Original scientific paper

UDK: 637.065

Occurrence and regulatory compliance of aflatoxin M1 in milk and dairy products on the Serbian market

DOI: 10.15567/mljekarstvo.2026.0405

Dragana Tomanić, Bojana Perduh, Radmila Radović, Jelena Miljanić, Ljubiša Šarić, Olja Todorić, Jovana Kos*

University of Novi Sad, Institute of Food Technology in Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

Received: 02.03.2026. **Accepted:** 01.09.2026.

*Corresponding author: dragana.tomanic@fins.uns.ac.rs

Abstract

Contamination of milk and dairy products with aflatoxin M1 (AFM1), a toxic metabolite of aflatoxin B1 from animal feed, continues to pose a persistent food safety concern. Monitoring the prevalence and concentration of AFM1 in dairy products is critical for public health protection, regulatory compliance, and consumer safety. This study aimed to assess the occurrence and concentration of AFM1 in milk and selected dairy products available on the Serbian market in 2024. A total of 96 samples, including milk, yoghurt, sour milk, kefir, sour cream, cheeses, milk-based spreads, goat milk products, and imported dairy products, were collected and analysed using an ELISA method. AFM1 was detected across all analysed product categories, with prevalence ranging from 50 % in cheeses and milk-based spread products to 100 % in milk, yoghurt, kefir, whey, and goat milk products. Mean concentrations ranged from 0.058 µg/kg in milk to 0.226 µg/kg in cheese and milk-based spreads, with the highest individual value (0.573 µg/kg) detected in a cheese spread. Although all milk samples complied with the Serbian limit (0.25 µg/kg), 63 % exceeded the stricter European Union reference value of 0.05 µg/kg. Concentrations above 0.05 µg/kg were also frequently observed in fermented products, particularly sour cream (78 %), yoghurt (62 %), and kefir (50 %), whereas imported products showed generally lower concentrations. Two-way ANOVA of $\log_{10}$-transformed data showed a significant effect of product origin ($p<0.001$), with lower AFM1 levels in imported compared to domestic products. These findings confirm that AFM1 contamination persists as a food safety concern in Serbia and highlight the need for continuous monitoring and improved control of aflatoxin contamination in animal feed, especially under conditions of increasing climatic variability.

Keywords: aflatoxin M1; dairy products; ELISA; milk; Serbia

Introduction

Milk and dairy products are a cornerstone of human nutrition worldwide, providing high-quality proteins, essential fatty acids, calcium, and vitamins (Škrbić et al., 2014; Malissiova et al., 2024). In European diets, milk and dairy products represent an important component of daily food consumption, accounting for approximately 15 % of total dietary intake (Jahromi et al., 2025). While dairy products are highly valued for their nutritional qualities, they may also be exposed to contamination by mycotoxins, toxic metabolites produced by fungi that represent a threat to consumer health (Heidari and Nematollahi, 2025).

Among the many mycotoxins that may contaminate dairy and animal feed, aflatoxins are of particular concern due to their high toxicity, widespread occurrence, and significant public health and economic implications. Aflatoxin B1 (AFB1), the most toxic and prevalent variant, is metabolized in the liver of lactating animals to aflatoxin M1 (AFM1), which is subsequently excreted into milk, making dairy products a primary route of human exposure (Duarte et al., 2013; Malissiova et al., 2022; Kos et al., 2023). AFM1 represents a potential health concern as a hydroxylated metabolite of aflatoxin B1, a compound classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC, 2012). Of additional concern is the high thermal stability of AFM1, as it is relatively heat-stable and conventional thermal treatments such as pasteurization or sterilization generally result in only limited reductions. As a result, AFM1 can persist in a wide range of dairy products, including powdered milk, yoghurt, cheeses, and other dairy products (Duarte et al., 2013).

AFM1 occurrence in dairy products is influenced by multiple factors, including feed contamination, climatic conditions, livestock species, production practices, and dairy processing methods (Poroșnicu et al., 2024). For monitoring purposes, ELISA is widely used as a rapid screening method suitable for routine analysis, while confirmatory chromatographic methods, particularly LC-MS/MS, provide higher specificity and sensitivity for AFM1 determination (Vaz et al., 2020). Regulatory authorities have therefore established maximum limits (ML) for AFM1 in milk in order to protect consumer health. In Serbia, the current ML for AFM1 in milk is 0.25 µg/kg (Serbian Regulation, 2022), which remains higher than the European Union (EU) ML of 0.05 µg/kg established under Regulation (EU) 2023/915 (European Commission, 2023). This discrepancy reflects the long-standing challenges associated with controlling aflatoxin contamination in feed and dairy production systems in Serbia (Kos et al., 2024a).

AFM1 contamination of milk in Serbia has been extensively documented over the past decade, particularly following the severe aflatoxin crisis in 2013 (Kos et al., 2014, Škrbić et al., 2014, Torović, 2015, Djekic et al., 2020, Kos et al., 2024b, Krstović et al., 2025). However, considerably fewer data are available for fermented dairy products such as yoghurt, kefir, sour cream, and soft cheeses, despite their widespread consumption (Tomašević et al., 2015; Djekic et al., 2020; Kos et al., 2024b). These products may retain or even concentrate AFM1 due to its affinity for milk proteins during fermentation and coagulation processes (Abi Rizk et al., 2026).

Recent studies have linked AFM1 occurrence to climatic factors, particularly elevated temperatures and drought conditions that favour AFB1 contamination of maize, a major component of dairy animal feed (Krstović et al., 2025). In Serbia and neighbouring Southeast European countries, recurrent aflatoxin contamination of maize has been reported since 2012, especially during years characterized by high temperatures and reduced precipitation (Kos et al., 2017; Janić Hajnal et al., 2023; Pleadin et al., 2023). These findings highlight the growing influence of climate variability on aflatoxin occurrence and the consequent risk of AFM1 contamination in milk and dairy products (Kos et al., 2023; Kos et al., 2024a).

Given the persistence of AFM1 in dairy products, its associated health risks, and the influence of climatic factors on contamination patterns, continuous monitoring remains essential for food safety and consumer protection. Therefore, the aim of this study was to evaluate the occurrence and concentration of AFM1 in milk and a variety of dairy products available on the Serbian market in 2024, including fermented dairy products, cheeses, goat milk products, and imported dairy products. To the best of our knowledge, this represents one of the few recent studies from Southeast Europe encompassing both milk and a broad range of processed dairy products currently available on the Serbian market.

Material and methods

Sampling

A total of 96 milk and dairy samples were collected and analysed for the presence of AFM1. The analysed samples included milk and various dairy products, namely yoghurt (n=13), sour cream and cream products (n=9), sour milk (n=7), kefir (n=2), cheeses and milk-based spreads (n=22), fluid milk (n=16), and whey (n=1). In addition, seven goat milk and goat dairy products were included. Furthermore, 19 internationally available products from the Serbian market were analysed. These included products from Croatia (yoghurt, n=2; kefir, n=2; cheeses and spreads, n=1; milk; n=2), Slovenia (milk, n=3), Germany (yoghurt, n=1; kefir, n=1; cheeses and spreads, n=4; milk, n=1), and Italy (cheeses, n=2). All imported dairy products available on the Serbian market at the time of sampling were included in the study.

Milk and dairy samples were purchased from supermarkets and local retail markets in Serbia, with the aim of covering the widest possible range of products available to consumers. All samples were collected in their original packaging. Sampling was conducted during the period October-December 2024. For each product, an aggregate sample of 1 L (for liquid products) or 1 kg (for solid products) was collected. Aggregate samples were obtained by combining incremental samples collected from the same lot and were considered representative of the lot. After sampling, all samples were transported to the laboratory of the Institute of Food Technology in Novi Sad and stored at 4 °C until analysis. Sampling procedures followed

the principles outlined in Regulation (EC) No 401/2006 (European Commission, 2006).

Chemicals and reagents

The determination of AFM1 was performed using a validated ELISA method in an ISO/IEC 17025:2017 accredited laboratory (ISO, 2017) at the Institute of Food Technology in Novi Sad. Analyses were conducted using a commercial ELISA test kit (I´Screen AFLA M1, Gold Standard Diagnostics, Trieste, Italy). All reagents required for the analysis (AFM1 standards, enzyme conjugate, extraction buffer, washing buffer, developing solution, stop solution and sample diluent) were supplied with the test kit. Methanol, dichloromethane and hexane (Fisher Chemical, USA) were used for sample extraction and preparation. Distilled water was produced using a laboratory water purification system (Crystal EX Laboratory Water Purification System, Adrona, Latvia).

Sample preparation

Milk samples were centrifuged at 3000 × g for 10 min, and the upper fat layer was subsequently removed. A 0.1 mL aliquot of the lower aqueous phase was used directly for analysis. For yoghurt and sour milk, 1 g of sample was extracted with 5 mL of methanol, shaken for 5 min (Griffin Flask Shaker, Griffin and George, Wembley, England), and centrifuged (Boeckel + Co GmbH + Co, Hamburg, Germany) at 5000 × g for 5 min. A 0.25 mL aliquot of the supernatant was evaporated under a nitrogen stream at 40 °C (Reacti-Vap, Thermo Fisher Scientific, Waltham, MA, USA), and the residue was reconstituted in 0.25 mL of sample diluent and vortexed (Velp Scientifica, Usmate, Italy) prior to analysis. For cheese and sour cream, 2 g of sample was extracted with 15 mL of dichloromethane and shaken for 15 min. After filtration, 3.75 mL of the extract was evaporated under nitrogen at 60 °C and reconstituted in 0.75 mL of extraction buffer. Subsequently, 0.75 mL of hexane was added, vortexed, and the upper hexane layer discarded. A 0.050 mL aliquot of the remaining phase was diluted with 0.20 mL of dilution buffer for ELISA analysis.

Aflatoxin M1 analysis in milk and dairy products

All analyses in this study were conducted in the laboratory of the Institute of Food Technology in Novi Sad, University of Novi Sad. AFM1 concentrations were determined using the ELISA method according to the manufacturer's instructions, as previously described by Kos et al. (2024b). Absorbance was measured at 450 nm using a microplate reader (Thermo Labsystems 354 Multiskan Ascent Microplate Reader Finland). AFM1 concentrations were calculated using the original spreadsheet provided with the ELISA kit (Eurofins Techna; Spreadsheet for I'Screen AFLA M1, IS Afla M1, rev. 6), based on a calibration curve constructed using seven standards (0, 5, 10, 25, 50, 100, and 250 ng/L). Colour development was inversely proportional to the AFM1 concentration in the samples. All analyses were performed in duplicate.

Method validation

The ELISA method used for the determination of AFM1 was previously validated in accordance with European requirements for screening and confirmatory analytical methods established by Regulation (EC) No 2002/657 (European Commission, 2002) and Technical Report 16059 (CEN, 2012), as described by Kos et al. (2024b).

The method demonstrated satisfactory sensitivity, precision, and trueness for milk and dairy matrices. Limits of quantification provided by the manufacturer were 0.005 µg/kg for milk, 0.025 µg/kg for fermented dairy products, and 0.037 µg/kg for cheese. Method performance was evaluated using milk powder reference material (04370, FAPAS, York, UK), as well as naturally contaminated and spiked dairy matrices. In addition, reference materials were routinely analysed within each analytical batch to ensure the validity of the obtained results. Precision parameters, expressed as relative standard deviations for repeatability and reproducibility, complied with the acceptance criterion of <20 %, while trueness and recovery values ranged between 80 % and 120 %. The suitability of the reported LOQ levels was additionally confirmed by spiking blank samples, which demonstrated acceptable recovery and repeatability. In addition, the performance of the ELISA method is regularly verified through participation in proficiency testing schemes.

Statistical analysis

The collected data were systematized in Microsoft Excel (v. 2019) and analysed through descriptive statistical analysis performed with Tibco Statistica (v. 14). Results were expressed as mean values, standard deviation, minimum and maximum concentrations, as well as the percentage of contaminated samples.

Differences in concentration levels (µg/kg) across product groups and origin were evaluated using a two-way analysis of variance (ANOVA), with product group (milk, fermented dairy products, soft cheeses) and origin (Serbian/domestic vs. imported) included as fixed factors, along with their interaction term (product group × origin). Given the positively skewed distribution of concentration values, the dependent variable was $\log_{10}$-transformed prior to analysis to improve normality and homoscedasticity assumptions. The normality of residuals was evaluated using the Shapiro–Wilk test, while the homogeneity of variance was assessed using Levene's test. No major violations of ANOVA assumptions were observed after $\log_{10}$ transformation ($p > 0.05$). When a statistically significant interaction was observed, simple main effects were examined by comparing Serbian and imported products within each product group. Adjustment for multiple comparisons was performed using the Holm correction method.

Results and discussion

Occurrence and regulatory compliance

The analysis of milk and dairy products collected in 2024 confirms the persistence of AFM1 contamination in Serbia, consistent with a long-standing trend reported over the past decade. AFM1 was detected in all analysed categories of milk and dairy products (Table 1). The highest mean concentration was observed in cheeses and spreads (0.226 µg/kg), followed by kefir (0.156 µg/kg), sour cream (0.120 µg/kg), yoghurt (0.083 µg/kg), sour milk (0.066 µg/kg) and milk (0.058 µg/kg). The highest individual concentration was detected in a cheese and spread sample (0.573 µg/kg), while the lowest concentrations were observed among imported products, particularly imported milk samples (0.006 µg/kg).

According to the current Serbian regulation, which sets the ML of AFM1 in milk at 0.25 µg/kg, all analysed milk samples complied with the national regulatory limit, and no exceedances were recorded. However, when the same results were evaluated against the stricter EU ML for AFM1 in milk (0.05 µg/kg), 10 out of 16 milk samples (63 %) exceeded the EU threshold. This substantial discrepancy between Serbian and EU regulatory standards suggests that, despite compliance with national legislation, consumers may still be exposed to AFM1 at levels considered unacceptable under EU criteria, potentially posing a public health concern, particularly for children and high dairy consumers. The proportion of dairy samples exceeding 0.05 µg/kg is presented in Figure 1.

Compared with previously published Serbian data, the mean AFM1 concentration in milk observed in 2024 indicates a marked reduction relative to the severe contamination episodes reported during and immediately after the 2013 aflatoxin crisis. The widespread aflatoxin contamination of maize in 2012-2013 triggered a national food safety crisis in Serbia, underscoring the vulnerability of the dairy supply chain to mycotoxin contamination and resulting in elevated AFM1 levels in milk, significant economic impacts, and regulatory responses (Kos et al., 2024a). During that period, AFM1 contamination was detected in nearly all milk samples, with mean concentrations around 0.13 µg/kg and approximately 70 % of samples above the EU limit

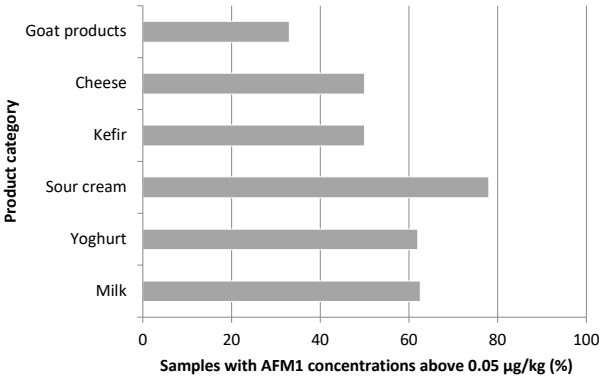


Figure 1. Percentage of milk and dairy product samples with AFM1 concentrations above 0.05 µg/kg, corresponding to the European Union maximum limit for AFM1 in milk. Product categories with a very small number of samples, such as kefir, should be interpreted with caution

of 0.05 µg/kg (Škrbić et al., 2014, Torović, 2015). Similarly high contamination levels were reported by Kos et al. (2014) in 2013, with 86 % of samples exceeding the EU limit and concentrations reaching up to 1.2 µg/kg, resulting in elevated dietary exposure, particularly among children. This trend was further confirmed by a systematic review of Serbian milk from 2015-2016, which reported AFM1 detection in 70-85 % of raw milk and up to 98 % of heat-processed milk samples, while exceedances of the Serbian limit were rare, compared with substantially higher exceedances of the EU limit (17-30 %), a pattern consistent with the present study (Milićević et al., 2017).

Subsequent monitoring studies demonstrated that, although average AFM1 concentrations declined after the crisis, contamination remained frequent. Between 2013 and 2018, AFM1 was detected in approximately 70-85% of milk samples in Serbia, with 22-31 % exceeding the EU limit (Jajić et al., 2018; Milićević et al., 2019). Importantly, AFM1 occurrence was consistently higher in raw milk than in processed dairy products, where exceedances were rare (<2%) and mean concentrations generally remained below 0.02 µg/kg, indicating effective control measures at the processing stage (Djekic et al., 2020).

Table 1. Prevalence and concentration of AFM1 (µg/kg) in milk and dairy products

Product category	n	Positive samples (%)	Mean (µg/kg)	SD	Min - Max (µg/kg)
Milk	16	100	0.058	0.020	0.012–0.091
Yoghurt	13	100	0.083	0.060	0.030–0.181
Sour cream	9	78	0.120	0.100	0.050–0.315
Sour milk	7	86	0.066	0.030	0.030–0.099
Kefir	2	100	0.156	0.150	0.052–0.260
Whey	1	100	0.014	n.a.	0.014
Cheeses and spreads	22	50	0.226	0.150	0.043–0.573
Goat products	7	100	0.059	0.020	0.008–0.073
Imported products	19	16	0.017	0.010	0.006–0.025

n - number of samples; SD - standard deviation; n.a. - not applicable (n=1); positive samples refer to samples with AFM1 concentrations above the limit of quantification (LOQ)

More recent large-scale studies confirm the persistence of AFM1 contamination in Serbia. Udovicki et al. (2023) reported AFM1 detection in approximately 78 % of milk samples collected between 2015 and 2022, while Krstović et al. (2025) documented AFM1 presence in nearly 70 % of raw milk samples during 2021–2025, with pronounced seasonal and interannual variability. These findings are supported by data from 2022 showing widespread aflatoxin B1 contamination of maize and high prevalence of AFM1 in milk, highlighting the strong link between climatic conditions, feed contamination and subsequent AFM1 transfer into milk (Kos et al., 2024b). Overall, while average AFM1 concentrations were lower than those reported during the 2013 aflatoxin crisis, the high prevalence of contamination and the substantial proportion of samples exceeding the EU MLs indicate that AFM1 remains a relevant food safety concern. Serbia is formally committed to harmonising its food safety legislation with EU standards. However, the multi-year inconsistencies in MLs for AFM1, persisting for over a decade, reflect a continuous challenge in controlling contamination, which is clearly confirmed by the high prevalence and widespread occurrence observed in the present study.

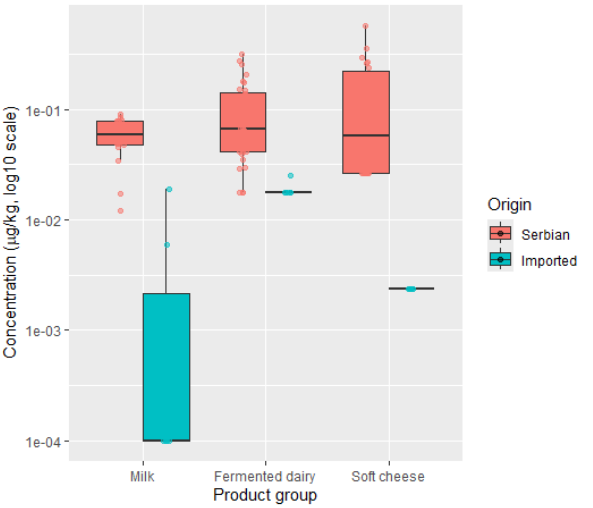


Figure 2. Distribution of AFM1 concentrations (µg/kg) by product group and origin (Serbian vs. Imported products), presented on a log_{10} scale. Boxes represent the interquartile range, horizontal lines indicate median values, and points indicate individual observations

Differences according to product group and origin

To further evaluate whether the observed regulatory asymmetry was reflected in measurable differences between domestically produced and imported dairy products, AFM1 concentrations were analysed according to product group (milk, fermented dairy products, soft cheeses) and origin (Serbian vs. Imported). A two-way analysis of variance (ANOVA) was performed on log_{10}-transformed concentration values. The results are presented in Table 2.

The two-way ANOVA revealed no statistically significant main effect of product group ($F(2,81)=4.73$, $p=0.063$), while origin ($F(1,81)=134.82$, $p<0.001$) and the interaction between product group and origin were statistically significant ($F(2,81)=13.49$, $p<0.001$). The presence of a significant interaction indicates that differences between Serbian and imported products varied across product categories; therefore, interpretation focused on the analysis of simple effects. Due to the significant interaction, additional analyses were conducted to examine differences between product groups separately for Serbian and imported samples. Although the interaction term was statistically significant, post hoc comparisons performed within each origin did not reveal statistically significant differences between product groups (all $p > 0.05$). This finding suggests that the interaction was primarily driven by differences between Serbian and imported products within product categories, rather than by pronounced differences among product groups within the same origin. Subsequently, comparisons between Serbian and imported products were performed within each product group using t-tests on log_{10}-transformed concentrations, with Holm adjustment for multiple comparisons (Table 3).

Statistically significant differences between origins were observed in all three product categories. AFM1 concentrations were significantly higher in Serbian compared with imported products in milk ($p_{adj}=0.004$), fermented dairy products ($p_{adj}<0.001$), and soft cheeses ($p_{adj}<0.001$). For visual confirmation of the ANOVA findings and simple effects analysis, the distribution of concentrations (µg/kg) within

Table 2. Results of the two-way ANOVA

Effect	Sum of Squares	df	F	p
Product group	2.92	2	4.732	0.063
Origin	25.50	1	134.815	<0.001
Product group × Origin	5.10	2	13.489	<0.001
Residuals	15.32	81	–	–

Table 3. Comparison of Serbian vs. imported products within each product group

Product group	Group 1	Group 2	n1	n2	t	p	p_adj
Milk	Serbian	Imported	16	6	4.67	0.005	0.004
Fermented dairy	Serbian	Imported	31	6	8.51	0.000	<0.001
Soft cheeses	Serbian	Imported	22	7	14.60	0.000	<0.001

each product group is presented separately according to origin (Serbian vs. Imported).

The graphical representation clearly demonstrates that concentrations in imported samples were generally lower and clustered at the lower end of the scale across all product groups, whereas Serbian samples exhibited higher values and greater variability, particularly among fermented products and soft cheeses (Figure 2). The consistently lower AFM1 concentrations observed in imported products may reflect stricter feed quality control systems and closer alignment with EU regulatory requirements, suggesting that current preventive measures in domestic production remain insufficient to consistently minimize contamination. This pattern is consistent with the results of the simple effects analysis (Table 3), confirming statistically significant differences between Serbian and imported products within each category. Notably, the higher AFM1 concentrations observed in fermented dairy products and cheeses in the present study are consistent with observations that AFM1 binds strongly to casein and is concentrated during coagulation and fermentation processes (Škrbić et al., 2015, Goncalves et al., 2021, Jauković et al., 2024). Numerous studies report higher contamination levels in cheeses and fermented products compared with raw or fluid milk (Škrbić et al., 2015; Sarmast et al., 2021; Abi Rizk et al., 2026).

Due to export requirements and the obligation to comply with EU legislation, Serbian dairy products intended for export must meet the EU MLs for AFM1 (0.05 µg/kg). Consequently, milk exceeding the EU threshold but remaining below the national ML of 0.25 µg/kg may legally remain on the domestic market. This regulatory asymmetry potentially creates a disparity between products intended for export and those available for domestic consumption. As an EU candidate country, Serbia is formally committed to harmonising its food and feed safety framework with EU legislation. However, European Commission progress reports under Chapter 12 - Food Safety, Veterinary and Phytosanitary Policy (European Commission, 2025) have repeatedly highlighted gaps in legislative alignment, enforcement of risk-based controls, and institutional capacity, including laboratory resources and inter-institutional coordination. In this context, the long-standing divergence between Serbian and EU MLs for AFM1 reflects not only regulatory differences but also structural challenges in achieving full implementation of EU-compliant food safety systems.

In a global context, the prevalence and distribution of AFM1 observed in the present study are consistent with international findings reported across diverse dairy production systems. For example, high prevalence has been reported in Bangladesh, where AFM1 was detected in 70-100 % of raw, pasteurized and UHT milk samples, with up to 97 % of samples exceeding the EU MLs in some surveys (Tarannum et al., 2020; Sumon et al., 2021). Similarly elevated contamination has been documented in several African countries, including Ghana, where AFM1 was detected in 55.8-94.6 % of raw milk samples, with concentrations reaching 1606.8 ng/L and exceedance rates up to 100 % (Kortei et al., 2022; Nuhu et al., 2025), as well as in Nigeria, where AFM1 concentrations in goat milk reached up to 3108 ng/L (Akinyemi et al., 2022). In contrast, countries with well-established monitoring systems and strict feed control measures are generally associated with lower AFM1 prevalence and fewer exceedances of regulatory limits. For instance, studies from Spain and Italy reported no detectable AFM1 or exceedance rates below 1% in milk samples (Flores-Flores and González-Peñas, 2018; Roila et al., 2021; Rodríguez-Cañás et al., 2024). Studies from neighbouring countries in Southeast Europe report heterogeneous contamination patterns, with a pronounced regional peak during the 2013 crisis followed by partial improvement in some countries, while sporadic exceedances and seasonal peaks persist, underscoring the importance of feed management and monitoring intensity rather than agro-climatic factors alone (Bilandžić et al., 2016; Kesić et al., 2021; Ilievska et al., 2022). Recent Croatian monitoring data indicate that approximately 5-15 % of raw milk samples exceeded the EU MLs (Bilandžić et al., 2025). Moreover, comparative analyses have shown a substantially higher proportion of AFM1-positive samples in dairy products of Croatian origin (55.1 %) compared with imported products (21.9 %), which may reflect differences in regional feed quality and production practices, although the observed variation could also be influenced by sampling characteristics and product comparability (Kovač Tomas et al., 2025).

This persistent occurrence of AFM1 in Serbian milk, particularly in samples exceeding the EU MLs, likely reflects the underlying AFB1 contamination in maize (Kos et al., 2013; Janić Hajnal et al., 2017; Janić Hajnal et al., 2020; Kos et al., 2020; Kos et al., 2024b). While official data for 2024 are not yet available, the extreme heat and drought during the summer months likely promoted AFB1 formation, which subsequently contributed to the AFM1 levels observed in milk and dairy products analysed in this study. These findings support growing evidence that climate variability, particularly elevated temperatures and drought conditions, may increasingly contribute to aflatoxin contamination in feed and consequently to AFM1 occurrence in dairy products across Southeast Europe. Persistent AFM1 contamination in Serbian milk may represent an early indicator of a broader regional trend, as rising temperatures, altered precipitation patterns, and increasing climate variability are expected to intensify mycotoxin risk across the Balkan region (Kos et al., 2023).

Certain limitations of the present study should also be considered. Some dairy products were grouped into broader categories for statistical analysis, which may have masked potential differences among individual product types. In addition, several categories, particularly kefir and whey, included a limited number of samples. Nevertheless, despite the small number of analysed samples, the obtained findings are consistent with previous reports. Milićević et al. (2021) also reported the occurrence of AFM1 in whey samples from Serbia, indicating that AFM1 may persist in whey as a consequence of toxin transfer during dairy processing. Similarly, the occurrence of AFM1 in kefir and other fermented dairy products has been documented in previous studies (Khorshidi et al., 2022), supporting the observation that AFM1 can persist in different fermented dairy matrices. Furthermore, AFM1 occurrence was not correlated with

feed contamination data, and confirmatory chromatographic analysis was not performed. Future research should address these limitations through the inclusion of a larger number of representative samples, feed contamination analysis, and confirmatory LC-MS/MS analysis.

Conclusion

This study shows that AFM1 is still frequently present in milk and dairy products available on the Serbian market in 2024. Although average AFM1 concentrations were lower than those reported during the major contamination episodes in 2013 and generally remained below the current Serbian MLs, a substantial proportion of samples showed AFM1 concentrations above 0.05 µg/kg, particularly among fermented dairy products and cheese and spread products. Statistical analysis revealed a pronounced effect of product origin, with significantly lower AFM1 concentrations observed in imported products across all product categories. These findings suggest that differences in regulatory standards and feed control practices may influence consumer exposure patterns.

Overall, the findings underline the importance of continuous monitoring of AFM1 in milk and dairy products, as well as improved control of aflatoxin contamination in animal feed. Strengthening preventive measures throughout the dairy production chain remains essential to ensure consumer safety and maintain milk quality under conditions of increasing climatic variability.

Acknowledgment

This research was supported by the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia [Grant No. 451-03-33/2026-03/200222].

Conflicts of interest

None of the authors have any conflict of interest to declare.

Author contributions

DT, LjŠ, and JK were involved in conceptualization of the study. Methodology, investigation, analysis and validation were performed by DT, BP, RR, JM, OT, JK. All authors were involved in writing - review and editing. All authors have read and agreed to the published version of the manuscript.

ORCID ID

Dragana Tomanić https://orcid.org/0009-0002-6826-0442
Bojana Perduh https://orcid.org/0000-0003-0403-3662
Radmila Radović https://orcid.org/0000-0002-1282-0263
Jelena Miljanić https://orcid.org/0000-0001-7348-3961
Ljubiša Šarić https://orcid.org/0000-0003-2484-485X
Olja Todorić https://orcid.org/0000-0002-1447-603X
Jovana Kos https://orcid.org/0000-0003-4035-9694

Pojavnost aflatoksina M1 u mlijeku i mliječnim proizvodima na tržištu Srbije i ocjena sukladnosti s propisima

Sažetak

Kontaminacija mlijeka i mliječnih proizvoda aflatoksinom M1 (AFM1), toksičnim metabolitom aflatoksina B1 koji potječe iz stočne hrane, i dalje predstavlja trajan problem sigurnosti hrane. Praćenje pojavnosti i koncentracije AFM1 u mliječnim proizvodima od ključne je važnosti za zaštitu javnog zdravlja, usklađenost s propisima i sigurnost potrošača. Cilj ovog istraživanja bio je procijeniti pojavnost i koncentraciju AFM1 u mlijeku i odabranim mliječnim proizvodima dostupnim na tržištu Srbije tijekom 2024. godine. Ukupno je prikupljeno i analizirano 96 uzoraka, uključujući mlijeko, jogurt, kiselo mlijeko, kefir, kiselo vrhnje, sireve, mliječne namaze, proizvode od kozjeg mlijeka te uvezene mliječne proizvode, primjenom ELISA metode. AFM1 je detektiran u svim analiziranim kategorijama proizvoda, pri čemu se pojavnost kretala od 50 % u sirevima i mliječnim namazima do 100 % u mlijeku, jogurtu, kefiru, sirutki i proizvodima od kozjeg mlijeka. Prosječne koncentracije kretale su se od 0,058 µg/kg u mlijeku do 0,226 µg/kg u sirevima i mliječnim namazima, dok je najveća pojedinačna vrijednost (0,573 µg/kg) utvrđena u sirnom namazu. Iako su svi uzorci mlijeka bili u skladu s najvećom dopuštenom razinom u Srbiji (0,25 µg/kg), 63 % uzoraka premašilo je maksimalno dopuštenu razinu prema propisima Europske unije od 0,05 µg/kg. Koncentracije veće od 0,05 µg/kg često su zabilježene i u fermentiranim proizvodima, osobito u kiselom vrhnju (78 %), jogurtu (62 %) i kefiru (50 %), dok su uvezeni proizvodi općenito sadržavali niže koncentracije AFM1. Dvosmjerna analiza varijance (ANOVA) $\log_{10}$-transformiranih podataka pokazala je statistički značajan učinak podrijetla proizvoda ($p<0,001$), pri čemu su uvezeni proizvodi sadržavali niže razine AFM1 u odnosu na domaće proizvode. Dobiveni rezultati potvrđuju da kontaminacija AFM1 i dalje predstavlja značajan problem sigurnosti hrane u Srbiji te naglašavaju potrebu za kontinuiranim praćenjem i unaprjeđenjem kontrole kontaminacije stočne hrane aflatoksinima, osobito u uvjetima sve izraženijih klimatskih promjena.

Ključne riječi: aflatoksin M1; mlijeko; mliječni proizvodi; ELISA; Srbija

References

1. Abi Rizk, M., Nehme, L., Snini, S.P., Hassan, H.F., Mathieu, F., El Rayess, Y. (2026): Mapping global research trends on aflatoxin M1 in dairy products: An integrative review of prevalence, toxicology, and control approaches. *Foods* 15 (1), 166. https://doi.org/10.3390/foods15010166
2. Akinyemi, M. O., Braun, D., Windisch, P., Warth, B., Ezekiel, C.N. (2022): Assessment of multiple mycotoxins in raw milk of three different animal species in Nigeria. *Food Control* 131, 108258. https://doi.org/10.1016/j.foodcont.2021.108258
3. Bilandžić, N., Varenina, I., Kolanović, B.S., Luburić, Đ., Benić, M., Cvetnić, L., Tanković, S., Cvetnić, Ž. (2016): Monitoring of aflatoxin M1 in raw cow milk in Croatia during winter 2015. *Mljekarstvo* 66 (1), 81-85. https://doi.org/10.15567/mljekarstvo.2016.0109
4. Bilandžić, N., Varga, I., Čalopek, B., Kolanović, B.S., Varenina, I., Đokić, M., Sedak, M., Cvetnić, L., Pavliček, D., Končurat, A. (2025): Occurrence of aflatoxin M1 over three years in raw milk from Croatia: exposure assessment and risk characterization in consumers of different ages and genders. *Foods* 14 (3), 2396. https://doi.org/10.3390/foods14132396
5. Djekic, I., Petrovic, J., Jovetic, M., Redzepovic-Djordjevic, A., Stulic, M., Lorenzo, J.M., Iammarino, M., Tomasevic, I. (2020): Aflatoxins in milk and dairy products: Occurrence and exposure assessment for the serbian population. *Applied Sciences* 10 (21), 7420. https://doi.org/10.3390/app10217420
6. Duarte, S., Almeida, A., Teixeira, A., Pereira, A., Falcão, A., Pena, A., Lino, C. (2013): Aflatoxin M1 in marketed milk in Portugal: Assessment of human and animal exposure. *Food Control* 30 (2), 411-417. https://doi.org/10.1016/j.foodcont.2012.08.002

7. European Commission (2002): Regulation 2002/657/EC of 14 August 2002 implementing council directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Official Journal of the European Communities* 221, 1-29.
8. European Commission (2006): Regulation (EC) No 401/2006 of 23 February 2006, laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Luxembourg: *Official Journal of the European Union* L 70/12.
9. European Commission (2023): Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006. *Official Journal of the European Union* 119**,** 103-157.
10. European Commission (2025): Staff Working Document (2014-2025) for Chapter 12, Brussels.
11. Flores-Flores, M.E., González-Peñas, E. (2018): Analysis of mycotoxins in Spanish milk. *Journal of Dairy Science* 101 (1), 113-117. https://doi.org/10.3168/jds.2017-13290
12. Goncalves, B.L., Ulliana, R.D., Ramos, G.L., Cruz, A.G., Oliveira, C.A., Kamimura, E.S., Corassin, C.H. (2021): Occurrence of aflatoxin M1 in milk and Minas Frescal cheese manufactured in Brazilian dairy plants. *International Journal of Dairy Technology* 74 (2), 431-434. https://doi.org/10.1111/1471-0307.12772
13. Heidari, E., Nematollahi, A. (2025): Key factors influencing aflatoxin M1 concentration in dairy products: A comprehensive review. *Environmental Science and Pollution Research* 1-24. https://doi.org/10.1007/s11356-025-37131-7
14. International Agency for Research on Cancer - IARC (2012): Chemical agents and related occupations, a review of human carcinogens in IARC monograph on the evaluation of carcinogenic risk to humans. Ed.; International Agency for Research on Cancer: Lyon, France, 2012; Volume 100 F, ISBN 978-92-832-1323-9.
15. Ilievska, G., Stojanovska-Dimzoska, B., Koceva, D., Stojković, G., Angeleska, A., Dimitrieska-Stojković, E. (2022): Dietary exposure and health risk assessment of aflatoxin M1 in dairy products consumed by population of North Macedonia. *Journal of Food Quality and Hazards Control* 9 (1), 14-22. https://doi.org/10.18502/jfqhc.9.1.9686
16. International Organization for Standardization (ISO) (2017): ISO/IEC 17025:2017. General requirements for the competence of testing and calibration laboratories. Geneva, Switzerland: ISO; 1-30.
17. Jahromi, A.S., Jokar, M., Abdous, A., Rabiee, M.H., Biglo, F.H.B., Rahmanian, V. (2025): Prevalence and concentration of aflatoxin M1 in milk and dairy products: an umbrella review of meta-analyses. *International Health* 17 (4), 403-415. https://doi.org/10.1093/inthealth/ihaf002
18. Jajić, I., Glamočić, D., Krstović, S., Horvatović, M.P. (2018): Aflatoxin M1 occurrence in Serbian milk and its impact on legislative. *Journal of the Hellenic Veterinary Medical Society* 69 (4), 1283-1290. https://doi.org/10.12681/jhvms.19618
19. Janić Hajnal, E., Kos, J., Krulj, J., Krstović, S., Jajić, I., Pezo, L., Šarić, B., Nedeljković, N. (2017): Aflatoxins contamination of maize in Serbia: The impact of weather conditions in 2015. *Food Additives & Contaminants: Part A* 34 (11), 1999-2010. https://doi.org/10.1080/19440049.2017.1331047
20. Janić Hajnal, E.J., Kos, J., Malachová, A., Steiner, D., Stranska, M., Krska, R., Sulyok, M. (2020): Mycotoxins in maize harvested in Serbia in the period 2012-2015. Part 2: Non-regulated mycotoxins and other fungal metabolites. *Food Chemistry* 317, 126409. https://doi.org/10.1016/j.foodchem.2020.126409
21. Janić Hajnal, E., Kos, J., Radić, B., Anić, M., Radović, R., Kudumija, N., Vulić, A., Đekić, S., Pleadin, J. (2023): Impact of climate changes on the natural prevalence of Fusarium mycotoxins in maize harvested in Serbia and Croatia. *Foods* 12 (5), 1002. https://doi.org/10.3390/foods12051002
22. Jauković, M.M., Roković, N.I., Vuksan, A.D. (2024): Recent aflatoxin levels in maize, feed mixtures, milk and cheese in Serbia. *Matica Srpska Journal for Natural Sciences* 146, 81-89. https://doi.org/10.2298/ZMSPN2446081J
23. Kesić, A., Hodžić, M., Mehmedinović, N.I., Šestan, A. (2021): Comparative analysis of milk samples from Bosnia and Herzegovina contaminated with aflatoxin M1. *European Journal of Food Science and Technology* 9 (3), 1-15.

24. Khorshidi, M., Heshmati, A., Hadian, Z., Smaoui, S., Mousavi Khaneghah, A. (2022): The occurrence of aflatoxin M1 in doogh, kefir, and kashk in Hamadan, Iran. *Food Science and Technology* 42, e42022.
25. Kortei, N.K., Annan, T., Kyei-Baffour, V., Essuman, E.K., Boakye, A.A., Tettey, C.O., Boadi, N.O. (2022): Exposure assessment and cancer risk characterization of aflatoxin M1 (AFM1) through ingestion of raw cow milk in southern Ghana. *Toxicology Reports* 9, 1189-1197. https://doi.org/10.1016/j.toxrep.2022.05.015
26. Kos, J., Mastilović, J., Hajnal, E.J., Šarić, B. (2013): Natural occurrence of aflatoxins in maize harvested in Serbia during 2009-2012. *Food Control* 34(1), 31-34. https://doi.org/10.1016/j.foodcont.2013.04.004
27. Kos, J., Lević, J., Đuragić, O., Kokić, B., Miladinović, I. (2014): Occurrence and estimation of aflatoxin M1 exposure in milk in Serbia. *Food Control* 38, 41-46. https://doi.org/10.1016/j.foodcont.2013.09.060
28. Kos, J., Hajnal, E.J., Šarić, B., Jovanov, P., Nedeljković, N., Milovanović, I., Krulj, J. (2017): The influence of climate conditions on the occurrence of deoxynivalenol in maize harvested in Serbia during 2013-2015. *Food Control* 73, 734-740. https://doi.org/10.1016/j.foodcont.2016.09.022
29. Kos, J., Hajnal, E.J., Malachova, A., Steiner, D., Stranska, M., Krska, R., Poschmaier, B., Sulyok, M. (2020): Mycotoxins in maize harvested in Republic of Serbia in the period 2012-2015. Part 1: Regulated mycotoxins and its derivatives. *Food Chemistry* 312, 126034. https://doi.org/10.1016/j.foodchem.2019.126034
30. Kos, J., Anić, M., Radić, B., Zadravec, M., Janić Hajnal, E., Pleadin, J. (2023): Climate change - A global threat resulting in increasing mycotoxin occurrence. *Foods* 12 (14), 2704. https://doi.org/10.3390/foods12142704
31. Kos, J., Radić, B., Lešić, T., Anić, M., Jovanov, P., Šarić, B., Pleadin, J. (2024a): Climate change and mycotoxins trends in Serbia and Croatia: A 15-year review. *Foods* 13 (9), 1391. https://doi.org/10.3390/foods13091391
32. Kos, J., Radić, B., Radović, R., Šarić, B., Jovanov, P., Šarić, L. (2024b): Aflatoxins in maize, milk and dairy products from Serbia. *Food Additives & Contaminants: Part B* 17 (4), 296-307. https://doi.org/10.1080/19393210.2024.2335656
33. Kovač Tomas, M., Rot, T., Arnautović, L., Lenardić Bedenik, M., Jurčević Šangut, I. (2025): Occurrence and Risk assessment of aflatoxin M1 in Fermented dairy products from the Croatian market. *Foods* 14 (24), 4354. https://doi.org/10.3390/foods14244354
34. Krstović, S., Jakšić, S., Miljanić, J., Iličić, B., Živkov Baloš, M., Guljaš, D., Damjanović, M., Jajić, I. (2025): Annual and seasonal variations in Aflatoxin M1 in milk: Updated health risk assessment in Serbia. *Toxins* 17(11), 544. https://doi.org/10.3390/toxins17110544
35. Malissiova, E., Soultani, G., Tsokana, K., Alexandraki, M., Manouras, A. (2022): Exposure assessment on aflatoxin M1 from milk and dairy products-relation to public health. *Clinical Nutrition ESPEN* 47, 189-193. https://doi.org/10.1016/j.clnesp.2021.12.017
36. Malissiova, E., Tsinopoulou, G., Gerovasileiou, E.S., Meleti, E., Soultani, G., Koureas, M., Maisoglou, I., Manouras, A. (2024): A 20-year data review on the occurrence of aflatoxin M1 in Milk and dairy products in Mediterranean countries - Current situation and exposure risks. *Dairy* 5 (3), 491-514. https://doi.org/10.3390/dairy5030038
37. Milićević, D.R., Spirić, D., Radičević, T., Velebit, B., Stefanović, S., Milojević, L., Janković, S. (2017): A review of the current situation of aflatoxin M1 in cow's milk in Serbia: risk assessment and regulatory aspects. *Food Additives & Contaminants: Part A* 34 (9), 1617-1631. https://doi.org/10.1080/19440049.2017.1363414
38. Milićević, D., Petronijević, R., Petrović, Z., Đjinović-Stojanović, J., Jovanović, J., Baltić, T., Janković, S. (2019): Impact of climate change on aflatoxin M1 contamination of raw milk with special focus on climate conditions in Serbia. *Journal of the Science of Food and Agriculture* 99 (11), 5202-5210. https://doi.org/10.1002/jsfa.9768
39. Milićević, D.R., Milešević, J., Gurinović, M., Janković, S., Đinović-Stojanović, J., Zeković, M., Glibetić, M. (2021): Dietary exposure and risk assessment of aflatoxin M1 for children aged 1 to 9 years old in Serbia. *Nutrients* 13 (12), 4450. https://doi.org/10.3390/nu13124450

40. Nuhu, A.H., Dorleku, W.-P., Blay, B., Derban, E., McArthur, C.O., Alobuia, S.E., Incoom, A., Dontoh, D., Ofosu, I.W., Oduro-Mensah, D. (2025): Exposure to aflatoxins and ochratoxin A from the consumption of selected staples and fresh cow milk in the wet and dry seasons in Ghana. *Food Control* 168, 110968. https://doi.org/10.1016/j.foodcont.2024.110968
41. Pleadin, J., Kos, J., Radić, B., Vulić, A., Kudumija, N., Radović, R., Janić Hajnal, E., Mandić, A., Anić, M. (2023): Aflatoxins in Maize from Serbia and Croatia: Implications of climate change. *Foods* 12 (3), 548. https://doi.org/10.3390/foods12030548
42. Poroșnicu, I., Ailincăi, L.-I., Ariton, A.-M., Neculai Văleanu, A.-S., Borș, S.-I., Vintilă, V., Mareș, M. (2024): The importance of aflatoxin M1 in dairy products - a mini-review. *Scientific Papers Journal Veterinary Series* 1, 67-75. https://doi.org/10.61900/spjvs.2024.01.12
43. Rodríguez-Cañás, I., González-Jartín, J.M., Alvariño, R., Alfonso, A., Vieytes, M.R., Botana, L.M. (2024): Identification of mycotoxins in yogurt samples using an optimized QuEChERS extraction and UHPLC-MS/MS detection. *Mycotoxin Research* 40 (4), 569-579. https://doi.org/10.1007/s12550-024-00547-0
44. Roila, R., Branciari, R., Verdini, E., Ranucci, D., Valiani, A., Pelliccia, A., Fioroni, L., Pecorelli, I. (2021): A study of the occurrence of Aflatoxin M1 in Milk supply chain over a seven-year period (2014–2020): human exposure assessment and risk characterization in the population of Central Italy. *Foods* 10 (7), 1529. https://doi.org/10.3390/foods10071529
45. Sarmast, E., Fallah, A.A., Jafari, T., Khaneghah, A.M. (2021): Impacts of unit operation of cheese manufacturing on the aflatoxin M1 level: A global systematic review and meta-analysis. *LWT* 148, 111772. https://doi.org/10.1016/j.lwt.2021.111772
46. Serbian Regulation (2022): Maximum allowed contents of contaminants in food and feed. *Official Bulletin of the Republic of Serbia* 127, 1-528.
47. Sumon, A.H., Islam, F., Mohanto, N.C., Kathak, R.R., Molla, N.H., Rana, S., Degen, G.H., Ali, N. (2021): The presence of Aflatoxin M1 in milk and milk products in Bangladesh. *Toxins* 13(7), 440. https://doi.org/10.3390/toxins13070440
48. Škrbić, B., Živančev, J., Antić, I., Godula, M. (2014): Levels of aflatoxin M1 in different types of milk collected in Serbia: Assessment of human and animal exposure. *Food Control* 40, 113-119. https://doi.org/10.1016/j.foodcont.2013.11.039
49. Škrbić, B., Antić, I., Živančev, J. (2015): Presence of aflatoxin M1 in white and hard cheese samples from Serbia. *Food Control* 50, 111-117. https://doi.org/10.1016/j.foodcont.2014.08.031
50. Tarannum, N., Nipa, M.N., Das, S., Parveen, S. (2020): Aflatoxin M1 detection by ELISA in raw and processed milk in Bangladesh. *Toxicology Reports* 7, 1339-1343. https://doi.org/10.1016/j.toxrep.2020.09.012
51. Technical report CEN/TR 16059 (2012): Food analysis-performance criteria for single laboratory validated methods of analysis for the determination of mycotoxins. Brussels, Belgium: European Committee for Standardization, Management Centre. 1-14.
52. Tomašević, I., Petrović, J., Jovetić, M., Raičević, S., Milojević, M., Miočinović, J. (2015): Two year survey on the occurrence and seasonal variation of aflatoxin M1 in milk and milk products in Serbia. *Food Control* 56, 64-70. https://doi.org/10.1016/j.foodcont.2015.03.017
53. Torović, L. (2015): Aflatoxin M1 in processed milk and infant formulae and corresponding exposure of adult population in Serbia in 2013-2014. *Food Additives & Contaminants: Part B* 8 (4), 235-244. https://doi.org/10.1080/19393210.2015.1063094
54. Udovicki, B., Keskic, T., Aleksic, B., Smigic, N., Rajkovic, A. (2023): Second order probabilistic assessment of chronic dietary exposure to aflatoxin M1 in Serbia. *Food and Chemical Toxicology* 178, 113906. https://doi.org/10.1016/j.fct.2023.113906
55. Vaz, A., Cabral Silva, A.C., Rodrigues, P., Venâncio, A. (2020): Detection methods for aflatoxin M1 in dairy products. *Microorganisms* 8 (2), 246. https://doi.org/10.3390/microorganisms8020246