kg, (Mohammadi, 2011).

#### **4.1. AFM1 in milk**

Milk, as a liquid, is a highly variable product that rapidly loses its quality and spoils if not to be treated. Since milk may be processed in numerous ways, the effects of storage and processing on stability and distribution of AFM1 are of great concern. Choudhary *et al.*, (1998) studied the effect of various heat treatments on AFM1 content of cow's milk and reported that sterilization of milk at 121 °C for 15 min caused 12.21% degradation of AFM1, whereas boiling decreased AFM1 by 14.5 %. They concluded that destruction of AFM1 depends on time and temperature combination of the heat treatment applied. In an investigation Conducted by Bakirci, (2001), it was observed that pasteurization caused a decrease in the level of AFM1 at the rate of 7.62 %. Fluctuation in data reported in literature could be attributed to the wide range of temperature, different analytical methods, and employment of both naturally and artificially contaminated milk. AFM1 distribution in milk is not homogeneous. Cream separation can affect AFM1 distribution, since 80% is partitioned in the skim milk portion, because of AFM1 binding to casein. An amount of 30% of AFM1 is indeed estimated to be associated with the nonfat milk solids and in particular with casein. Many authors showed that Seasonal effect influences concentration of Aflatoxin M1. They reported higher concentration of AFM1 in cold seasons as compared to hot seasons (Fallah, 2010), the reason being in winters mostly milking animals are fed with compound feeds and thus concentration of Aflatoxin B1 increases which in turn enhances AFM1 concentration in milk. Moreover, temperature and moisture contents also affect the presence of Aflatoxin B1 in feeds. *A. flavus* and *A. parasiticus* can easily grow in feeds having moisture between 13% and 18% and environmental moisture between 50% and 60%, furthermore, they can produce toxin.

Another reason of low AFM1 level in summer may be attributed to out-pasturing of milking cattle.

Aflatoxin M1 (AFM1) in milk and milk products is considered to pose certain hygienic risks for human health. These metabolites are not destroyed during the pasteurization and heating process. In (Çelik *et al.*, 2005) study, the contamination level of AFM1 in pasteurized milk that all age groups, including children, consume worldwide is defined. A total of 85 pasteurized milk samples were analyzed for AFM1 with the ELISA technique. Seventy-five samples (88.23%) were found to be contaminated with AFM1, and 48 samples (64%) exceeded the legal level of AFM1 in milk according to the Turkish Food Codex and Codex Alimentarius limit (50 ng/kg). Serious risks for public health exist from milk consumption. Thus, milk and milk products have to be controlled periodically for AFM1 contamination. Also, dairy cow feeds should be stored in such a way that they do not become contaminated. As a result of their study, 48 samples (64%) exceeded the regulatory limits, ranging from 50 to 127.6 ng/kg. Rastogi *et al.*, (2004) reported that 75% of liquid milk samples exceeded ECI Codex Regulations. Also, some studies indicated that contamination by AFM1 was relatively much higher, ranging from 28 to 1012 ng/kg in some European countries. During the period of October–July 2000, 240 samples of dairy ewes milk, obtained by (Bognanno *et al.*, 2006) from farms of Enna (Sicily, Italy), were checked for Aflatoxin M1 (AFM1) by HPLC using a fluorimetric detector. The limit of detection and the limit of quantification were 250 ng/L for AFM1. All the positive milk samples for AFM1 were confirmed by LC-MS. AFM1 was detected in 81% of milk samples, ranging from 2 to 108 ng/L. Three samples were over the legal limits (50 ng/L). Mean contamination of samples obtained from stabulated ewes was higher than that from grazing ewes (35.27 vs. 12.47 ng/L). Furthermore, samples collected in the period September–October showed higher contamination than samples collected during the other months (42.68 vs. 10.55 ng/L). Both differences are related to the administration of compound feed. Based on current toxicological knowledge they concluded that the AFM1 contamination levels recorded in ewe milk did not present a serious human health hazard. However, as ewe milk is exclusively used to produce cheese due to its higher protein content, and also considering the preferential binding of AFM1 to casein during coagulation of milk, a potentially high concentration effect could occur, thus the surveillance of contamination levels should be more continuous and widespread.

On the other hand **Abdallah *et al.*, (2012)** studies aimed to evaluate the concentrations of Aflatoxin M1 in full fat, cow's UHT milk solid in Najran–Saudi Arabia with regard to its public health significance. 96 samples of different brands full fat, cow's UHT milk were randomly punched from different supermarkets at Najran city during the period of September 2011 to January 2012. The samples were examined for AFM1 using the competitive enzyme-linked immunosorbent assay (ELISA), AFM1 residues were detected in 79 samples (82.30% of total). Data also indicated that AFM1 residues concentrations detected in all the positive samples were below the tolerated level of AFM1. This finding agrees with **Mahdavi *et al.* (2010)** in Iran who established the local milk as a main source of AFM1 exposure for lactating women. Whereas in Egypt, raw milk was recurrently a cause of many public health problems due to the lack of the hygienic measures and investigations. The consumers are depending only on heat treatment of this milk; however AFM1 is resistant to thermal inactivation (**Park, 2002**). Therefore, raw milk may be regarded as a serious risk factor for AFM1 exposure. In spite of the significance of promoting the sanitary measures of raw milk, the animal feed should be free of fungal growth especially in current screened area which has high temperature and humidity conditions. Meanwhile, **Polychronaki *et al.*, (2007)** previously evaluated the level and frequency of AFM1 in breast milk in a group of Egyptian mothers attending the New El-Qalyub Hospital, Qalyubiyah governorate, Egypt. In their study, fifty of those women who were AFM1 positive were revisited monthly for 12 months to assess the temporal variation in breast milk AFM1. AFM1 was detected in 248 of 443 (56%) samples. The identification and understanding of factors determining the presence of toxicants in human milk is important and may provide a knowledge driven basis for controlling the transfer of chemicals to infants. In total 443 breast milk samples were collected during the 12 months follow up period. AFM1 was detected in 248 of 443 (56%) of the samples with higher rates of detection during summer months. On the other hand occurrence of Aflatoxin M1 (AFM1) in infant formula milk powder (IFMP) and maternal breast milk (MBM) was investigated by (**El-Tras *et al.*, 2011**) as a risk factor affects the health of newborns in Egypt. A total of 125 IFMP and 125 MBM samples were collected and examined for the presence of AFM1 using competitive ELISA test. The results indicated that the relative risk (RR) of exposure to AFM1 via consumption of MBM was higher than IFMP (RR; 1.6, 95% CI; 1.28–2.03,  $p = 0.0001$ ). The mean concentrations of AFM1 were significantly differed ( $p < 0.0001$ )



between MBM ( $74.413 \pm 7.070$  ng/l) and IFMP ( $9.796 \pm 1.036$  ng/l). High frequency distributions were detected within the range of 5–25 ng/l and >50–100 ng/l in IFMP and MBM, respectively. The average daily exposure of newborns to AFM1 via consumption of MBM and IFMP was 52.684 and 8.170 ng, respectively, with a significant difference at  $p < 0.0001$ . Consumption of raw milk by lactating mothers exhibited a significant correlation ( $p < 0.0001$ ) with the presence of AFM1 in their milk, this work established a pioneering concept that AFM1 may be considered as an etiological factor for a novel food-borne zoonosis identified as Aflatoxicosis M1. From the results of their study, animal milk could be regarded as a hazardous source of AFM1 for infants even the toxin is occurred in low levels. The percentage of AFM1 in the branded IFMP was 43.2% of 125 examined samples and none of the positives were exceeded EC limit (25 ng/l). Another studies such as **Oveisi *et al.*, (2007)** who examined 120 infant formula milk samples in Iran, they found that 116 (96.6%) were positive with range of 1–14 ng/kg. The low levels of AFM1 in branded IFMP may be attributed to strict inspections applied during the production system and control precautions of the animal feeding before milk production. On the other hand, MBM examination indicated that AFM1 percentage in 69.6% of samples; 52% of them were above EC limit. Although breastfeeding generally provides babies with many immunological and nutritional beneficial components, breast milk may contain contaminants related to the maternal dietary exposures. The calculated average of infant daily and 6 months exposure to AFM1 indicated highly exposure values with a significant difference in MBM than IFMP. So, further studies should be carried out to detect the absorption frequency of AFM1 in infants. Also, the body weight of infants may affect the concentration of AFM1 in their tissues. Some results show that, the presence of AFM1 in MBM is significantly associated with mothers' consumption of raw milk and some contaminated food with Aflatoxins.

#### **4.2. AFM1 in cheese**

Occurrence of Aflatoxins in cheese can be owing to three possible causes:

1. AFM1 present in raw milk as a consequence of carry over of AFB1 from contaminated animal feed to milk.
2. Synthesis of Aflatoxins (B1, B2, G1, and G2) by fungi that grow on cheese (although the low level of carbohydrate does not make it a very suitable substrate).

3. The use of powdered milk contaminated with AFM1 for cheese production

Contrasting data have been reported on the influence of cheese preparation on AFM1 recovery. Studies performed in the early years showed variable losses of AFM1 during cheese production ranged from 15 to 65%, according to many studies. In contrast, later investigations of several authors, reported increases in AFM1 concentration in cheese as a function of cheese type, technologies, and the amount of water eliminated during processing. For example, **Mohammadi *et al.*, (2008)** investigated some factors, which are involved in the process of making Iranian white brine cheese. They reported that some factors such as renneting temperature, press time, and saturated brine pH affected the amount of water eliminated and in turn the content of AFM1 in the cheese curds. However, many results have been drawn from experiments in which the processed milk contained the toxin at high levels, which seldom appear in the practice. Therefore, additional investigations should verify the influence of cheese making on AFM1 occurrence to avoid uncertainty in actual practice when the concentration of the toxin in the processed milk is at around the maximum permissible level of 0.05 mg/kg that is frequently recorded in monitoring programmes. The increase in AFM1 concentration in cheese has been ascribed to the affinity of AFM1 for casein, AFM1 is a water-soluble component and due to the hydrophobic sides of the casein molecule, AFM1 has affinity to casein of milk. Therefore, they defined a factor named “Enrichment Factor” (EF) for cheeses. Further surveys should be done to find as for cheese manufacture influences on AFM1 distribution. Some tests have been carried out on several kinds of cheeses as to overall stability of AFM1 during ripening and storage, reported that the concentrations of AFM1 in Camembert cheese were higher at the beginning than at the later time of ripening. These results were in agreement with studies by **Govaris *et al.*, (2001)**. Such results however, conflict with reports of earlier studies that indicate different behaviour of AFM1 in various other types of cheeses. Thus, in Camembert and Tilsit Cheddar and Brick cheeses stored for 3, 14 and 6.5 months, respectively, the concentration of the toxin increased during the early stage of their ripening to decrease thereafter to reach about its initial concentration at the beginning of ripening. On the other hand, the concentration of AFM1 in Parmesan cheese started high at the beginning of the ripening period, decreased until about the fifth month and then slowly increased up to the tenth month of storage. In contrast, the AFM1 content of Mozzarella remained almost constant during storage of 4.5 months. These different

profiles of AFM1 in various cheese products may be the result of several factors such as heat treatment, proteolysis, exposure of contaminated milk to light, and especially to an inadequate method of analysis, (**Mohammadi, 2011**). Several investigations on the partitioning of AFM1 during cheese production starting with different milk contamination levels reported a wide range of distribution of AFM1 between whey and curd. On the other hand **Kaniou-Grigoriadou *et al.*, (2005)** observed that enrichment factor in the production of Feta cheese made from naturally contaminated milk ranged between 4.3 and 5.6. Meanwhile, **Kamakar *et al.*, (2008)** showed that the mean concentration of toxin in curd and cheese was 3.12 and 3.65-fold more than that in whey and 1.68 and 1.80 fold more than that in cheese milk, respectively. Neither ultra-filtration, nor acidic or enzymatic treatments were able to influence the toxin's interaction with casein or whey proteins. Only the combined action of heat and low pH (as used in ricotta cheese production) was able to denature whey proteins to a point where they lost their AFM1-binding capacity. As regards the contamination level, several authors, found a maximum contamination level over 1000 ng of AFM1 per kg. This latter contamination level could be hazardous, (**Fallah, 2010**).

#### **4.3. AFM1 in yogurt**

Several studies have been conducted regarding the effect of yogurt manufacturing on AFM1 content. Some authors reported no influence on Aflatoxin M1 content. In contrast, **Bakirci, (2001)** detected variable increases of AFM1 content in yogurt related to the milk. The effect of fermentation was assessed by **Govaris *et al.*, (2002)**. They reported that AFM1 levels in all yoghurt samples showed a significant decrease from those initially present in milk. This decrease in AFM1 was attributed to factors such as low pH, formation of organic acids or other fermentation by-products, or even to the presence of lactic acid bacteria. The low pH during fermentation alters the structure of milk proteins such as the caseins leading to formation of yoghurt coagulum. The change in casein structure during yoghurt production may affect the association of AFM1 with this protein, causing adsorption or occlusion of the toxin in the precipitate, during refrigerated storage, AFM1 was rather more stable in the yoghurts with pH 4.6 than with pH 4.0. The percentage loss of the initial amount of AFM1 in milk was estimated at about 13 and 22% by the end of the fermentation, and 16 and 34% by the end of storage for yoghurts with pHs 4.6 and 4.0, respectively

(Govaris *et al.*, 2002). Unlike cheese and milk samples, the presence of AFM1 in yogurt has not frequently been studied. Thus, more investigations are needed because:

1. Currently, human consumption of yogurt has greatly increased
2. There are contradictory data on AFM1 stability over manufacture and storage in the literature
3. The presence of Aflatoxins in yogurt could reduce the nutritional values of its consumption.

#### **4.4. AFM1 in other milk products**

Many other milk products such as cream, butter, ice cream may contain AFM1. The presence of AFM1 in these products has rarely been investigated and could be of interesting aspects for study. Some surveys conducted on the occurrence of AFM1 in milk products are reported. In a study by Bakirci, (2001), the levels of AFM1 in the products made from contaminated milk namely butter, butter milk, cream, skim milk was investigated. The mean AFM1 level found in cream samples was 64.4% of AFM1 concentration of bulk-tank milk. Whereas, mean AFM1 level of skim milks was 3% higher than those of bulk-tank milk. Levels of AFM1 in butter samples in the study were less, and they were as 33.80% of AFM1 amounts of bulk-tank milk. Mean AFM1 levels obtained from buttermilk samples were similar to those of bulk-tank milk (mean 83% of it). During butter processing, protein membrane around fat globules is broken down and serum phase is separated. Due to the chemical structure of AFM1 and its affinity to casein, it adsorbs on this fraction of protein, therefore, cream contained less AFM1 than milk, and butter contained less amount of AFM1 than cream. As a result of the associate effects of these factors, AFM1 concentration occurs in lipid phase (like butter and cream) less than serum phase and protein fraction, (Mohammadi, 2011).

AFM1 is frequently observed in the Aflatoxin exposed individuals and in the breast milk. AFM1 toxicity in this respect is important as it is known that within Aflatoxin exposed nursing mothers it can provide a source of Aflatoxin exposure to the infant. The occurrence of AFM1 in breast milk has been investigated in some regions. There is increased awareness of the link between growth and health of the fetus and infant, and disease risk in later life. Long term pre and postnatal exposure to Aflatoxins could be one of the factors contributing to growth faltering and/or the early onset of hepatocellular carcinoma (HCC) in countries with a high incidence of the disease.

Additionally, the presence of other Aflatoxins, B1, B2, G1, G2 and M2, has also been reported in breast milk. The identification and understanding of factors determining the presence of toxicants in human milk is important and may provide a strong basis for controlling the transfer of chemicals to the infants through breast milk, (Mohammadi, 2011).

## **5. How to manage Aflatoxins?**

### **(Legislation - Controls - Good practices) :**

AFM1 is relatively stable in raw and processed milk products, pasteurization, sterilization and ultra-high-temperature (UHT) treatment or processing result in negligible destruction of AFM1. It has been reported that AFM1 was a resistant to thermal inactivation during food processing for procedures such as pasteurization and autoclaving. However, frequent analytical surveillance by food control agencies is highly recommended to control the incidence of Mycotoxins contamination, especially in dairy products. Implementing a food control system, such as the Hazard Analysis and Critical Control Point (HACCP) system in the food industries, suggest an efficient means for limiting Mycotoxins contamination in the Saudi's food supply. The most effective way of controlling Aflatoxin M1 in food supply is to reduce contamination of raw material and supplementary feedstuffs for dairy cattle with Aflatoxin B1. Specific regulation exist in many countries, and practical programs are being developed as the Codex Committee on Food Additives and Contaminants has developed, a code of practice for reducing Aflatoxin B1 in raw materials. Reduction can be achieved by good manufacturing practices and good storage practices.

### **Legislation: Maximum levels of Aflatoxins:**

The European Union Commission regulations (EC) standardized the maximum level of AFM1 in infant milk to be under 25 ng/l (EC, 2006).

### **Controls**

Recalled food products are subsequently sampled and tested for Aflatoxins.

### **Good practices**

Chemical methods of detoxification are practiced as a major strategy for effective detoxification most are impractical or potentially unsafe because of the formation of toxic residues or the perturbation of nutrient content and the organoleptic properties of the product. Two chemical approaches to the detoxification of Aflatoxins: are ammoniation and reaction with sodium bisulfite.

### **Good practices**

The key to preventing storage mold problems is detecting them early, in the field and in the bin. Applying HACCP to prevent Mycotoxins contamination, and Good Practices can reduce Aflatoxins production in grain:

1. Control insects in the field.
2. Scout.
3. Adjust the combine to minimize grain damage. Fungi infect damaged grains more easily than intact ones.
4. Clean bins and grain-handling equipment and remove fines from the grain before storing. Old grain residue is frequently a source of contamination.
5. After the harvest, clean grain can be kept at very low moisture during the winter.
6. Cool grain after drying.
7. Control storage insects.
8. Check grain every 2 weeks in storage
9. Antifungal agents can be applied to grain.
10. Also, AFM1 can be reduced through feed decontamination using chemical, physical or biological treatments. Also, using of non-nutritionally inert adsorbents can sequester the Aflatoxins and reduce the absorption of toxins from the intestinal tract.

### **6. Conclusions:**

In conclusion, these studies has shown the serious risk for public health since all age groups, including infants and children, consume milk and dairy products worldwide. For this reason, milk and milk products have to be controlled continuously by accurate and reliable analytical techniques for presence of AFM1 contamination. It is also extremely important to maintain low levels of AFM1 in the feeds of dairy animals. In order to achieve this, dairy cow feds should be kept away from contamination as much as possible. Therefore, animal feeds should be checked regularly for Aflatoxin and, particularly important, storage conditions of feeds must be strictly controlled. At present, since it considers that there is not enough information to establish a reasonable exposure level, The World Health Organization (WHO) recommends the reduction of AFM1 consumption to a minimum so as to minimize AFM1 potential risks. The regulatory limits are widely variable and there has been little scientific basis in their setting. Efforts should be made in attempting to provide further and extensive scientific information on human health hazards related

to low-level long term Aflatoxins exposure and to standardize the already existing regulatory limits for Aflatoxins. Future studies should verify the effect of milk storage and processing on AFM1 occurrence to avoid actual uncertainty. However, since it is generally assumed that neither storage nor processing determine reduction of AFM1 content, further information on possible AFM1 concentration following milk processing should be furnished. The occurrence of AFM1 in cow milk and milk products is widespread and the occurrence of Aflatoxin and their metabolites in human breast milk is of great concern. Since serious health hazards to the mother, fetus, and infant could occur. Therefore extensive and periodic surveys should be performed. Additionally, the incidence and occurrence of AFM1 in dried milk infant formula should be more investigated.

## **7. Recommendations:**

The above data showed that the potential hazard associated with Aflatoxins in food has been serious. The risks posed to health can be further lowered by reduced exposure. To minimize the risk of Aflatoxins exposure, close tripartite cooperation among the trade, the public and the government is essential. The followings are some recommended risk reduction measure for the trade and the consumers.

### **Advice To Trade**

The prime responsibility to ensure the wholesomeness of the foods lies with the trade. They are advised to adopt the Good Manufacturing Practice (GMP) and integrate it with HACCP based safety programme. The following measures are useful:

- (a) Obtain raw materials from reliable and reputable suppliers.
- (b) Verify the specifications for quality product.
- (e) Decontamination process for reduction of Aflatoxin level.
- (c) Maintain good storage conditions: -dry and cool environment and -stock rotation should be on a first-in first out basis.
- (d) Keep documentation well in place.

### **Advice To Consumers**

Consumers are advised to take the following measures to reduce the risk of Aflatoxins exposure.

### **Upon Purchase**

- (a) Purchase from reliable and reputable retailers.
- (b) Observe whether foods are stored in ventilated cool condition.

(b) Reject any unclean, opened or damaged package.

### **Storage**

(a) Maintain at dry and cool environment (temperature preferably below 20°C and relative humidity below 80%).

(b) Avoid direct sunlight.

(c) Watch out the durability of the products.

(d) Avoid stocking up excessive foods Consumption.

(a) Consume foods within the designated "best before date".

(b) Discard any foods that look mouldy, damped, shriveled and discoloured.

Finally, Milk that is sold commercially must be checked for Aflatoxin M1. When Aflatoxin M1 is found at concentrations of 0.5 parts per billion (ppb) or greater, the milk is discarded because it cannot be used for products that go in to the human food supply. If the level of milk contamination exceeds 0.5 ppb on a second test, a special dietary chemisorbent should be added to the diet at recommended levels. These compounds include clays (bentonites) at 1 percent of the diet, activated carbon at 1 percent of the diet and glucomannan (Mycosorb®) at 0.05 percent of the diet on a dry matter basis. Limited data is available on the numerous compounds that are available to absorb the Aflatoxins in the digestive system. However, in one study, about 1/4 pound of hydrated sodium calcium aluminosilicate (HACA—a compound approved for feed as an anti caking agent) was shown to reduce Aflatoxin M1 in milk about 50 percent when cattle consumed feed containing 200 ppb Aflatoxins.

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### سموم الأفلاتوكسينات م1 و م2 في منتجات الألبان

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#### الملخص العربي :

يشق مصطلح السموم الفطرية من الكلمة اليونانية (ميكوس) بمعنى الأعفان، ومن الكلمة اللاتينية (توكسين) وهو ما يعني السم . السموم الفطرية هي مركبات ذات وزن جزيئي منخفض نسبياً تنتجها بعض الفطريات وتضر الحيوانات والإنسان. السموم الفطرية مثل الأفلاتوكسينات ب1، ب2، ج1، ج2، م1 و م2 وأيضاً الأوكراتوكسينات والزيرالينون هي مركبات أيضاً ثانوية سامة تنتجها الفطريات المختلفة مثل الأسبرجلس، البنسليوم والفيوزاريوم والتي تصيب مجموعة واسعة من المنتجات الزراعية المخصصة للإستهلاك الآدمي والعلف الحيواني. إن السموم الفطرية الموجودة في المنتجات الغذائية والأعلاف الحيوانية هي مشكلة هامة في الأغذية وسلامة الأعلاف وتسبب خسائر اقتصادية كبيرة بالإضافة لتأثيرها على صحة الإنسان والحيوان. والسموم الفطرية التي تلوث الأغذية والأعلاف لا تزال تمثل مشكلة في جميع أنحاء العالم، وتقدر منظمة الأغذية والزراعة التابع للأمم المتحدة (الفاو) أن ما يقارب 25 ٪ من المحاصيل الغذائية في العالم ملوثة بشكل كبير بالسموم الفطرية. الأفلاتوكسين ب1، هو سم يصيب الكبد وهو الأقوى وله مجموعة كبيرة ومتنوعة من الآثار البيولوجية، مثل السرطنة و التسبب بطفرات وراثية في البشر وحيوانات المزرعة وتم تصنيفه في المجموعة ب1 من قبل الوكالة الدولية لبحوث السرطان. الأفلاتوكسين م1 والأفلاتوكسين م2 هي النواتج الهيدروكسيلية للسم الكبدى من الأفلاتوكسين ب1 و ب2، على التوالي . ولقد تم العثور على الأفلاتوكسين م1 في الحليب و منتجات الألبان التي تم الحصول عليها من الماشية التي تناولت الأعلاف الملوثة بسموم الأفلاتوكسين ب1. ولقد وجد أن إمكانية التسبب بالسرطان من الأفلاتوكسين م1 هي أقل بحوالي عشر مرات من الأفلاتوكسين ب1، و لهذا السبب فقد أدرج في الفئة ب2 من قبل الوكالة الدولية لبحوث السرطان. وكما وجد أنه في حيوانات الحليب يكون معدل التحويل من الأفلاتوكسين ب1 إلى الأفلاتوكسين م1 يتراوح بين 0.5 و 6 ٪. ويرجع ذلك إلى عوامل مختلفة مثل الاستجابة الفردية، وكمية الأفلاتوكسين ب1 المتناولة، ومرحلة إنتاج الحليب وكذلك نظام

التمثيل أو الميثابولزم. ولقد ذكر العديد من الباحثين أن هناك علاقة خطية بين كمية الأفلاتوكسين م 1 في الحليب و كمية الأفلاتوكسين ب 1 في العلف الذي تستهلكه الأبقار الحلوب.

إن الأفلاتوكسين م 1 في الحليب و منتجات الألبان يشكل مخاطر صحية على صحة الإنسان. حيث لا يتم تحطيم مثل هذه المركبات أثناء عملية البسترة والمعاملات الحرارية الأخرى. كما إنه قد ينتج تلوث الأفلاتوكسين في الحليب و منتجاته بطريقتين. إما بمرور السموم إلى الحليب عند تناول الأعلاف الملوثة بسموم الأفلاتوكسين، أو أنه قد يحدث التلوث بالفطريات في المراحل اللاحقة من تصنيع وتدوال الحليب ومنتجات الألبان. وكما في السموم الفطرية الأخرى، يمكن الكشف عن سموم الأفلاتوكسينات م 1 وم 2 باستخدام أجهزة الكروماتوجرافي أو تقنية الإليزا. وإن حدود المعايير والمواصفات في العديد من البلدان لوجود الأفلاتوكسين م 1 وم 2 هي بين 0.5-0 جزء في المليون، في الحليب و منتجات الألبان الأخرى. وفي بعض الدول الأوروبية وهيئة الدستور الغذائي التي تفرض أقصى مستوى من الأفلاتوكسين م 1 في الحليب السائل و منتجات الحليب المجفف أو المعامل بالتصنيع يجب أن لا تتجاوز 50 نانوجرام / كجم. ولهذا، فإننا في هذه الورقة العلمية نريد تسليط الضوء على هذه السموم الفطرية الخطيرة في منتجات الألبان في بلادنا من خلال الإستعراض لكافة المعلومات والبيانات التي تتوفر في المراجع العلمية.

**الكلمات المفتاحية :** السموم الفطرية – سموم الأفلاتوكسينات م 1 و م 2 – منتجات الألبان .