



Detection of aflatoxins, ochratoxins and some chemical adulterants in raw Milk

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ABSTRACT

Milk is essential for the nourishment of children and adult life providing their daily food requirements. Aflatoxins are mycotoxins have been produced by some species of *Aspergillus*. Ingested Aflatoxins B1 (AFB1) are metabolised into carcinogenic Aflatoxins M1 (AFM1) which are eliminated through milk. Also, seller and producer add chemical substances (adulterants) to milk to increase its shelf life. Adulteration is defined as removal or replacement of milk components and an addition of substances without a consumer's knowledge which is banned. The presence of mycotoxins or chemical adulterants has serious health risk. The present study evaluated 60 samples of cow's raw milk in El- Minufia governorate for the presence of aflatoxins, ochratoxins and some chemical adulterants. Also, its chemical composition (fat, S.N.F and protein). The result indicated that 16.7%, 8.33% of tested samples contained aflatoxins M1 and aflatoxins M2 respectively. All the tested samples were free from ochratoxins. Also, 88.33% of collected milk samples contained different chemical adulterants; inhibitory substances (70%), formalin (41.67%), water (37.5%), hydrogen peroxide (20%), boric acid (16.70%), carbonate and bicarbonate (8.30%), nitrate (5%). Moreover, 50% and 54.17% of milk samples were less than the legal requirement for fat and S.N.F respectively; then protein was decreased in 41.67% of samples. The present study recommended to monitor the marketing of milk by instructions and rules, which include the standards of the sold milk and to control the milk quality to be safe for the consumer.

Keywords: milk, aflatoxins, ochratoxins, chemical adulterants, Minufia.

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1. INTRODUCTION

Milk as a natural product is a complete food and can supply infants and adult with the daily requirement of nutrition. Mycotoxins are toxic substances produced by moulds that growing on agricultural products; some have mutagenic or carcinogenic effects, others are toxic for specific organs, and others have health risks (Bezerra da Rocha et al., 2014). Aflatoxin, ochratoxin, fumonisin, T-2 toxin, vomitoxin, and zearalenone are mycotoxins that have the most attention by industry and academic research (Chi and Broomhead, 2009). Aflatoxins produced by some species of *Aspergillus* that have hepatotoxic, mutagenic and carcinogenic effect (Bennett and Klich, 2003). Dairy cows are sensitive to aflatoxin as young animals; not for the toxicity of AFB1 to the cow but because of the resulting AFM1 in the milk as AFB1 is metabolised into carcinogenic AFM1 which is eliminated through milk (Chi and Broomhead, 2009) and have a risk for consumer health. Aflatoxin M1 is a probable human hepatocarcinogen found in the milk of animals that consume feeds contaminated with aflatoxin B1

(AFB1) which produced by fungi of genus *Aspergillus* (Sacca et al., 2007). Indeed, untreated mycotoxin contaminated feeds fed to dairy cattle may reduce milk production, alter milk compositions, or produce toxins in milk (Chi and Broomhead, 2009). On the other hand, Ochratoxins (OTA) A and B are produced by several fungal species, even though only low concentrations of OTA may have existed in milk, these small amounts may be important to consumers of large quantities of this product, particularly children (Skaug, 1999). On the other hand, milk considered as a good environment for bacteria and can be changed. So, sellers and producers add some chemicals to delay this change without awareness its health risk; to increase income and to mask these added chemicals. Undoubtedly all these make changes to the milk nature and known as adulterants which had been reported in different countries (Embrapa, 2014). Water, whey, sucrose, starch, salt, sodium hydroxide and formaldehyde are substances used for milk adulteration (Bansal and Singhal, 1991; Santos et al., 2013). Also,

sodium Citrate, sodium hydroxide, sodium chloride, sucrose, phosphates, carbonates, bicarbonates and hydrogen peroxide were used to correct milk defects, such as high acidity and microbial growth, and to increase its volume (Hoorfar J., 2012). Using of urea and formaldehyde to cover water addition to milk was reported (UOL, 2014). Milk adulteration is banned owing to its hazard effect on health (Beall and Scofield, 1995). Therefore, this study was undertaken to evaluate the presence of aflatoxins, ochratoxins and some chemical adulterants in cow's milk and its chemical composition.

2. MATERIAL AND METHODS

2.1. Collection of samples

A total of 60 random samples of fresh raw milk were collected from supermarkets and dairy milk shops from El- Minufia Governorate. The fresh raw milk was collected and transferred into an ice box to the laboratory directly without undue delay to be immediately examined for detection of aflatoxins and ochratoxins, chemical adulterants and chemical composition.

2.2. Quantitative estimation of mycotoxins

2.2.1. Aflatoxins

The presence of aflatoxin M1 and M2 were detected in samples by HPLC after post-column derivatization with the electrochemical generation of bromine (KOBRA cell – Rhone diagnostic technologies, UK) with a current of 100 μ A and a fluorescence detector (Shimadzu LC-10 AD Model; 360 nm excitation wavelength; 435 nm emission wavelength; with Shim-Pack CLC – ODS column, 5 μ m, 4.6 \times 250 mm, preceded by a guard column Shim – Pack G – ODS, 5 μ m, 4 \times 10 mm). The mobile phase was deionized water-acetonitrile-methanol (60:20:20, v/v/v) with the addition of 350 μ L of 4M HNO₃ and 120 mg of K Br at a flow rate of 1 ml/min. The injection volume was 50 μ L. The quantification of aflatoxin was performed by measuring its peak areas at each retention time and comparing it with the calibration curve (Galvano et al., 2001). The performance of the method, aflatoxin recovery and effectiveness of the clean-up procedure, was evaluated by the samples spiked with the aflatoxin.

2.2.2. Ochratoxins

The samples were extracted according to the method as described by Iqbal et al. (2013) with few modifications. The sample 15 ml was blended (15 min) in 50 ml of acetonitrile - water (45:05, v/v), using high speed blending and then the extract was

filtered through filter paper. About 5 ml of the filtrate was mixed with 50 ml of phosphate buffer saline (PBS) and filtered through a glass microfiber. Then 10 ml of the filtrate was passed through immunoaffinity columns. OTA was eluted from the column by passing 1.5 ml of methanol (HPLC grade) and collected in a vial. The eluate was evaporated until dryness at 40°C and residues were re-dissolved in 1 ml of mobile phase i.e. acetonitrile: water: acetic acid (47/51/2, v/v/v) for HPLC analysis. Calibration standards were prepared by combining standard solutions into the neat solvent and blank matrix extracts (matrix-matching) to yield the desired concentrations in the range of 10–500 μ g/L for each analyte.

2.3. Detection of inhibitory substances

Qualitative *B. subtilis* disc assay method (American Public Health Association "APHA", 1992) was used as follow: Each milk sample was heated at 80°C for 5 min to inactivate the naturally occurring inhibitory substance in milk and to eliminate the possibility of false-positive results. After cooling, 0.1 ml of each milk sample was applied in a circular well in Bacto-Pm indicator agar inoculated with *B. subtilis* organism. The plates were examined for violet coloured inhibition zones after 2.5-3.0 hours' incubation at 65°C. The presence of inhibition zone was recorded as a positive result.

2.4. Chemical adulterants

2.4.1. Detection of preservatives

Formalin, Hydrogen peroxide, Salicylic acid, Boric acid, carbonate & bicarbonate, starch and nitrate were detected according to Draaiyer et al. (2009).

2.4.2. Detection of urea

The classical spectrophotometric method recommended by Bector et al. (1998) was applied.

2.5. Chemical composition

Analysis of milk for determination of its fat, solid not fat (S.N.F) and protein, was performed according to the techniques recommended by FSSAI (2015).

3. RESULT

3.1. Prevalence of aflatoxins and ochratoxins in milk

Table (1) revealed that 10 samples were positive for AFM1 with a range of 0.021 – 0.095 (μ g/kg) and 5 samples were positive for AFM2 with a range

of 0.010 – 0.034 (µg/kg). meanwhile, ochratoxins were nil for all samples.

3.2. Chemical adulterants

Figure 1 showed that inhibitory substances were present in 70 % of milk samples. The prevalence of formalin was 41.67% followed by water 37.5% then hydrogen peroxide with 20 %, then boric acid 16.70%, after that carbonate and

bicarbonate came with 8.30%, finally nitrate with 5%. Meanwhile, starch, urea and salicylic acid were not detected.

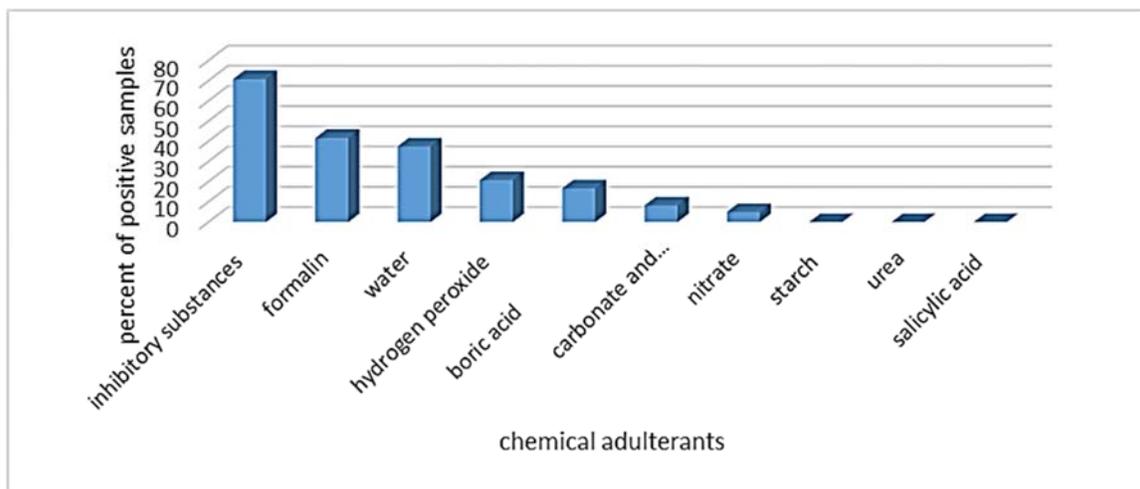
3.3. Chemical composition

Table 2 and 3 showed that 50% and 54.17% of samples were below the Egyptian Standards (2005) for fat and S.N.F respectively. Protein ranged from 2.5% to 3.4% with average of 2.9%

Table 1: prevalence of aflatoxins and ochratoxins in milk samples

	Aflatoxins (µg/kg)	Ochratoxins (µg/kg)
M1	M2	
10 samples were positive with 16.7% and ranged from 0.021 – 0.095 (µg/kg)	5 samples were positive with 8.33% and ranged from 0.010- 0.034 (µg/kg)	-

Figure 1: chemical adulterants in milk samples



Chemical adulterants in milk samples: values as a mean (n = 60).

Table 2: chemical composition of milk sample

Parameters	Range	Minimum	Maximum	Average
Fat %		2.6	3.5	3.00
S.N.F %		7.82	8.53	8.21
Protein %		2.5	3.4	2.90

Table 3: milk sample below legal requirement for fat %, S.N.F % and protein %

Fat%	S.N.F%	Protein %
Fat % for 50% of samples < 3%*	S.N.F% for 54.17% of samples < 8.25*	Protein % for 41.67% of samples ≤ 2.90

* Egyptian regulated standards

4. DISCUSSION

In the present study, our result data in table 1 revealed that 16.7 % and 8.33 % of collected samples contaminated with AFM1 and AFM2, respectively with a range of 0.021 – 0.095 ($\mu\text{g}/\text{kg}$) for AFM1 and 0.010- 0.034 ($\mu\text{g}/\text{kg}$) for AFM2 respectively. According to European commission regulation (2006), the MRL for AFM1 in milk is 0.05 ($\mu\text{g}/\text{kg}$), 8.4 % of samples exceed the established limit of EC, which come in accordance with Rouhi et al. (2015) and Jawaid et al. (2015) who stated contamination of milk samples with AFM1 that exceeded than standard levels established by United States regulation. On the other hand, Elzupir and Elhussein (2010) found that the percentage of AFM1 contamination in milk is 95.45% and also Nemati et al. (2010) who stated that all of the milk samples (100%), were contaminated with AFM1. The aflatoxins production in grains is mostly influenced by harvest time; fertilization; irrigation; pest control; silage moisture; and storage practices. Therefore, the incidence and occurrence of AFM1 contamination in milk and dairy products depend on the country of origin (Prandini et al., 2009). The risk of AFM1 for human makes aflatoxin in dairy feed a constant concern as the concentration of AFM1 in milk is extremely dependent upon the dietary content of aflatoxin (Chi and Broomhead, 2009).

Figure (1) showed that 70% milk samples contained inhibitory substances. The presence of inhibitory substances as antibiotics, or antibiotic residues in the milk; because of their usage for prevention or treatment of various diseases of a bacterial cause (Petrovic et al., 2008). Non-compliance for period of antibiotic excretion (Kirbiš, 2006) the overdose of antibiotics, use of antibiotics that are banned or addition of antibiotics in milk in order to prevent multiplication of microorganisms, are the common cause of the presence of antibiotic residues in milk (McEwen et al., 1992; Nikolic et al., 2011). Either the producer or seller adds formalin, hydrogen peroxide, boric as a preservative to the milk for increasing its shelf life (Abbas et al., 2013). Formalin was the highest preservative used in our data. determination of formalin in milk samples was revealed (Abbas et al., 2013; Chanda et al., 2012). Formaldehyde decreased serum/tissue total antioxidant levels and it increased total oxidant level, oxidant and apoptosis index in the cell. Moreover, damages of liver and lung tissues and increasing of total oxidant capacity by formalin was stated (Aydin et al., 2015). While, Hydrogen peroxide followed formalin as preservatives. Hydrogen peroxide

added to milk to delay microbial growth (Hoorfar J., 2012). Hydrogen peroxide adulteration disturbs the antioxidants activity in the body that causes a disturbance in natural immunity, which leads to increase ageing (Clare et al., 2003). On the other hand, boric acid is the least preservative used. Abbas et al. (2013) revealed the using of boric as for milk adulteration. Also, carbonate and bicarbonate were added to adulterated milk for neutralizing the developed acidity resulting from adulteration (Hoorfar J., 2012). Our result revealed the addition of carbonate and bicarbonate to adulterated milk. Adulteration of milk with carbonate was stated by Sanjeevani et al. (2011), Chanda et al. (2012) and Abbas et al. (2013). Gastrointestinal problems like gastric ulcer, colon ulcer, diarrhoea, and electrolytes disturbance may be caused by carbonates in milk (Beall and Scofield, 1995). Nitrates as oxidizing agents were used as preservative in milk ((Kamthania et al., 2014), our reported data in figure 1 revealed presence of nitrate in 5% of samples. Foods contain nitrate and nitrite may be considered hazardous after ingestion in the gastrointestinal tract as they react with naturally occurred secondary amines to form potentially carcinogenic nitrosamines (Chamandust et al., 2016).

Table 2 and 3 showed that fat content of cow's milk samples ranged from 2.6% to 3.5% with an average of 3.00% and 50% of samples fat content were below the legal requirement of the Egyptian Standards (2005) for cow's milk (not less than 3%). Adulteration of milk by partial skimming or addition of water lower its fat (Eman et al., 2015). Meanwhile, milk solids ranged from 7.82% to 8.53% with an average of 8.21% and 54.17 % of samples, and were below the legal requirement of the Egyptian standard for cow's milk (not less than 8.25%). The lower S.N.F content could be attributed mainly to adulteration by the addition of water (Harding, 1995) as it decreases only by the addition of water and not affected by partial skimming (Eman et al., 2015).

Our result in figure 1 revealed that 37.5% of samples were adulterated with water. Adulteration of milk with the water was stated by Ramya et al. (2016). On the other hand, Cow's milk protein ranged from 2.98% to 3.87% (Mahmood and Usman, 2010) and data in table 2 and 3 showed that 41.67% of examined samples decreased in their protein content. Milk Adulteration with water decreases its protein content as the S.N.F % decreased along with protein % (Karthek et al., 2011).

5. CONCLUSION

The present study concluded that the collected milk samples contained aflatoxins but were free from ochratoxins. Moreover, some chemical adulterants were added to milk; all of them have a health risk. So, it is an obligatory to introduce regular systematic control of inhibitory substances and other adulterants in raw milk. Moreover, to Monitor the mycotoxin in animal feed.

6. REFERENCES

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