International Scientific – Practical Conference
FOOD, TECHNOLOGIES & HEALTH

UNIVERZITET U NOVOM SADU
TEHNOLOŠKI FAKULTET NOVI SAD

AFLATOXIN M1 IN DAIRY PRODUCTS AVAILABLE IN SERBIAN MARKET

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Novi Sad, Serbia

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Plovdiv

This study was conducted in order to monitor of the natural occurrence of AFM1 in different types of milk and white cheese available in Serbian market...

... Important in a light of recent outbreak of milk contamination by AFM1 that occured at the end of February in Serbia...

ПОЛИТИКА ONLINE

31. oktobar 2013.
poslednja izmena u 13:00

eПОЛИТИКА електронско издање

Beograd 16°C

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MOJ ŽIVOT U INOSTRANSTVU

EKONOMIJA

Alarm zbog mleka u prahu

Ako se zagađenost mleka označava faktorom 1, onda prerađeno mleko u prahu ima najvišu moguću koncentraciju zagađenja – faktor 7, kažu u Saveznom institutu za procenu rizika u ishrani u Berlinu

Mleko u prahu ima najvišu koncentraciju aflatoksina – faktor 7

Afera mleko – mlečni proizvodi, meso i iznutrice zatrovani kancerogenim gljivičnim otrovom aflatoksin B1 i M1 – izvesno će na duži period o(p)stati u srpskom lancu ishrane. To je posledica zagađenja stočne hrane kontaminiranim kukuruzom.

- Strani investitori primetili reforme
- Krstić: Stabilizacija javnog duga u 2016.
- Ilić: Građevinska dozvola za 30 dana i u startu besplatno
- Vučić:Niko ne sme da ucenjuje državu, posebno ne FAP
- Blokada SNS-a, kontrablokada URS-a
- Radulovićev recept za kraj privatizacije
- Blokada pruge na jubilej FAP-a
- Privilegije sapliću lične karte
- Privatizacija u Srbiji oročena na 31. decembar 2014.
- Srbija 93. na svetu po uslovima poslovanja
- Radnici FAP-a blokirali prugu Beograd – Bar
- Pošalji SMS za krađu struje

RAZGOVOR NEDELJE: SAŠA RADULOVIĆ, ministar privrede

- Lična karta pa pomoć od države
- Poletela Er Srbija – prvo za Abu Dabi
- Restrukturiranje Srbijagasa najkasnije za godinu i po
- „Južni tok" teče po planu
- Zbogom, Jate, dobar let, Er Srbijo
- Državne banke koštale nas milijardu evra

Till now, there have not been available studies reporting the AFM1 content in milk and dairy products from the Serbian market

and

this study presents the first **comprehensive** survey of the occurrence of AFM1 in different types of milk and white cheese available in Serbia.

The presence of AFM1 in cow's milk is the consequence of the consumption of feed contaminated by aflatoxin B1 (AFB1), a secondary metabolite produced of some molds during the crop growth, harvest and/or storage since…

...

AFB1 is known to be metabolized to form the monohydroxy derivative - AFM1.

The AFM1 derivative can be detected in milk within 12–24 h after the first intake of AFB1, while its concentration decreases to an undetectable level 72 h after the initial intake is stopped.

- AFM1 is also known to be hepatotoxic and carcinogenic compound.

- International Agency for Research on Cancer classified AFM1 in Group 1 toxin as possibly carcinogenic for humans.

International Agency for Research on Cancer
World Health Organization

The AFM1 is not destroyed by pasteurization of milk, and
thus can be transferred into yoghurt,
powdered milk and other milk based products.

Biljana Škrbić, Jelena Živančev, Igor Antić | *Faculty of Technology, University of Novi Sad*

The contamination of milk and milk products with AFM1 displayed variations according to:

- geography,
- country,
- season,
- environmental conditions,
- inability of certain agricultural systems,
- low availability of green fodder,
- excessive use of concentrated feed, cottonseed cake, corn, soybean, threshed wheat straw, paddy straw, wheat bran and
- contamination of the feed and the grain with AFs during storage.

THUS, milk has the greatest potential demonstrated for introducing AFM1 into human diet.

The frequency of occurrence of AFM1 in commercially available milk and dairy products, the high intake of these products by human population,

especially by infant and young children and its probable carcinogenic effect,

led to an increased concern about the establishment of measures to control AFM1 contamination.

In the light of these concerns, several countries have established regulatory limits for AFM1 in milk and derivative products, with values varying according to national legislation.

According to the Food and Agriculture Organization,

sixty countries have established regulatory limits for AFM1.

The European Commission Regulation 1881/2006 sets a maximum limit of 0.05 μg/kg for AFM1 in raw milk, heat-treated milk and milk for the manufacture of milk based products.

20.12.2006 EN Official Journal of the European Union L 364/5

COMMISSION REGULATION (EC) No 1881/2006

of 19 December 2006

setting maximum levels for certain contaminants in foodstuffs

(Text with EEA relevance)

EUROPEAN COMMISSION

It should be noted that regulations regarding AFM1 toxin in cheese have not yet been established by European Commission.

The same limit was also valid in Serbia from 2011 till the end of February 2013.

However, after the outbreak of milk contamination by AFM1 that occurred in Serbia in the very end of February 2013,

the Serbian Government has established new maximum level of 0.5 µg/kg,

seemed to be a practical compromise between the need to control the AFM1 and the economic consequences of the setting regulatory limit.

Samples

Sampling period:
February,
April and May

42 samples of commercial milk

6 milk samples produced in households

2 milk samples were taken from small private farm

Total of 50 milk samples were collected

4 cheese samples produced in household

13 samples of commercial cheese

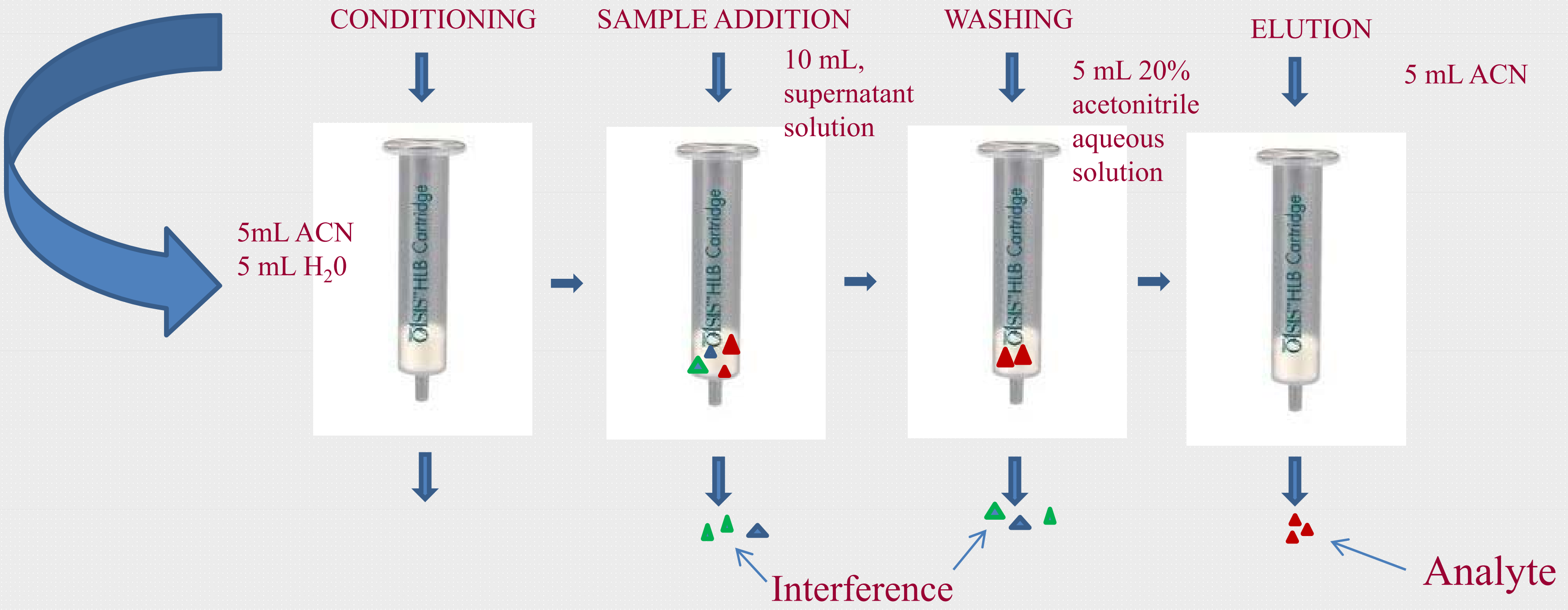
Total of 17 white cheese samples were collected

The sample preparation procedure for milk based on solid phase extraction (Oasis HLB, Waters).

20 g milk

Centrifugation 10 min (8000 rpm)

fat layer was removed and the supernatant was collected



Eluate was collected and evaporated to dryness using gentle stream of nitrogen. The residue was reconstituted with 1 ml of 20% aqueous acetonitrile and the obtained solution was passed through the 0.2 μm nylon syringe filter.

The simple sample preparation technique with only one step of extraction was used for isolation of AFM1 from white cheese.

cheese

Homogenized sample 5g

↓

Extraction 1 h (shaking) — **20 ml ACN/H_2O (84:16, v/v)**

↓

Filtration

↓

Dilution (1:3) ← **with UHPLC mobile phase of the initial content (95% A and 5% B)**

↓

<u>0.2 µm nylon syringe filter</u>

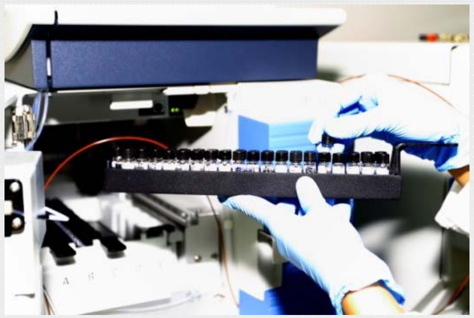
↓

UHPLC-HESI-MS/MS

The selected samples

were analyzed by

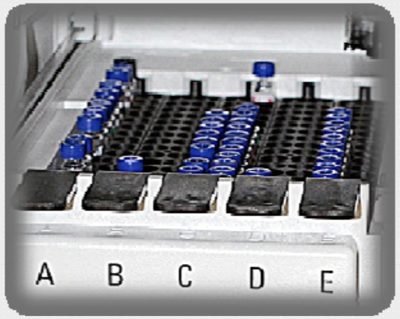
ultra-high performance liquid chromatography with heated electrospray ionization triple quadrupole mass spectrometry (UHPLC/HESI-MS/MS).



UHPLC/HESI-MS/MS parameters of AFM1 separation and identification under optimized conditions on Accela-TSQ Vantage (Thermo Fisher Scientific, USA)

Mycotoxin	Retention time, min	Dwell time, s	Precursor ion, m/z	Product ions: quantifier/qualifier, m/z	CID [a], eV
AFM1	2.86	0.1	329 $[M+H]^+$	259.1/273.1	25/23

[a] Collision-induced dissociation energy for quantifier/qualifier ion.



Validation data of UHPLC-HESI-MS/MS for AFM1 determination

Samples	AFM1 spiked level (µg/kg)	Recovery (%)	RSD (%)
milk	0.05	69	10
	0.5	71	7
white cheese	0.25	110	9
	0.5	72	7

Milk
$R^2 > 0.990$
LOD =0.0002 µg/kg

White cheese
$R^2 > 0.990$
LOD = 0.02µg/kg

Recovery values for **milk and white cheese** were within the range of 69-110% recommended by the Commission Regulation (EC) 401/2006.

UHPLC-MS/MS chromatograms for AFM1 corresponding to:

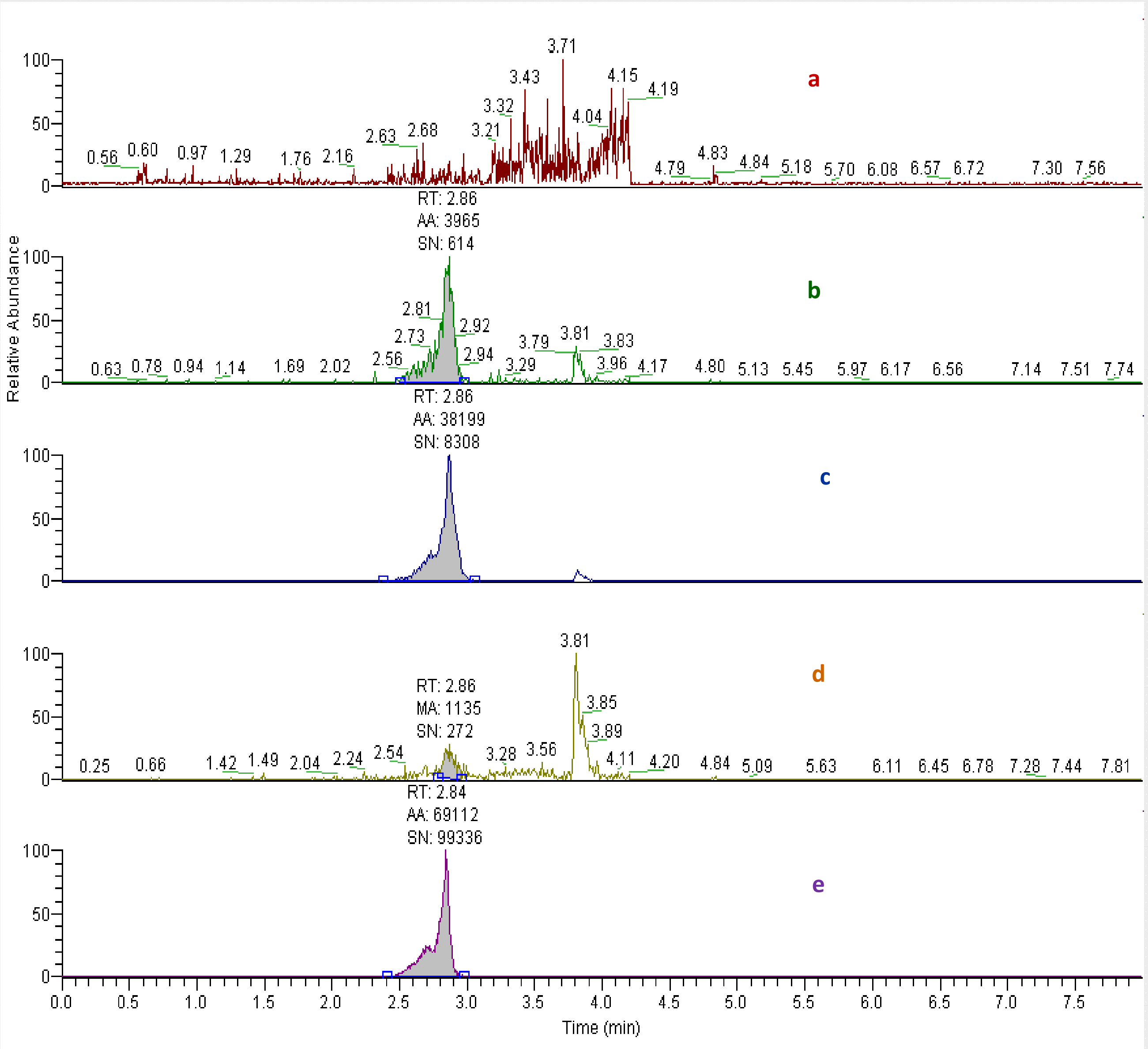
a) uncontaminated sample (□LOD),

b) uncontaminated milk sample spiked at level of 0.05 µg/kg,

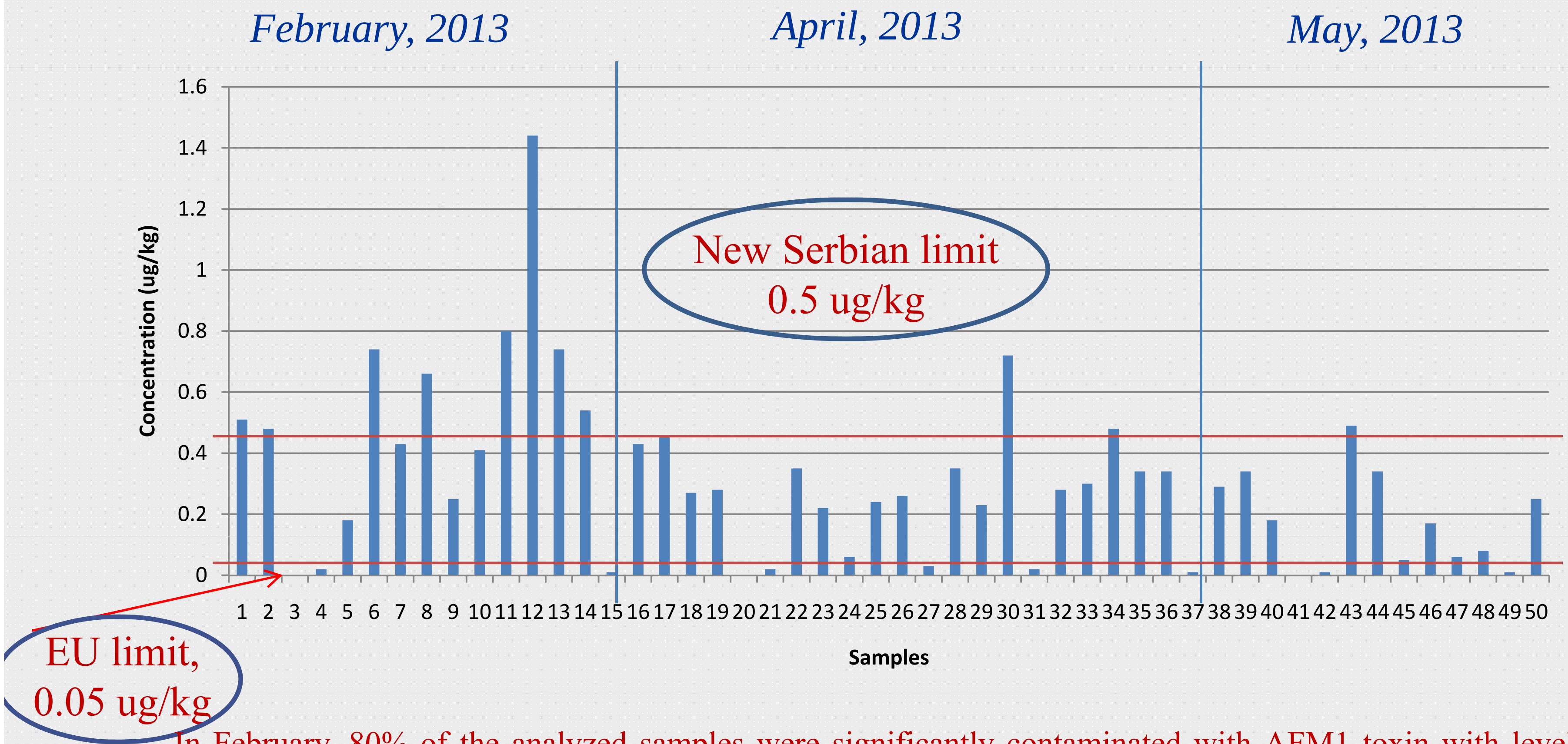
c) uncontaminated milk sample spiked at level of 0.5 µg/kg

d) the real sample with the lowest level of AFM1 (0.01 µg/kg) and

e) the real sample with the highest level of AFM1 (1.44 µg/kg).



Occurrence of Aflatoxin M1 in all milk samples analyzed in this study

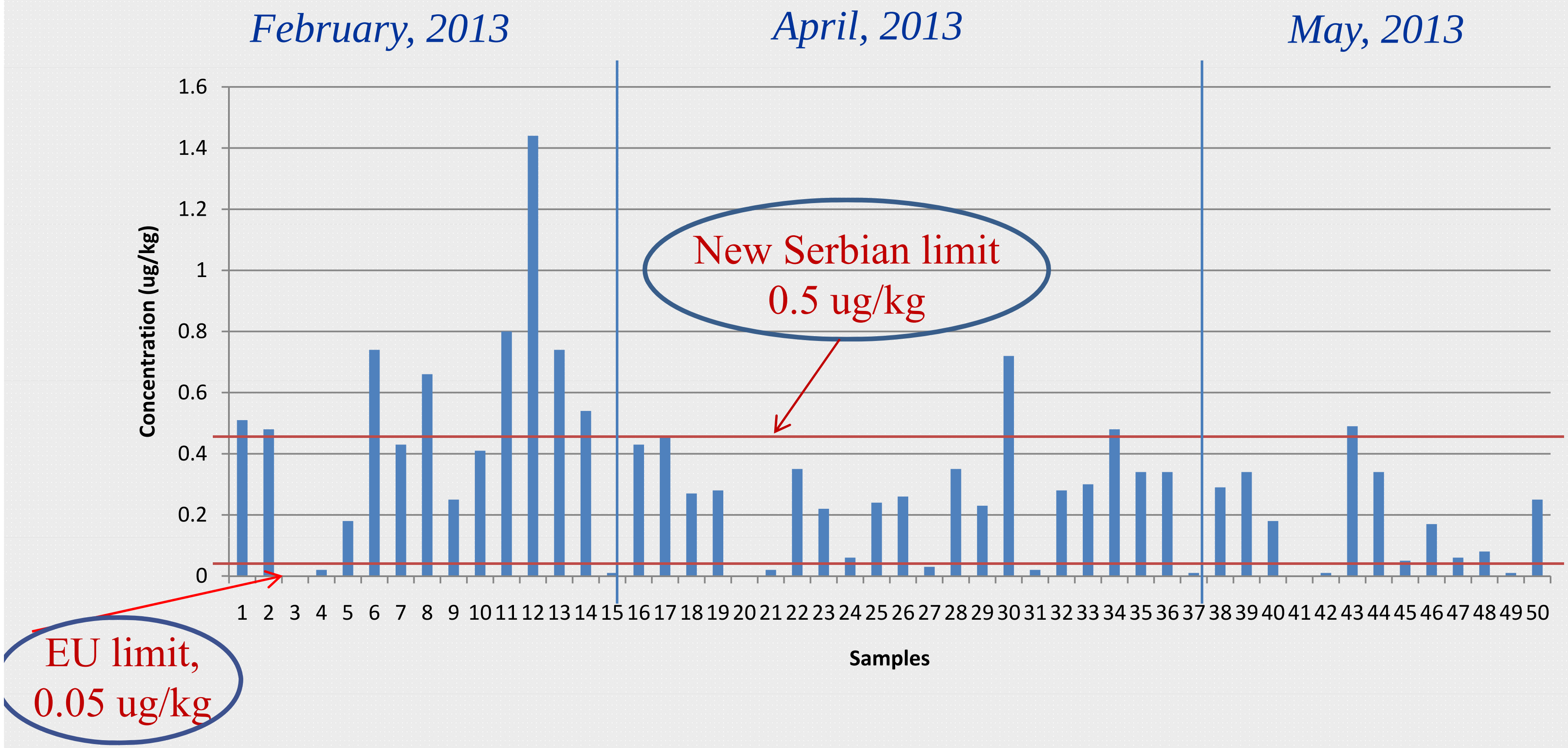


In February, 80% of the analyzed samples were significantly contaminated with AFM1 toxin with levels above 0.05 µg/kg set as maximum allowable concentration by EU.

Almost the same percentage of the positive samples with AFM1 above EU limit was obtained for samples taken in April.

Frequency of occurrence of AFM1 (69% of the analyzed samples) in investigated samples of milk collected in May was lower than for the investigated in February and April.

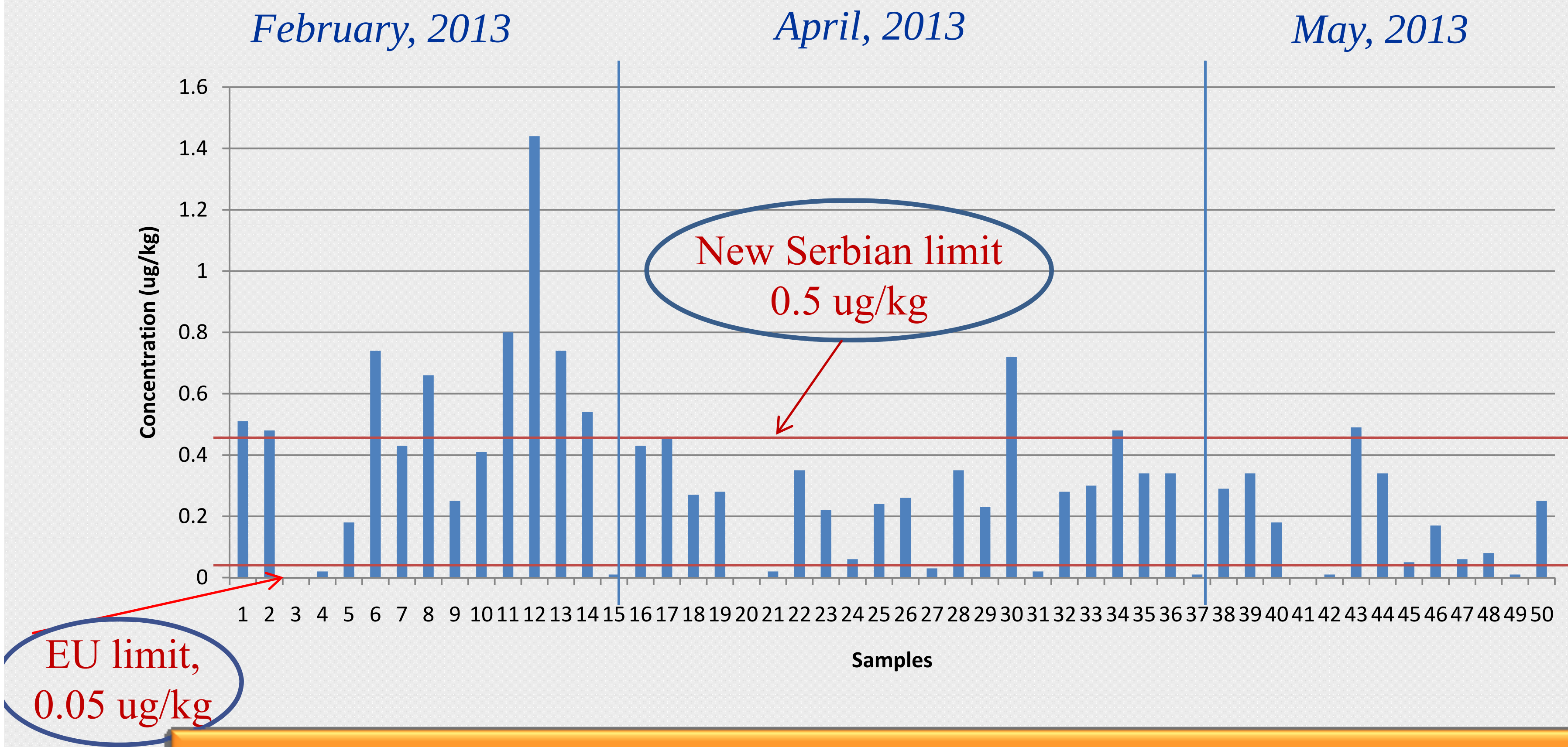
Occurrence of Aflatoxin M1 in all milk samples analyzed in this study



The contamination levels of the AFM1 toxin in milk samples collected in February ranged from < LOD to 1.44 µg/kg, while concentration range of selected toxin in investigated samples of milk collected in April was from < LOD to 0.72 µg/kg.

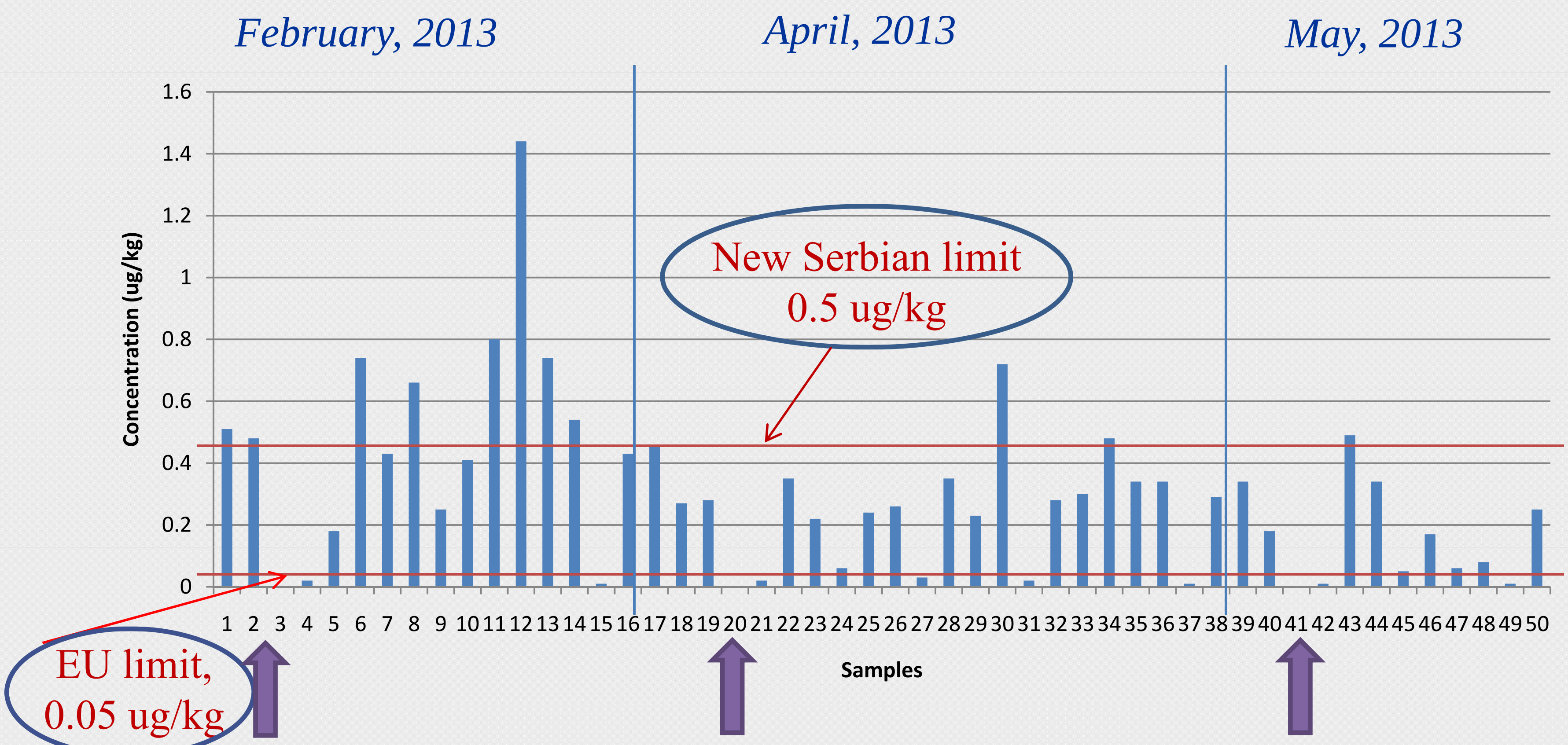
Remarkably, lower concentration range of AFM1 (< LOD – 0.49 µg/kg) in milk samples collected in May was obtained.

Occurrence of Aflatoxin M1 in milk samples analyzed in this study



In total, only 12 samples out of 50 investigated milk samples were not contaminated taking into consideration level of 0.05 µg/kg sets by EU regulation.
Considering the new value set for AFM1 by the Serbian regulation, only one of the investigated milk samples produced in Serbia and/or found in domestic market in April exceeded the limit of 0.5 µg/kg.

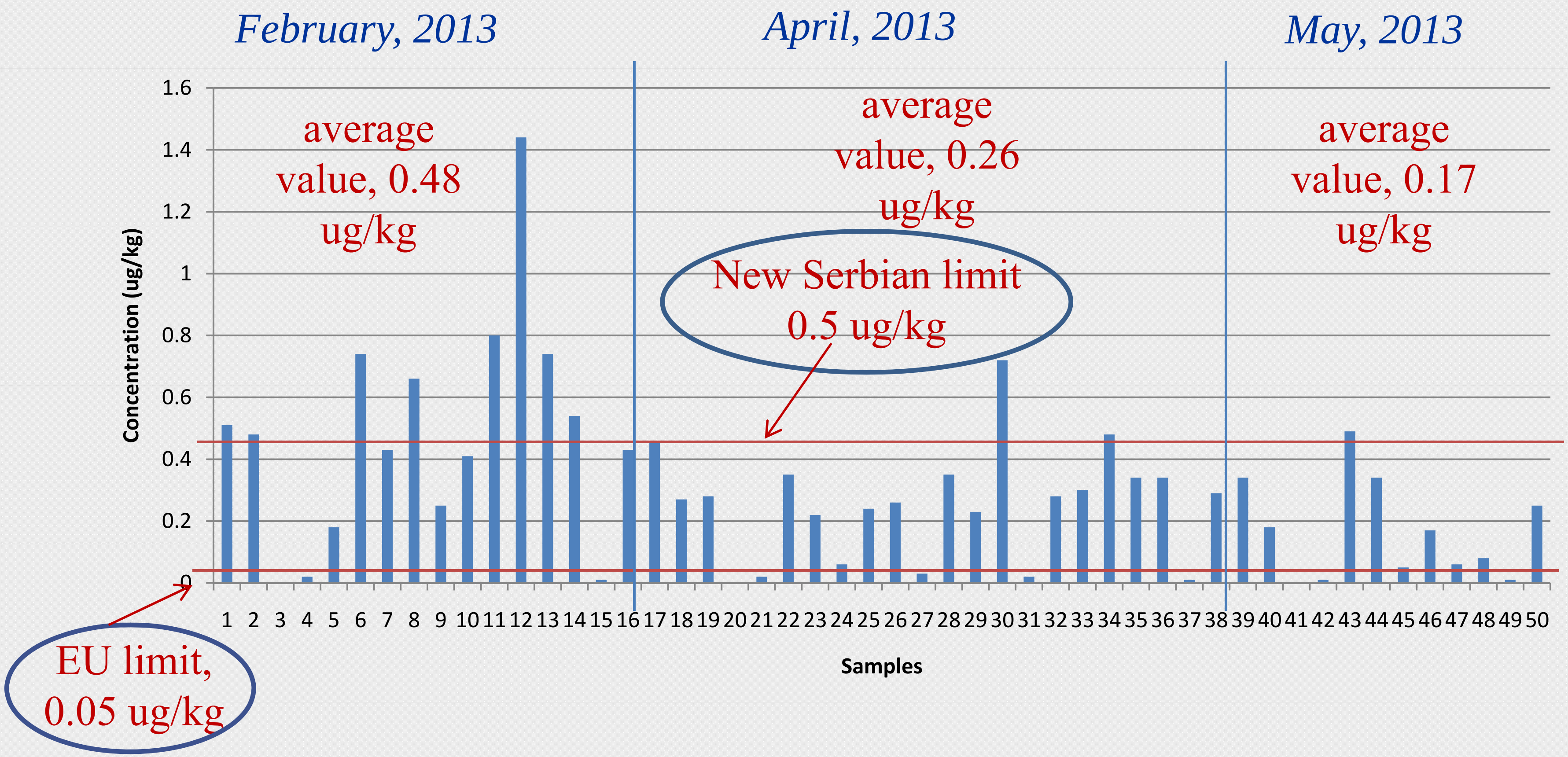
Occurrence of Aflatoxin M1 in all milk samples analyzed in this study



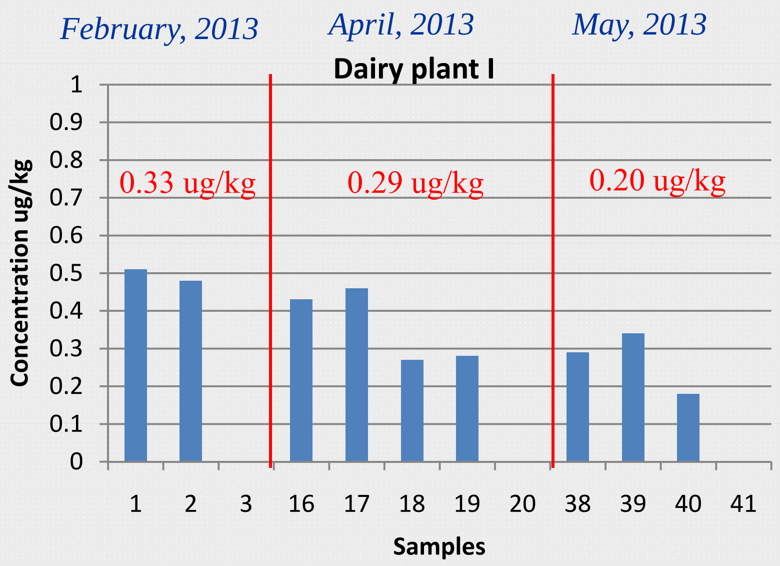
The lowest levels of AFM1 were determined in samples (no. 3, 20 and 41) of organic milk produced at the dairy plant I.

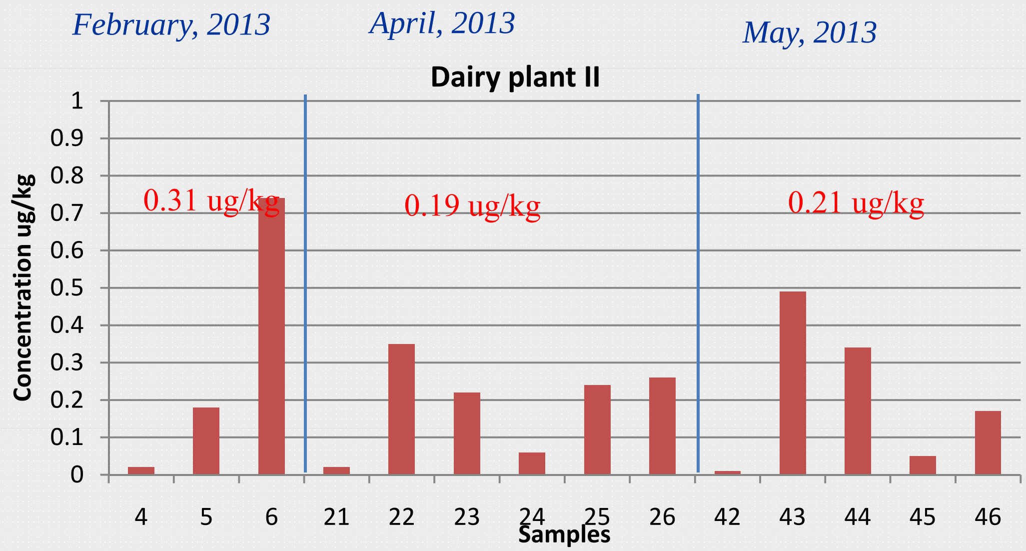
It is worth to note that organic milk from this dairy plant is the only commercially available organic milk in Serbia.

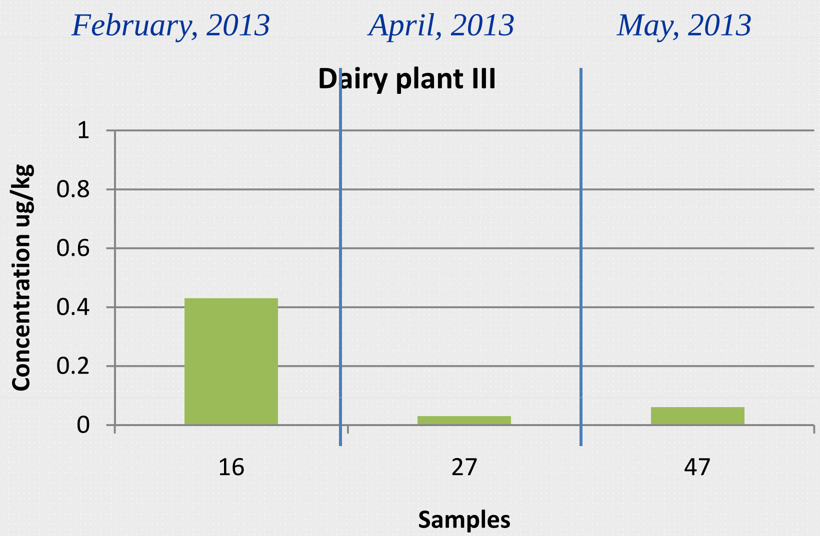
Occurrence of Aflatoxin M1 in all milk samples analyzed in this study



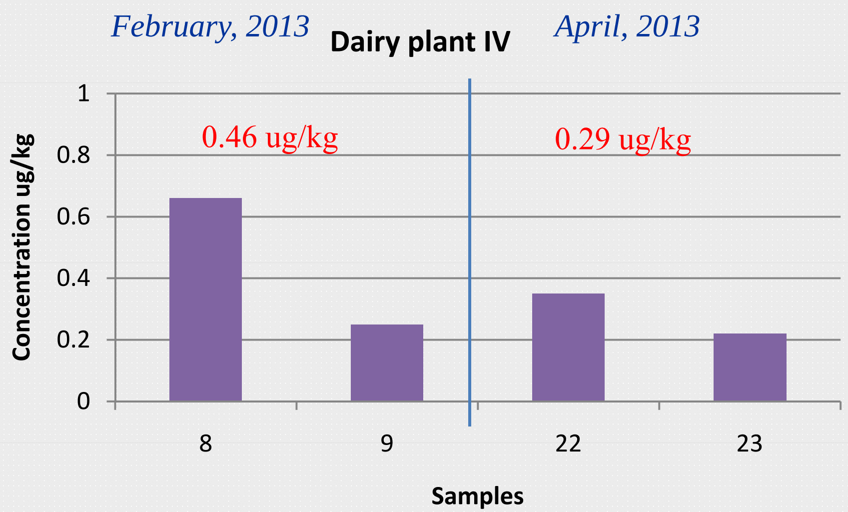
The occurrence of AFM1 indicated that the level of this toxin was the highest during February followed by April and May;
the average value of AFM1 in February samples was 0.48 μg/kg, while in April and May, it was 0.26 and 0.17 μg/kg, respectively.

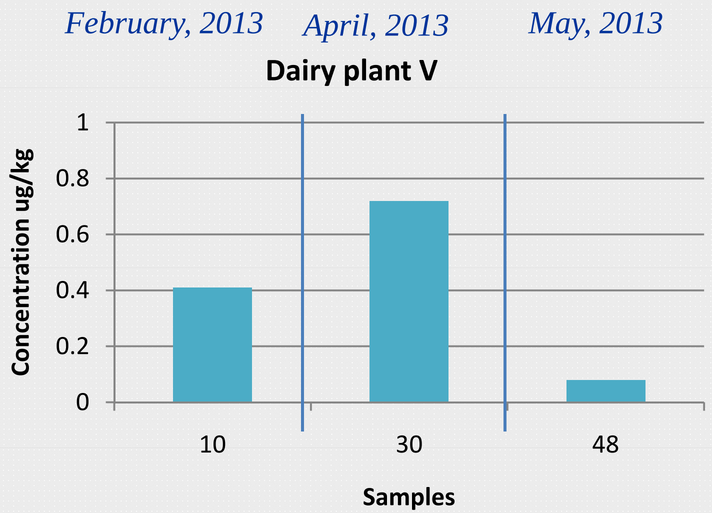


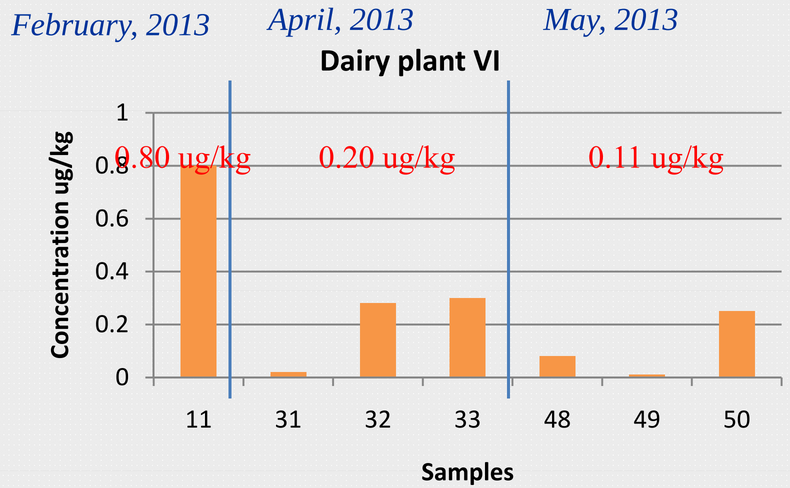




The similar trend of reducing average value from February to May could be seen also when the samples originating from each dairy plant were taken into account.

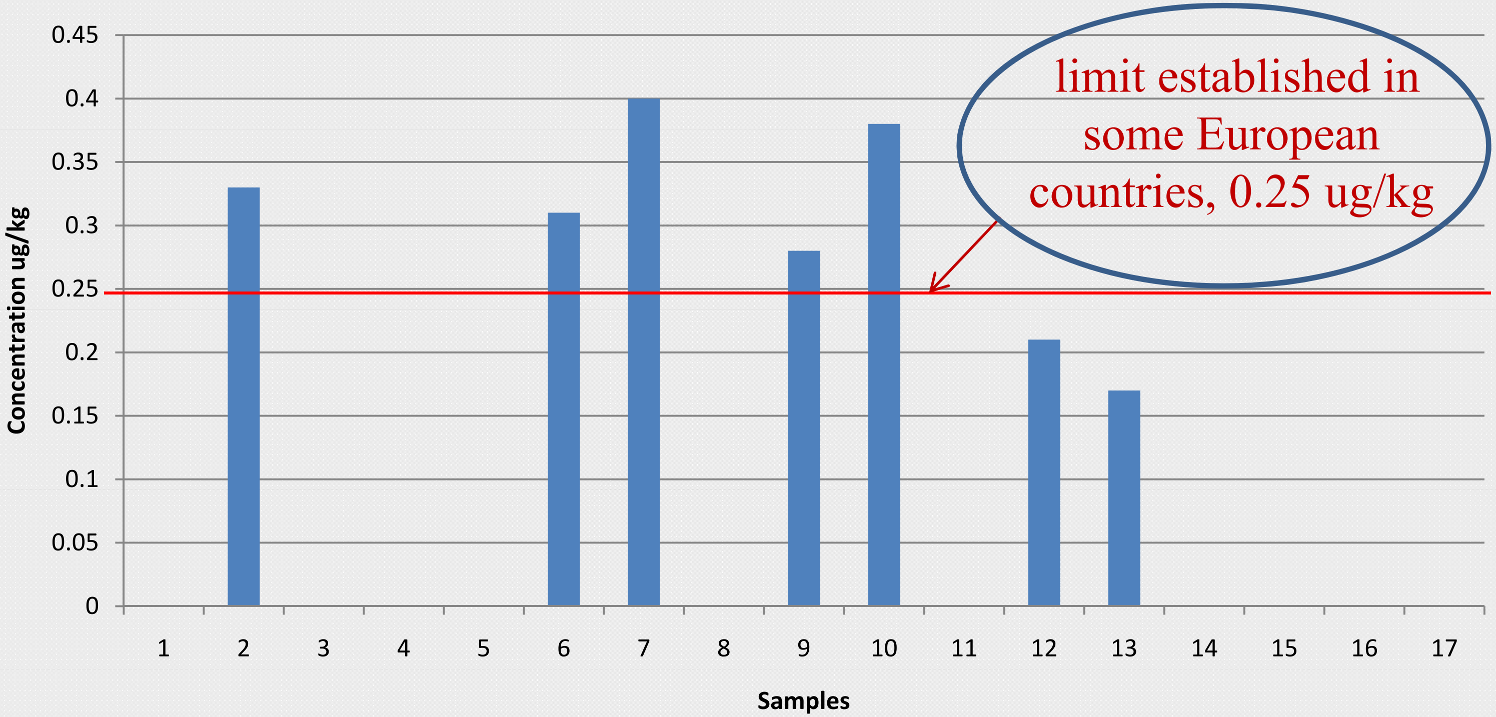






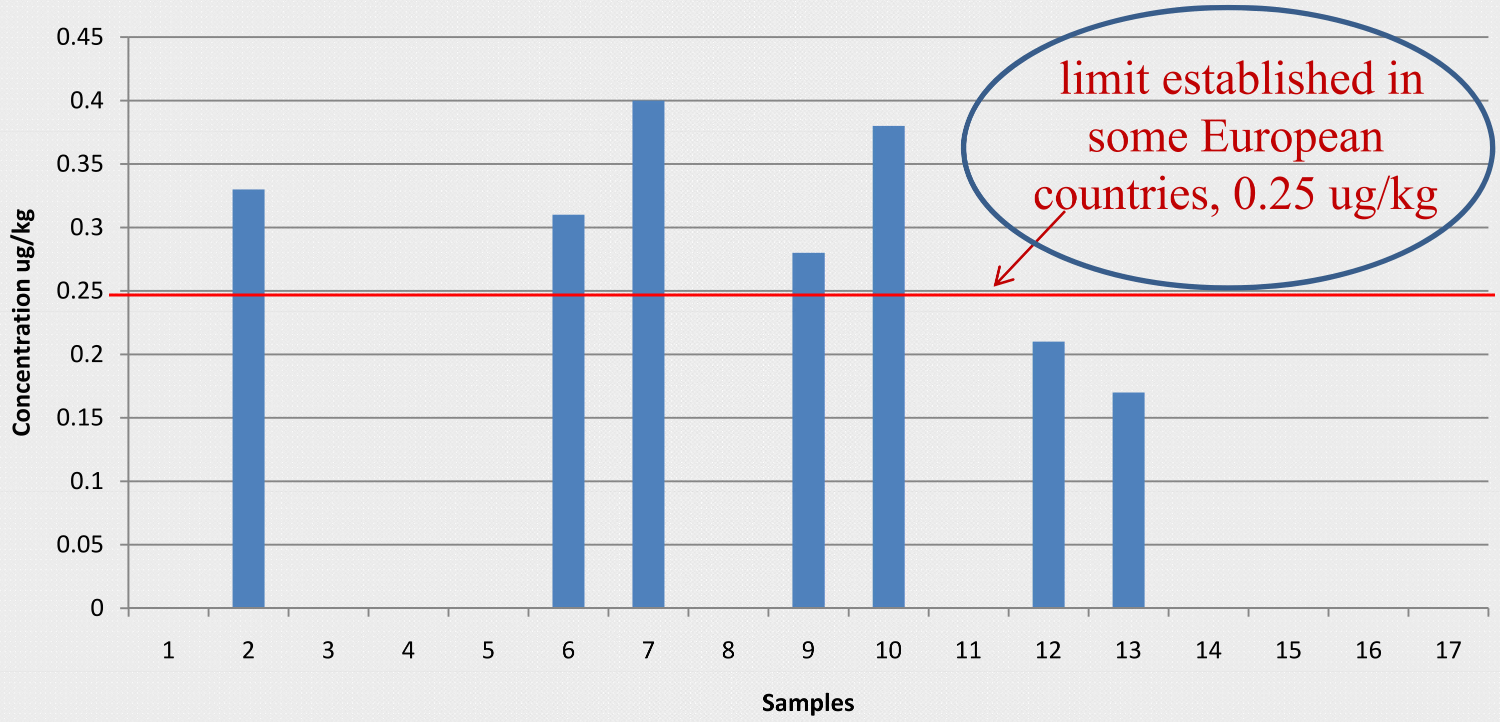
The only exception could be seen for dairy plant V but it could not be taken as representative for the following of the trend because only one sample per period was available.

Occurrence of Aflatoxin M1 in white cheese samples analyzed in this study



- ✓ It should be noted that regulations regarding AFM1 toxin in cheese have not yet been established by European Commission.
- ✓ However, the maximum acceptable level of AFM1 in cheeses in some European countries including Switzerland, France, Austria and Turkey has been established as 0.25 μg/kg.

Occurrence of Aflatoxin M1 in white cheese samples analyzed in this study



The contamination levels of the AFM1 toxin in white cheese samples collected in Serbian market ranged from < LOD to 0.40 μg/kg.

Frequency of occurrence of AFM1 in investigated samples of cheese with level above 0.25 ug/kg was 39%.

CONCLUSIONS

- ***The obtained results indicate that the contamination of milk with AFM1 has the potential to be a serious public health problem in Serbia,***

particularly if the EU regulation would be taken into account.

CONCLUSIONS

- ***The high level of AFM1 in investigated samples of milk confirmed that constant monitoring throughout the milk production chain is necessary in order to minimize health risks related to the presence of this toxin in milk.***

CONCLUSIONS

- ***Reducing the levels of AFM1 in milk can be achieved by the implementation of good agricultural and storage practices to control of AFs contamination feed supply chain***

and

- ***also by regulating stringent limits for AFs in feed and milk in Serbia.***

Thank you for your kind attention!