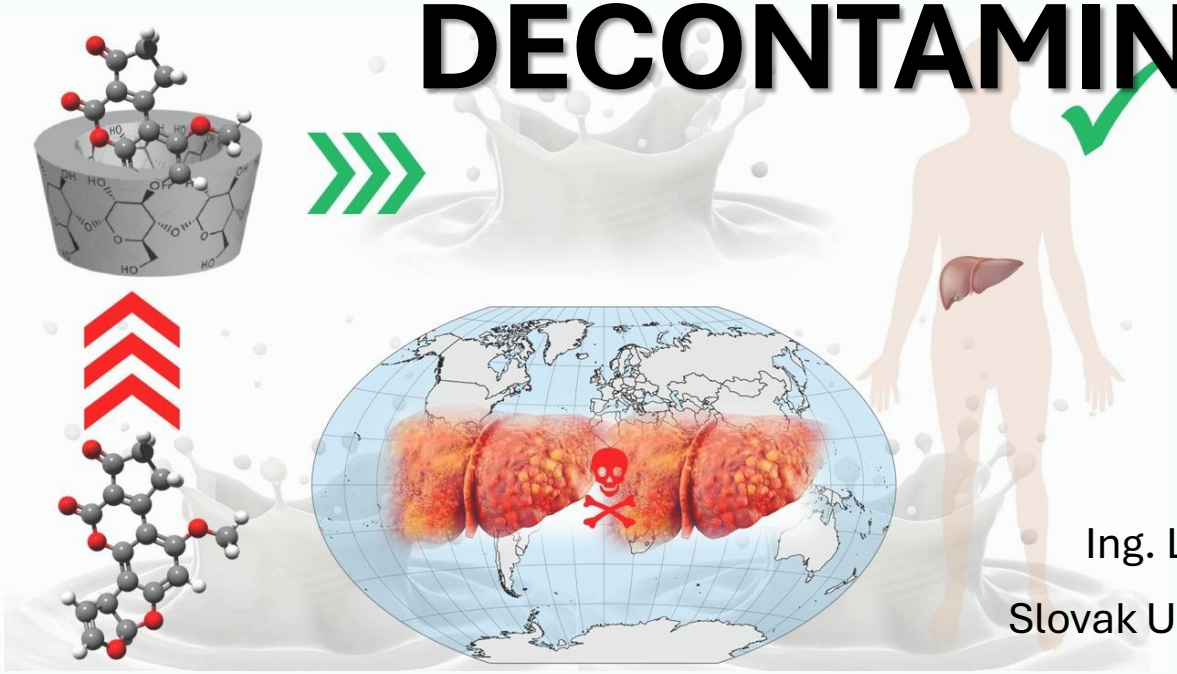
THE OCCURRENCE OF AFLATOXIN M1 IN MILK AND THE CHARACTERIZATION OF DECONTAMINATION METHODS



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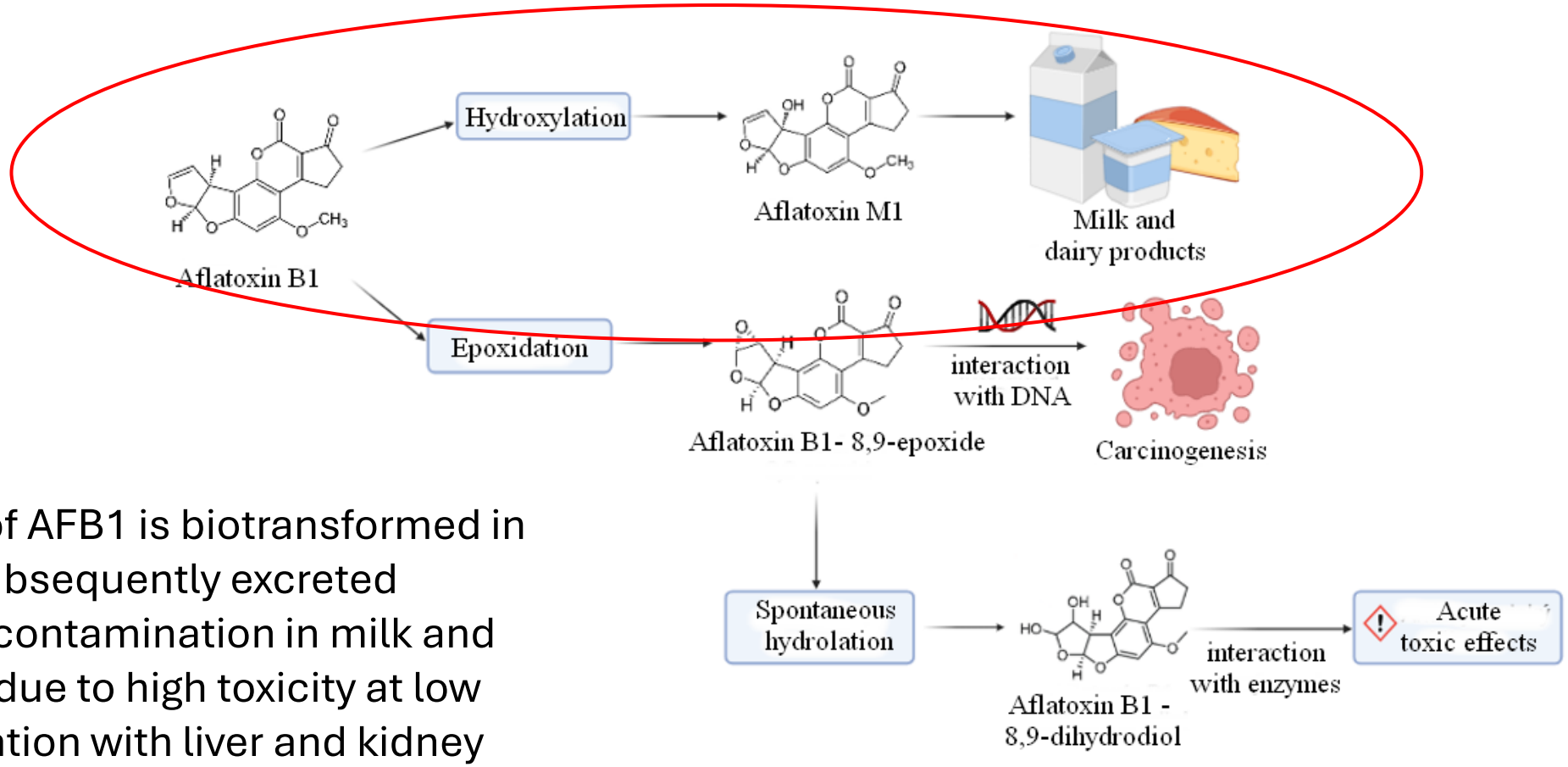
2025

Aflatoxins

- The most frequently monitored mycotoxins, due to their **high toxicity** – the most important producers of aflatoxins are two closely related fungi of the genus ***Aspergillus***, *A. flavus* and *A. parasiticus*
- The highest amount of aflatoxins (in some cases hundreds of thousands of mg/kg) - **corn**, peanuts, pistachios, Brazil nuts, cottonseeds
- Lower levels of aflatoxins - almonds, pecans, walnuts, raisins, figs and various spices
- **Rapid Alert System for Food and Feed (RASFF)** – aflatoxins are the contaminants most commonly found in the control of food imported into the EU (most commonly nuts and nut products)
- *A. flavus* - aflatoxins B1 and B2
- *A. parasiticus* - aflatoxins G1 and G2



- Between 0.5% and 5% of AFB1 is biotransformed in the liver to **AFM1** and subsequently excreted
- **Concerns** about AFM1 contamination in milk and dairy products - mainly due to high toxicity at low concentrations, association with liver and kidney cancer, and thermal stability during pasteurization and sterilization
- The carcinogenic efficacy of AFM1 is lower than that of AFB1 (about 1-tenth of AFB1) - AFM1 is also classified as a **group 1** compound carcinogenic to humans by the International Agency for Research on Cancer (IARC)

Several organisations and countries have restricted the maximum tolerable level of AFM1 residues in milk and milk products:

- **50 ng/kg** for raw milk or heat-treated milk and **25 ng/kg** for infant food in the European Union (Commission Regulation, 2023)
- **500 ng/kg** for milk in the USA
- **50 ng/kg** for milk and **250 ng/kg** for cheese in Turkey
- **100 ng/kg** for different types of milk in Iran

5.5.2023 EN Official Journal of the European Union L 119/103

COMMISSION REGULATION (EU) 2023/915

of 25 April 2023

on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006

(Text with EEA relevance)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Council Regulation (EEC) No 315/93 of 8 February 1993 laying down Community procedures for contaminants in food ([1]), and in particular Article 2(3) thereof,

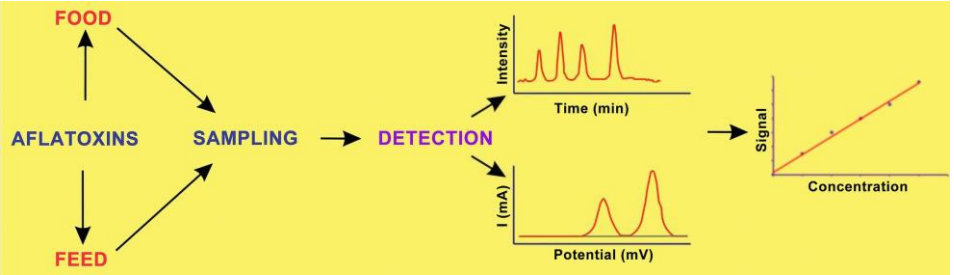
The AFM1 content in dairy products is closely related to:

- Geographical location
- The level of development of the country
- Season
- Economic management
- Milk and Dairy Processing Systems

Long periods of high temperatures and prolonged dry summers in European countries with temperate climates over the past decade have influenced the increased occurrence of AFB1 in dairy feed and the occurrence of increased levels of AFM1 in milk and dairy products

- AFM1 is combined with different parts of milk, mainly milk serum and casein, while the fat fraction may contain smaller amounts
- The level of AFM1 in cheese can be approximately 3-5 times higher than in original milk due to its high affinity for the hydrophilic parts of casein or whey protein due to its hydrophilic nature

Analysis of AFM_1



Analytical techniques used for the detection of aflatoxins

Chromatographic methods with different detection techniques

GC-MS, GC-FID, GC-TCD

LC-MS

HPLC-MS, HPLC-FLD

TLC

Optical methods based on UV-VIS or fluorescence detection

Simple UV-VIS and fluorescence spectroscopy

Fluorescence polarization immunoassay

Enzyme linked immunosorbent (ELISA)

Fourier transform infrared spectroscopy

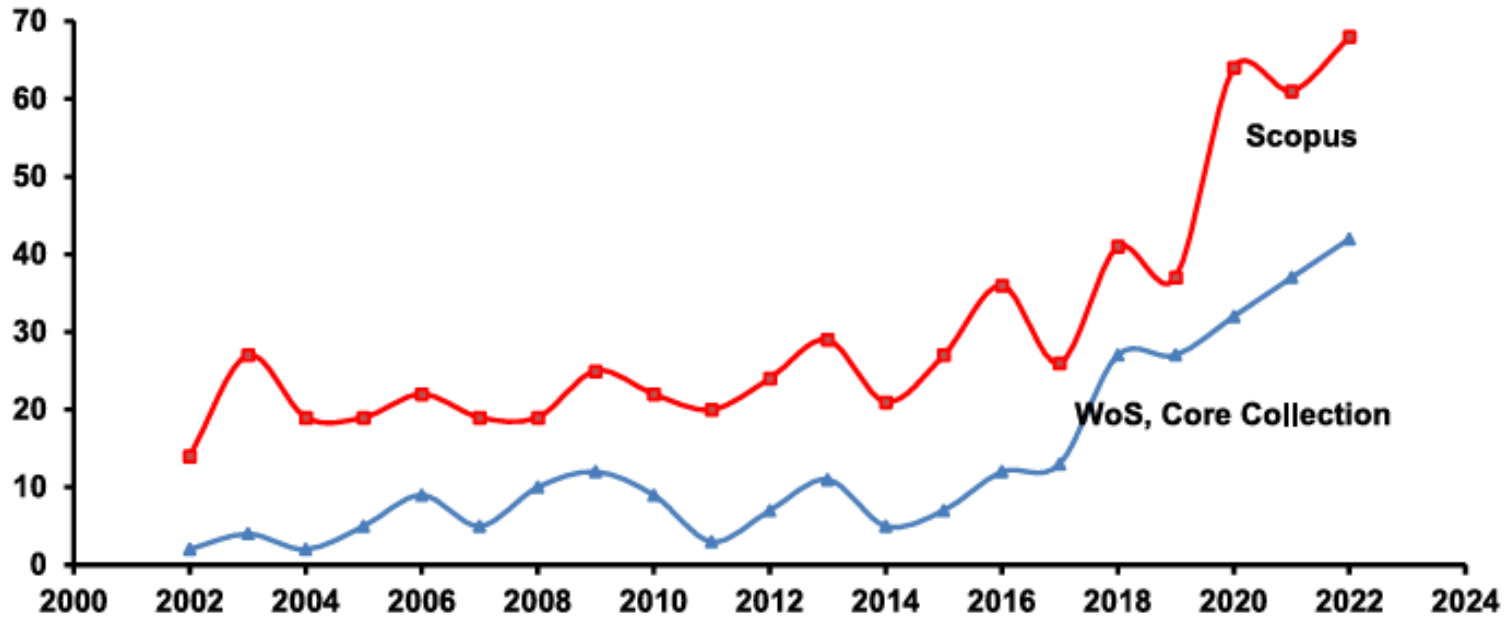
Infrared spectroscopy

Electrochemical Detection Techniques

Voltammetric and amperometric chemical, enzyme, immunosensors

Most applied

- **Chromatographic methods** - mainly used to confirm the results obtained from rapid screening tests or to accurately quantify the content of aflatoxins
- **Immunochemical methods** - used for rapid screening of aflatoxins in various sample matrices
- **HPLC with fluorescence (FLD) detection** using possible post-column derivatization - reference method and currently the most commonly used method for qualitative and quantitative determination of AFM1 content in milk



The number of reviews yearly, related to aflatoxin analysis, from the Clarivate Analytics, Web of Science Core Collection (▲) and Scopus (■).

(Pisoschi, A.M., Iordache, F., Stanca, L., et al. (2023). Comprehensive overview and critical perspective on the analytical techniques applied to aflatoxin determination – A review paper. Microchemical Journal, 191, 108770.)

Selected findings of AFM1 in during 2018–2024.

Country	Milk type	Positive/total samples	Concentration of AFM_1 [ng/L]	Reference
Bangladesh	Raw milk	75/105	5.0–198.7	Sumon et al. (2021)
	Pasteurized milk	15/15	17.2–187.7	
	UHT milk	15/15	12.2–146.9	
	Powdered milk	4/15	5.9–7.0	
	Yogurt	5/5	8.3–41.1	
Bangladesh	Raw milk	39/90	23.08–533.83	Ali et al. (2023)
Brazil	UHT	15[a]/34	150–560	Conteçotto et al. (2021)
	Powdered milk	1[a]/10	1020	
	Infant formulas	1[a]/16	320	
Brazil	Cheese	28/28	26–132	Silva et al. (2023)
Croatia	Raw milk	109[a]/5817	50.3–1100.0	Bilandžić et al. (2022)
China	Pasteurized milk	40/93	5.1–11.3	Xiong et al. (2022)
	Extended-shelf-life milk	44/96	5.1–16.5	
	Donkey raw milk	0/70	<5	
Ghana	Raw milk	67[a]/120	60–3520	Kortei et al. (2022)
Greece	Infant/toddler milk	31/52	0.23–9.38	Malissiova et al. (2022)
	Pasteurized milk	22/32	0.20–17.84	
	Feta cheese	7/25	0.54–4.09	
Hungary	Raw milk	191/278	5–173	Buzás et al. (2023)
	Drinking milk	155/196	5.3–100.0	
India	Raw milk	70[a]/100	10–1116	Sharma et al. (2020)
	Pasteurized milk	40[a]/50	27–2281	
Indonesia	Raw milk	18/20	24–449	Sumantri et al. (2019)
	Pasteurized milk	16/16	100–570	

Iran	Raw milk	62/67	2.02–189.81	Ghaffarian-Bahraman et al. (2023)
	Pasteurized milk	33/33	5.40–125.37	
Italy	Raw milk	667[a]/95 882		Ferrari et al. (2023)
Lebanon	Raw milk	412/701	11–440	Daou et al. (2020)
	Milk (UHT + pasteurized)	10/11	13–219	
Mexico	Milk (UHT + pasteurized)	33[a]/84	510–1270	Quevedo-Garza et al. (2018)
	Infant formula	7[a]/49	40–420	Quevedo-Garza et al. (2020)
	Follow-on formulae	4[a]/6	40–450	
Pakistan	Raw milk	50/72		Ullah et al. (2023)
Qatar	Milk (UHT + pasteurized)	61/72	3.34–48.68	Hassan et al. (2019)
	Yogurt	16/21	4.16–38.21	
	Cheese	39/46	2.53–217.15	
	Butter	12/18	3.49–41.54	
Serbia	Raw milk	245/305	5–5078	Jajić et al. (2018)
	Pasteurized milk	41/57	7–1215	
	UHT milk	51/55	7–411	
	Organic milk	5/6	8–101	
Tanzania	Fresh milk	11/25	10–230	Robert et al. (2023)
Turkey	UHT milk	7[a]/13	25.97–80.00	Yeşil et al. (2019)
	Butter	15[a]/18	50.00–308.13	
	Cheese	162[a]/217	50.00–595.31	

Occurrence of AFM_1 in cheese reported in some countries in 2010.

Country/Region	Cheese type	Positive/Total sample	Detected range of AFM_1 [ng/kg]	Reference
Slovakia	Different kinds of cheese	0/36	–	This study
Serbia	Commercial white cheese samples	10/23	130–550	Škrbić et al. (2015)
	Hard cheese samples	10/10	80–2230	
	White cheese samples produced in households and small private farms	9/21	130–220	
Italy	Different kinds of cheese	85/102	50–250	Anfossi et al. (2012)
Serbia	Different kinds of cheese	42/60	26–591	Torović et al. (2021)
Greece	Feta cheese	7/25	2–4	Malissiova et al. (2022)
Spain	Grated, fresh and semi-dried cheese samples	0/72	–	Cano-Sancho et al. (2010)
North Macedonia	Brined white cheese	31/36	< 11–795	Ilievska et al. (2022)
	Hard yellow cheese	58/65	< 11–366	
Turkey	Ezine cheese	60/120	19–158	Eker et al. (2019)
	Halva cheese	7/120	50–213	
Spain	Manchego cheese	–/82	2–522	Rubio et al. (2011)
Turkey	Different kinds of cheese	217/217	50–595	Yeşil et al. (2019)

Slovakia

- 2023 – number of analysed milk samples: 25

  - number of positive samples: 0

(Kolarič, L., & Šimko, P. (2023). Development and validation of HPLC-FLD method for aflatoxin M1 determination in milk and dairy products. Acta Chimica Slovaca, 16(1), 99-108.)

- 2024 – number of analysed cheese samples: 36

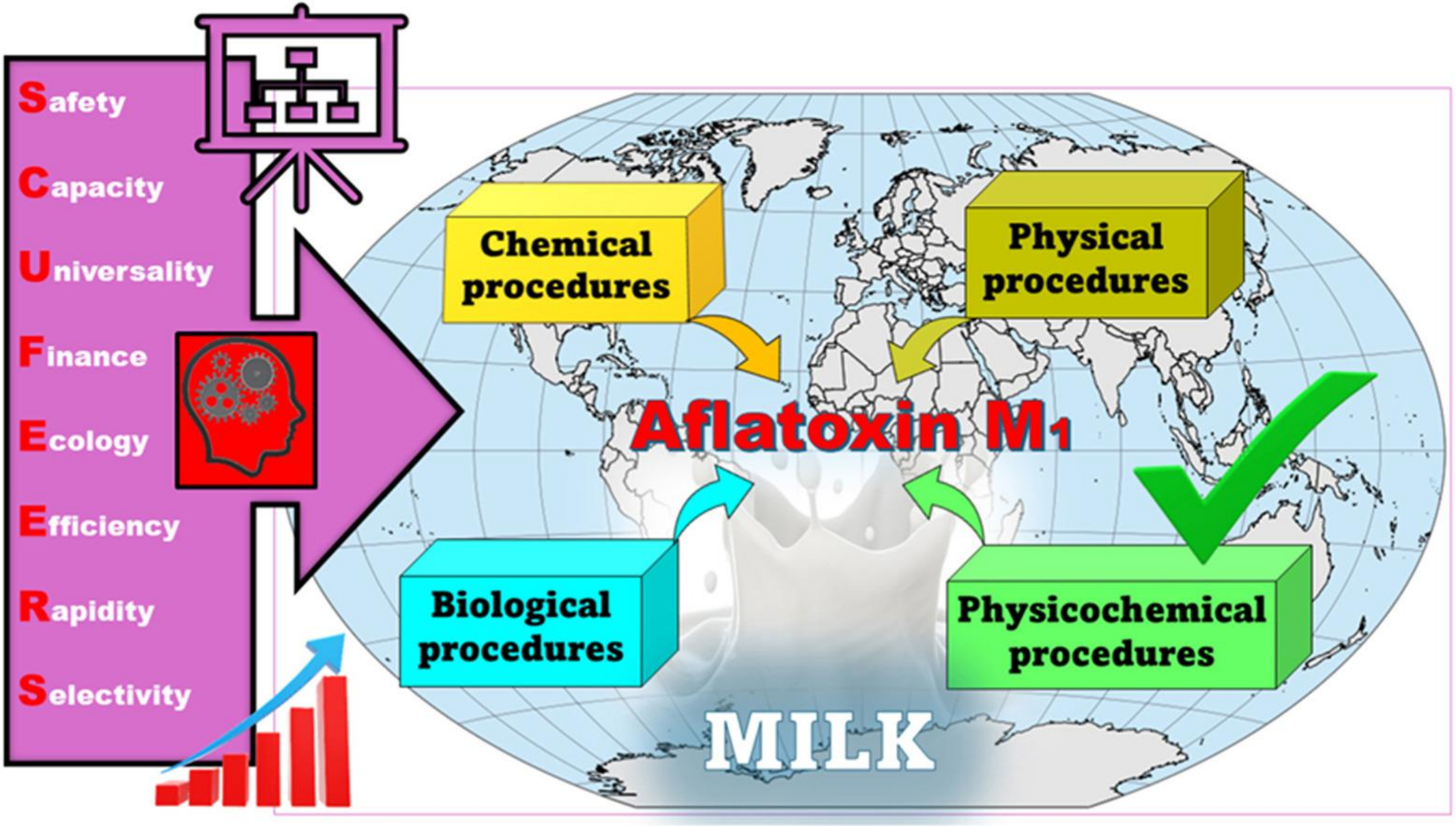
  - number of positive cheese samples: 0

(Kolarič, L., Vinohradská, E., & Šimko, P. (2025). Aflatoxin M1 in cheese: situation on the Slovak market. Acta Chimica Slovaca, 18(1), 32-40.)

- 2024/2025 – number of analysed milk samples: 22

  - number of positive milk samples: 5 (15,1 – 57,3 (526,2) ng/l)
  - **526,2 ng/l** – sample of goat milk from domestic farm

Decontamination methods



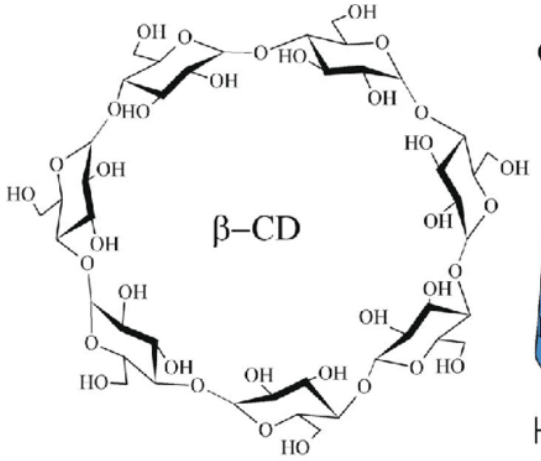
Characterization and summarisation of SCUFEERS criteria of elimination procedures

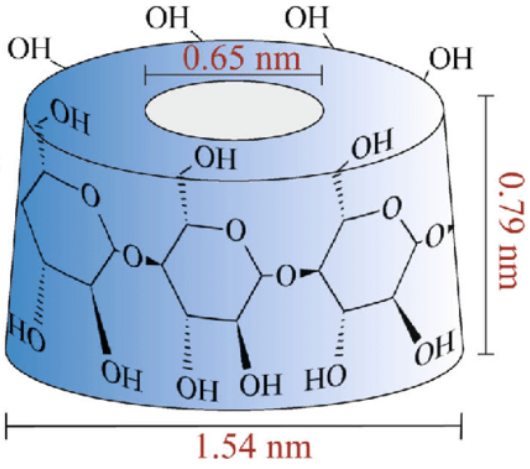
Criterion	Description of the criterion	CP	PP	BP	PCP
Safety	Compliance with food safety legislation and Codex Alimentarius standards (e.g. good manufacturing practice), maximum acceptable limits for food contaminants, use of approved chemicals, permitted package materials, microorganisms, and approved enzymes, official technology, and treatments	x	x	x	+
Capacity	Ability to treat a regular volume of daily production	+	x	+	+
Universality	Applicable for all kinds of milk, i.e. raw milk, thermally processed milk, homogenized milk, reconstituted milk from dried matrix	+	x/+	+	+
Finance	Applicable for existing production lines including production capacity, i. e. zero need for investment in new technology equipment	x/+	x	x/+	x/+
Ecology	Carbon neutral approach based on using renewable natural materials and low/zero energy consumption, spontaneously degraded/zero wastes, or fully recycled	x	x/+	+	+
Effectivity	A measure of elimination should be significant in comparison with the original concentration	x/+	x	x/+	x/+
Rapidity	The procedure should be integrated into production lines/storage without overlimited delays	+	x/+	x/+	x/+
Selectivity	Sufficient selectivity of AFM_1 elimination, without a negative impact on nutritive value, organoleptic profiles, and technological properties of milk	x	x	+	+

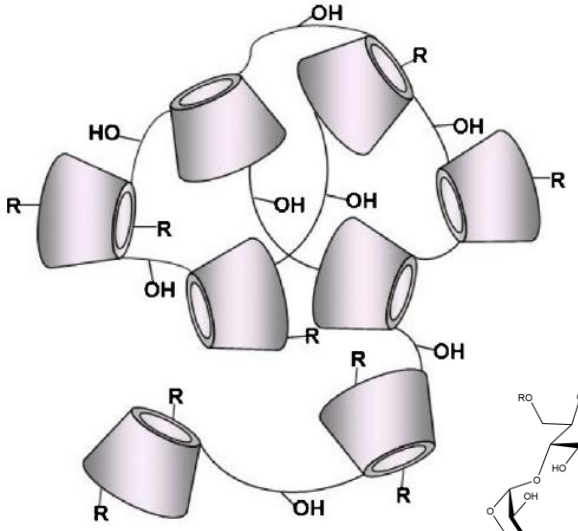
+ - without negative effects.
x - with negative effects.
x/+ - with neutral effects.

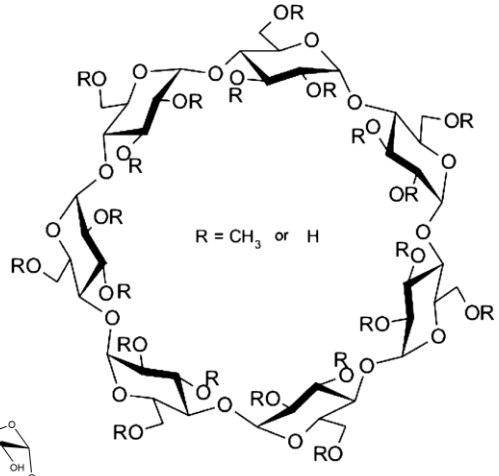
Experimental work

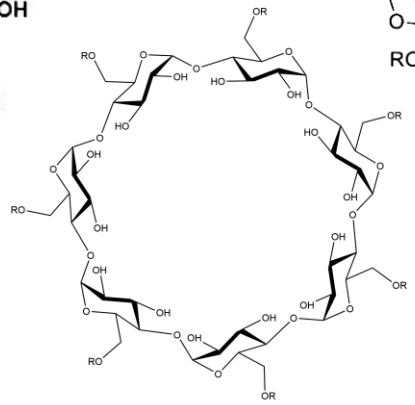
- To study the effectiveness of physicochemical methods for AFM1 elimination from model water solutions and milk using different types of β-cyclodextrin (β-CD)
- Tested β-CDs: native, crosslinked with adipic and tartaric acid, insoluble bead polymer, heptakis(2,3,6-tri-O-acethyl)-β-CD, succinyl-β-CD, triacethyl-β-CD a methyl-β-CD

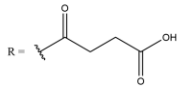












Methodology of AFM1 elimination from milk by β-CD

Milk + β-CD

Mixing

- Time
- Temperature
- Speed

Settling

- Time
- Temperature

Centrifugation

- Time
- Temperature
- Speed

Results

Measure of cholesterol and aflatoxin M_1 removal from milk using β-cyclodextrin

Sample (Milk, 3.5% fat)	Initial Concentration of Cholesterol [mg/kg]	Measure of Cholesterol Removal [%]	Initial Concentration of AFM_1 [µg/kg]	Concentration of AFM_1 after Spiking [µg/kg]	Measure of AFM_1 Removal (%) [%]
1	129.04 ±2.13	92.0	<LOQ*	0.20	35
2	135.78 ±6.01	95.2	<LOQ*	0.40	38
3	150.39 ±0.64	96.5	<LOQ*	0.60	40
4	113.32 ±6.30	92.1	<LOQ*	0.80	41
5	123.01 ±2.21	98.8	<LOQ*	1.00	45
6	103.92 ±0.43	90.9	<LOQ*	1.20	33
7	122.33 ±1.45	80.8	<LOQ*	2.00	42
Priemer	**125.40 ±2.74**	**92.3**	**<LOQ***	**0.89**	**39.1**

Measure of AFM1 removal from model Ringer solution using insoluble β-cyclodextrin bead polymer

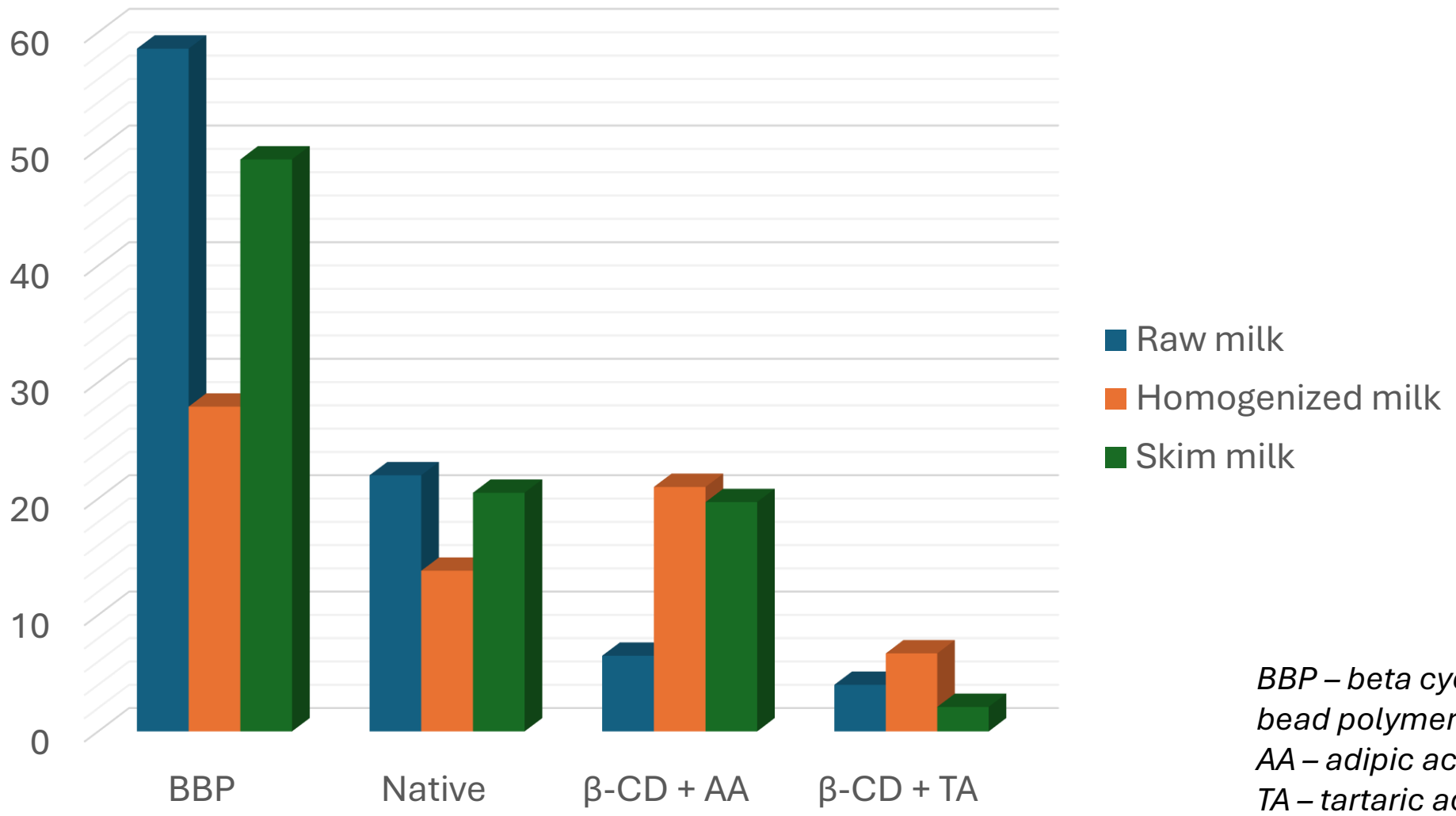
Initial concentration of AFM_1 [ng/ml]	Addition of BBP [%]	Mixing		Centrifugation	Final concentration of AFM_1 [ng/ml]	Measure of AFM_1 removal [%]
		Time [min]	Speed [rpm]			
0.50	2	40	600	1500/15 min	0.29 ±0.00	**42.11 ±0.71**
			800		0.30 ±0.00	**38.36 ±0.04**
			1000		0.28 ±0.00	**44.46 ±0.58**
			1200		0.26 ±0.00	**47.36 ±0.26**
			1400		0.26 ±0.00	**46.48 ±0.59**
0.50	2	40	1000	500/15 min	0.23 ±0.01	**50.52 ±0.47**
				1000/15 min	0.27 ±0.01	**47.96 ±2.89**
				1500/15 min	0.28 ±0.00	**44.46 ±0.58**
				2000/15 min	0.27 ±0.01	**47.08 ±2.90**
				2500/15 min	0.22 ±0.00	**52.54 ±1.06**
				3000/15 min	0.23 ±0.00	**50.44 ±0.61**

Measure of AFM1 removal from model Ringer solution using different type of β-CD

β-CD level [%, w/v]	Type of β-CD	Initial AFM1 concentration [ng/mL]	Final AFM1 concentration [ng/mL]	% of AFM1 removal
1.0	BBP	0.50	0.33 ±0.001	33.6 ±0.2
2.0	BBP	0.50	0.27 ±0.005	46.5 ±1.1
4.0	BBP	0.50	0.19 ±0.006	63.1 ±1.2
6.0	BBP	0.50	0.14 ±0.004	**71.9 ±0.8**
1.0	β-CD + AA	0.50	0.42 ±0.005	15.7 ±1.0
2.0	β-CD + AA	0.50	0.39 ±0.010	**21.1 ±2.0**
4.0	β-CD + AA	0.50	0.43 ±0.004	14.2 ±0.8
6.0	β-CD + AA	0.50	0.44 ±0.001	12.2 ±0.2
1.0	β-CD + TA	0.50	0.45 ±0.001	9.5 ±0.3
2.0	β-CD + TA	0.50	0.43 ±0.006	**14.7 ±1.1**
4.0	β-CD + TA	0.50	0.44 ±0.004	12.7 ±0.8
6.0	β-CD + TA	0.50	0.44 ±0.002	11.2 ±0.3

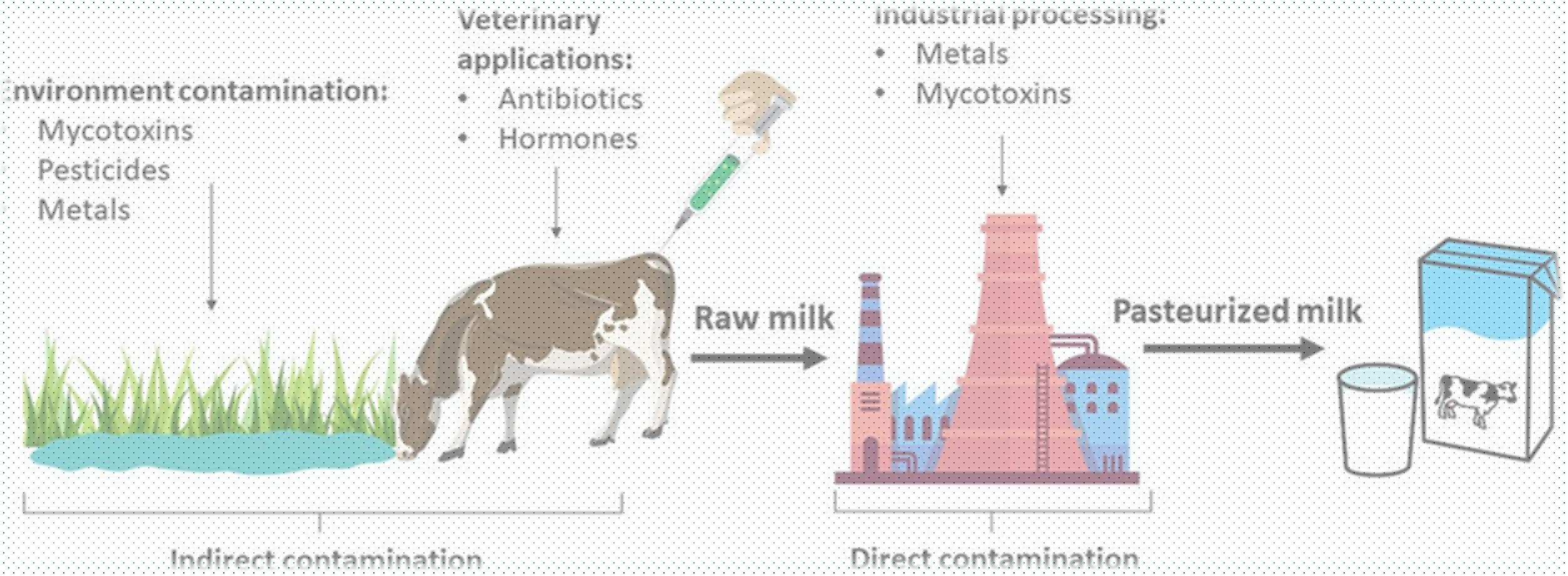
BBP – beta cyclodextrin bead polymer
AA – adipic acid
TA – tartaric acid

Measure of AFM1 removal from different type milk using different type of β-CD



BBP – beta cyclodextrin bead polymer
AA – adipic acid
TA – tartaric acid

Conclusions

- Dairy products are often contaminated with AFM1 (it has serious health consequences for consumers, economic losses in both domestic and international trade)
- Due to increased global warming, the occurrence of aflatoxins is projected to become a serious food safety concern also in Europe in the +2 ◦C scenario
- Procedures for the removal of AFM1 from milk can currently be divided into four groups: chemical, physical, biological and physico-chemical; However, all of them have only been tested at the laboratory level, so none is ready for the industry
- Physicochemical processes generally have the highest usable potential due to the fulfilment of the SCUFEERS criteria
- Future opportunities are also based on the application of an active packaging that can remove AFM1 from packaged milk due to its adsorption properties on the surfaces of the packaging material
- The elimination rate of AFM1 can be positively influenced by modification of the original packaging materials using a selective adsorption matrix (e.g., chitin, β-cyclodextrin, clay sorbents) as well as artificial receptors with specific affinity binding sites for specific molecules integrated into most molecularly imprinted polymers

Thank you for your attentation