



Short Communication

Assessment of Milk Quality by Analyzing Aflatoxin M1 in Branded and Non Branded Milk Samples Collected from Different Shops/Areas in Lahore, Pakistan

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ABSTRACT

In the present study a total of 80 samples were analyzed for Aflatoxin M1 using competitive ELISA kit method. Branded samples (n=40) were collected from different shops in Lahore city of Punjab Province while non-branded samples (n=40) collected from four selected towns of Lahore. Overall 58% and 100% of aflatoxin contamination in branded and non-branded milk samples were found, respectively. The quantities of AFM1 in 58% of branded milk samples and 95% of non-branded milk samples were found beyond permissible limits of 50 ppt as set by European Union. The most pathetic condition of non-branded milk samples was found in Shalimar Town of Lahore which showed highest levels of aflatoxin M1 *i.e.* 2735.08 ppt.

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Authors' Contributions
NZ, NJ and SRA designed the study. NZ and IK conducted the experiments. MKS and IA collected the samples. QAS reviewed the article. SM analyzed the data. NZ wrote the article.

Key words
Aflatoxin B1, M1, Milk, Contamination, ELISA.

Aflatoxins are toxins produced by different species of *Aspergillus* especially *flavus* and *parasticus*. International Agency of Research on Cancer (IARC, 2002) has also placed aflatoxins among powerful carcinogens. Aflatoxin M1 is a hydroxylated metabolite of aflatoxin B1. Cytochrome oxidase system of P₄₅₀ present in the microflora and animal cells is responsible for conversion of aflatoxin B1 to M1 (Fallah *et al.*, 2009; Unusan, 2006).

In European Union countries, the allowable limits of AFM1 in milk are 0.05 ppb and 0.025 ppb consumed by adults and infants, respectively while in the United States, the permissible limit of aflatoxin M1 in milk is 0.5 ppb (Kyprianou, 2007; Battacone *et al.*, 2009).

In present study the objective was to check the levels of aflatoxin M1 in different branded and non branded milk samples by using ELISA method.

Materials and methods

The non-branded samples were collected from retail milk shops of city Lahore, Pakistan. Forty samples of raw milk (500 ml each) were collected randomly from different markets of Samanabad Town, Iqbal Town, Shalimar Town

and Gulberg Town during October, 2016 to March, 2017. 10 samples from each town were collected. Similarly, branded samples were also collected from different shops in Lahore. The milk samples were transported in sterilized polythene bags in ice-packed cooler to the aflatoxin laboratory at PCSIR Laboratories Complex, Lahore, where samples were kept at -20°C until analyzed for AFM1 detection (Muhammad *et al.*, 2010). Samples were prepared by using Romer Labs Method #: PI-000047-1. Fresh milk sample (5 ml) was taken into test tubes and incubated at 4°C for 30 min. The samples were centrifuged at 3000 g for at least 10 min. Milk serum (0.4 ml) below fat layer was taken and mixed with 0.1mL of 100% methanol *i.e.* in the ratio is 4:1. ELISA kit (Romer Labs, Singapore), was used for the examination of M1 in milk samples (Kamkar *et al.*, 2011).

Results and discussion

Aflatoxin M1 was determined by ELISA method in branded and non-branded milk samples supplied in Lahore city of Pakistan. The percent maximum absorbance at 450 nm was observed for aflatoxin M1.

In present study it was determined that 58% of branded samples collected from Lahore shops were infected with aflatoxin M1, respectively. In a similar studies the occurrence of AFM1 contamination in the raw

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milk (non branded) samples collected from Gulberg Town, Ravi Town, Gunjbaksh Town and Shalimar Town was 71%, 86%, 81% and 86%, respectively (Muhammad *et al.*, 2010). The results of aflatoxin M1 detection in different 40 branded milk samples are given in Table I.

Table I.- Quantity of aflatoxin M1 in branded milk samples.

Sample ID	MQ (ppt) \pm SD	Sample ID	MQ (ppt) \pm SD
B1	ND	B21	163.79 \pm 0.19
B2	99.85 \pm 0.053	B22	117.63 \pm 0.19
B3	ND	B23	ND
B4	ND	B24	577.97 \pm 0.89
B5	ND	B25	104.24 \pm 0.05
B6	ND	B26	245.40 \pm 0.37
B7	176.31 \pm 0.18	B27	285.29 \pm 0.09
B8	223.95 \pm 0.041	B28	122.64 \pm 0.04
B9	148.89 \pm 0.09	B29	605.90 \pm 0.51
B10	ND	B30	526.27 \pm 0.07
B11	54.30 \pm 0.05	B31	ND
B12	254.92 \pm 0.07	B32	ND
B13	163.57 \pm 0.08	B33	274.81 \pm 0.15
B14	ND	B34	ND
B15	140.47 \pm 0.11	B35	ND
B16	ND	B36	196.67 \pm 0.18
B17	208.09 \pm 0.03	B37	ND
B18	163.51 \pm 0.12	B38	ND
B19	148.86 \pm 0.10	B39	176.59 \pm 0.40
B20	ND	B40	ND

*ND, not detected; MQ (ppt) \pm SD, mean quantity \pm standard deviation.

The branded sample (B29) was found with maximum concentration (605.90 ppt) of aflatoxin M1 beyond EU permissible limits *i.e.* 50 ppt. It was observed that all 40 raw milk (non-branded) samples collected from different shops of selected Towns were contaminated with aflatoxin M1. 100% contamination of milk samples show miserable conditions of supplied milk at Lahore shops. The results of aflatoxin M1 analysis in non-branded milk samples are given in Table II.

The sample with maximum concentration 2735.08 ppt (exceeding permissible limits set by EU) of aflatoxin M1 was also collected from Shalimar Town. In comparison with present study of aflatoxin M1 in non-branded milk samples range between 17.34-2735.08 ppt, the analysis of randomly selected raw cow milk samples in North African countries (Elgerbi *et al.*, 2004), were contaminated with AFM1 (range between 30 and 3130 ppt). These results are

in accordance with Elgerbi *et al.* (2004) and El-Sayed *et al.* (2000) who reported high levels of AFM1 in bovine raw milk samples. The percentage analysis of non-branded milk samples regarding aflatoxin M1 contamination showed that all the samples were highly infected with aflatoxin M1 among which 95% of the samples had aflatoxin M1 levels beyond permissible limits *i.e.* 50 ppt.

Table II.- Quantity of aflatoxin M1 in non-branded milk samples.

Sample ID	MQ (ppt) \pm SD	Sample ID	MQ (ppt) \pm SD
Samanabad Town		Shalimar Town	
N1	193.19 \pm 0.17	N21	2231.75 \pm 0.04
N2	186.28 \pm 0.03	N22	2545.27 \pm 0.02
N3	83.79 \pm 0.05	N23	2100.63 \pm 0.06
N4	137.85 \pm 0.09	N24	2378.86 \pm 0.03
N5	414.13 \pm 0.23	N25	2545.22 \pm 0.03
N6	163.56 \pm 0.06	N26	2231.74 \pm 0.03
N7	1877.57 \pm 0.36	N27	2735.08 \pm 0.03
N8	1299.27 \pm 0.11	N28	1299.09 \pm 0.09
N9	943.79 \pm 0.04	N29	943.85 \pm 0.03
N10	110.74 \pm 0.08	N30	110.58 \pm 0.33
Iqbal Town		Gulberg Town	
N11	860.38 \pm 0.06	N31	169.79 \pm 0.01
N12	219.84 \pm 0.07	N32	381.82 \pm 0.03
N13	115.78 \pm 0.38	N33	193.32 \pm 0.03
N14	2378.81 \pm 0.03	N34	160.52 \pm 0.01
N15	886.77 \pm 0.02	N35	52.75 \pm 0.05
N16	345.61 \pm 0.05	N36	81.93 \pm 0.06
N17	835.14 \pm 0.13	N37	25.30 \pm 0.01
N18	526.13 \pm 0.13	N38	17.34 \pm 0.02
N19	338.95 \pm 0.04	N39	72.70 \pm 0.06
N20	706.48 \pm 0.07	N40	236.75 \pm 0.04

MQ (ppt) \pm SD, mean quantity \pm standard deviation.

The percentage analysis of branded milk samples showed that 42% samples were not contaminated and found no aflatoxin M1 while 58% samples were aflatoxin M1 contaminated. Among these contaminated samples all the samples contained aflatoxin M1 levels beyond permissible limits.

In a study conducted on buffalo and cow milk samples collected from dairy farms of Faisalabad, Pakistan. 84% buffalo milk samples and 72% cow milk samples were exceeded the European Commission MRL of 50 ppt (Sajid *et al.*, 2015). Fungal metabolite AFM1 is carcinogenic, hepatotoxic and immunosuppressive and is accountable for injurious effects on human as well as animal's health

(Williams *et al.*, 2004). This adverse scenario has dragged the international trepidation over AFM1 contamination in milk supplied.

Conclusion

In current study the analysis depicts that 58% of branded samples while 100% of non-branded milk samples were found contaminated with Aflatoxin M1, respectively. HACCP system and analytical inspection are highly recommended to limit aflatoxin M1 contamination in order to fearless consumption of milk in Pakistan.

Statement of conflict of interest

The authors declare no conflict of interest.

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