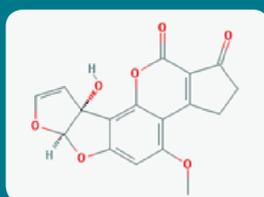


Development of a matrix proficiency test material for the measurement of Aflatoxin M1 in cheese

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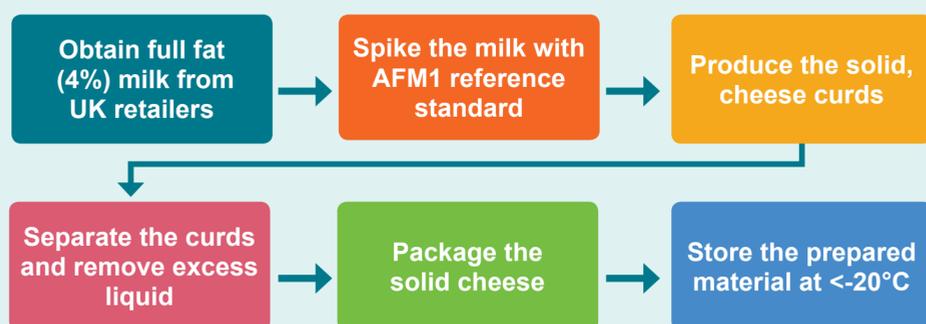
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Fig 1. Structure of Aflatoxin M1 (AFM1)



Introduction

Aflatoxins are a family of toxins produced by certain fungi which can contaminate agricultural crops such as maize (corn), groundnuts, tree nuts, spices and other dried foods. Exposure to aflatoxins is associated with an increased risk of liver cancer. Aflatoxin M1 (AFM1) is the principal hydroxylated metabolite of aflatoxin B1, which can be present in milk from animals fed with contaminated feed. AFM1 can subsequently be found in a range of dairy products, posing a significant potential risk to human health and as a result Maximum Residue Levels (MRLs) have been established for AFM1 in milk and related products, e.g. the MRLs applied by a number of countries for AFM1 in cheese is 0.25µg/kg.



Trial batch production

A 'target' concentration of, approximately 0.5 µg/kg was chosen and the material produced according to the established method. Homogeneity and stability testing were carried out by an external sub-contractor on 5 randomly selected samples, analysed in duplicate. An average recovery of 105% and a measurement uncertainty of 15% of the measurement result (K=2) were reported. The power of the statistical test and the calculated homogeneity of the sample was acceptable at a value of 0.054 µg/kg, which corresponds to 10% of the AFM1 concentration in the sample.

Table 1. Calculated factors for the assessment of Homogeneity for AFM1 in cheese (trial batch).

Mean result	0.535	
Standard deviation	0.0227	
Analytical variance	0.00053	
Sufficient precision (10% of mean)	0.43	PASS
Cochran's test, critical value	0.841	
Cochran's value	0.679	PASS
Homogeneity critical value	0.0016	
Sampling variance	0.0000	PASS

Table 2. Stability assessment of AFM1 in cheese (trial batch).

Date	Measurement	Result µg/kg	
15/11/2016	Mean result, homogeneity	0.535	
26/01/2017	Mean result, stability*	0.520	
	Allowable difference (SDPA = 10% of mean)	0.016	
	Difference	0.015	PASS

*Materials were stored frozen at <-20°C prior to dispatch and for assessment of stability.

Production of Sample 62 for the QDCS scheme, Round 252

Table 3. Calculated factors for assessment of Homogeneity AFM1 in cheese (QDCS Rd252).

Mean result	0.318	
Standard deviation	0.0159	
Analytical variance	0.000263	
Sufficient precision (11% of mean)	0.46	PASS
Cochran's test, critical value	0.602	
Cochran's value	0.315	PASS
Homogeneity critical value	0.0008	
Sampling variance	0.0000	PASS

Table 4. Recovery of AFM1 in cheese

Volume of milk	13.5	litre
AFM1 spike	0.144	µg/l
Weight of cheese solids	2.51	kg
AFM1 concentration	0.318	µg/kg
Total weight of AFM1	0.796	µg
AFM1 Recovery	39.80	%

Results

The majority of participants used HPLC for the analysis of AFM1, which is the most common internationally recognised official method. A relatively large robust standard deviation was observed for the data, although this was not surprising given the average measurement uncertainty reported was approximately 30% of the measured value.

Table 5. Results of participant analysis

PT Round	Sample	Homogeneity result	Homogeneity SD	Participant median	Participant robust SD
QDCS 252	Sample 62	0.318 µg/kg	0.016 µg/kg	0.28 µg/kg	0.12 µg/kg

Conclusions

- The stability of aflatoxin M1 during milk fermentation processes has been studied and AFM1 concentrations above EU MRLs have been reported.
- A 'soft cheese' containing AFM1 has been produced from milk, entirely in the laboratory.
- A predictable recovery of AFM1 was obtained in the cheese, enabling levels of interest to be achieved.
- The material was sufficiently homogeneous and stable for use as a PT sample, with an acceptable SDPA (potentially in the range of 10-20%, relative to the assigned value).