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# Detection of Mold and Aflatoxin B1 in Mayonnaise Product from Egyptian Markets by HPLC

**Neveen S. M. Soliman[1*], Fatma H. Amro[2], Alaa A. algabaly[3], Ayah B. Abdelsalam[1]**
[1]Food Hygiene Department, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
[2]Food Hygiene Department, Animal Health Research Institute, Agriculture Research Center, Giza 12619, Egypt
3Microbiology Department, Central Laboratory of Residue Analysis of Pesticides and Heavy Metals in Food, Agriculture Research center, Dokki, Giza, Egypt
***Corresponding Author:** Neveen S. M. Soliman, **E-Mail:** neveen.soliman@vet.cu.edu.eg

## ABSTRACT

Egyptian consumers' demand and preference for sauces like mayonnaise have increased lately. The processing and packaging techniques of such products may safeguard the consumer from bacterial hazards, although mold and/or mycotoxins are still expected hazards in such products. Therefore, the current investigation is intended to determine the incidence of mold and aflatoxin B1 in commercial mayonnaise sold in Egyptian markets. A total of thirty mayonnaise samples were arbitrarily gathered from Cairo and Giza governorates in order to determine the presence of mold using cultivation techniques and AFB1 using a low-cost high-recovery fluorescence detector (FLD) in combination with an easy-to-use, highly specific and specially developed High-Pressure Liquid Chromatography (HPLC) assay that adhered to green chemistry principles. About 53.33% of the examined samples were positive for AFB1, while mold couldn't be detected in any of the examined samples. It was also discovered that 43.33% of AFB1 in total samples was below the maximum permitted threshold. Therefore, more attention is required from the authorities for continuous examination of such products that are present in the market for the incidence of chemical contamination with aflatoxin.

***Keywords:*** Aflatoxin B1, Chemical hazard, Mayonnaise, Mold.

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## INTRODUCTION

Mayonnaise is an oil-in-water emulsion with a thick texture and a rich flavor prepared mainly from a mixture of egg yolk, vegetable oil, vinegar and mustard with the addition of salt and sugar that is formulated to develop special characteristics like mouthfeel and spreadability desirable by the consumer. Recently, there has been an increase in demand for it in markets **(Chivero *et al*., 2016; Alvarez-Sabatel *et al*., 2018)**.

Mayonnaise is prone to microbiological spoilage caused by aciduric microorganisms, such as molds like Geotrichum and Aspergillus spp., yeasts like Saccharomyces spp., and various species of Lactobacillus and Bacillus. (**Ray and Bhunia, 2013**; **Teneva *et al.*, 2021)**. During the storage of mayonnaise, factors such as pH value, acid type, storage duration and temperature play a significant role in reducing its stability and increasing the risk of spoilage (**Yolmeh *et al.*, 2014; Mirzanajafi-Zanjani *et al.*, 2019)**.

Throughout the different stages of the food chain, including pre- and post-harvest, processing and storage, filamentous fungi play a role as food contaminants, leading to food spoilage **(Sadiq *et al.*, 2019)**. In developing countries, spoilage of foods by fungi is a major problem **(Asiye, 2019).** Their presence and growth on food can reduce the quantity and quality of food **(Sanchez *et al.*, 2005; Razaghi-Abyaneh et al., 2006)**. It is also a risk to human health because some species of fungi are mycotoxins (**Alla, 1997**; **Kabak *et al.*, 2006)**.

Around the globe, various types of fungi, specifically Aspergillus, Penicillium, and Fusarium, produce mycotoxins that can be present in both food and animal feed **(Bibani *et al.*, 2019; Ismael *et al.*, 2022).** The mycotoxins commonly known as aflatoxins include AFB1, AFB2, AFG1, and AFG2. Ingesting food that is contaminated with these toxins can lead to a range of harmful effects, such as cytotoxicity, genotoxicity, nephrotoxicity, reproductive disorders, teratogenicity, hepatotoxicity, immune toxicity and carcinogenicity

(**Eaton and Gallagher, 1994; Lee *et al.*, 2004; Cimbalo *et al.*, 2020; de Souza *et al*., 2021)**. In addition, the detrimental impact of mycotoxins on oxidative stress is evident in their disruption of the neuroimmune response as well as the body's translation and transcription mechanisms. Epidemiological studies have indicated a correlation between regions with elevated levels of aflatoxin and a heightened prevalence of liver cancer (**Da Silva *et al*., 2018)**.

As stated by FAO (the Food and Agriculture Organization), about 25% of food production contains at least one mycotoxin (**CAST, 1989)**. Mycotoxins can come into direct contact with humans through the consumption of plant-derived foods contaminated with mycotoxigenic fungi.

Additionally, humans can also indirectly encounter mycotoxins by consuming animal-derived products from animals that have consumed rations containing mycotoxins **(Zain, 2011)**. Mycotoxins pose a significant global threat due to their ability to remain stable and toxic even when exposed to various chemical and physical treatments (**Alshannaq and Yu, 2017)**. Recently, there have been about 500 species of mycotoxins, and another 1000 have been discovered yet. Masked mycotoxin poses a great risk because of a lack of routine methods for their determination (**Berthiller *et al.*, 2016)**.

The Aspergillus genus is primarily responsible for the production of aflatoxins, which are secondary compounds known for their teratogenic, mutagenic, hepatic, immunosuppressive, and carcinogenic effects **(Yaling *et al.*, 2008**; **Morteza *et al*., 2013)**. As stated by the International Agency for Research on Cancer (**IARC, 2002)**, Aflatoxin B1 (AFB1) is a dangerous mycotoxins found in Group 1 carcinogens. In general, AFB1 contaminates feeds that contaminate animals and animal products. Ingestion of contaminated food causes human infection (**Qi *et al*., 2019)**. AFB1 intoxications depend on the level of ingestion dose and exposure time. Acute and chronic aflatoxicosis has been reported in many studies in humans and animals **(Pleadin *et al.*, 2019)**.

Mycotoxins contamination occurs mainly in hen feeds that include maize and other cereals **(Thirumala-devi *et al.*, 2002; Jang *et al.*, 2007a, 2007b;** and **Greco *et al.*, 2014)**. Aflatoxins (AFs), zearalenone (ZEA), fumonisins, and ochratoxin (OTA) are the most frequently identified mycotoxins found in eggs **(Greco *et al.*, 2014; Iqbal *et al.*, 2014;** and **Jia *et al.*, 2016)**.

The most widely used traditional methodology for determining the levels of aflatoxins in feed and food is the HPLC method. It is an accurate and specific quantitative method for mycotoxin level determination in contaminated feed and food based on the physical and chemical features of the mycotoxins. Several studies provide clarification on the analytical and genetic processes (**Hassan *et al*., 2015)**. There is little information on the presence of AFs in mayonnaise (**Iqbal *et al*., 2014)**. The purpose of the current investigation was to detect mold contamination in commercial mayonnaise and the degree of AFB1 presence that could pose a risk to public health in order to provide safe goods that are fit for human consumption.

## MATERIALS AND METHODS

### Samples collection

Thirty commercial mayonnaise samples representing different brands were randomly collected from markets in Cairo and Giza governorates, Egypt. The samples were stored at 4 °C for analysis.

### Mycological examination

Samples were examined for the detection of mold incidence using Dg18 media (Dichloran 18 Glycerol Agar) (oxoid). Ten-fold serial dilutions were done, and 0.1 ml of each dilution was spread on DG-18 agar plates and incubated at 25 oC for 5 days, according to **ISO (2008).**

### Investigation of mycotoxins

HPLC was used in combination with FLD to analyze different samples in order to assess the presence of aflatoxin (AFB1), according to the AOAC the AOAC (11995).

### Chemicals

Sigma-Aldrich, Steinhaus, and Merck Germany provided the ascertained references for aflatoxin B1 in acetonitrile solution (3µg/ml), as well as Nacl, Pb $(CH_3Coo)_2$, acetic acid, acetone, diatomaceous earth, peteroleum ether, dichloromethane, methanol, acetonitrile chromatography ultrapure grade, nitric acid, and trifluoroethanoic acid (TFA). For the saturated Nacl solution, 100 milliliters of deionized water were used to dissolve 40 grams of Nacl.

200 g of Pb $(CH_3Coo)_2$*3 $H_2O$ and 1 l of deionized water were used to make Pb $(CH_3Coo)_2$. The mixture was heated until the salt was dissolved, at which point 3 ml of acetic acid was added.

By diluting 1.4 ml of nitric acid (65%) with 5 ml of deionized water, 4 M of nitric acid was created.

R-Biopharm Rhône Ltd., UK, is the supplier of immunoaffinity cartridges (IAC) for the clean-up process (AFLAPREP® and OCHRAPREP®). Methyl cyanide (MeCN), deionized water (DW) from a Milli-Q system (Millipore, Mosheim, France), and MeCN, MeOH, and DW (20:20:60) made up the mobile phase (Iqbal *et al.*, 2014).

### Sample extraction

With minor modifications, the extraction processes were carried out in accordance with (Iqbal *et al*., 2014). Prior to LC injection, the extraction was completed in three stages: derivatization, purification, and sample preparation.

- Preparation: A 3 g sample was centrifuged at 3000 rpm for 2 minutes at ambient temperature after being homogenized for 10 minutes with 0.3 g sodium chloride and 10 mL of MeCN:DW (45:55). For the extraction of AFs, 2 mL of the filtrate was mixed with 2 mL of DW.
- Purification: The sample was poured slowly through a particular immunoaffinity cartridge at a flow rate of one drop per second, and then it was sprayed with one milliliter of water at the same flow rate. Elusion in 1 milliliter of MeOH. Finally, under a nitrogen stream at 40 °C, the elute evaporated.
- Derivatization: Following drying, 100 µl of TFA was added to AFB1 vials, which were then sealed and left in a dark, ambient-temperature environment for 15 minutes. Next, fill the vials with 500 µl of the MeCN:DW (1:9) mixture.

### Chromatography Separation

An HPLC system (Agilent, 1200, USA) was injected with 40 µl. The isocratic Mph flow rate was 1 milliliter per minute. The mycotoxins were separated using an Agilent 1200 C18 ODS column (250 mm, 4.6 mm i.d., 5 µm particle size) in reverse phase at 40 °C with a FLD (Japan). There were 360 nm excitation wave lengths and 425 nm emission wave lengths.

## RESULTS

### 1. Mycological results

It was revealed that mold couldn't be detected in all examined mayonnaise samples.

### 2. Mycotoxin B1 detection:

Data presented in **table 1** showed that the prevalence of mycotoxin AFB1 residues in the tested Mayonnaise samples were high, as it was found in 53.33% of the examined samples with concentrations ranged from 0.0895 to 2.321µg/kg, and a mean value of 1.0627 ± 0.804 µg/kg.

Table 1: Prevalence of AFB1 (µg/kg) in mayonnaise samples:

| Total No. of samples | No. of positive samples | % of positive samples | Minimum | Maximum | Mean ± SEM |
|---|---|---|---|---|---|
| 30 | 16 | 53.33% | 0.0895 | 2.321 | 1.0627 ± 0.804 |

Our results revealed that 43.3% of AFB1 from total samples were found to be below the maximum permitted threshold, as in **(table, 2)** showed that 3 out of 16 positive samples were above maximum permitted threshold (unacceptable) ** EU MRL 2 ppb for AFB1 **(EC 1881/2006)**.

Table 2: Frequency distribution of AFB1 in positive samples:

| Range | No. | % | Acceptability level |
|---|---|---|---|
| <0.3 | 3 | 18.75 | acceptable |
| 0.3- >0.8 | 5 | 31.25 | acceptable |
| 0.8- >1.2 | 2 | 12.5 | acceptable |
| 1.2- >1.7 | 1 | 6.25 | acceptable |
| 1.7->2 | 2 | 12.5 | acceptable |
| >2 | 3 | 18.75 | unacceptable |
| Total | 16 | 100 | |

From total examined samples 43.3% AFB1 was found to be below the highest permitted level **(Fig., 5)**. Caliberation plots for AFB1 (**Fig., 2). chromatogram of** aflatoxin b1**(Fig.,1,3,4).**

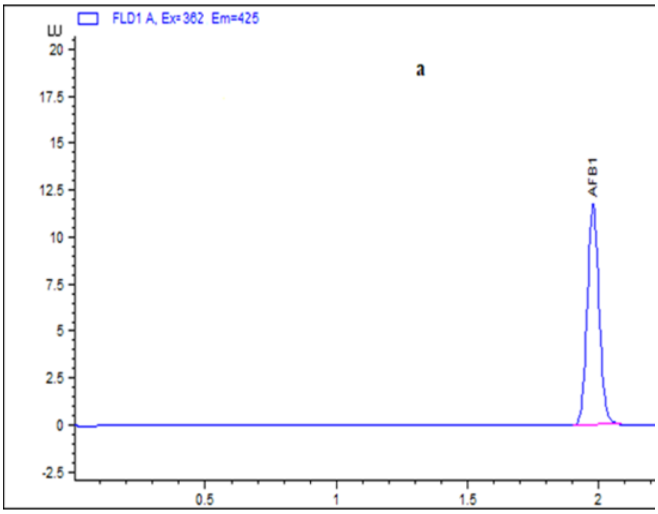


**Fig. 1:** chromatogram of aflatoxin b1 standard at concenteration 0.15µg/kg.

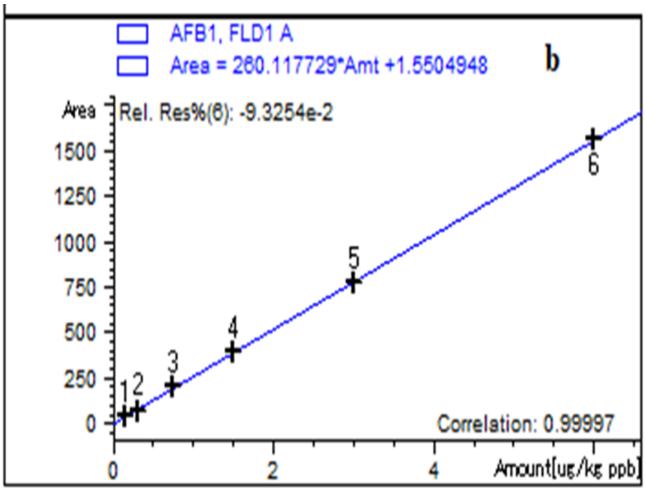


**Fig. 2:** Caliberation plots for AFB1

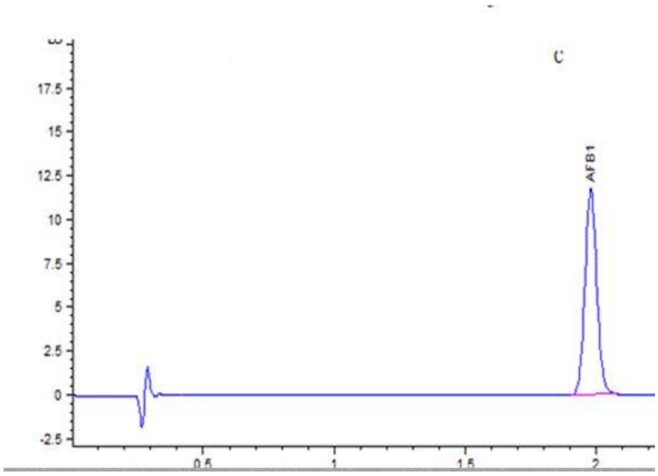


**Fig. 3:** Chromatograms of aflatoxins at a concentration of (0.15 µg/kg) in blank.

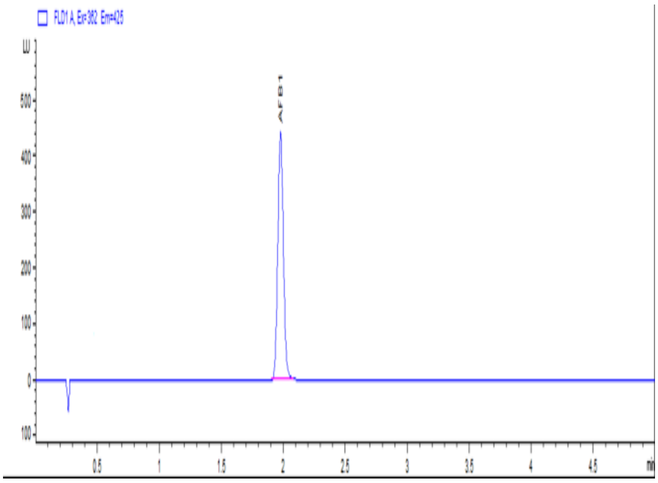


**Fig. 4:** Chromatogram of mayonnaise sample with 2 µg/kg

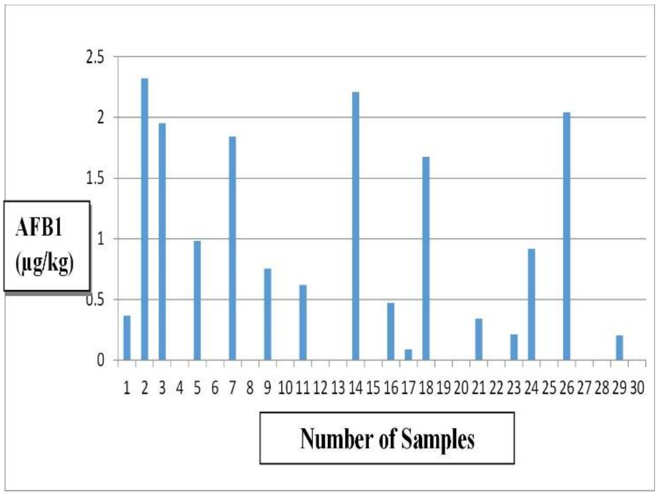


**Fig 5:** Amount of AFB1 (µg/kg) in positive mayonnaise samples

## DISCUSSION

### Mycological results

In our study, mold couldn't be detected in all examined mayonnaise samples. These results could be attributed to that: food manufacturers use the labels to ensure the use of natural ingredients with no chemical additives in the formulation of their products in order to gain customer trust. Depending on their homogenization process, these products produce quality, stability and viscosity **(Aganovic *et al.*, 2018).** Preservatives used in many foods and drinks are benzoic acid (E210), sorbic acid (E200), and their salts **(Mischek and Krapfenbauer-Cermak, 2012; Piper and Piper, 2017).** In commercial mayonnaise production, the risk of pathogenic contamination can be reduced through the use of pasteurized eggs and the incorporation of acidic ingredients, which are the two primary factors contributing to this decrease **(Muhialdin *et al.*, 2021 ; Ozdemir *et al.*, 2021).**

According to Ferial *et al*. (2008, the presence of mold and yeast in mayonnaise samples was not detected during the initial 5 weeks of storage. However, as time progressed, the detection of these microorganisms increased, with the highest counts observed at 20 weeks. It was found that pasteurized mayonnaise had a lower rate of mold growth compared to unpasteurized mayonnaise. Additionally, mayonnaise made from ostrich eggs had lower contamination levels than those made from chicken eggs. After 20 weeks of storage, the counts of mold and yeast in pasteurized mayonnaise made from ostrich and chicken eggs were 1.1 x $10^2$ and 2.1 x $10^2$, respectively. In unpasteurized mayonnaise, the corresponding values were 1.7 x $10^2$ and 2.9 x $10^2$, respectively. On the other side, **Paivaa *et al.*, (2023)** found that the collected samples of mayonnaise and acai were contaminated with molds and yeasts above the established limit of $10^3$ CFU/g.

### Concentrations of mycotoxins

Contamination of the food supply by aflatoxin can occur in two ways: either directly through the growth of mold on the food or indirectly through the use of contaminated ingredients in processed food or the feeding of moldy feed to animals. The indirect contamination of food is particularly concerning in regions where food undergoes extensive processing **(Bahagt *et al.*, 1999).** The contamination of food and feedstuffs with aflatoxins (AFTs) poses a significant health challenge for both humans and animals in developing nations **(Silvia, 2007; Ghazvini *et al.*, 2016).**

The presence of mycotoxigenic molds in food poses a significant threat to public health and the economy, making it a pressing issue. **(Dalié *et al.*, 2010).** Foods such as meat, milk, eggs, vegetables, fruits, cereals, and their derived products are susceptible to contamination by mycotoxins **(Capriotti *et al.*, 2012; Mir *et al.*, 2021).** In addition, the majority of mycotoxins exhibit resistance to heat and physicochemical treatments commonly used in food processing, including cooking, baking, boiling, roasting, frying, and pasteurization **(Alizadeh *et al.*, 2020).** Thus, they cannot be removed from food by conventional techniques **(Akhila *et al.*, 2021).**

The main ingredient formulations used in mayonnaise production are egg yolk or whole egg, oil, mustard, vinegar, sugar, and salt **(Fialova *et al.*, 2008).** Aflatoxin presence in eggs has an influence on consumer health, especially in children, who are more susceptible than adults. The occurrence and quantities of mycotoxins in eggs vary depending on the type of fungus and environmental factors such as moisture, temperature, and oxygen presence **(Frenich *et al.*, 2011).** The feeding ratio of birds affects the kind and amount of mycotoxin in the eggs. The most important elements in chicken diets are grains and legumes, which determine the concentration level of mycotoxin contamination **(Adegbeye *et al*., 2020).**

In this aspect, Herzallah 2009 found that AFs had an average of 1.23 µg/kg in contaminated eggs. As stated by **Iqbal *et al.*, (2014),** 28 out of 80 eggs that were collected from Pakistan had AFs. Also, **Shehata *et al.*, (2014**) stated that AFT residues were detected in 16.6%, 20% and 30% of brown farm eggs, white farm eggs and baladi eggs, respectively, with an average level of 6.7 µg/kg in baladi eggs, 3.2 µg/kg in farm brown eggs and 4.34 µg/kg in farm white eggs. In the **Amirkhizi *et al.*, (2015**) study, 58% of Iranian egg samples were found to contain AFB1 at an average value of 0.30 µg/kg.

The inclusion of edible vegetable oils in our diet is crucial for obtaining necessary energy and essential fatty acids, as well as serving as carriers for fat-soluble vitamins. Palm, soybean, olive, sunflower, corn, and rapeseed are significant contributors to the production of edible oils **(Hashemi *et al.*, 2017).** Throughout the pre- and post-harvest periods, oilseeds and fruits are at risk of being contaminated by a multitude of molds that produce toxins **(Fernández-Cruz *et al.*, 2010; Bhat and Reddy, 2017).** When fungal-infected oil seeds are extracted, mycotoxins are transferred to vegetable oils. These mycotoxins, including aflatoxin B1 (AFB1), ochratoxin A, fumonisin B1 (FB1), trichothecenes, zearalenone (ZEA), and citrinin (CTN), are contaminants found in edible oils **(Bao *et al.*, 2010).**

Given the unavoidable presence of aflatoxins in animal diets, it becomes imperative to have protection against aflatoxicosis, making the inclusion of microorganisms in the diet capable of removing AFB1 the most suitable alternative **(Romina *et al.*, 2011).**

To mitigate the significant health risks posed to humans, the content of mycotoxins in foods is strictly regulated through the implementation of maximum permissible limits **(Claeys *et al.*, 2020).** Various international organizations, including the World Health Organization (WHO), Food and Agriculture Organization (FAO), Codex Alimentarius Commission (CODEX), and EU Commission, have established regulations regarding different mycotoxins in various food products to ensure consumer safety **(Adeyeye, 2016).** Even though in our study, from the total examined samples, 43.3% of AFB1 was found to be below the highest permitted level.

## CONCLUSION

Exposure to AFB1 increased the risk of health issues in both adults and children, according to the health risk assessment. By increasing the consumption of mayonnaise in Egypt and the potential risks of mycotoxin exposure, especially for children. It is advised to put control and monitoring mechanisms in place to lower the amount of mycotoxins that may be present. Furthermore, the presence of toxigenic mold strains did not correlate with mycotoxin contamination in the samples that were analyzed. This clearly indicates that the aflatoxin found in the sample was not generated during the processing stage but rather existed prior to processing as a residual level originating from the ingredients.

### Conflicts of interest

There are no conflicts of interest, according to the authors.

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