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Generation of occurrence data on citrinin in food

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Abstract

A total of 1195 samples of industry cereals, plant derived raw materials and foods including 92 samples of Red Yeast Rice food supplements (RYR), available on the European market were analysed for the presence of the mycotoxin citrinin (CIT). The samples, of which 13% came from organic production, were collected from industrial premises and retail stores, between September 2015 and November 2016, in eight European countries (France, Germany, Italy, Lithuania, the Netherlands, Poland, Spain and Sweden). These samples comprised 390 industry cereals (wheat, barley, rye, oats, rice), 510 cereal-based products from retail (flour, rice retail, bread and bread rolls, pasta (dry), breakfast cereals (flakes & muesli)), 92 RYR and 203 other products (beans, and fruit and vegetable juices). The 92 RYR samples were retrieved from retail stores and internet in Italy, the Netherlands, Poland and Spain. Samples were analysed by liquid chromatography-tandem mass spectrometry. The methods were subsequently implemented and in-house validated by each participant and were considered fit for purpose. The limits of quantification (LOQ) for the various food groups were: 10 µg/kg for RYR and 1 µg/kg for the rest of matrices. Citrinin was detected at concentrations above the LOQs in 6% of the industry cereals, 3% of the cereal-based products from retail, and in 26% of RYR samples. No citrinin was detected in beans and (fruit and vegetable) juices. RYR food supplements were the most prone samples to contamination with citrinin. Citrinin was detected above the EU legal limit of 2,000 µg/kg in three of the RYR samples.

Key words: citrinin, liquid chromatography tandem mass spectrometry, red yeast rice, cereals, juices, cereal-based products

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Amendment: This external scientific report replaces the original report published on 28 January 2017. With respect to the original external scientific report, editorial corrections were inserted in Tables 14 – 18 for empty cells and for table footnotes. In addition, in Table 15 refined values were inserted for the three columns related to average values in the last two rows. In Table 18, some of the concentrations were corrected. No changes were made to the Abstract, Summary or Conclusions sections. The original version is available on request as well as the version showing the changes made.

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Summary

Citrinin is a mycotoxin that is produced by several species of the fungal genera *Aspergillus*, *Penicillium* and *Monascus*. Occurrence of citrinin has been reported in grains and grain-based products, beans, fruits, fruit and vegetables juices, herbs and spices, olives and mouldy cheese. Citrinin can co-occur with other mycotoxins such as ochratoxin A and patulin. For the EU only limited occurrence data of citrinin in food and RYR are available.

The EFSA Panel on Contaminants in the Food Chain (CONTAM) assessed the risk for public and animal health related to the presence of citrinin in food and feed in 2012. The CONTAM Panel concluded that more data regarding the occurrence of citrinin in food are needed in Europe to enable refinement of the risk assessment. In 2015, the EFSA published a call for proposals to investigate the concentrations of citrinin in food samples with special focus on grains and grain-based products from different geographic regions in Europe.

This report describes the outcome of project GP/EFSA/CONTAM/2015/01, 'Occurrence of Citrinin in food' carried out in accordance with Article 36 of Regulation (EC) No 178/2002 (EU, 2002), which was designed to obtain representative data on the occurrence of citrinin in food and Red Yeast Rice (RYR) in Europe, using validated state-of-the-art analytical methods.

All samples were analysed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Two sample preparation methods were developed. The first method was developed to analyse citrinin in RYR, according to the procedure in Regulation (EU) No 519/2014 and based on a Standard Operational Procedure (SOP) from RIKILT. The second extraction method included a clean-up step using immunoaffinity columns. Limits of quantification (LOQ) for the various food groups were: 10 µg/kg for RYR and 1 µg/kg for the rest of matrices under scope. The results of the in-house validations showed that the methods were fit-for-purpose.

A total of 1195 industry cereals and food samples were collected from September 2015 till November 2016 from eight EU countries (France, Germany, Italy, Lithuania, the Netherlands, Poland, Spain and Sweden) and analysed for citrinin, 13% of which was from organic production. Citrinin was detected above the LOQ in 6% of the industry cereals, 3% of the cereal based products and 26% of RYR samples. In general, the levels of citrinin in manufactured products were lower than those in grains. The maximum concentration of citrinin detected in industry cereals and cereal-based samples from retail was 155 and 5.7 µg/kg, respectively.

The 92 RYR samples were retrieved from retail stores and internet in Italy, the Netherlands, Poland and Spain. Citrinin was detected above the LOQ in 26% of samples and in three samples above the legal limit of 2,000 µg/kg.

Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Citrinin is a mycotoxin that is produced by several species of the fungal genera *Aspergillus*, *Penicillium* and *Monascus*. Occurrence of citrinin has been reported in grains and grain-based products, beans, fruits, fruit and vegetables juices, herbs and spices, olives, mouldy cheese and red yeast rice. Citrinin can co-occur with other mycotoxins such as ochratoxin A and patulin. For the EU only limited occurrence data are available.

Another route of exposure of the population to citrinin is via consumption of Red Yeast Rice (RYR) food supplements. In 2014 the concentration of citrinin in RYR was set at a limit of 2,000 µg/kg by Regulation (EC) 212/2014 (EU, 2014a) with a corresponding sampling procedure described in Regulation (EU) No 519/2014 (EU, 2014b).

EFSA´s Panel on Contaminants in the Food Chain (CONTAM) assessed the risk for public and animal health related to the presence of citrinin in food and feed in 2012 (EFSA, 2012). The CONTAM Panel concluded that due to the limitations and uncertainties in the database, the derivation of a health-based guidance value was not considered appropriate but a level of no concern for nephrotoxicity of 0.2 µg/kg b.w. per day was determined. However, a concern for genotoxicity and carcinogenicity cannot be excluded at this level of no concern for nephrotoxicity. The available occurrence data were not adequate to assess the dietary exposure to citrinin. Instead, the CONTAM Panel estimated the citrinin concentrations in grains and grain-based products that would result in an exposure equal to the level of no concern for nephrotoxicity. This concentration is between 9 and 53 µg citrinin/kg for high consuming toddlers, other children and adults. The CONTAM Panel concluded that more data regarding the occurrence of citrinin in food are needed in Europe to enable refinement of the risk assessment.

The European Food Safety Authority (EFSA) wishes to co-finance a study on the occurrence of citrinin in food with special focus on grains and grain based products for human consumption from different geographic regions in Europe, to possibly serve as supporting information to the CONTAM panel for future exposure assessment for citrinin.

This grant was awarded by EFSA to:	Stichting Landbouwkundig Onderzoek DLO-RIKILT; Akkermaalsbos 2; 6708 WB Wageningen; the Netherlands
Beneficiary:	RIKILT Wageningen University & research
Grant title:	Generation of occurrence data on citrinin in food
Grant number:	GP/EFSA/BIOCONTAM/2015/01

The aim of this grant is to generate representative occurrence data of citrinin in food samples with special focus on grains and grain-based products for human consumption from different geographic regions in Europe by using a validated, sensitive method such as liquid chromatography tandem mass spectrometry (LC-MS/MS) or high-performance liquid chromatography with fluorescence detection (HPLC-FLD).

The beneficiary shall perform the following tasks, in order to achieve the objectives:

1. To elaborate a protocol for collecting in total at least 1000 samples that is in accordance with the Commission Regulation (EC) No 401/2006 (EU, 2006) and that shall take into account the following requirements;
    a. the samples shall be taken from at least 3 different European countries (preferably not from neighbouring countries) ensuring a representative geographical coverage of Europe;
    b. the following samples of grains for human consumption and food products shall be analysed:
        i. at least 300 samples of grains including wheat, barley, rye, oats and rice;

ii. at least 400 samples of grain-based products for human consumption including flour, bread and rolls, pasta, cereal flakes and muesli;

iii. at least 300 samples of other foods such as beans, fruit and vegetable juices, herbs and spices, olives, food supplements based on rice fermented with red yeast *Monascus purpureus*.

c. the samples shall also include organic grains for human consumption and organic food products.

2. To collect the samples as described above.

3. To analyse the collected samples using a validated, sensitive method such as LC-MS/MS or HPLC-FLD that complies with the requirements of the Commission Regulation (EC) No 401/2006 and more precisely with the performance criteria for ochratoxin A and that has a sensitivity comparable to methods that have been published in the literature (Polisenska et al., 2010; Zaied et al., 2012; Arroyo-Manzanares et al., 2013; Yogendrarajah et al., 2013). To use appropriate storing procedures for the samples before and after sample pre-treatment. To apply appropriate sample pre-treatment (e.g. milling) and sample preparation steps.

4. To prepare a Final External Scientific Report and a database providing the results of the analyses performed. The database as well as the interim and Final External Scientific Reports will be prepared in line with the time schedule reported in the call for proposals.

1.2. Objectives

The main objective of this study is to provide representative data on the occurrence of citrinin in food samples from different geographic regions in the EU. The quality of the delivered data must be suitable for use in future exposure assessments performed by the EFSA CONTAM Panel.

2. Data and Methodologies

2.1. Sampling plan

The survey, as stated in the EFSA GP/EFSA/BIOCONTAM/2015/01 call, focussed on cereals and cereals-based products for human consumption, as well as on other products such as beans, juices (from fruit and vegetables) and red yeast rice food supplements.

It was of great importance that the samples collected were representative of the situation in the EU with regard to the production of the different target products and to the consumption habits in the different regions. The Netherlands, Germany, Poland, Lithuania, Sweden, Spain, France and Italy were sampled, which represents around 65% of the European Union population. Furthermore, different sampling countries are included covering the EU according to the classification of the World Health Organization (WHO, 2012).

The sampling was carried out in two sampling periods in order to coincide with the preparation of the Interim Report and the Final Report. During the first sampling period, from September 2015 to April 2016, the sampling in Germany and France was completely conducted, while around 40% for the rest of the countries. The second sampling period, which ran from May 2016 till November 2016, continued with the remaining samples. Nevertheless, the items to sample during the second period were subjected to modifications, depending on the outcomes of the first sampling period that was discussed during the Interim Meeting in May 2016.

Sampling was performed according to the methods of sampling for official control laboratories described in the Commission Regulation (EC) No 401/2006 (EU, 2006) and its amendment Commission Regulation (EC) No 519/2014 (EU, 2014b).

Industry cereal samples

The sampling protocol for industry cereal samples is described in Regulation (EC) No 401/2006 (EU, 2006).

Grain-based products

The sampling protocol for this type of samples is also described in the epigraph A of Annex A of Regulation (EC) No 401/2006 (EU, 2006). In case of retails samples at least three different samples from the same lot should be taken to form an aggregate of at least 1 kg.

Flour

There are many different entries for flour in the FOODEX database. In order to provide data with enough statistically consistency, three groups were selected: wheat flour, mix flour (preferably coming from whole grains) and other type of flours.

Bread and bread rolls

Among the different entries for this product in the FOODEX database, the products wheat bread and rolls, multigrain bread and rolls and other type of breads and rolls were selected for the purpose of this survey.

Breakfast cereals and muesli

Three different products within this category were selected: cereals flakes, porridge and muesli.

Other products

Beans

There is no specific protocol describing the sampling procedure for beans. Therefore, 3 different retail samples belonging to the same lot were collected to form an aggregate of approximately 1 kg. In this project canned beans (broad beans and peas) without pods and dry beans were sampled.

Fruit and vegetable juices

Sampling of fruit juices is described in epigraph H of Regulation (EC) No 401/2006 (EU, 2006), while epigraph I describes the sampling of apple products, including apple juices (amended by Regulation (EU) No 519/2014 (EU, 2014b). As there is no specific legislation regarding vegetable juices, their sampling was conducted according to guidelines defined for fruit juices. In case of retail samples, three different packages of the same lot were taken to form an aggregate of 1 litre.

The FOODEX database contains a lot of entries for juices. In order to obtain representative data, the sampling was focused on orange, apple and other (depending on the country) fruit juices and on tomato, carrot and other vegetable juices.

Red yeas rice (RYR) food supplements

Regulation (EU) No 519/2014 (EU, 2014b) describes the sampling protocol for red yeast rice supplements. For this project, three samples from the same lot were taken and used to form the aggregate sample. Samples were purchased either in retail stores (pharmacies, drugstores) or via internet.

2.1.1. Actual sampling

A total of 1195 samples both industrial and available at retail stores were collected. A summary of the collected samples is presented in Table 1, showing the planned and the actual number of samples.

The total number of samples collected was slightly (9%) more than the 1,100 planned. The number of samples collected per item was also slightly higher than planned. On overage, the samples collected for the food category rye was slightly less (-5%) than planned. However, wholemeal rye flour samples were taken additionally.

Table 1: Overview of the collected samples (September 2015-November 2016)

Food category	Planned	Actual	Actual vs Planned
Industry cereals			**107%**
Wheat	120	140	117%
Barley	50	57	114%
Rye	75	71	95%
Oats	70	70	100%
Rice	48	52	108%
Cereal-based products (retail)			**111%**
Flour	72	89	124%
Rice retail	97	102	105%
Bread and bread rolls	110	122	111%
Pasta (dry)	100	105	105%
Breakfast cereals (flakes&muesli)	80	92	115%
Other products			**106%**
Beans	66	68	103%
Fruit juices	66	66	100%
Vegetable juices	66	69	105%
Red yeast rice supplements	80	92	115%
TOTAL	1,100	1,195	**109%**

Reg: regular production. Org: organic production.

Table 2 shows the number of samples collected per country. In general, more samples than planned were collected in all countries.

Sample collection covered a period of more than one year, with emphasis on 2016 (see Figure 1). In 2015, the samples in France were collected, as well as rice from industrial origin in Italy and the RYR by RIKILT. The production of red yeast rice does not follow the harvest period, so differences due to sample collection dates were not expected. Sampling in Germany was conducted in January 2016 and in Lithuania in June 2016. Industry cereal samples from Sweden were collected in February, April and May 2016. Although often it is not indicated on the package, it can be assumed based on the time of sampling that the ingredients of the processed cereal products most likely originated from cereals grown in 2015. A production year of 2014 can be assumed for products with a long shelf life.

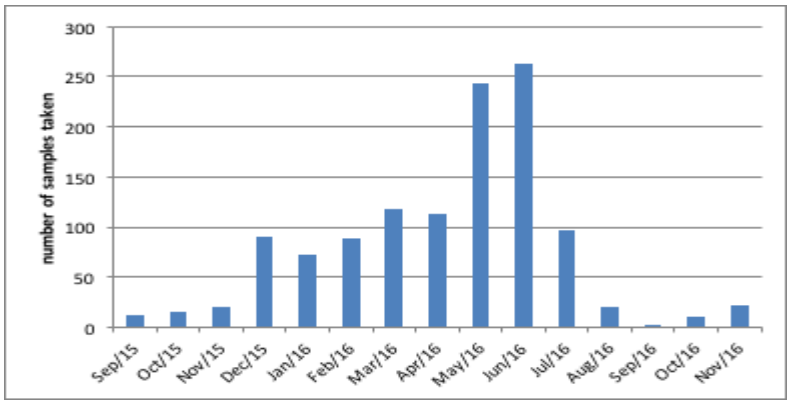

Figure 1: Overview of time of sampling of the products analysed in this survey

Industry cereal samples were collected from April 2016 on in Spain and Poland. In case of Spain, farmers are local producers and did not have product from the previous harvested period. In case of Poland, its official sampling in storage houses, mill and factories depends on the State Sanitary Inspection and consequently, NIPH-NIH had to follow their deadlines. Nevertheless, the levels of citrinin in samples might have been influenced more by the storage conditions rather than the harvest period (EFSA, 2012).

The samples were collected at various sampling points (Figure 2). Industry samples were mainly taken at processing plant, farming and storage facilities. A minor number of industry samples was taken from mills, wholesales or growing crops. Cereal-based products, beans, juices and RYR were all obtained from retail.

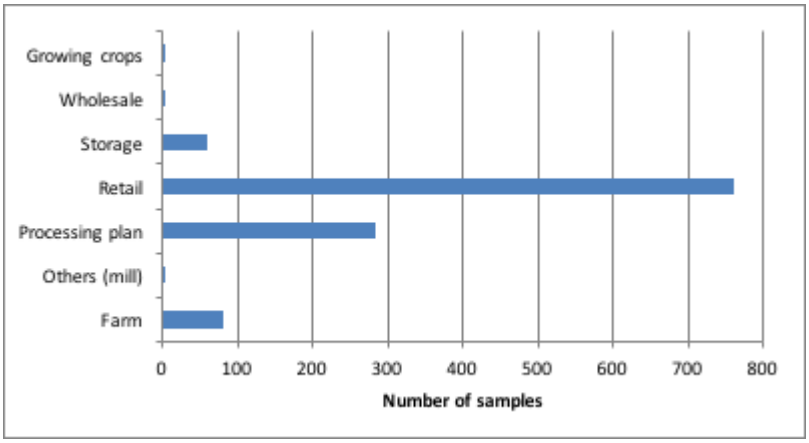

Figure 2: Overview of sampling point where the samples of this survey were collected

The industrial sampling in the Netherlands was conducted by the Netherlands Food and Consumer Product Safety (NVWA) and included not only grain samples, but also cereal-based products. The industrial sampling by UCSC was carried out with the support of the company Barilla G. e R.F. Ill S.p.A (Parma, Italy), Lameri (Cremona, Italy), Ente Nazionale Risi (Pavia, Italy) and the Swedish National Food Agency (Uppsala, Sweden), which provided cereal samples from other places across Europe, such as Slovenia and Hungary. UCSC collected all the industry samples of wheat and rice during the first sampling period, but they experienced problems to find industry rye samples. On the other hand, NIPH-NIH encountered problems to find industry rice samples. After discussion, it was agreed that the industry samples of rice, scheduled from Poland, would be collected in Italy and Spain, while NIPH-NIH would collect a higher number of rye samples. The industry samples of rice by IRTA were purchased in retail from the Protected Designation Areas (PDAs) of Calasparra, Albufera and Ebro Delta in Spain.

Table 3 shows the number of organic samples collected per country. In total, 160 samples out of the 1,195 were from organic origin. Although the overall percentage of organic samples (13%) is accordance to planned, some deviations can be observed as far as country and product distribution are concerned. 40% of the German samples and 21% of the Dutch samples were of organic origin, while 6% and 2% were from organic origin in France and Lithuania, respectively. It has been observed that some products, such as carrot juices or breakfast cereals, were much easier to find in their organic version.

Samples originated from many other countries in and outside Europe, and therefore the survey adequately covers the food supply in the EU in 2016. An overview of countries of origin of the various products is provided in Table 4.

Table 2: Detailed overview of the sampling plan (September 2015-November 2016) per country

Food category	TOT		NL		DE		IT		SE		SP		FR		PL		LT	
	Plan	Act	Plan	Act	Plan	Act	Plan	Act	Plan	Act	Plan	Act	Plan	Act	Plan	Act	Plan	Act
Industry cereals																		
Wheat	120	140	25	25			25	41	20	20	25	25			25	29		
Barley	50	57	10	13			10	10	10	10	10	10			10	14		
Rye	75	71	15	15				1	17	17	10	10			33	28		
Oats	70	70	15	15			15	18	10	10	15	15			15	12		
Rice	48	52	20	21			28	31										
Cereals-based products																		
Flour	72	89	15	15	4	6	15	16			15	15	4	4	15	26	4	7
Wheat flour		*44*	*8*	*6*	*2*	*4*	*8*	*10*			*8*	*10*	*2*	*2*	*8*	*11*	*2*	*3*
Mix flour		*18*	*7*	*6*	*2*	*2*	*7*	*2*			*7*	*3*	*2*	*2*	*7*	*1*	*2*	
Other		*18*		*3*				*4*				*2*				*14*		*4*
Rice retail	97	102	10	11	10	12	10	10			32	35	10	10	10	12	10	12
Bread & bread rolls	110	122	20	21	10	12	20	20			20	20	10	10	20	35	10	4
Wheat		*54*	*10*	*11*	*5*	*6*	*10*	*11*			*10*	*7*	*5*	*5*	*7*	*14*	*5*	
Multigrain		*51*	*10*	*10*	*5*	*6*	*10*	*6*			*10*	*9*	*5*	*5*	*7*	*14*	*5*	*1*
Other type cereals		*17*						*3*				*4*			*6*	*7*		*3*
Pasta (dry)	100	105	15	15	10	10	15	17			20	20	10	10	20	20	10	13
Breakfast cereals	80	92	10	12	10	13	10	10			15	15	10	10	15	19	10	13
Muesli		*31*	*3*	*3*	*4*	*5*	*3*	*4*			*5*	*8*	*4*	*4*	*5*	*6*	*4*	*1*
Flakes		*45*	*4*	*5*	*3*	*4*	*4*	*5*			*5*	*3*	*3*	*3*	*5*	*13*	*3*	*12*
Porridge		*16*	*3*	*4*	*3*	*4*	*3*	*1*			*5*	*4*	*3*	*3*	*5*		*3*	
Others																		
Beans	66	68	15	15	2	3	15	15			15	15	2	2	15	14	2	4
Dry		*34*	*11*	*11*	*1*	*2*	*11*	*15*			*11*	*11*	*1*	*1*	*11*	*10*	*1*	*2*
Peas		*11*	*2*	*2*	*1*	*1*	*2*				*2*	*2*	*1*	*1*	*2*		*1*	*2*
(Broad) beans		*10*	*2*	*2*			*2*				*2*	*2*			*2*	*4*		
Fruit juices	66	66	18	16	2	2	18	15			18	15	2	2	18	14	2	2
Orange juice		*34*	*8*	*8*	*1*	*1*	*8*	*8*			*8*	*8*	*1*	*1*	*8*	*7*	*1*	*1*
Apple juice		*32*	*7*	*8*	*1*	*1*	*7*	*7*			*7*	*7*	*1*	*1*	*7*	*7*	*1*	*1*
Vegetable juices	66	69	15	16	2	2	15	15			15	15	2	2	15	13	2	6
Tomato juice		*36*	*8*	*8*	*1*	*1*	*8*	*8*			*8*	*8*	*1*	*1*	*8*	*7*	*1*	*3*
Carrot juice		*33*	*7*	*8*	*1*	*1*	*7*	*7*			*7*	*7*	*1*	*1*	*7*	*6*	*1*	*3*
Red yeast rice	80	92	20	23			20	20			20	20			20	29		
TOTAL	**1,100**	**1,195**	**220**	**233**	**50**	**60**	**213**	**239**	**54**	**57**	**227**	**230**	**50**	**50**	**228**	**265**	**50**	**61**
Actual vs planned %		***109***		***106***		***120***		***112***		***106***		***101***		***100***		***116***		***122***

Act: actual; DE: Germany; FR: France; IT: Italy; LT: Lithuania; NL: The Netherlands; PL: Poland; Plan: planned; SE: Sweden; SP: Spain; TOT: total.

Table 3: Detailed overview of the organic samples collected per sampling country (September 2015-November 2016)

	T#	O*	NL T#	NL O	DE T#	DE O	IT T#	IT O	SP T#	SP O	FR T#	FR O	PL T#	PL O	LT T#	LT O
Industry cereals																
Wheat	140	5	25	4			41		25				29	1		
Barley	57	6	13	2			10	3	10				14	1		
Rye	71	4	15	3			1		10				28	1		
Oats	70	10	15	6			18	2	15				12	2		
Rice	52	2	21	2			31									
Cereals-based products																
Flour	89	19	15	5	6	1	16	1	15	3	4		26	9	7	
Wheat flour	*46*	*5*	*6*		*4*	*1*	*10*		*10*	*1*	*2*		*11*	*3*	*3*	
Mix flour	*16*	*3*	*6*	*2*	*2*		*2*		*3*	*1*	*2*		*1*			
Other	*27*	*11*	*3*	*3*			*4*	*1*	*2*	*1*			*14*	*6*	*4*	
Rice retail	102	18	11	3	12	6	10		35	4	10	1	12	4	12	
Bread & bread rolls	122	9	21	1	12	2	20		20	2	10	1	35	3	4	
Wheat	*54*	*4*	*11*		*6*	*1*	*11*		*7*	*1*	*5*	*1*	*14*	*1*		
Multigrain	*51*	*3*	*10*	*1*	*6*	*1*	*6*		*9*	*1*	*5*		*14*		*1*	
Other	*17*	*2*					*5*		*4*				*7*	*2*	*3*	
Pasta (dry)	104	14	15	4	10	1	17	2	20	2	10	1	20	4	13	1
Breakfast cereals	93	26	12	3	13	10	10	1	15	5	10		19	6	13	1
Muesli	*31*	*8*	*3*	*1*	*5*	*4*	*4*		*8*	*1*	*4*		*6*	*2*	*1*	
Flakes	*45*	*11*	*5*	*2*	*4*	*3*	*5*		*3*	*1*	*3*		*13*	*4*	*12*	*1*
Porridge	*16*	*7*	*4*		*4*	*3*	*1*	*1*	*4*	*3*	*3*					
Others																
Beans	68	11	15	2	3		15	7	15	1	2		14	**1**	**4**	
Dry beans	*34*	*9*	*11*	*2*				*5*	*11*	*1*			*10*	*1*	*2*	
Broad beans	*9*		*2*		*2*				*2*		*1*				*2*	
Peas	*10*	*2*	*2*		*1*			*2*	*2*		*1*		*4*			
Fruit juices	66	8	16	4	2		15	1	15	1	2		14		2	
Orange juice	*34*	*4*	*8*	*2*	*1*		*8*		*8*	*1*	*1*		*7*		*1*	
Apple juice	*32*	*4*	*8*	*2*	*1*		*7*	*1*	*7*		*1*		*7*		*1*	
Vegetable juices	69	21	16	8	2		15	6	15	3	2		13	2	6	
Tomato juice	*36*	*6*	*8*	*2*	*1*		*8*	*2*	*8*	*1*	*1*		*7*		*3*	
Carrot juice	*33*	*13*	*8*	*6*	*1*		*7*	*4*	*7*	*2*	*1*		*4*	*2*	*3*	
Red yeast rice	92	7	23	3			20		20	4			29			
TOTAL	1,195	160	233	50	60	24	239	23	230	25	50	3	265	34	50	1
%Organic		***13***		***21***		***40***		***10***		***11***		***6***		***13***		***2***

T=total number of samples;
*O=organic;
DE: Germany; IT: Italy; LT: Lithuania; NL: The Netherlands; PL: Poland; SP: Spain.

Table 4: Overview of sample numbers by country of origin

Country	Number of samples	% of total samples	Industry cereals	Cereal-based products from retail	Beans	Juices	Red Yeast Rice
Austria	1	0.1%	-	-	-	1	-
Belgium	11	0.9%	3	4	1	-	3
Czech Republic	1	0.1%	1	-	-	-	-
Denmark	2	0.2%	-	-	-	1	1
Estonia	3	0.3%	1	1	-	1	v
EU	6	0.5%	-	6	-	-	-
France	42	3.5%	8	26	-	3	5
Finland	2	0.2%	-	2	-	-	-
Germany	79	6.6%	22	42	1	14	-
Greece	1	0.1%	-	1	-	-	-
Hungary	3	0.3%	2	-	1	-	-
Italy	248	20.8%	89	99	15	28	17
Latvia	10	0.8%	-	9	-	1	-
Lithuania	27	2.3%	-	24	-	3	-
Netherlands	83	6.9%	24	30	4	11	14
Poland	253	21.2%	82	101	13	28	29
Slovakia	1	0.1%	-	-	1	-	-
Slovenia	1	0.1%	1	-	-	-	-
Spain	108	9.0%	60	37	5	6	-
Sweden	60	5.0%	60	-	-	-	-
United Kingdom	5	0.4%	-	3	-	2	-
Total EU Member States	**947**	**79.2%**	**353**	**385**	**41**	**99**	**69**
Argentina	6	0.5%	-	-	6	-	-
Brazil	1	0.1%	-	-	-	1	-
Cambodia	9	0.8%	-	9	-	-	-
Canada	1	0.1%	-	-	1	-	-
China	3	0.3%	-	-	3	-	-
Egypt	2	0.2%	-	-	2	-	-
India	10	0.8%	8	2	-	-	-
Kyrgyzstan	1	0.1%	-	-	1	-	-
Myanmar	2	0.2%	-	2	-	-	-
Non EU	7	0.6%	-	6	1	-	-
Pakistan	8	0.7%	3	5	-	-	-
Russian Federation	1	0.1%	1	-	-	-	-
Surinam	1	0.1%	-	1	-	-	-
Switzerland	1	0.1%	-	-	-	-	1
Thailand	2	0.2%	-	2	-	-	-
Turkey	4	0.3%	3	1	-	-	-
United States	2	0.2%	-	-	-	-	2
Uruguay	1	0.1%	1	-	-	-	-
Vietnam	0	0.0%	-	1	-	-	-

Country	Number of samples	% of total samples	Industry cereals	Cereal-based products from retail	Beans	Juices	Red Yeast Rice
Total non-EU countries	**62**	**5.2%**	**16**	**28**	**14**	**1**	**3**
Unknown	**186**	**15.6%**	**21**	**97**	**13**	**35**	**20**
Total number of samples	**1,195**		**390**	**510**	**68**	**135**	**92**

2.1.2. Collection, transport and storage of the samples

The samples were purchased in supermarkets, shops, other retail outlets and on internet.

As described in Commission Regulation (EC) No 401/2006 (EU, 2006), of each product three items with the same expiry date and the same lot number were collected. The combined amount of product collected should be sufficient to prepare an aggregate sample of at least 1 kg or 1 L. As for food supplements, the three items were restricted by commercial format.

The purchased products were transported and stored at the usual storage temperature of the product in the retail shop. Products with an extended shelf-life were either stored at room temperature or under cooled conditions. The products were not frozen before preparation of the aggregate sample.

All relevant information regarding the sample (as described on the product label) as well as the place and date of collection was collected and recorded in the format of the EFSA Sample Description Database. The original packing and/or labels were kept as a back-up of the available product information. Alternatively, or additionally, scans and/or photos were taken of the sample for the same purpose.

2.2. Preparation of the aggregate and subsamples

Aggregate samples were prepared by mixing the contents of the three subsamples followed by homogenization by milling. Subsamples were prepared by transferring an amount of the homogenized aggregate sample into three plastic Greiner tubes. Aggregate and sub-samples were prepared as soon as possible after collection, and always before the expiration date.

An aggregate sample of the red yeast rice food supplements was prepared by combining all tablets or capsules of the three packages according to Regulation (EU) No 519/2014 (EU, 2014b). In the case of capsules, the coatings were removed before homogenisation. The aggregate samples were mixed and homogenised by overhead-shaking for 30 minutes. Finally, three sub-samples of the aggregate sample were transferred to polypropylene tubes of 50 mL. The respective three sub-samples and aggregate sample were appropriately coded and stored at -20 °C until analysis.

2.3. Standards, chemical and reagents, and materials (QC samples)

2.3.1. Standards

Citrinin standard and internal standard were purchased by each participant. Table 5 shows information regarding the quality of the standards used during the project.

The commutability of the standards was evaluated during the inter-laboratory comparison.

Table 5: Citrinin standards used by each laboratory

	Name	Supplier	µg/mL	Solvent	Purity	Lot number
RIKILT	Citrinin $^{13}C_{13}$-Citrinin	Sigma-Aldrich Romer Labs	powder 10.6	- Acetonitrile	100% -	124M4013V I14125C
UCSC	Citrinin $^{13}C_{13}$-Citrinin	Sigma-Aldrich Orsell	powder 10.6	- Acetonitrile	>98% -	124M4013V OR15125
IRTA	Citrinin $^{13}C_{13}$-Citrinin	Analytical Standard LGC	1,000 10,6	Acetonitrile Acetonitrile	>99% >99%	C226A151013HP I15125C
NIPH-NIH	Citrinin $^{13}C_{13}$-Citrinin	Biopure (Romer Labs) Biopure (Romer Labs)	100.1±0.6 10.3±0.46	Acetonitrile Acetonitrile	99.6% 99.0%	L15231C I15361C

2.3.2. Chemical and reagents

RIKILT: The chemicals and solvents used for sample preparation were 'pro-analysis' quality or better. Methanol, acetonitrile and LC–MS grade water were purchased from Actu-Alls (Oss, the Netherlands). Magnesium sulphate and ammonium formate were from Sigma-Aldrich (Zwijndrecht, the Netherlands). Hydrochloric acid, acetic acid and sodium chloride were supplied by Merck (Amsterdam, the Netherlands).

IRTA: The chemicals and solvents used for sample preparation were 'pro-analysis' quality or better. Methanol, acetonitrile and LC–MS grade water were purchased from Merck (VWR - Barcelona, Spain). Magnesium sulphate, hydrochloric acid, acetic acid, sodium chloride and ammonium formate were from Sigma-Aldrich (Madrid, Spain).

NIPH-NIH: The chemicals and solvents used for sample preparation were 'pro-analysis' or 'trace-analysis' quality. Acetonitrile p.a. and methanol were purchased from J.T. Baker (Avantor™ Performance Materials). Water for LC–MS was prepared in the laboratory and the conductivity (< 18.2 MΩ) was checked. Magnesium sulphate anhydrous, acetic acid and ammonium acetate were from Sigma-Aldrich (Poznań, Poland). Hydrochloric acid p.a. 36% and sodium chloride were purchased from Purchem (Poland).

2.3.3. Materials: preparation of the QC and stability samples

QC and stability samples were prepared for three matrices: wheat, fruit juices and red yeast rice (RYR) (Table 6). These QC samples were used for the limited inter-laboratory comparison study, stability tests and were included in each run by all partners. The results were also used to evaluate reproducibility.

Blank and incurred materials for wheat and red yeast rice were obtained from previous analyses carried out in RIKILT in 2013. Blank material for fruit juice was purchased from local retail supermarkets.

Table 6: Materials used to prepare QC samples

	Blank/incurred	Type of sample used
Wheat	Blank Incurred samples	RIK0351077 and RIK0351078 RIK0351098
Fruit juice	Blank	Fruit juice purchased from supermarket
RYR	Blank samples Incurred samples	RIK0343865, RIK0343872, RIK0343873, RIK0343875 RIK0343880

The QC samples were for: (i) use as QC samples in each analysis run by all four partners (based on the sampling plan and the number of samples to be analysed during the project by the 4 partners, the number of QC samples was estimated (Table 7)); (ii) stability testing; (iii) as samples for the limited

inter-laboratory comparison study; and (iv) a back-up series were prepared. Stability of the QC was assessed at -80°C, -20°C and at room temperature at 6 months (before Interim report) and after 1 year of storage (at the end of the project)

Table 7: Overview of the number of QC samples

	Number of QC	Number of stability	TOTAL
Wheat	60 Blank 60 Spiked at 5 μg/kg 60 Incurred samples	8 Blank 8 Spiked at 5 μg/kg 8 Incurred samples	204
Fruit juice	16 Blank samples 16 Spiked at 5 μg/kg	8 Blank samples 8 Spiked at 5 μg/kg	48
RYR	16 Blank samples 16 Spiked at 100 μg/kg 16 Incurred samples	8 Blank samples 8 Spiked at 100 μg/kg 8 Incurred samples	72

The QC samples were prepared by making slurries in water, followed by freeze-drying and grinding of the materials.

For the stability tests, 6 sets were stored at the indicated temperatures and the other two were used as reference at the starting point of the project. After 6 months, by the end of the reporting period 1 (March 2016) for each matrix one set of 8 samples stored at each temperature was analysed for stability. After 1 year, by the end of the project (October 2016) for each matrix the second set of 8 samples stored at each temperature were analysed for stability (Table 8).

Table 8: Overview of the number of samples used for stability testing

	-80 °C stability	-20 °C stability	Room temperature
Wheat	2 sets (blank + spiked +incurred)	2 sets (blank + spiked +incurred)	2 sets (blank + spiked +incurred)
Fruit juice	2 sets (blank + spiked)	2 sets (blank + spiked)	2 sets (blank + spiked)
RYR	2 sets (blank + spiked +incurred)	2 sets (blank + spiked +incurred)	2 sets (blank + spiked +incurred)

Before shipment of the QC samples to the partners, spiked and incurred samples were analysed in triplicate at RIKILT. QC samples were sent on dry-ice. Each partner received all QC samples (15 sets of wheat, 4 sets of juice and 4 sets for RYR). One set was used for the limited inter-laboratory comparison study.

2.4. Methodologies

2.4.1. Sample preparation

RIKILT and IRTA:

All matrices except for RYR: 5±0.5 g of aggregate samples were weighed and extracted with: i) 12.5 mL of the saturated aqueous solution with 1% acetic acid, ii) 125 μL of hydrochloric acid 37% and iii) 25 mL of the extraction solution of acetonitrile acidified with 1% of acetic acid. The extracts were mixed for 1 hour head-over-head, after which 7.5 g of magnesium sulphate and 1.87 g of sodium chloride were added to induce phase separation. The extracts were hand-shaken for 1 min and centrifuged for 10 min at 3,500 rpm. Finally, 490 μL of supernatant and 10 μL of internal standard ($^{13}C_{13}$-Citrinin at 50 ng/mL) were mixed and filtered in mini-uniprep PTFE filter vials.

RYR: 1±0.15 g of aggregate samples were weighed and extracted with: i) 2.5 mL of the saturated aqueous solution with 1% acetic acid, ii) 25 μL of hydrochloric acid 37% and iii) 5 mL of the extraction solution of acetonitrile acidified with 1% of acetic acid. The extracts were mixed for 1 hour head-over-head, after which 1.5 g of magnesium sulphate and 0.38 g of sodium chloride were added to induce phase separation. The extracts were hand-shaken for 1 min and centrifuged for 10 min at 3,500 rpm. Finally, 490 μL of supernatant and 10 μL of internal standard ($^{13}C_{13}$-Citrinin at 50 ng/mL) were mixed and filtered in mini-uniprep PTFE filter vials. Ten-fold diluted extracts were also prepared by mixing in mini-uniprep PTFE filter vials 50 μL of supernatant, 440 μL of dilution solution and 10 μL of internal standard. Extracts were stored at 4 °C and analysed within 24 hours. The extracts were analysed by LC-MS/MS.

UCSC:

For cereals, beans and derived products, citrinin was extracted from a 20 g sample with 100 mL of a mixture of 10 mM phosphoric acid-methanol (30 + 70 v/v) for 45 min using a rotary-shaking stirrer.

RYR: citrinin was extracted from a 1 g of sample with 20 mL of a mixture of 10 mM phosphoric acid-methanol (30 + 70 v/v).

Juices: 10 mL of sample was diluted with 35 mL of methanol and 5 mL 10 mM of phosphoric acid.

After filtration through a folded filter paper, an aliquot of the filtrate (2 mL) was diluted with phosphate buffer solution (PBS) (18 mL) and purified through an immunoaffinity column (Easi-extract Citrinin, r-Biopharm). The column was washed with PBS (2 mL) and citrinin was slowly eluted (0.5 mL/min) with methanol (4 mL) into a graduated glass vial; the eluate was concentrated under a gentle stream of nitrogen, brought to 1 mL with methanol:water (30 + 70 v/v) and vortex-mixed for a few seconds. The extract was filtered (Millex HV 0.45 μm, Millipore), then 100 μL of internal standard solution (^{13}C-CIT, 10.6 μg/L) was added to 400 μL of purified extract. The extracts were analysed by LC-MS/MS.

NIPH-NIH:

The sample preparation was similar to that followed by RIKILT and IRTA with some differences. In case of beans, the amount of hydrochloric acid was doubled (250 μL instead of 125 μL). For all matrices after centrifugation, 2.5 mL of supernatant were transferred into a glass tube, 50 μL of internal standard $^{13}C_{13}$-citrinin at 100 ng/ml were added and then evaporated till dryness in a gentle stream of nitrogen at 40°C. The extract was reconstituted with 0.5 mL of dilution solution and then transferred into an Eppendorf-type PP tube. The extract was centrifuged for 5 min at 14,000 rpm and the supernatant was transferred into a LC vial for analysis.

2.4.2. Instrumental analysis

RIKILT: 5 μL of the extract were injected into an UHPLC-MS/MS system. The system consisted of a Waters Acquity LC-system (degasser, pumps, autosampler, column, oven) and a Xevo TQ-S triple quadrupole mass spectrometer from Waters. Separation was performed on Acquity UPLC HSS T3 100 x 2 mm 1.8 μm (Waters) maintained at 40°C, with a mobile-phase gradient of water (phase A) and methanol (phase B), both containing 5 mM of ammonium acetate and 0.05% of acetic acid. The gradient elution was applied as follows: after an initial hold time of 0.1 min at 95% eluent A, 85% eluent B was reached within 4 min and 100% eluent B within the next 0.1 min. This composition was kept for 2.1 min, after which 95% eluent A was reached within the next 0.1 min and kept for 2.3 min for column re-equilibration. The flow rate was 0.4 mL/min.

MS/MS measurements were performed using negative electrospray ionisation. The general source settings were as follows: source temperature 150°C, desolvation temperature 600°C, cone gas flow 150 L/h, desolvatation gas 800 L/h and cone voltage 50V.

For citrinin, using [M-H+OH]- (m/z 281.2) as precursor ion, three transitions were measured: m/z 249.2 (collision energy 20V) [quantification ion], m/z 205.2 (25 V) and m/z 177.1 (30 V). For the

isotopic label ($^{13}C_{13}$), 294.2 m/z was used as precursor ion with m/z 262.2 (20 V) as product ions. All transitions were measured in one event using dwell times of 80 ms.

Mass Lynx software version 1.4 (Waters) was used for data-evaluation. Peak assignment and integration were manually verified by the operator. Quantification was based on multi-level calibration using solvent standards of citrinin (concentrations corresponding to 0.1, 0.2, 0.4, 1.0, 2.0, 10, 20, 40 and 60 ng/mL in the vial, internal standard at 1 ng/mL, which were injected prior and after the extracts.

Responses in extracts and standards were normalised to the internal standard. Since the internal standard was added after extraction, it corrected for matrix effects only, not for recovery. In case of positive samples, the concentration found was corrected for the recovery calculated from the QC samples analyse during the series.

UCSC: Analysis was carried out using a HPLC-MS/MS system, consisting of a LC 1.4 Surveyor pump, a Quantum Discovery Max triple-quadrupole mass spectrometer (Thermo-Fisher Scientific, San Jose, CA, USA) and a PAL 1.3.1 sampling system (CTC Analitycs AG, Zwingen, Switzerland); the system was controlled by an Excalibur 1.4 software (Thermo-Fisher). Citrinin was separated on a XBridge BEH RP-18 column (2.5 µm particle size, 100 x 3.0 mm, Waters Corporation, Milford, Massachusetts, USA) with a mobile-phase gradient methanol-water (both acidified with 0.2% formic acid) from 45:55 to 75:25 in 3 min, then isocratic for 4 min; gradient to 45:55 in 1 min and re-equilibration for 7 min. The flow rate was 0.2 mL/min; the injection volume was 20 µL. The ionisation was carried out with an ESI interface (Thermo-Fisher Scientific) in positive mode as follows: spray capillary voltage 4.0 kV, sheath and auxiliary gas 35 and 8 psi, respectively, temperature of the heated capillary 270°C. For fragmentation of [M+H]+ ion (251 m/z), the argon collision pressure was set to 1.5 mTorr; the selected fragment ions were: 233 m/z (20 V), 205 and 191 m/z (28 V). Quantitative determination was performed using a LC-Quan 2.0 software.

Citrinin (5 mg, Sigma) was dissolved in ethanol (25 mL) and the solution, after dilution, was calibrated spectrophotometrically at 319 nm using the value 4,710 L/(mol cm) for the absorption coefficient (Neely et al., 1972) and stored at –20°C when not in use. Working standards (between 0.10 and 5.0 µg/L) were prepared by dilution with methanol-water 30+70 v/v.

IRTA: 4 µL of the extract were injected into an UHPLC-MS/MS system. The system consisted of a Waters Acquity LC-system (degasser, pumps, autosampler, column, oven) and a TQD triple quadrupole mass spectrometer from Waters. Separation was performed on Aquity UPLC BEH C18 100 x 1 mm internal diameter (Ø of the particle 1.7 µm) maintained at 40°C, with a mobile-phase gradient of water: methanol 95:5 v/v (phase A) and methanol (phase B), both containing 5 mM of ammonium acetate and 0.05% of acetic acid.

The gradient elution was applied as follows: initial time 100% eluent A, 100% eluent B was reached within 4 min. This composition was kept for 2.5 min, after which 100% eluent A was reached within the next 0.1 min and kept for 3.0 min for column re-equilibration. The flow rate was 0.1 mL/min.

MS/MS measurements were performed using negative electrospray ionisation. The general source settings were as follows: source temperature 135°C, desolvation temperature 370°C, cone gas flow 25 L/h, desolvatation gas 400 L/h and cone voltage 50V.

For citrinin, using [M-H+OH]- (m/z 281.2) as precursor ion, three transitions were measured: m/z 249.2 (collision energy 20V) [quantification ion], m/z 205.2 (25 V) and m/z 177.1 (30 V). For the isotopic label ($^{13}C_{13}$), 294.2 m/z was used as precursor ion with m/z 262.2 (20 V) as product ions. All transitions were measured in one event using dwell times of 80 ms.

Mass Lynx software version 1.4 (Waters) was used for data-evaluation. Peak assignment and integration were manually verified by the operator. Quantification was based on multi-level calibration using solvent standards of citrinin (concentrations corresponding to 0.5, 1.0, 2.0, 10, 20 and 40 ng/mL in the vial, internal standard at 2 ng/mL, which were injected prior and after the extracts.

NIPH-NIH: The analysis of the samples was carried out by injecting 25 µL of extract into a HPLC-MS/MS system, consisting of a Waters Alliance HPLC 2695 with column oven coupled to a Waters Quattro micro mass spectrometer. Citrinin was separated on a XBridge BEH C18 column (2.5 µm particle size, 100 x 3.0 mm, Waters) maintained at 40°C, with a mobile-phase gradient of methanol/water 95/5 (v/v) (phase A) and water (phase B), both containing 5 mM of ammonium acetate and 0.1% of glacial acetic acid. The gradient elution was applied as follow: initial composition 40% A, which reached 100% A in 4 min. This composition was kept for 10 min to come back to the initial conditions 40% A in one minute. The initial composition was kept for 7 min for column re-equilibration. The flow rate was set at 0.2 mL/min.

MS/MS measurements were performed using negative electrospray ionisation. The general source settings were as follows: source temperature 120°C, desolvation temperature 400°C, cone gas flow 50 L/h, desolvatation gas 400 L/h, capillary voltage 2.8 kV and cone voltage 30 V.

For citrinin, using [M-H+OH]- (m/z 281.0) as precursor ion, three transitions were measured: m/z 249.1 (quantification ion, collision energy 18 V), m/z 205.1 (qualification ion, 24 V) and m/z 177.2 (30 V). For the isotopic label $^{13}C_{13}$-CIT, 294.0 m/z was used as precursor ion with m/z 262.1 (18 V) as product ions. All transitions were measured in one event using dwell times of 80 ms.

Peak assignment and integration were manually verified by the operator. Quantification was based on multi-level calibration using solvent standards of citrinin (concentrations 1, 2, 5, 10, 20, 50 ng/mL in dilution solvent, internal standard at 10 ng/mL).

Responses in extracts and standards were normalised to the internal standard. Since the internal standard was added after extraction, it corrected for matrix effects only, not for recovery. In case of positive samples, the concentration found was corrected for the recovery calculated from spiked sample analyse during the series. Results above LOQ were confirmed during second analysis with different series.

2.5. Method validation

2.5.1. Validation method

The analytical methods described in Section 2.4 were in-house (re)-validated by each partner using their own method for 13 matrices: wheat, barley, rye, oats, rice, pasta, bread and bread rolls, breakfast cereals, beans, fruit juice, vegetable juices and red yeast rice supplements.

For wheat, barley, rye, oats, rice, pasta, bread and bread rolls, breakfast cereals, beans, fruit juice and vegetable juices, the validation was performed by spiking each matrix in duplicate at two levels (1 µg/kg and 10 µg/kg), i.e. a total validation consisting of 22 samples was obtained.

For RYR, the validation was conducted by spiking in duplicate at 10 µg/kg and at 2,000 µg/kg). All matrices included in the validation were also analysed without spiking, as well as a reagent blank.

From these initial in-house validations, the linearity, recovery, repeatability, selectivity, LOQ, and LOD were derived. The use of $^{13}C_{13}$-citrinin when applying LC-MS/MS corrects the suppression or enhancement of the signal due to matrix effects. In addition, the stability of retention time and ion ratios in solvent standards and extracts for LC-MS/MS-based methods were determined.

2.5.2. Validation criteria

As requested in the call, to demonstrate the fitness-for-purpose of the analytical method(s) for citrinin in food, the criteria for ochratoxin A, as specified in Regulation (EC) No 401/2006 (EU, 2006) and its amendment Regulation (EU) No 519/2014 (EU, 2014b), were applied (see Table 9). Performance criteria for citrinin are available in the Regulation (EU) No 519/2014 (EU, 2014b)), but can only be calculated from the Horwitz equation since inter-laboratory comparison studies have not yet been conducted to determine $RSD_{(R)}$.

Table 9: Validation criteria for ochratoxin A, as described in Regulation (EC) No 401/2006 and its amendments

Criterion	< 1 μg/kg	≥ 1 μg/kg
Recovery	50-120	70-110
Repeatability $RSD_{(r)}$ %	≤ 40	≤ 20
Reproducibility $RSD_{(R)}$ %	≤ 60	≤ 30

The linearity for all the matrices, except for RYR, was evaluated in a range of 10 to 100 µg/kg, while for RYR the linearity was evaluated in a range 10 to 2,000 µg/kg. The method will be considered as linear if correlation coefficients are greater than 0.99 and the back-calculated concentrations of the calibration standards do not exceed ± 20% of theoretical.

The recommended value for average recovery was 70-110% (derived from Commission Regulation (EC) No 401/2006 for ochratoxin A).

The recommended repeatability (RSDr), derived from Commission Regulation (EC) No 401/2006 for ochratoxin A, should not be greater than 20%.

The limit of quantification (LOQ) was here defined as the lowest spiked level for which the criteria for average recovery and repeatability were met. Therefore, the method aimed a LOQ of 10 μg/kg for RYR and of 1 μg/kg for the rest of the matrices.

The limit of detection (LOD) was defined as the level corresponding to a signal-to-noise ratio (S/N) of three. In case of MS/MS determination, the response should be taken for the transition with the lowest S/N (i.e. the qualifier ion). RIKILT decided to define LOD as 0.5 x LOQ.

The selectivity was assessed based on the analysis of the blank samples, which meant that the response for the blank samples was not greater than 30% of the response of the samples spiked at LOQ level.

Matrix effects were not assessed in those methods using the isotopically labelled internal standard, since they are compensated.

2.6. Quality control

2.6.1. On-going quality control

With each batch of survey samples, at least two types of recovery samples were included: one set (blank, spiked, incurred) of the QC samples prepared at the beginning of the project and one recovery prepared *in-situ* by the laboratory itself. In this way, method performance in time and extension of applicability to other matrices from the same product group were assessed. These data were also used to establish the long-term within-laboratory reproducibility.

2.6.2. Inter-laboratory comparison

The comparability of results between the four laboratories performing the analyses was verified by exchanging solvent standards used for spiking and calibration, and by the analysis of one set of each of the three types of QC samples (Section 2.3.3) prepared and distributed at the beginning of the project: wheat, fruit juice and red yeast rice.

2.6.3. Storage stability

The stability of standards was assessed by comparison of newly purchased stock solutions against the ones already available in the laboratory, and by comparison of freshly prepared working solutions from the existing stock against older working solutions.

The storage stability of citrinin in wheat, fruit juice and RYR at three different temperatures was assessed at medium-term (after 6 months of preparation) and at long-term (by month 14).

2.6.4. Identification criteria

Using LC-MS/MS, the identification of citrinin was based on retention time and the presence of coinciding peaks for at least 2 product ions in the correct abundance ratio. In general, the retention time of citrinin in samples should not deviate more than 0.20 minutes from the reference retention time in solvent standards. The ion ratio of the product ions (least abundant/most abundant) of citrinin in the samples should be consistent with that obtained during validation and in any case should not deviate more than 30%.

2.7. Reporting results

The results obtained for the survey samples were only considered valid when the linearity and recoveries obtained for a particular batch complied with the criteria mentioned in Section 2.5.1 and when the criteria for identification mentioned in Section 2.6.4 were met.

The results obtained were corrected for recovery, except when the internal standard was added to the sample before extraction. In the latter case recovery correction was inherent to the procedure.

Taking the EFSA reporting format into account, there were three possible types of analysis result:

i) 'VAL': interpreted here as samples in which citrinin was found at levels equal to or higher than the lowest validated level (≥ 1.0 µg/kg).

ii) 'LOQ': interpreted here as samples in which citrinin was identified but below the lowest validated level (< 1.0 µg/kg).

iii) 'LOD': interpreted here as samples in which no citrinin was identified.

In the EFSA SSD database, values below the lower limit of the working range (< 1.0 µg/kg) are included as 'Comment to the result'. The value provided here should be regarded as indicative because no data on recovery and precision were generated below that level, and the response may be below the lowest calibration level. The LOD value depended on the method for analysis.

To calculate average, median and 95th percentiles, a substitution method with three different approaches was followed: the lower bound (LB), middle bound (MB) and upper bound (UB). For the lower bound values, zero was used for '< LOD', and the LOD value was used for those samples in which citrinin was identified, but below the LOQ. For the middle bound, half of LOD was used for '< LOD', and half the LOQ in case citrinin was identified below the LOQ. For the upper bound scenario, LOD-value and the LOQ values were used for '<LOD' and '<LOQ', respectively.

3. Results

3.1. In-house method validation

When using LC-MS/MS methods the linearity was guaranteed within the range of 0.1-60 ng/mL, which in sample corresponds to 0.5 - 3,000 μg/kg. However, for quantification purposes the calibration line was split for low concentration and high concentration.

An overview of the individual recoveries of all samples analysed during initial in-house validation by each of the four laboratories, and the average recovery and repeatability (RSDr) for each food group are given in Table 10 and Table 11. The average recovery and repeatability were compliant with the validation requirements, from which it was concluded that the methods used by the different laboratories were fit-for-purpose for quantitative analysis of citrinin in food.

Based on the results of recovery and repeatability, LOQ was set at 1 μg/kg for all matrices except for RYR, for which LOQ was set at 10 μg/kg.

The LOD was derived from the signal-to-noise ratio observed in the (extracted) ion chromatograms. Since it is affected by the matrix and the instrumental conditions of the LC-MS/MS systems at the time of analysis, it is considered less meaningful to give an exact fixed value. Therefore, a range is given here, based on the observations during validation and the analysis of the survey samples: 2.0-5.0 µg/kg for red yeast rice and 0.2–0.5 µg/kg for the rest of the food commodities under study

Table 10: Recoveries (%) and repeatability ($RSD_{(r)}$) obtained during the in-house validation

Food category	Spiking concentrations (μg/kg)	Laboratory			
		RIKILT	UCSC	IRTA	NIPH-NIH
Wheat	1	70	107	84	98
Wheat	1	83	109	117	95
Wheat	10	89	88	89	93
Wheat	10	91	99	89	96
Barley	1	96	92	99	76
Barley	1	99	103	83	86
Barley	10	92	79	84	70
Barley	10	88	84	90	78
Rye	1	85	100	82	77
Rye	1	94	106	87	75
Rye	10	86	79	85	78
Rye	10	80	89	82	81
Oats	1	97	100	114	79
Oats	1	104	91	94	84
Oats	10	87	100	82	90
Oats	10	88	86	86	89
Rice	1	99	104	117	80
Rice	1	99	99	104	88
Rice	10	98	87	94	84
Rice	10	98	93	85	87
Bread and bread rolls	1	108	103	77	80
Bread and bread rolls	1	108	103	88	88
Bread and bread rolls	10	89	81	74	85
Bread and bread rolls	10	91	91	85	96
Pasta	1	109	103	80	77
Pasta	1	98	97	102	71
Pasta	10	95	80	102	81
Pasta	10	100	70	107	81
Breakfast cereals	1	82	98	63	84

Food category	Spiking concentrations (µg/kg)	Laboratory			
		RIKILT	UCSC	IRTA	NIPH-NIH
Breakfast cereals	1	82	106	73	88
Breakfast cereals	10	90	85	85	87
Breakfast cereals	10	95	85	89	85
Beans	1	104	104	89	83
Beans	1	104	112	82	97
Beans	10	91	107	72	97
Beans	10	96	110	84	82
Fruit juices	1	94	103	71	84
Fruit juices	1	86	85	87	89
Fruit juices	10	81	107	82	87
Fruit juices	10	81	108	81	84
Vegetable juices	1	104	106	69	83
Vegetable juices	1	102	120	77	73
Vegetable juices	10	85	84	87	77
Vegetable juices	10	97	89	85	78
Average	1	96	102	88	83
$RSD_{(r)}$	1	11	7	17	9
Average	10	90	90	87	85
$RSD_{(r)}$	10	7	11	9	8

Table 11: Recoveries (%) and repeatability ($RSD_{(r)}$) for RYR obtained during the in-house validation

Matrix	Spiked (µg/kg)	Laboratory			
		RIKILT	UCSC	IRTA	NIPH-NIH
RYR	10	79	115	104	78
RYR	10	75	113	86	86
RYR	2,000	71	95	73	69
RYR	2,000	75	94	75	71
Average	10	77	114	95	82
$RSD_{(r)}$	10	3	1	13	7
Average	2,000	73	94	74	70
$RSD_{(r)}$	2,000	4	1	2	2

Chromatograms from the validation studies of the laboratories are shown in Figures 3-6.

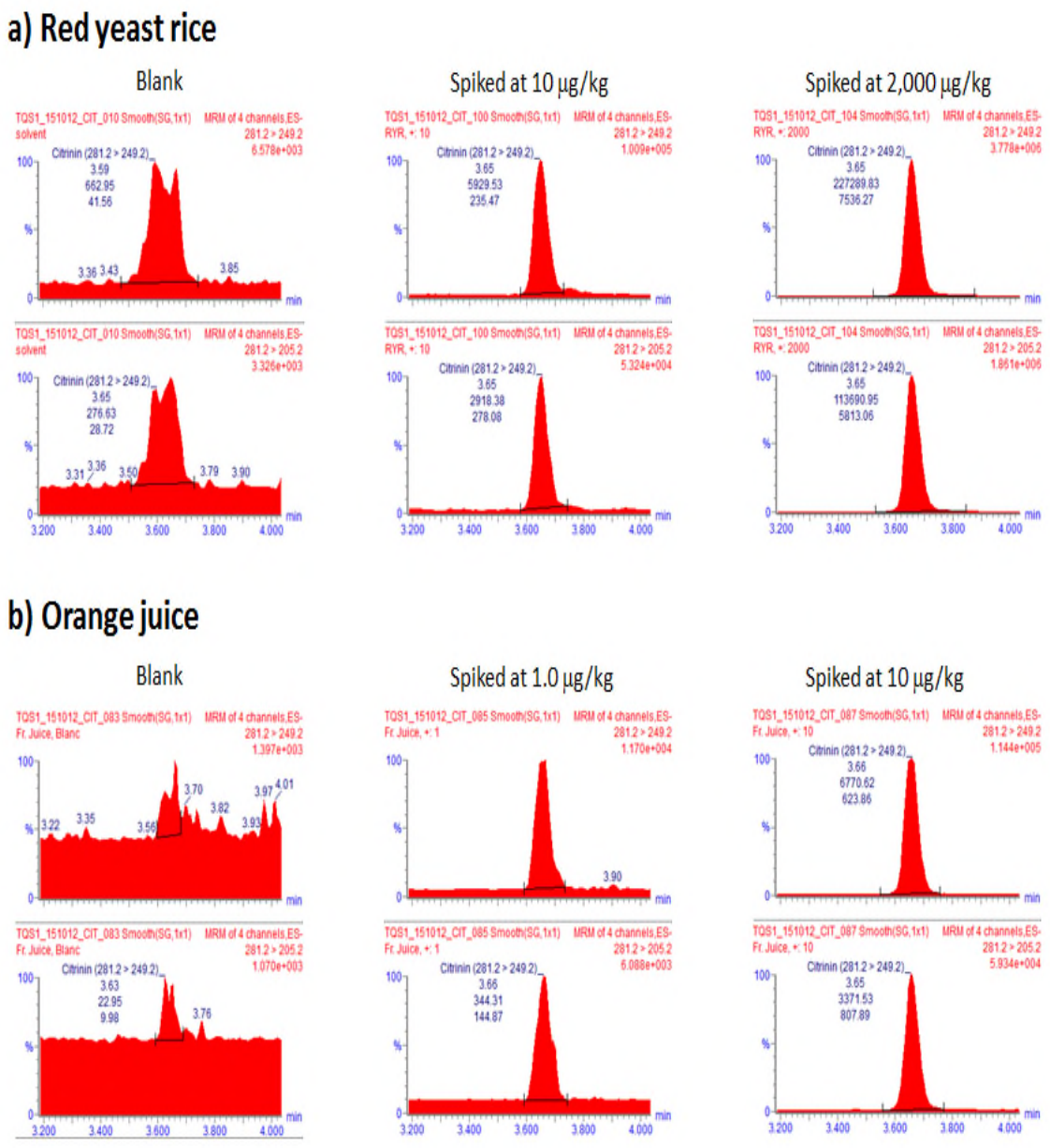

Figure 3: Chromatograms obtained during the validation of citrinin in red yeast rice and orange juice by RIKILT

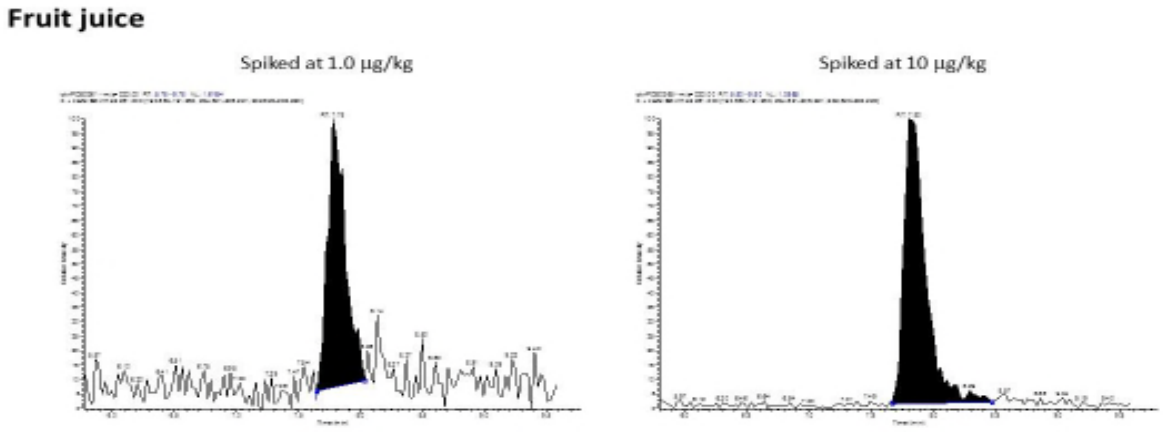

Figure 4: Chromatograms obtained during the validation of citrinin in fruit juice by UCSC

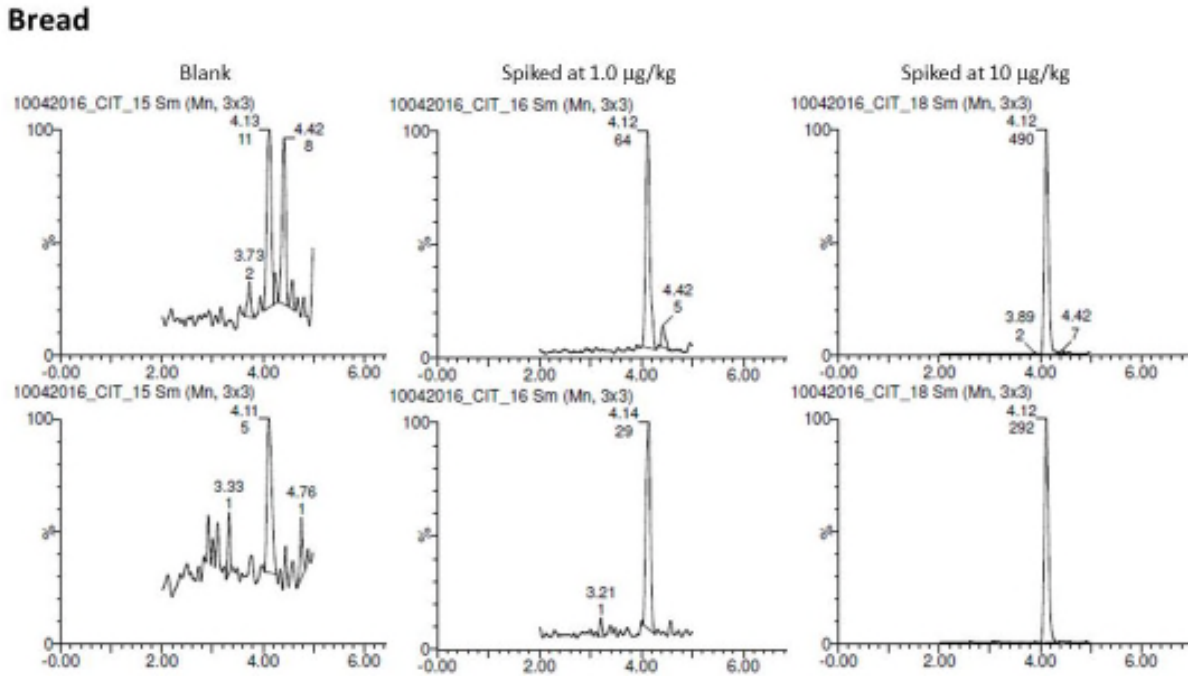

Figure 5: Chromatograms obtained during the validation of citrinin in bread by IRTA

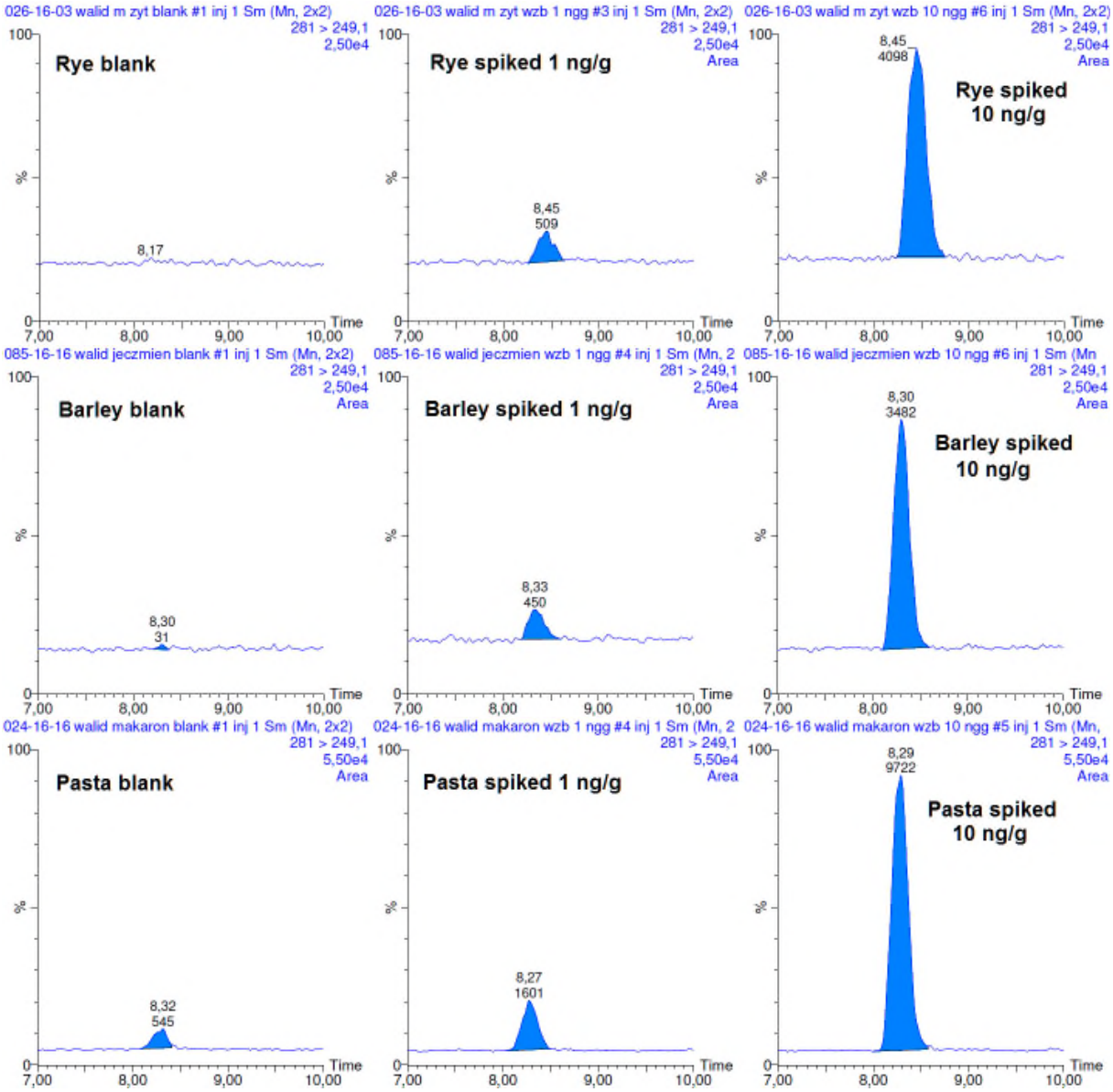

Figure 6: Chromatograms obtained during the validation of citrinin in rye, barley and pasta (blank sample, spiked at 1.0 μg/kg and spiked at 10 μg/kg) by NIPH-NIH

3.2. Limited inter-laboratory study

With the purpose of assessing the commutability of results among the methods run in the four laboratories, a solvent standard with concentrations of citrinin ranging from 1 to 60 ng/mL, and samples (blanks, incurred and spiked) of wheat, red yeast rice and orange juice were distributed.

The solvent standard and the samples were analysed by each lab, using their own methods and calibration solutions.

The RYR incurred sample had been analysed by RIKILT for research activities in 2014. The spiked samples were prepared and analysed by RIKILT, just before shipment. The amount of material

available was insufficient to perform a full homogeneity study. In Table 12, the results obtained by the different laboratories are given.

Table 12: Results for the limited inter-laboratory study

Concentration (μg/kg)		Laboratory				AVERAGE	%RSD_R[(a)]
		RIKILT	UCSC	IRTA	NIPH-NIH		
Standard	20 ng/mL	21.4	19.8	19.9	19.5	20.1	4%
Wheat	Blank	<LOD	<LOD	<LOD	<LOD		
	Spiked	5.4	4.3	6.1	6.2	5.5	16%
Orange juice	Blank	<LOD	<LOD	<LOD	<LOD		
	spiked	4.9	3.0	6.3	4.7	4.7	29%
RYR	Blank	<LOD	<LOQ	<LOD	<LOD		
	Spiked	83.8	102.4	79.9	83.8	87.5	12%
	Incurred	51.4	32.9	50.6	45.7	45.1	19%

(a): RSD_R: reproducibility.

The four laboratories were able to measure the solvent standard within a more than satisfactory deviation of 4% among their results.

As far as the samples were concerned, all four laboratories provided results below LOD for the blank samples, meaning that there was no inherent contamination in their methodology. Overall, results for spiked and incurred materials provided by UCSC were lower than those provided by other laboratories, except for the spiked RYR, while the results provided by IRTA for the spiked wheat and juice material were higher than average. Despite these observations, the performance of both laboratories was still acceptable and consistent.

3.3. Storage stability of the samples

The storage stability of citrinin in wheat (spiked), orange juice (spiked) and RYR (incurred and spiked) was evaluated at mid-term (five months after preparation, April 2014) and at long-term (October 2014). The samples were stored at -80°C, -20°C and +4°C. Results are shown in Table 13.

Table 13: Results for the storage stability of citrinin in incurred and spiked samples

Matrix	Reference (μg/kg) November 2015	Storage temperature	Mid-term (μg/kg) April 2016	Long-term (μg/kg) October 2016
Wheat spiked	5.0	-80 °C	6.4	6.7
		-20 °C	6.3	6.6
		+4 °C	5.9	6.2
Orange juice spiked	5.0	-80 °C	5.3	5.9
		-20 °C	4.6	5.0
		+4 °C	1.6	1.0
RYR spiked	100	-80 °C	106	97.2
		-20 °C	96.5	90.2
		+4 °C	87.8	72.0
RYR incurred	51.5	-80 °C	55.0	49.1
		-20 °C	55.5	53.5
		+4 °C	54.0	48.9

Results at mid-term showed that temperature and the type of matrix played a paramount role on the stability of citrinin in spiked samples. Whereas the difference of citrinin concentration in spiked wheat samples between -80°C and +4°C storage temperature was less than 10% and can even be

considered as constant, the concentration of citrinin in spiked orange juice stored at +4°C dropped 70% compared to the reference value. As for RYR, no differences were observed in the concentration of citrinin stored at either -80°C or -20°C, although it slightly decreased in comparison with the reference value (12%) if stored at 4°C. On the other hand, the concentration of citrinin was stable in incurred RYR samples, regardless of the storage temperature.

The same trends were confirmed when samples were long-term stored. Storage temperature did not affect the stability of citrinin in spiked wheat samples and incurred RYR. However, the concentration of citrinin decreased when spiked samples of RYR and orange juice were stored at 4°C. No significant differences were observed in the stability of citrinin at mid-term or long-term.

In summary, citrinin was stable in QC samples over the timeline of the project as long as they were stored at least at -20°C. Citrinin is susceptible to degrade, especially in spiked samples, if the storage conditions are not appropriate.

3.4. Quality control data obtained during the analysis of samples

The method performance during routine analysis was monitored by every partner by the analysis in each series of measurements of the QC samples, distributed at the beginning of the project and used for the interlaboratory comparison.

Figures 7-10 show the results of the QC samples by the different partners of the project.

Figure 7 shows the results obtained for the QC spiked wheat flour. As observed, results obtained by UCSC, with a method in which citrinin was cleaned up with immunoaffinity column, usually obtained concentrations lower than the spiking one (5 μg/kg), with an average recovery of 84%. RIKILT, IRTA and NIPH-NIH usually found in QC samples concentrations higher than 5 μg/kg, with an average recovery of 116%, 123% and 117% respectively. The fact that the recoveries were higher than the validated values did not entail that the quality of the measurements was compromised, but it may indicate a possible error regarding the preparation of the QC extract for analysis. In-house QC samples prepared for every batch gave recoveries lower than 120%. The between-lab reproducibility (RSD_R%) was 19%.

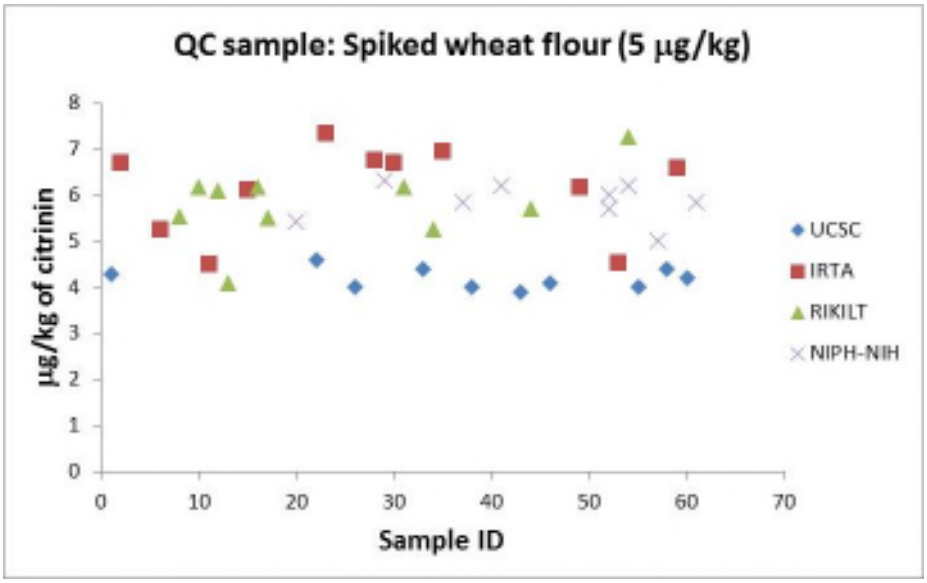

Figure 7: Results for the QC wheat spiked sample (5 μg/kg)

As observed from Figure 8, citrinin was only degraded in the QC samples sent to UCSC. The degree of degradation could not be completely explained by storage temperature since QC samples were kept at

-20°C upon arrival at UCSC. Transportation could have negatively affected the stability of citrinin. Average recoveries of citrinin for the spiked QC samples were 99% (RIKILT), 50% (UCSC), 110% (IRTA) and 90% (NIPH-NIH). The between-lab reproducibility (RSD_R%), excluding UCSC, was 10%.

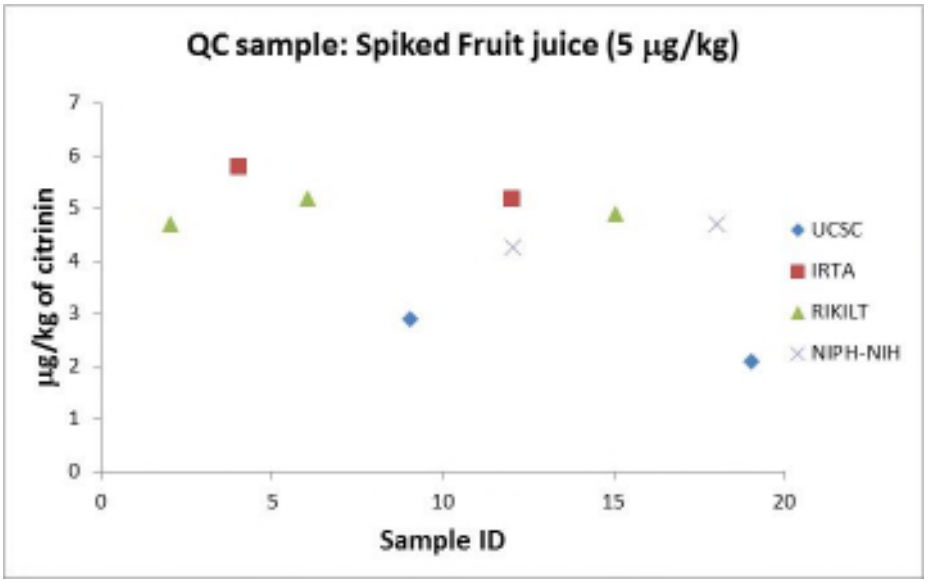

Figure 8: Results for the QC fruit juice spiked sample (5 µg/kg)

The results presented in Figure 9 show that the average recovery of citrinin in QC spiked RYR samples was 78% RIKILT, 107% UCSC, 86% IRTA and 85% NIPH-NIH. The between-lab reproducibility (RSD_R%) was 14%.

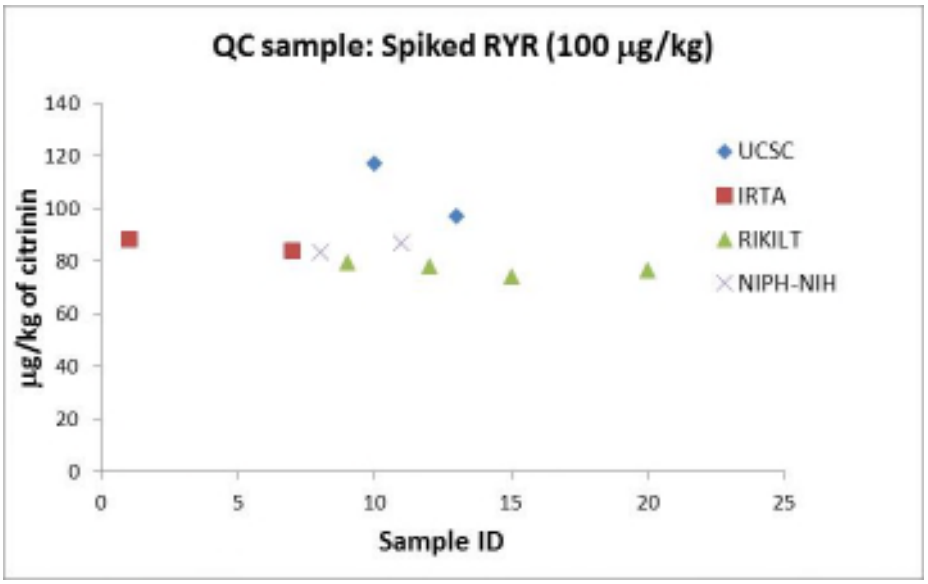

Figure 9: Results for the QC RYR spiked (100 µg/kg) sample

Figure 10 shows the results of the QC RYR incurred. As observed, concentration values provided by UCSC were slightly lower than those reported by the other three partners. The between-lab reproducibility (RSD_R%) was 18%.

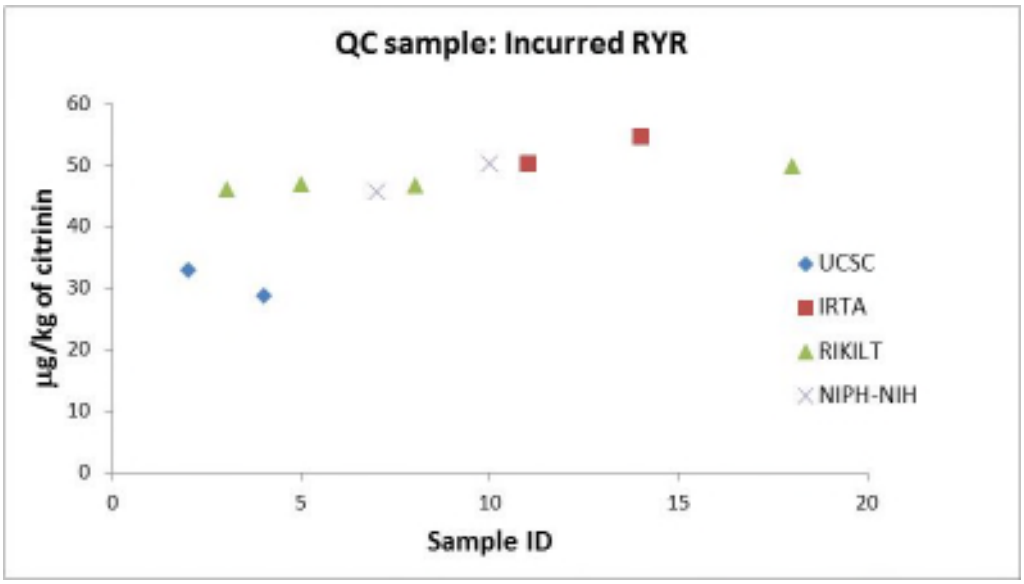

Figure 10: Results for the QC RYR incurred sample

3.5. Occurrence of citrinin in food samples

In total 1,195 samples were analysed by the four laboratories in multiple analytical batches run between January and November 2016. Representative examples of extracted-ion chromatograms for samples in which citrinin was blank or detected, both below and above the LOQ, are provided in Appendix A. An overview of the results is shown in Table 14. In total, citrinin was identified in 105 samples and quantified in 61. As observed, the RYR was the food category for which the major number of positives was obtained.

Table 14: Summary of the analysis result for citrinin

Food category	ANALYSED	DETECTED		LOD-LOQ		>LOQ	
	N	N	%	N	%	N	%
Industry cereals	**390**	**31**	**8%**	**9**	**2%**	**22**	**6%**
Wheat	140	8	6%	3	2%	5	4%
Barley	57	4	7%	0	0%	4	7%
Rye	71	12	17%	2	3%	10	14%
Oats	70	0	0%	0	0%	0	0%
Rice	52	7	13%	4	8%	3	6%
Cereal-based products	**510**	**37**	**7%**	**22**	**4%**	**15**	**3%**
Flour	89	15	17%	7	8%	8	9%
Rice retail	102	6	6%	5	5%	1	1%
Bread and bread rolls	122	6	5%	4	3%	2	2%
Pasta (dry)	105	6	6%	4	4%	2	2%
Breakfast cereals (flakes&muesli)	92	4	4%	2	2%	2	2%
Other products	**295**	**37**	**12%**	**13**	**4%**	**24**	**8%**
Beans	68	0	0%	0	0%	0	0%
Fruit juices	66	0	0%	0	0%	0	0%
Vegetable juices	69	0	0%	0	0%	0	0%
Red yeast rice supplements	92	37	40%	13	14%	24	26%
TOTAL	**1,195**	**105**	**9%**	**44**	**4%**	**61**	**5%**

N: number of samples; LOD: limit of detection; LOQ: limit of quantification.

Table 15 shows the average, median concentration and 95th percentiles of citrinin according to lower, middle and upper bound approaches.

Table 15: Average, median and 95th percentiles of citrinin concentration (µg/kg) in different food categories

Food category	Average[(a)]			Median[(a)]			95th percentile[(a,b)]		
	LB	MB	UB	LB	MB	UB	LB	MB	UB
Industry cereals	**1.2**	**1.4**	**1.6**	**0.0**	**0.3**	**0.5**	**1.2**	**1.2**	**1.2**
Wheat	0.7	1.0	1.2	0.0	0.3	0.5	0.1	0.3	0.6
Barley	2.1	2.3	2.6	0.0	0.3	0.5	(1.8)	(2.0)	(2.6)
Rye	3.2	3.4	3.6	0.0	0.3	0.5	5.1	5.1	5.1
Rice	0.3	0.5	0.7	0.0	0.3	0.5	(1.0)	(1.0)	(1.3)
Cereal-based products	**0.1**	**0.3**	**0.5**	**0.0**	**0.3**	**0.5**	**0.4**	**0.4**	**0.9**
Flour	0.3	0.4	0.7	0.0	0.3	0.5	1.5	1.5	1.5
Rice retail	0.0	0.3	0.5	0.0	0.3	0.5	0.4	0.5	1.0
Bread and bread rolls	0.0	0.3	0.5	0.0	0.3	0.5	0.0	0.3	0.5
Pasta (dry)	0.0	0.3	0.5	0.0	0.3	0.5	0.3	0.4	0.7
Breakfast cereals (flakes&muesli)	0.1	0.3	0.5	0.0	0.3	0.5	0.2	0.3	0.7
RYR	**162**	**164**	**166**	**0.0**	**2.5**	**5.0**	**830**	**830**	**830**
ALL	**12.9**	**13.2**	**13.6**	**0.0**	**0.3**	**0.5**	**2.0**	**2.5**	**5.0**

LB: lower bound; MB: middle bound; RYR: red yeast rice; UB: upper bound.
(a): Concentration values were corrected for recovery.
(b): when N < 60 then the calculated 95th percentile is in between brackets and should be considered as an indicative value only due to the limited number of data (EFSA, 2011).

3.5.1. Industry cereals samples

Citrinin was detected in 31 (8%) of the industry cereal samples and quantified in 22 (6%) samples above the LOQ (Table 19) ranging from 1.1 µg/kg to 155 µg/kg (see Table 16). Rye grain was the industry cereal samples in which citrinin occurred most frequently (17%), followed by rice (13%). Citrinin did not occur in any of the collected oat industry samples. One rye sample collected in Poland had high levels of citrinin (155 µg/kg), while two barley samples had levels of citrinin of 66 and 45 µg/kg. In general, the positive samples originating from Poland had the highest levels of citrinin.

These results were in agreement with those reported by EFSA in their Scientific Opinion on citrinin (EFSA, 2012). EFSA reported in the opinion that citrinin could only be reported above LOQ in less than 10% of the cereal-grain samples. In case of wheat, EFSA reported that citrinin occurred in two wheat Durum samples at levels below 1.0 µg/kg in UK and in one Czech wheat sample out of 11 at levels close to 1.5 µg/kg. Unlike in the present study, no citrinin was reported in the EFSA opinion in barley, oat or rye.

Table 16: Industry cereal samples contaminated with citrinin

CODE	Product description (EFSA coding)	µg/kg[(a)]	O/ NO	Country of origin	Country of sampling	Year of sampling
RIK200398343	Rye grain	1.2	O	Unknown	Netherlands	2015
RIK200416811	Spelt flour, wholemeal	21	NO	Unknown	Netherlands	2016
RIK200416812	Spelt milling products	28	NO	Unknown	Netherlands	2016
RIK200416818	Spelt flour, wholemeal	10	NO	Netherlands	Netherlands	2016
RIK200416819	Spelt flour, wholemeal	5.3	NO	Netherlands	Netherlands	2016
RIK200419980	Rye milling products	<LOQ[(b)]	NO	Unknown	Netherlands	2016
RIK200419982	Rye flour, wholemeal	4.7	NO	Netherlands	Netherlands	2016
RIK200419962	Rice, long-grain	<LOQ[(b)]	NO	India	Netherlands	2016
RIK200419964	Rice, long-grain	<LOQ[(b)]	NO	Unknown	Netherlands	2016
RIK200419970	Rice, parboiled	<LOQ[(b)]	NO	Unknown	Netherlands	2016
RIK200419971	Rice, brown	<LOQ[(b)]	O	Pakistan	Netherlands	2016
RIK200419974	Rice, long-grain	1.6	NO	India	Netherlands	2016
RIK200419975	Rice, long-grain	2.8	NO	India	Netherlands	2016
RIK200426983	Oat porridge	<LOQ[(b)]	O	Germany	Netherlands	2016
UCSC-39	Rice (Crop)	7.2	NO	Italy	Italy	2015
062/16/01/LHŻ/F	Wheat grain, soft	<LOQ[(b)]	NO	Poland	Poland	2016
084/16/05/LHŻ/F	Wheat grain, soft	<LOQ[(b)]	NO	Poland	Poland	2016
084/16/10/LHŻ/F	Wheat grain, soft	38	NO	Poland	Poland	2016
085/16/18/LHŻ/F	Barley grain, whole	66	NO	Poland	Poland	2016
088/16/02/LHŻ/F	Barley grain, whole	4.3	NO	Poland	Poland	2016
088/16/07/LHŻ/F	Barley grain, whole	45	NO	Poland	Poland	2016
088/16/10/LHŻ/F	Barley grain, whole	8.8	NO	Poland	Poland	2016
084/16/13/LHŻ/F	Rye grain	5.4	NO	Poland	Poland	2016
085/16/06/LHŻ/F	Rye grain	155	NO	Poland	Poland	2016
085/16/19/LHŻ/F	Rye grain	1.1	NO	Poland	Poland	2016
086/16/06/LHŻ/F	Rye grain	<LOQ[(b)]	NO	Poland	Poland	2016
086/16/08/LHŻ/F	Rye grain	4.1	NO	Poland	Poland	2016
087/16/20/LHŻ/F	Rye grain	3.1	NO	Poland	Poland	2016
088/16/05/LHŻ/F	Rye grain	12	NO	Poland	Poland	2016
088/16/13/LHŻ/F	Rye grain	33	NO	Poland	Poland	2016
088/16/15/LHŻ/F	Rye grain	1.6	NO	Poland	Poland	2016

O/NO: Organic/Non-organic.
(a): Concentration values are corrected for recovery.
(b): Trace level between limit of detection (LOD) and limit of quantification (LOQ).

3.5.2. Cereal-based products from retail

Citrinin was detected in 37 (7%) of the cereal-based products from retail and quantified in 15 samples (3%) at levels ranging from 1.0 µg/kg to 5.7 µg/kg (Table 17).

Citrinin occurred in the selected five food categories, being flour the food commodity with the highest incidence (17%). It was quantified in eight flour samples (1.0-5.7 µg/kg), one rice sample (2.6 µg/kg), two bread samples (1.5 and 2.3 µg/kg), two pasta samples (1.2 and 1.4 µg/kg) and two breakfast cereals (1.5 and 2.2 µg/kg) collected in the Netherlands, Italy, France, Spain, Poland and Lithuania. It is noteworthy to highlight the high incidence of citrinin in wholemeal rye flour or flour mix with rye as ingredient originated from Poland. This finding may be explained by the high levels of citrinin found in some rye grain samples, also collected in Poland.

The results in retail rice were in agreement with previous ones. Citrinin was not detected above 1.5 µg/kg in a recent study on retail rice in Spain (Huertas-Perez et al., 2015).

Citrinin degrades at temperatures above 175°C under dry conditions, and at temperatures above 100°C in the presence of water (Xu et al., 2006). Food processing involving high temperature (baking) might reduce the content of citrinin. The occurrence of citrinin in breakfast cereals products in the present study was lower than that found in France in 2005, in which citrinin ranged from 1.5 to 42 μg/kg (Molinie et al., 2005).

Table 17: Cereal based samples from retail containing citrinin

CODE	Product description (EFSA coding)	μg/kg[(a)]	O/ NO	Country of origin	Country of sampling	Year of sampling
RIK200398287	Rice grain	<LOQ[(b)]	NO	Surinam	Netherlands	2016
RIK200413230	Brown basmati rice	2.6	O	India	Netherlands	2016
IRTA-CIT-098	Rice basmati	<LOQ[(b)]	NO	Unknown	France	2015
025/16/08/LHŻ/F	Rice basmati	<LOQ[(b)]	NO	Pakistan	Poland	2016
067/16/08/LHŻ/F	Rice basmati	<LOQ[(b)]	O	Pakistan	Poland	2016
099/16/08/LHŻ/F	Rice parboiled	<LOQ[(b)]	NO	Vietnam	Lithuania	2016
RIK200401069	Muesli and similar (oat flakes)	2.2	NO	Great Britain	Netherlands	2015
IRTA-CIT-034	Porridge	1.5	O	France	France	2015
024/16/01/LHŻ/F	Wheat flakes	<LOQ[(b)]	NO	Poland	Poland	2016
127/16/07/LHŻ/F	Rye bran	<LOQ[(b)]	O	Poland	Poland	2016
RIK200398311	Flour mix (like wheat/rye/barley/oats and other)	<LOQ[(b)]	NO	Netherlands	Netherlands	2016
RIK200413244	Spelt flour, light	<LOQ[(b)]	O	Belgium	Netherlands	2016
UCSC-306	Flour mix (like wheat/rye/barley/oats and other)	1.2	NO	Italy	Italy	2016
IRTA-CIT-004	Wheat flour, white	<LOQ[(b)]	NO	Unknown	France	2015
IRTA-CIT-006	Flour mix (like wheat/rye/barley/oats and other)	1.7	NO	France	France	2015
IRTA-CIT-202	Wheat flour	<LOQ[(b)]	NO	Unknown	Spain	2016
IRTA-CIT-204	Wheat flour	1.1	NO	Unknown	Spain	2016
068/16/06/LHŻ/F	Wheat flour, white	<LOQ[(b)]	O	Poland	Poland	2016
068/16/08/LHŻ/F	Rye flour, wholemeal	5.1	NO	Poland	Poland	2016
068/16/11/LHŻ/F	Oat flour	<LOQ[(b)]	NO	Poland	Poland	2016
101/16/04/LHŻ/F	Wheat flour, white	2.0	NO	Lithuania	Lithuania	2016
127/16/02/LHŻ/F	Rye flour, wholemeal	2.0	NO	Poland	Poland	2016
127/16/03/LHŻ/F	Rye flour, wholemeal	<LOQ[(b)]	O	Poland	Poland	2016
127/16/05/LHŻ/F	Rye flour, wholemeal	5.7	O	Poland	Poland	2016
127/16/06/LHŻ/F	Rye flour, wholemeal	1.0	O	Poland	Poland	2016
RIK200416834	Wheat bread and rolls	<LOQ[(b)]	NO	Netherlands	Netherlands	2016
RIK200416836	Wheat bread and rolls	<LOQ[(b)]	NO	Netherlands	Netherlands	2016
IRTA-CIT-301	Bread crumbs	2.3	NO	Unknown	Spain	2016
098/16/01/LHŻ/F	Multigrain rolls	1.5	NO	Lithuania	Lithuania	2016
140/16/09/LHŻ/F	Rye bread, wholemeal	<LOQ[(b)]	O	Poland	Poland	2016
140/16/19/LHŻ/F	Rye-wheat bread, wholemeal	<LOQ[(b)]	O	Poland	Poland	2016
IRTA-CIT-306	Penne gluten free	<LOQ[(b)]	NO	Unknown	Spain	2016
024/16/10/LHŻ/F	Penne whole grain	<LOQ[(b)]	NO	Poland	Poland	2016
024/16/16/LHŻ/F	Pasta without eggs	<LOQ[(b)]	NO	Poland	Poland	2016
100/16/02/LHŻ/F	Fusilli	<LOQ[(b)]	NO	Lithuania	Lithuania	2016

CODE	Product description (EFSA coding)	µg/kg[(a)]	O/ NO	Country of origin	Country of sampling	Year of sampling
100/16/04/LHŻ/F	Corneti regatta	1.2	NO	Lithuania	Lithuania	2016
100/16/06/LHŻ/F	Fusilli	1.4	NO	Lithuania	Lithuania	2016

O/NO: Organic/Non-organic.
(a): Concentration values are corrected for recovery.
(b): Trace level between limit of detection (LOD) and limit of quantification (LOQ).

3.5.3. Red yeast rice food supplement samples

Red yeast rice (RYR) was clearly identified in this survey as the product with the highest incidence of citrinin contamination. Details on the contaminated samples are provided in Table 18. Citrinin was quantified in twenty-four RYR food supplements with concentrations ranging from 10 to 3,597 µg/kg and detected in thirteen samples below the quantification limit of 10 µg/kg. Due to confidentiality issues, the identification of the samples in Table 18 is omitted. The levels of citrinin were in accordance with finding in other publications (Liao et al., 2014; Kiebooms et al., 2016). Although it was labelled that the origin of the samples was Europe, it is not clear whether the raw material came originally from Europe or it was processed here.

As observed, three of the samples were above the legal limit of 2,000 µg/kg in the EU (EU, 2014a). Levels were confirmed by triplicate analysis in another laboratory. Literature does recall several cases of citrinin contaminated commercially available capsulated RYR, with concentrations ranging from 2.2 up to 114.2 µg of citrinin/capsule (Gordon et al., 2010).

Table 18: Red yeast rice samples contaminated with citrinin

Product description (EFSA coding)	µg/kg[(a)]	O/NO	Year of sampling
Yeast based formulations	864	NO	2015
Yeast based formulations	70	NO	2015
Yeast based formulations	615	NO	2015
Yeast based formulations	1,975	NO	2015
Yeast based formulations	52	NO	2015
Yeast based formulations	2,926	NO	2015
Yeast based formulations	25	NO	2015
Yeast based formulations	<LOQ[(b)]	NO	2016
Yeast based formulations	19	NO	2016
Yeast based formulations	234	NO	2016
Yeast based formulations	<LOQ[(b)]	NO	2016
Yeast based formulations	664	NO	2016
Yeast based formulations	250	NO	2016
Yeast based formulations	10	NO	2016
Yeast based formulations	<LOQ[(b)]	NO	2016
Yeast based formulations	3,597	NO	2016
Yeast based formulations	<LOQ[(b)]	NO	2016
Yeast based formulations	<LOQ	NO	2016
Yeast based formulations	802	NO	2016
Yeast based formulations	2,589	NO	2016
Yeast based formulations	11	O	2016
Yeast based formulations	<LOQ[(b)]	NO	2016
Yeast based formulations	<LOQ[(b)]	NO	2016
Yeast based formulations	<LOQ[(b)]	NO	2016
Yeast based formulations	22	NO	2016

Product description (EFSA coding)	µg/kg[(a)]	O/NO	Year of sampling
Yeast based formulations	27	NO	2016
Yeast based formulations	<LOQ[(b)]	NO	2016
Yeast based formulations	36	NO	2016
Yeast based formulations	24	NO	2016
Yeast based formulations	13	NO	2016
Yeast based formulations	<LOQ[(b)]	NO	2016
Yeast based formulations	<LOQ[(b)]	NO	2016
Yeast based formulations	21	NO	2016
Yeast based formulations	15	NO	2016
Yeast based formulations	<LOQ[(b)]	NO	2016
Yeast based formulations	14	NO	2016
Yeast based formulations	<LOQ[(b)]	NO	2016

O/NO: Organic/Non-organic.
(a): Concentration values are corrected for recovery.
(b): Trace level between limit of detection (LOD) and limit of quantification (LOQ).

4. Conclusions

- LC-MS/MS-based methods for the determination of citrinin in various food matrices were successfully validated at levels down to 1.0 µg/kg and down to 10 µg/kg for RYR food supplements.
- The methods enabled highly sensitive detection of citrinin in food with LODs in the range of 0.2-0.5 µg/kg for cereals, cereal-based products, juices and beans and in the range of 2.0-5.0 µg/kg for RYR.
- Sampling was successfully completed. The survey was comprised of 1195 samples, 9% more than planned.
- Red yeast rice food supplements were the most prone samples to contamination with citrinin, being detected in 40% of the samples. The EU legal limit of 2,000 µg/kg was exceeded in three samples.
- Citrinin was detected in 8% (thirty-one out of 390) of the industry cereal samples and quantified above 1 µg/kg in 6% (twenty-two samples). It was detected in wheat, barley and rye. The incidence of citrinin in rye grain originated from Poland was higher than that in the other sampling countries.
- Citrinin occurred in cereal-based products from retail in 7% (thirty-seven out of 510) of the samples. Concentration levels for the fifteen quantified samples ranged from 1.0 to 5.0 µg/kg. Flour was the food category with the highest incidence.
- No citrinin was detected in fruit or vegetable juices or in beans.

5. References

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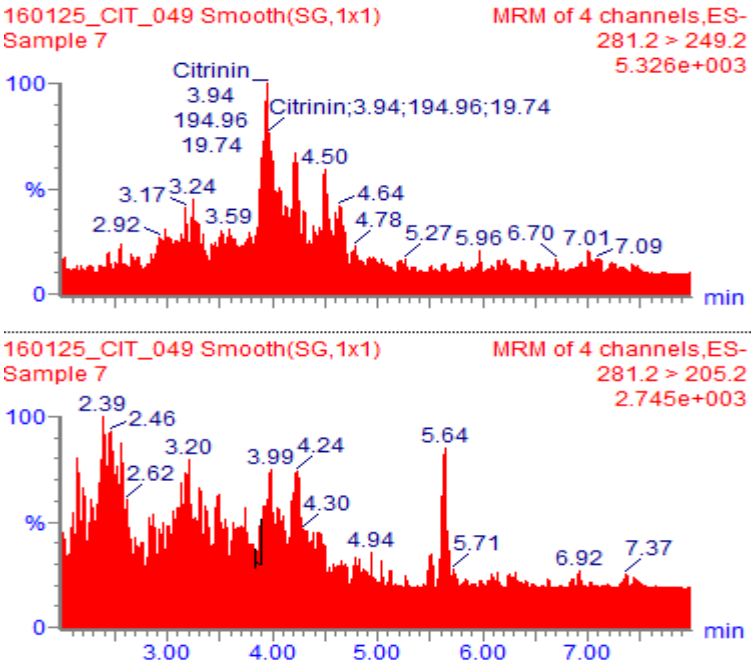
Abbreviations

CIT	Citrinin
EFSA	European Food Safety Authority
FLD	Fluorescence detector
(U)HPLC	(Ultra)High pressure liquid chromatography
IRTA	Institut de Recerca i Tecnologia Agroalimentàries
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
NIPH-NIH	National Institute of Public Health – National Institute of Hygiene
NVWA	Netherlands Food and Consumer Product Safety Authority
PBS	Phosphate-buffered saline
PDA	Protected Designation Area
PP	polypropylene
PTFE	polytetrafluoroethylene
QC	Quality Control
RIKILT	RIKILT Wageningen University & Research
RYR	Red yeast rice
UCSC	Università Cattolica del Sacro Cuore
WHO	World Health Organization

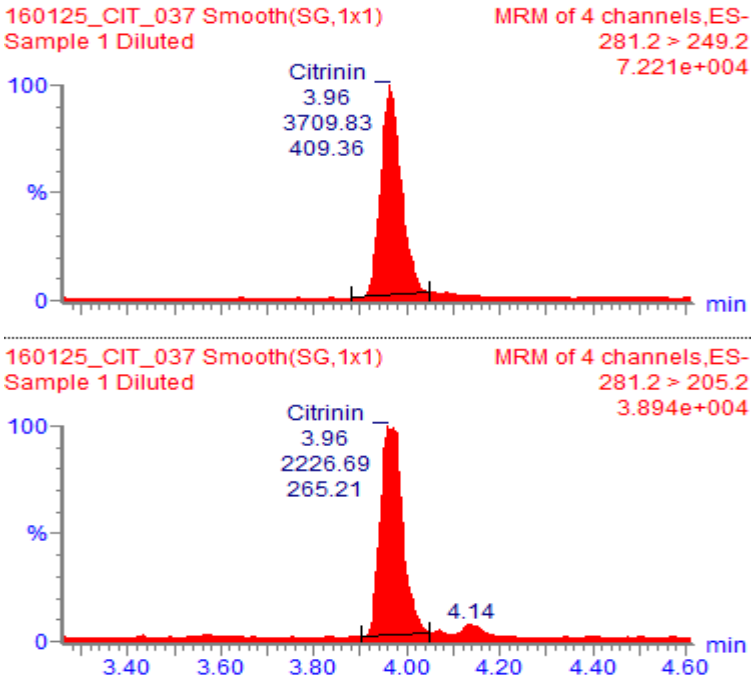
Appendix A – Chromatograms of positive samples

Red yeast rice

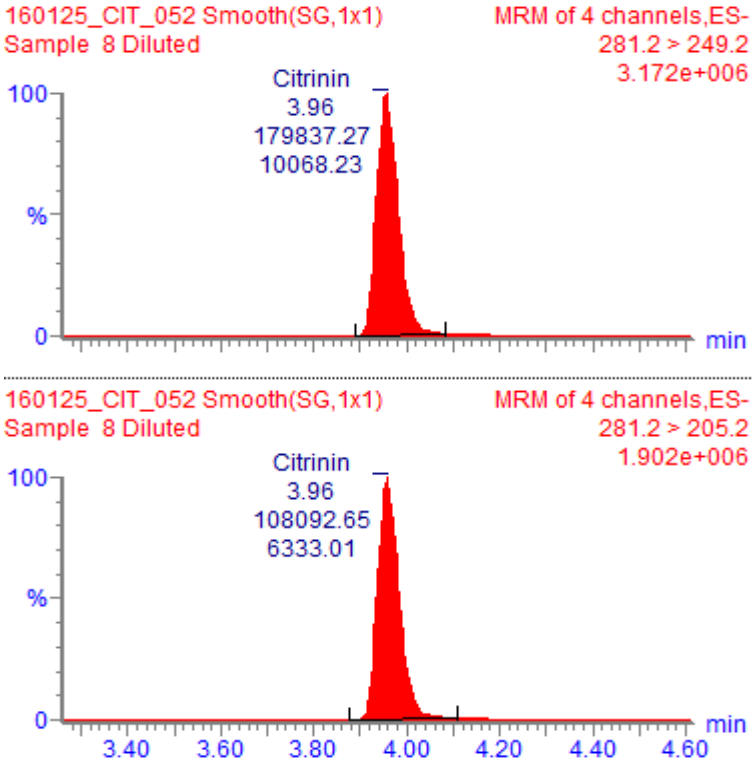
Citrinin <LOD



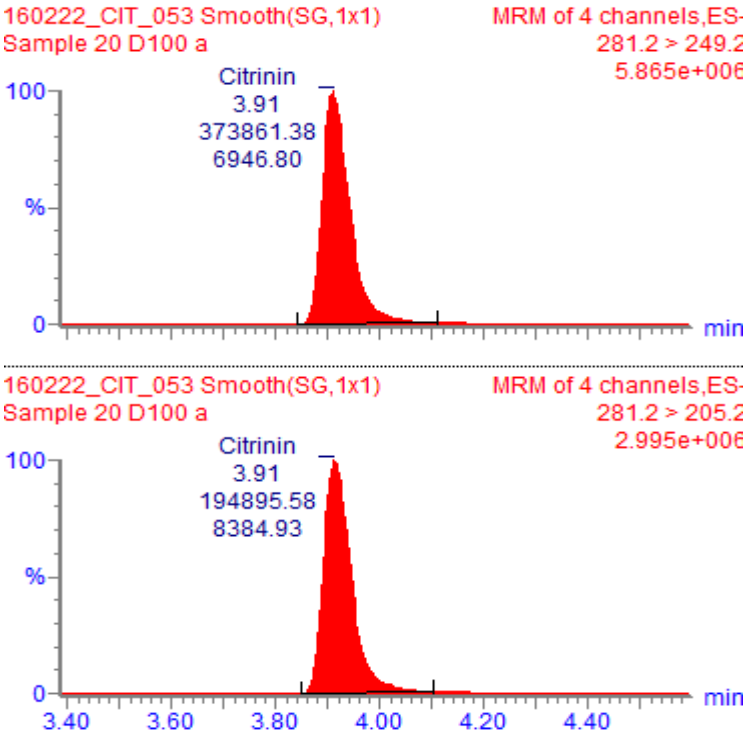
Citrinin 15.4 μg/kg



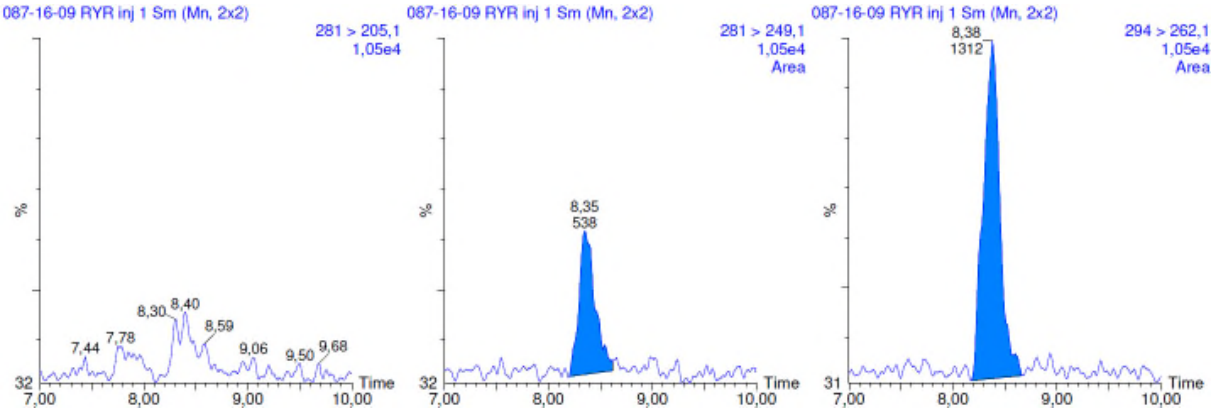
Citrinin 482 μg/kg



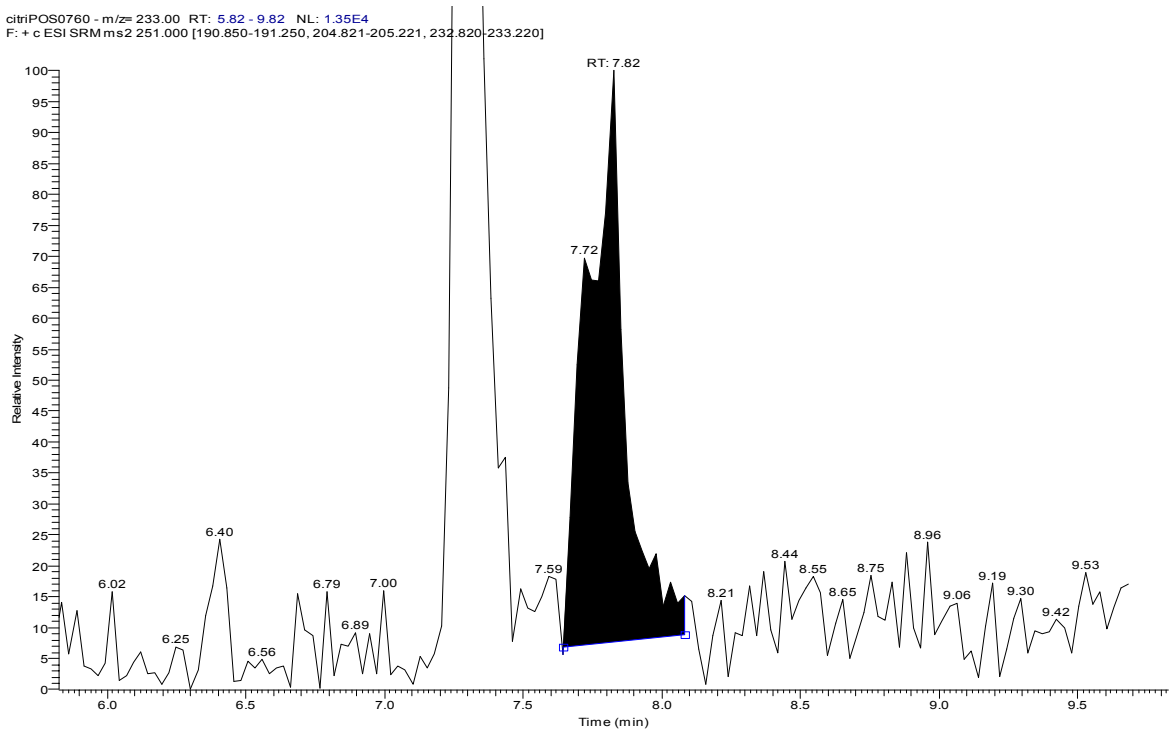
Citrinin 2,926μg/kg



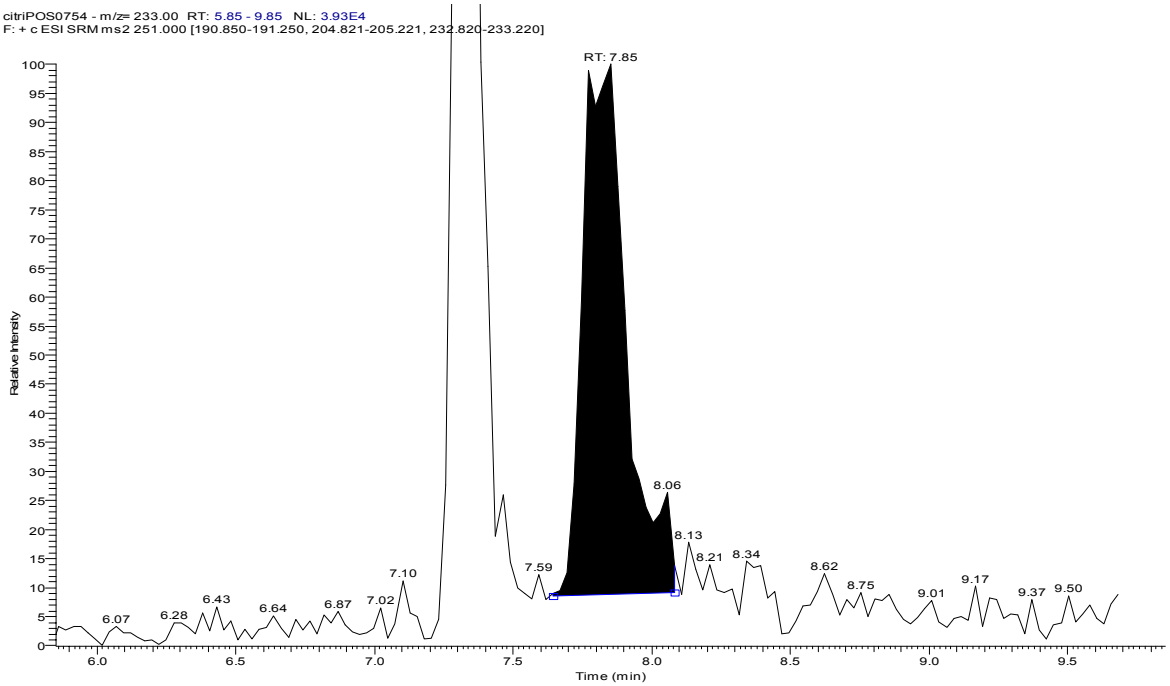
Citrinin 21.4 µg/kg



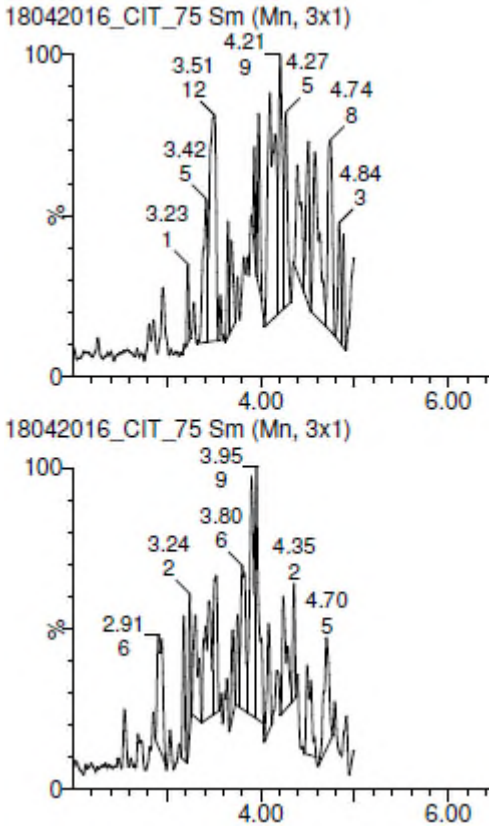
Citrinin <LOQ (6.2 µg/kg)



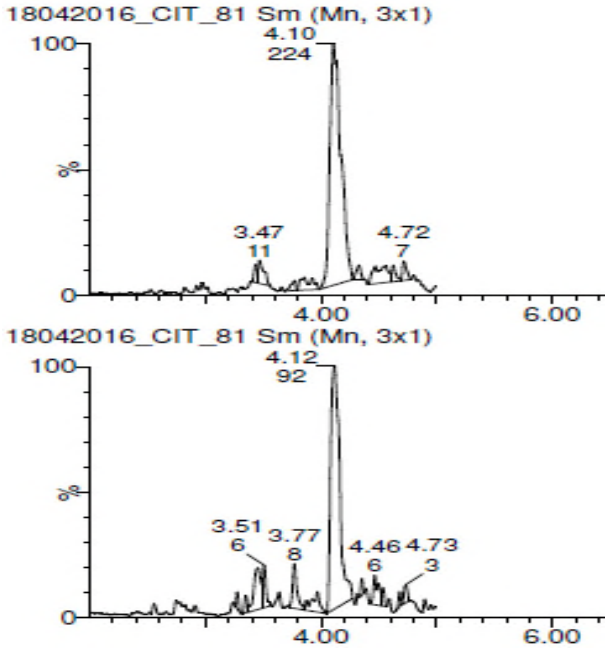
Citrinin 25.5 μg/kg



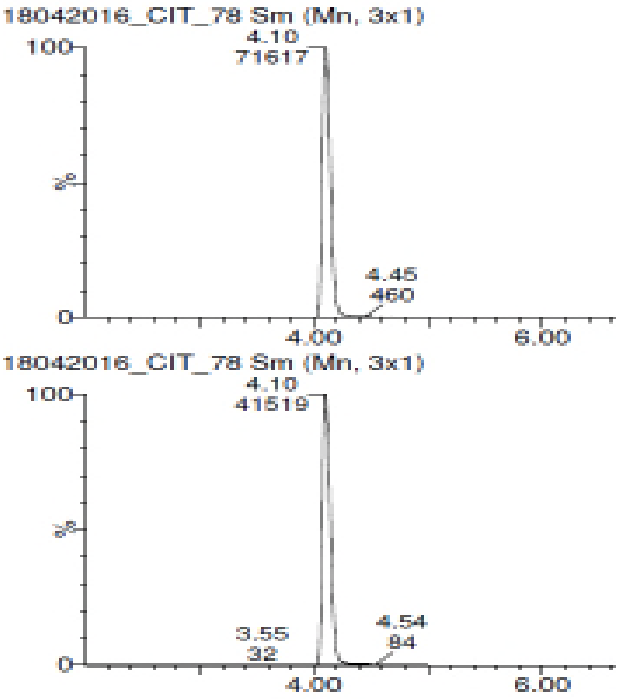
Citrinin <LOD



Citrinin <LOQ (5.3 μg/kg)

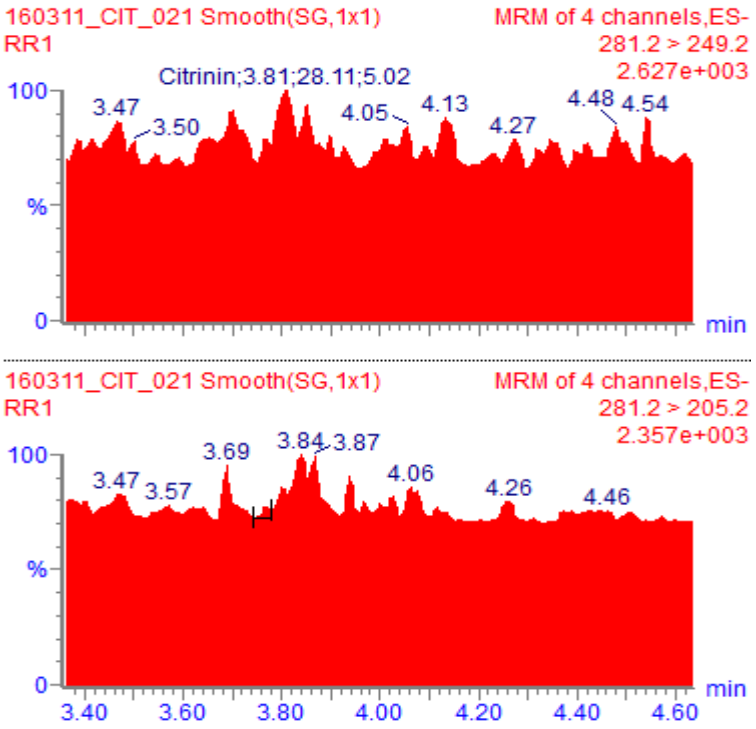


Citrinin 2,306 μg/kg

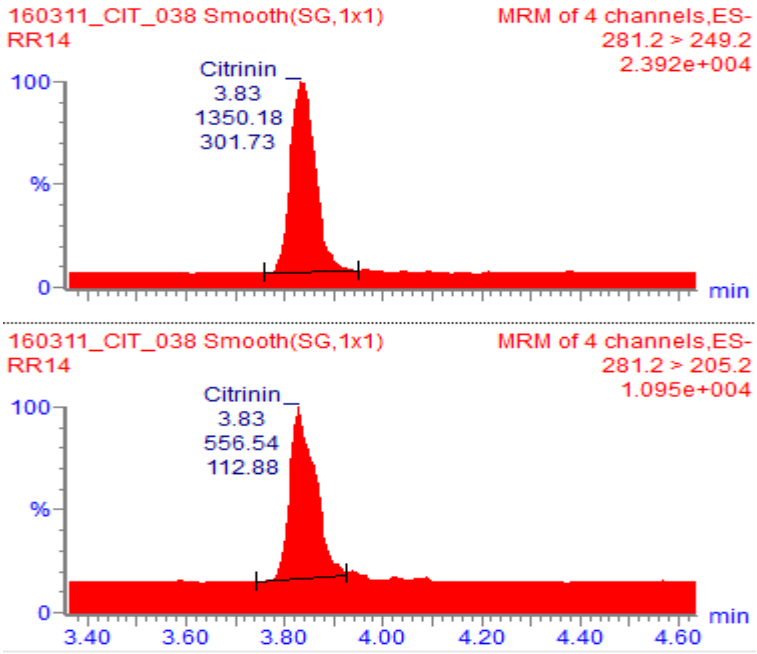


Rice retail

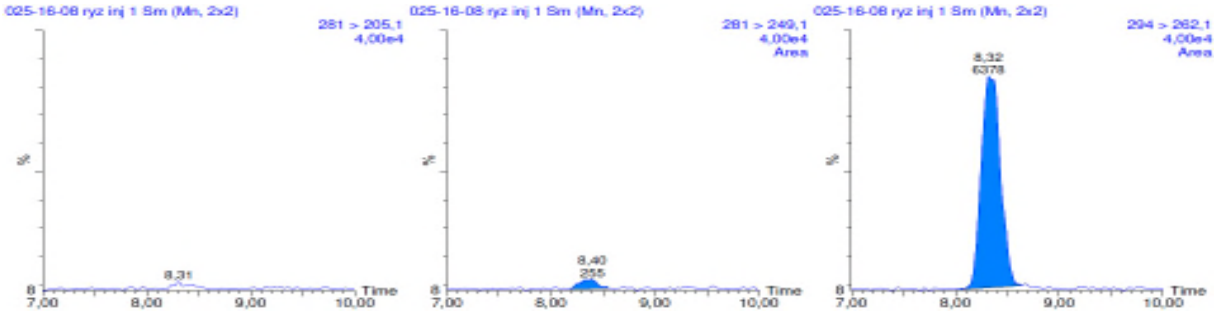
RIK200398274: Citrinin <LOD



RIK200398287: Citrinin <LOQ (0.92 μg/kg)

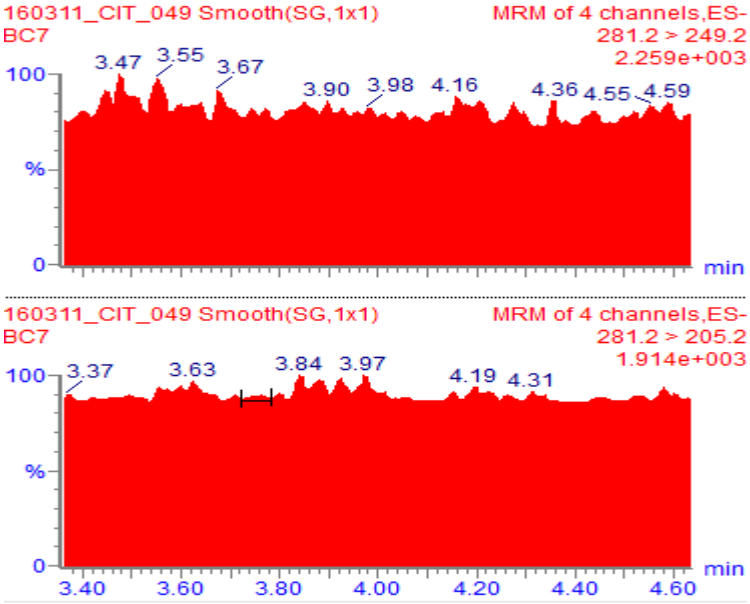


025/16/08/LHŻ/F Rice basmati <LOQ (0.45 µg/kg)

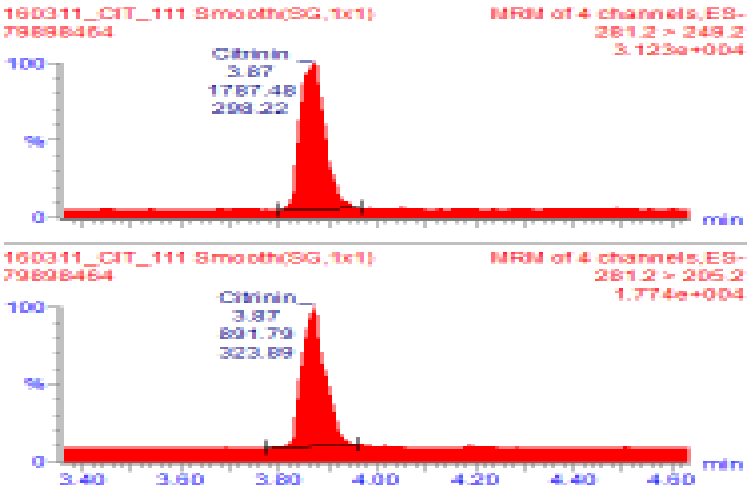


Breakfast cereals

RIK200398294: Citrinin <LOD

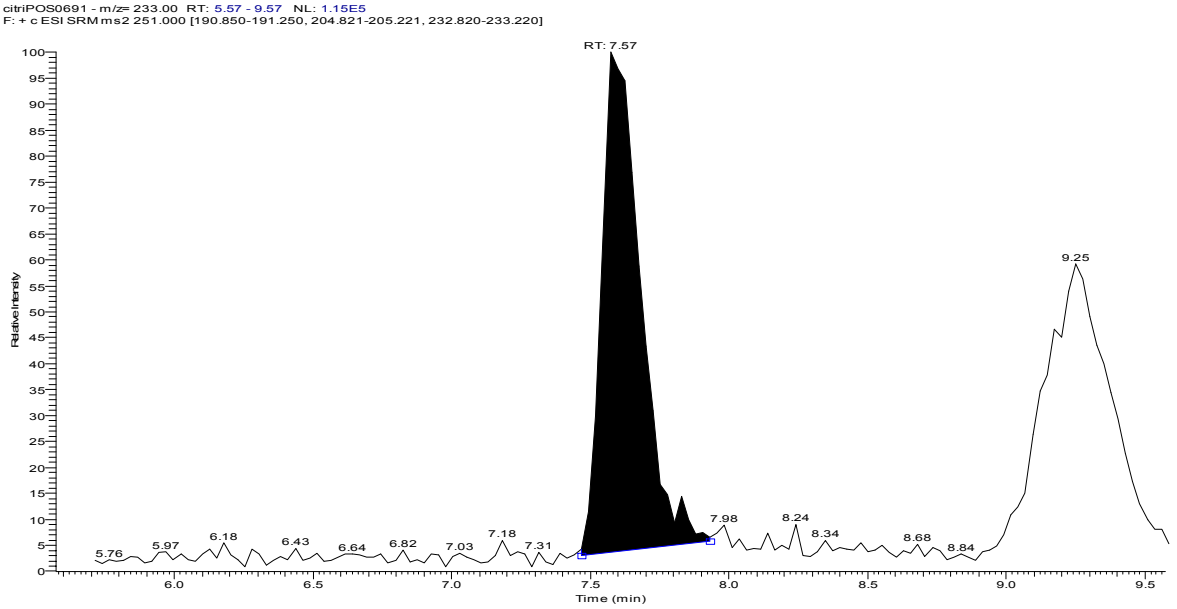


RIK200401069: Citrinin 2.2 µg/kg



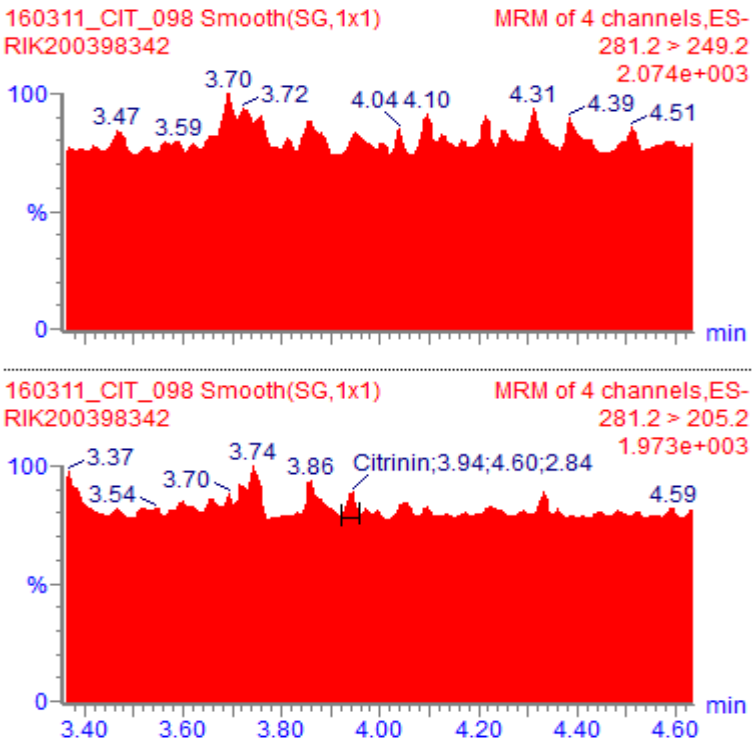
Rice crop

UCSC: Citrinin 6.9 μg/kg

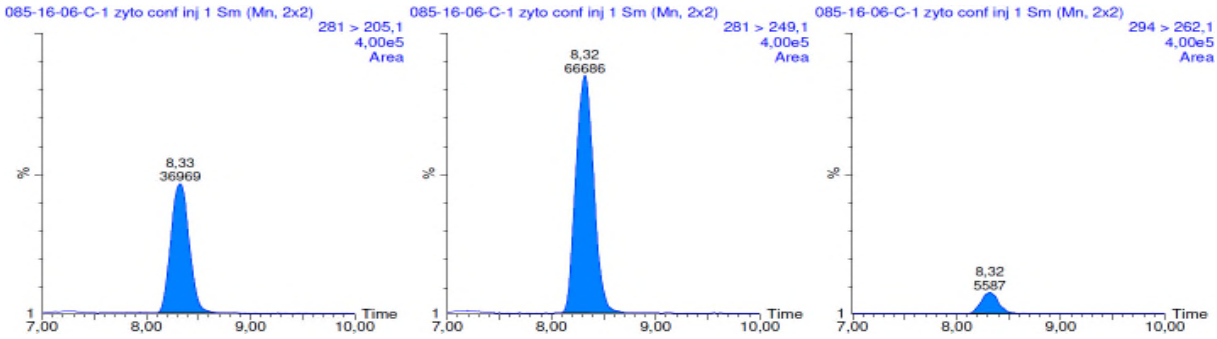


Rye grain

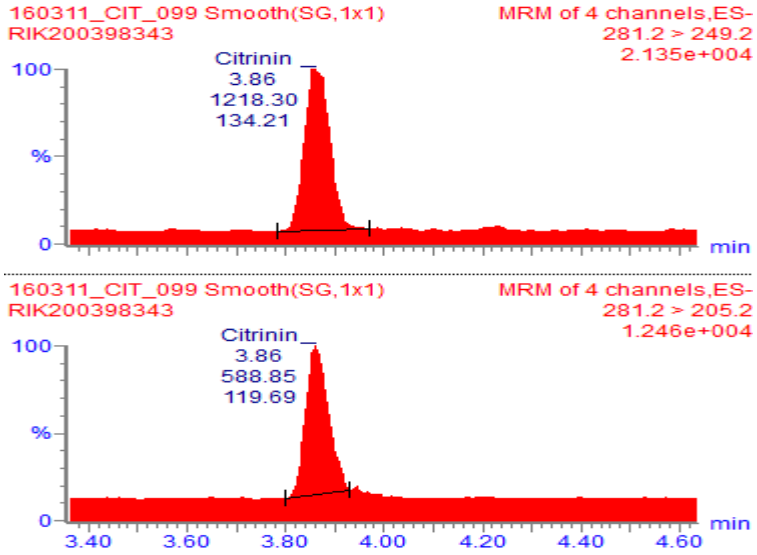
RIK200398342: Citrinin <LOD



085/16/06/LHŻ/F Rye grain 155.3 µg/kg

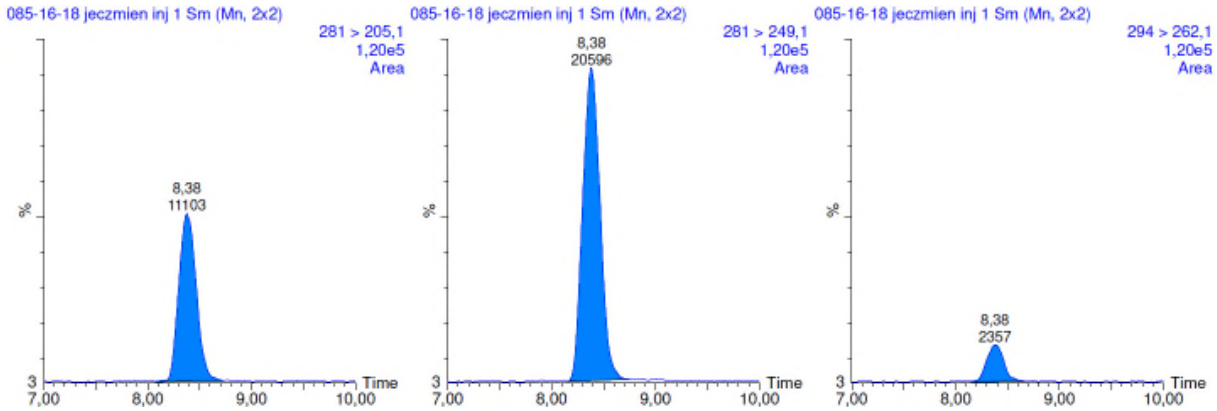


RIK200398343: Citrinin 1.2 µg/kg



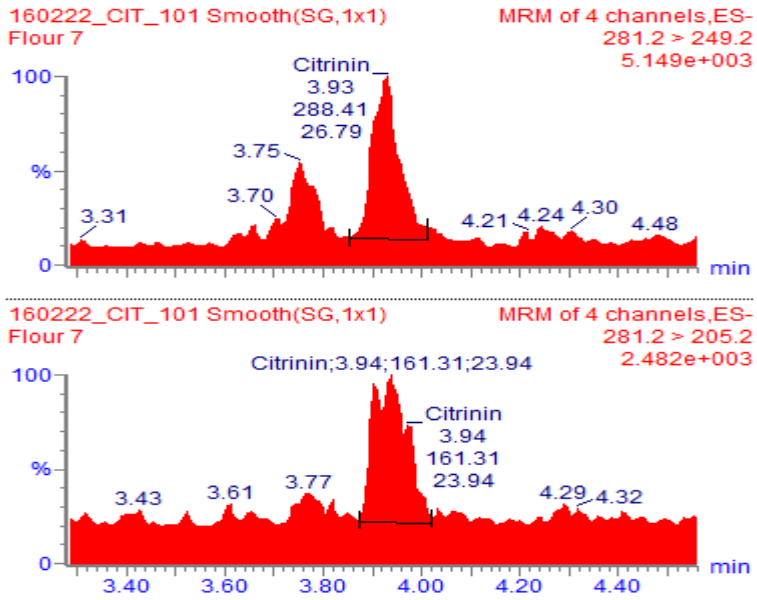
Barley grain

085/16/18/LHŻ/F Barley grain 66.2 µg/kg

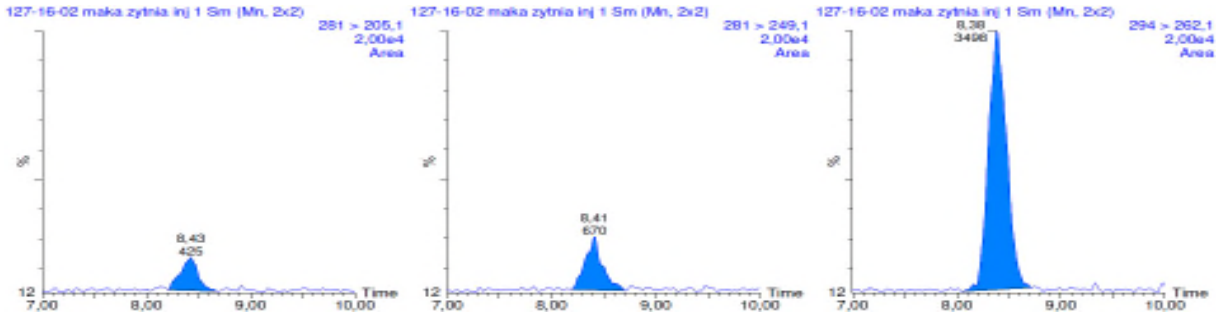


Flour

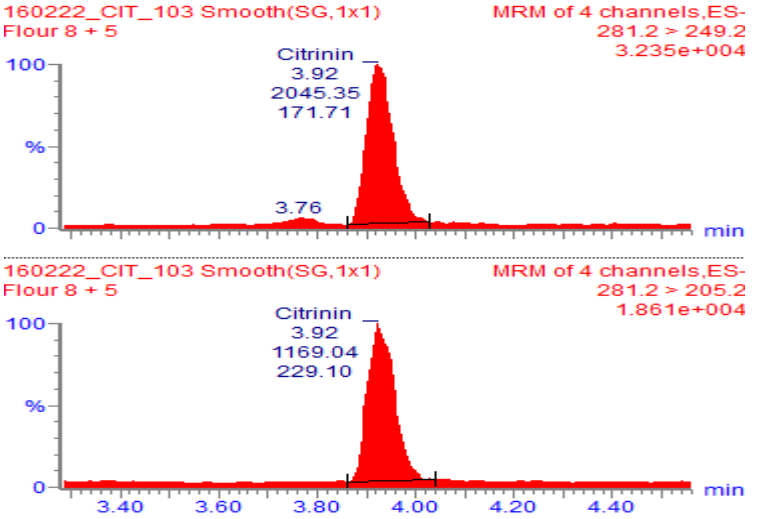
RIK200398310: Citrinin <LOD



127/16/02/LHŻ/F Wholemeal rye flour, type 2000; 2.0 µg/kg



RIK200398311: Citrinin <LOQ (0.5 µg/kg)



Pasta samples

100/16/04/LHŻ/F Pasta cornetti rigati, with eggs 1.2 µg/kg