Phomopsin A in food samples in The Netherlands

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Introduction

Phomopsin A (PHA) is a mycotoxin, mainly occurring in lupin, but contamination of chestnuts and mango's cannot be excluded[1]. Primary target organ for phomopsin A in sheep is the liver. Australia and New Zealand have set a regulatory limit of 5 µg phomopsin A/kg product [2].

Lupin has comparable nutritional characteristics to soy and can be used to replace genetically modified soy in foods. Numerous food products containing lupin are currently available on the market[3]. EFSA works on a risk assessment on phomopsins for the European situation.

A straightforward LC/MS-MS method has been developed to investigate several lupin-containing food products available in the Netherlands.

Principle

Phomopsin A is extracted by shaking with a solvent. The raw extracts are centrifuged and diluted 1:1 in water and analysed using a highly sensitive LC-MS/MS system.

Samples

Twenty samples, containing various levels of lupin as ingredient, were bought from grocery and internet shops in The Netherlands. The origin of lupin used for preparation of the food products was unknown.

Sample	#	Example
Lupin flour	3	
Flour for bread	1	
Bread	5	
Biscuit/cakes	4	
Frozen lupin flakes	3	
Lupin snacks	2	
Dry pasta products	2	

Experimental

Extraction

An aliquot of 20 ml of acetonitrile/water (80%/20%) with 1% acetic acid was added to 2.5 g test portion. The suspension was shaken for 30 minutes, centrifuged at 3500 RPM (10 min; RT) and the supernatant was diluted 1:1 in water. The extract was cooled (30 min), filtered (0.45 µm PTFE filter) and stored in refrigerator. PHA contamination of samples was determined by the external standard method based on average matrix matched standard measurements before and after samples.

LC-MS/MS analysis

An aliquot of 20 µL was injected on a reverse phase HPLC column for separation. Gradients of mobile phases A (5 mM ammonium formate buffer pH 5) and B (5 mM ammonium formate buffer in 95% ethanol) changing from 90% to 0% and 10% to 100% respectively over 8 min with flow rate of 400 µL/min. The Shimadzu HPLC system was coupled with a mass spectrometer (Applied Biosystems, MDS AB Sciex API Q-trap 5500) for detection.

Phomopsin A (PHA) MS/MS transitions

	[M+H]+	Product ion	CE* (eV)	CXP** (V)
PHA	789.2±0.5	226.1±0.5	47	16
PHA	789.2±0.5	323.1±0.5	35	22

* CE Collision Energy

** Cell Exit Potential

The product ion with the highest intensity, the quantifier, is underlined.

Results

Validation

The repeatability of the method was determined using blank lupin flour.

Parameter	Range / result
Validation range	5 and 25 µg PHA/kg
Average recovery	79%
Average RSD	9%
LOQ	5 µg PHA/kg product#
LOD	1.22 µg PHA/kg product^
Matrix effect	+34% (ion enhancement)

lowest concentration fulfilling reproducibility requirements

^ calculated according to standard line prepared in matrix

Example Chromatogram

Combined chromatogram of blank (red) and spiked sample 5 µg PHA/kg (blue) for quantifier 789.2> 226.1.

Samples

No phomopsin A was detected above LOD in any of the 20 samples.

Conclusion

The LC-MS/MS method performs well for the analysis of phomopsin A in lupin flour and lupin containing food.

No phomopsin A was detected above LOD (1.22 µg/kg product) in the food samples analysed.

References

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