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VIEW DATASET

Survey of T-2 and HT-2 toxins by LC–MS/MS in oats and oat products from European oat mills in 2005–2009

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T-2 and HT-2 toxins were analysed in oats ($n = 243$), oat flakes ($n = 529$), oat meal ($n = 105$) and oat by-products ($n = 209$) from 11 European mills during 2005–2009 by high-performance liquid chromatography with a triple quadrupole mass spectrometer. Limits of quantification were 5 $\mu g\,kg^{-1}$ for both T-2 and HT-2 toxins in oats. The incidence of T-2 + HT-2 ($> 5\,\mu g\,kg^{-1}$) in oats, oat flakes, oat meal and oat by-products was 93, 77, 34 and 99%, respectively. The mean values of T-2 + HT-2 were 94, 17, 11 and 293 $\mu g\,kg^{-1}$ for oats, oat flakes, oat meal and oat by-products, respectively. T-2 and HT-2 occurred together and the T-2 level was 52% of HT-2 in oats. Maximal T-2 and HT-2 concentration in oat flakes and oat meal were 197 and 118 $\mu g\,kg^{-1}$. The toxins were reduced by 82–88% during processing, but increased 3.1 times in oat by-products.

Keywords: cereals; cereals and grain; mycotoxins, trichothecenes, fusarium toxins; oats; T-2 toxin; HT-2 toxin; milling

Introduction

Fusarium fungi infest small-grain cereal crops worldwide, causing a destructive disease of cereal spikes and heads known as Fusarium head blight, also called scab, Fusarium ear blight or head fusarioses. In contrast to wheat and barley, this disease is rarely observed in a standing oat crop (Liu et al. 1997; Tekauz et al. 2004). Several different *Fusarium* species attack oats, reduce crop yield and also produce toxins in the grains. Different trichothecene mycotoxins are produced by the *Fusarium* fungi. Deoxynivalenol is the most commonly found trichothecene and is present at the highest concentrations in wheat and wheat products. T-2 and HT-2 toxins are more commonly found in oats (Langseth and Rundberget 1999) and have recently been detected at high concentrations in oats harvested in Europe (Edwards 2009; Edwards et al. 2009). The toxins are mainly produced by a newly described *Fusarium* species, *Fusarium langsethiae,* first isolated from Norwegian oats with high T-2 and HT-2 toxin concentrations (Torp and Langseth 1999; Torp and Nirenberg 2004). The correlation between *F. langsethiae* and the toxins in oats was later shown in other studies from UK and Sweden using quantitative PCR detection (Imathiu 2008; Fredlund et al. 2010).

There have been some surveys of T-2 and HT-2 toxins in oats harvested in Germany, Austria, Norway, Finland and Sweden in certain years during the period 1987–2001 (Hietaniemi and Kumpulainen 1991; Müller et al. 1998; Langseth and Rundberget 1999; Canady et al. 2001; SCOOP 2003; Hietaniemi et al. 2004; Schollenberger et al. 2006). Incidences of the toxins were below 30% during most years, but there was a higher occurrence in Germany in 1990 and in Norway in 1996, 1999 and 2000. In most surveys, the mean levels for the sum of the toxins were below 100 $\mu g\,kg^{-1}$, even during years with higher occurrence. A few samples with very high ($>1000\,\mu g\,kg^{-1}$) toxin levels were found in some years, which increased the mean levels. Surveys of T-2 and HT-2 toxins have also been conducted over several years during the period 2000–2009 in oats harvested in UK, Finland, Norway and Sweden (Edwards 2009; Edwards et al. 2009; Pettersson 2010). Incidences of the toxins seem to have increased and were between 50 and 94% in most years. High occurrence and high levels ($> 200\,\mu g\,kg^{-1}$) were found in most years and countries during the period 2002–2007. Some samples from UK were also found to contain very high toxin levels (6260–9990 $\mu g\,kg^{-1}$) during that period.

Oat products, mainly oat flakes, from the German market were analysed for T-2 and HT-2 toxins during 2005–2007 (Gottschalk et al. 2007, 2009; Usleber 2008). The toxin incidences were very high

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(98–100%), mainly due to the low detection limits for the methods used. The toxin levels in the analysed oat flakes were generally low (median 7–14 $\mu g\,kg^{-1}$).

Processing of oats reduce the T-2 and HT-2 toxin concentrations in oat products for human consumption. In the first stage, cleaning and screening of the oat grains reduce the toxin level, but the highest reduction is obtained during de-hulling (Pettersson et al. 2008; Edwards et al. 2009). In a study of industrial processing, the reduction was consistently above 90% (Scudamore et al. 2007). On the other hand, toxin concentrations increase 3–4-fold in by-products, which are mostly used in feed.

T-2 and HT-2 toxins are two of the most toxic trichothecenes. They are potent inhibitors of protein synthesis, highly cytotoxic, causing skin and mucosa erosions and reduction of lymphocytes, immune defence and growth in exposed animals.

The Scientific Committee for Food (SCF) of the European Commission and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) have evaluated the risk with T-2 and HT-2 toxins in food cereals (Canady et al. 2001; SCF 2001). They came to the same conclusions and established a provisional maximum tolerable daily intake (PMTDI) of 0.06 $\mu g\,kg^{-1}\,bw\,day^{-1}$ for the sum of T-2 and HT-2 toxins. EFSA has recently been asked by the European Commission to provide a scientific opinion on the risks for animal and public health related to the presence of T-2 and HT-2 toxins in food and feed, considering any new results of toxicological studies, deliver an updated dietary exposure assessment, and determine the daily exposure levels of the different animal species.

JECFA in 2001 and SCOOP in 2003 accumulated and assessed the occurrence and intake data for trichothecenes. For T-2 and HT-2, the data indicated that the PMTDI may be exceeded in many cases and that a high portion may have come from consumed oats. The calculations were based on limited occurrence database of the toxins and the concentrations had, in many cases, been determined with methods using a high limit of quantification (LOQ).

The European Commission introduced in 2005 maximum permitted levels for the *Fusarium* toxins deoxynivalenol, zearalenone and fumonisins in cereals and cereal products intended for human consumption (EC 2005). T-2 and HT-2 toxins were also included, but the level was not regulated. More occurrence data for the toxins in, particularly, oats and oat product was requested before a level could be fixed. In response, CEEREAL, the European Breakfast Cereal Association, initiated and planned this survey of T-2 and HT-2 toxins in oats and oat products from European mills. This survey of the toxins is the first and most extensive in oats and oat products sampled directly at European mills and interim reports have been given to the European Commission at their yearly *Fusarium* toxin Forum.

Materials and methods

Chemicals

Water for HPLC was produced by a Barnstedt/Thermolyne E-pure system (Braunschweig, Germany), Acetonitril (gradient grade) and methanol (gradient grade) were provided by Sigma-Aldrich (Seelze, Germany). Standard toxins were obtained in acetonitrile solutions from Biopure (Tulln, Austria): T-2 toxin 100 $\mu g\,ml^{-1}$ (Biopure BRM S02035), HT-2 toxin 100 $\mu g\,ml^{-1}$ (Biopure BRM S02036) and internal standard ^{13}C-labelled T-2 toxin 25 $\mu g\,ml^{-1}$ (Biopure BRM 002044). Reference oat meal material, FAPAS T2234, was obtained from FAPAS (CSL, York, UK).

Samples, sampling and sample preparation

Samples were taken from raw oats delivered to European oat mills during 2005–2009, and oat flakes, oat meal and oat by-products produced at the mills during the same period. Eleven oat mills from UK (6), Germany (2), Finland (1), Poland (1) and Ireland (1), represented by CEEREAL, participated in the survey. The materials were sampled at the individual mills according to the Directive EC 401/2006 (EC 2006). The whole aggregate samples of 1–6 kg, depending on sampled lot size, were sent to the analytical laboratory (Gesellschaft für Bioanalytik Hamburg GmbH, Germany). A total of 243 raw oat samples, 529 oat flakes, 105 oat meal and 209 oat by-products were taken for analysis.

A 25 g test portion of the ground sample was extracted with 100 ml acetonitrile/water (84 : 16, v/v) by shaking for 30 min. Then, 8 ml of the filtered extract was used for clean-up with a solid-phase extraction column (MultiSep 227 Trich+; Romer Labs, Tulln, Austria). A 4-ml aliquot of the eluate was evaporated under nitrogen gas to dryness and re-dissolved in 400 µl methanol/water (30 : 70, v/v) containing ^{13}C-labelled T-2 toxin as internal standard.

Method of analysis

LC–MS/MS analysis was performed with an Agilent HPLC system Series 1200 (Agilent, Waldbronn, Germany) and a triple-quadrupole mass spectrometer API 3200 Q Trap from AB Sciex (Foster City, CA, USA). The mass spectrometer was used in an electrospray ionization mode (ESI). The acquisition was done in multiple reaction monitoring (MRM) mode and determination of transition ions with a dwell time of 50 ms. LC separation was carried out on a Nucleodur Sphinx RP column (50 × 2 mm I.D., 3 µm) from

Table 1. MS/MS parameters for detection of trichothecenes in MRM mode.

Analyte	Precursor ion (m/z)	Product ion (m/z)	Declustering potential (V)	Collision energy (eV)	Ion ratio (Q/q)
T-2	489.2 $[M+Na]^+$	245.1 (Q)		37	
	489.2 $[M+Na]^+$	387.3 (q)	96	27	0.60
	489.2 $[M+Na]^+$	327.1 (q)		29	0.34
HT-2	446.9 $[M+Na]^+$	345.2 (Q)		23	
	446.9 $[M+Na]^+$	285.1 (q)	71	29	0.67
	446.9 $[M+Na]^+$	159.0 (q)		37	0.03
^{13}C-T-2	513.2 $[M+Na]^+$	406.2 (Q)		29	
	513.2 $[M+Na]^+$	260.2 (q)	61	31	0.75
	513.2 $[M+Na]^+$	138.2 (q)		99	0.24

Note: Q: transition used for quantification (quantifier); q: transition used for confirmation (qualifier).

Macherey-Nagel (Düren, Germany). A linear water/methanol gradient was applied at a flow rate of $0.25\,ml\,min^{-1}$ and an oven temperature of 25°C. The injection volume was 50 µl.

Measurements were carried out using the LC–MS/MS conditions presented in Table 1. Accepted tolerance for identification by qualifier/quantifier ratio was dependent on the ratio value, i.e. for ratio $\leq 0.1 = 50\%$, ratio 0.1–0.2 = 30%, ratio 0.2–0.5 = 25% and ratio $> 0.5 = 20\%$.

T-2 and HT-2 toxin-free oat meal or oat flakes were used for the matrix-assisted calibration with a linear range between 5 and $400\,ng\,ml^{-1}$ injection solution. The linear correlation was 0.999 or better. An oat reference material from FAPAS was used as control sample in the analysis.

All analyses in the survey were performed at the laboratory of Gesellschaft für Bioanalytik Hamburg GmbH (Germany).

Results and discussion

Method validation and quality assurance

The method has been validated for the analysis of T-2 and HT-2 toxins in both whole oat meal and oat flakes. Matrix-matched calibrations with T-2 and HT-2 toxin-free whole oat meal and oat flakes were used. The linear range was 5–400 and 2–500 $ng\,ml^{-1}$ for oat meal and oat flakes, respectively, with a correlation of 0.999 or better. Limit of quantification was $5.0\,\mu g\,kg^{-1}$ for T-2 toxin/HT-2 toxin/^{13}C T-2 toxin for oat meal and, in oat flakes, $3.8\,\mu g\,kg^{-1}$ for T-2 toxin and ^{13}C T-2 toxin, and $3.2\,\mu g\,kg^{-1}$ for HT-2 toxin. Limit of detection was $0.5\,\mu g\,kg^{-1}$ for T-2 toxin, HT-2 toxin and ^{13}C T-2 toxin in oat meal and, in oat flakes, $0.38\,\mu g\,kg^{-1}$ for T-2 toxin and ^{13}C T-2 toxin, and $0.32\,\mu g\,kg^{-1}$ for HT-2 toxin. Recovery of T-2 toxin in oat meal was in the range 81–105% and, in oat flakes, 80–95%; recovery of HT-2 toxin in oat meal was in the range 80–95% and, in oat flakes, 75–86%. The relative repeatability standard deviation (RSD_r) determined in oat meal at $300\,\mu g\,kg^{-1}$ was 8.9% for T-2 toxin and 5.2% for HT-2 toxin. The RSD_r determined in oat flakes at $25\,\mu g\,kg^{-1}$ was 18% for T-2 toxin and 23% for HT-2 toxin.

A reference material of oat meal from FAPAS was used as control sample. The assigned value for T-2 toxin and HT-2 toxin was 83 and $113\,\mu g\,kg^{-1}$, respectively. Analysed values were in the range 69–88 $\mu g\,kg^{-1}$ for T-2 toxin and 94–118 $\mu g\,kg^{-1}$ for HT-2 toxin.

The laboratory has participated in three proficiency tests on T-2 and HT-2 toxins in oat meal organised by FAPAS. The *Z*-scores in FAPAS 2243 for T-2 toxin were −0.4, FAPAS 2252 for T-2 toxin −0.5 and HT-2 toxin −1.1 and FAPAS 2261 for T-2 toxin −0.1 and HT-2 toxin −2.0.

The laboratory and the method are accredited according to ISO 17025.

Analytical results in the survey were not corrected for recovery, but matrix effects during the measuring with LC–MS/MS like ion-suppression have been considered by the addition of ^{13}C-labelled T-2 toxin before measuring. Samples with a concentration below the LOQ were assigned a concentration equal to half the LOQ in the calculations.

Occurrence study

The occurrence and levels of T-2 and HT-2 toxins in oats, oat flakes, oat meal and oat by-products from European oat mills in 2005–2009 are summarized in Table 2. T-2 and HT-2 toxins were found together in most contaminated samples and the incidence of the toxins (as $>5\,\mu g\,kg^{-1}$) in oats, oat flakes, oat meal and oat by-products were 93, 77, 34 and 99%, respectively. The concentration of HT-2 toxin was normally highest and the mean concentration of T-2 in raw oats was 52% of HT-2 or 1.9, if calculated as HT-2/T-2 ratio. The ratio in oat flakes was slightly higher at 2.4 and lower in oat meal and oat by-products at 1.8 and 1.7,

Table 2. Occurrence of T-2 and HT-2 toxins in oats, oat flakes, oat meal and oat by-products from European oat mills in 2005–2009.

Toxin product	Number of samples	Number of samples in the range ($\mu g\,kg^{-1}$)						Mean ($\mu g\,kg^{-1}$)	Median ($\mu g\,kg^{-1}$)	90th %ile ($\mu g\,kg^{-1}$)	Max ($\mu g\,kg^{-1}$)
		<5	5–50	50–200	200–500	500–750	>750				
T-2 toxin											
Oats raw	243	65	128	47	3	0	0	32	13	78	269
Oat flakes	529	402	127	0	0	0	0	5	3	10	38
Oat meal	105	87	18	0	0	0	0	4	3	8	34
Oat by-product	209	4	60	86	19	8	0	110	53	270	643
HT-2 toxin											
Oats raw	243	22	124	92	9	3	0	62	27	137	572
Oat flakes	529	167	349	13	0	0	0	12	8	26	159
Oat meal	105	69	35	1	0	0	0	7	3	21	84
Oat by-product	209	3	66	78	45	12	7	183	101	446	1068
T-2 + HT-2 toxin											
Oats raw	243	16	113	86	23	3	2	94	38	210	841
Oat flakes	529	122	377	30	0	0	0	17	12	41	197
Oat meal	105	69	35	1	0	0	0	11	5	28	118
Oat by-product	209	3	28	82	64	12	20	293	152	662	1711

respectively. This co-occurrence of T-2 and HT-2 toxins in oats has been reported in several other studies but a higher ratio (8.7–2.4) has often been found (Gottschalk et al. 2007, 2009; Scudamore et al. 2007; Edwards 2009; Schwake-Anduschus et al. 2010). A higher ratio may be explained by either under estimation of T-2 toxin or hydrolysis of the toxin in the oat–fungal material or the extract.

The average and median concentrations for the sum of the toxins in raw oats delivered to the mills are lower than those from most of the oat surveys during the same period (Edwards 2009; Pettersson 2010), but the incidence and level are higher than in the early surveys in 1987–2001. The occurrence and levels of T-2 and HT-2 toxins in oat flakes are similar to those found in oat flakes from Germany in 2005–2007 (Gottschalk et al. 2007, 2009; Usleber 2008).

The mean levels of T-2 and HT-2 toxins in oat flakes and oat meals were much lower, 82 and 88%, respectively, than the levels in the raw oats delivered to the mills. The T-2 and HT-2 mean and median concentrations in oat by-products were 3- and 4-fold higher, respectively, compared to levels in all oats delivered to the mills.

The effect of commercial oat processing on T-2 and HT-2 toxin levels in the products has been studied by Scudamore et al. (2007). They found a reduction in HT-2 toxin in oat flakes, on average 95%, compared to the concentration in the original oats. The reduction was higher at high toxin levels in the oat. De-hulling of oats is the main reducing step and can account for 75–98% of the reduction in laboratory trials (Edwards 2007; Pettersson 2010). In the study on commercial oat processing, it was also found that the T-2 + HT-2 concentrations in oat by-products were 4.2–4.6 times higher and, thus, similar to the increase found in this study.

In Table 3, the occurrence and levels of T-2 and HT-2 toxins in raw oats, oat flakes and oat by-products are presented by year of harvest. The highest mean and median concentrations in raw oats were found in 2006; levels were slightly lower in 2007, lowest in 2008 and increased in 2009 to similar levels as in 2007. The same pattern of toxin occurrence has also been seen in surveys of oats in UK, Sweden, Norway and Finland (Edwards et al. 2009; Pettersson 2010). Occurrence, mean and median concentrations of T-2 and HT-2 toxins in oat flakes and oat by-products follow the same yearly fluctuations, but at lower and higher levels, respectively, as raw oats.

The incidence and concentrations of T-2 and HT-2 in raw oats and oat flakes by country of harvest are presented in Table 4. Mean and median concentrations of the toxins in raw oats from Ireland and Sweden are higher, but the numbers of samples are too low to distinguish any difference between countries. In addition, the influence of harvest year may be too high. Edwards (2009) found statistically regional differences within the UK in the 2002–2007 surveys. Surveys in Finland, Sweden, Norway and UK in 2000–2009 showed a similar occurrence by year, but different sampling strategies and analytical methods make it difficult to draw any conclusions on differences in occurrence due to country of harvest. There is a wide variation in the results by harvest and country of origin, indicating that even higher results may be found

Table 3. Occurrence by harvest year of T-2 and HT-2 toxins, as the sum of their concentrations in raw oats, oat flakes and oat by-products from European oat mills in 2005–2009.

Year	Product	Number of samples	Number of samples in the range ($\mu g\,kg^{-1}$)						Mean ($\mu g\,kg^{-1}$)	Median ($\mu g\,kg^{-1}$)	90th %ile ($\mu g\,kg^{-1}$)	Max ($\mu g\,kg^{-1}$)
			<5	5–50	50–200	200–500	500–750	>750				
2006	Oats raw	32	0	3	15	10	2	2	261	189	664	841
2007	Oats raw	27	0	10	12	5	0	0	113	88	269	415
2008	Oats raw	99	16	70	11	2	0	0	27	15	65	283
2009	Oats raw	78	0	30	41	6	1	0	99	83	169	571
2006–2009	Oats raw	243	16	113	86	23	3	2	94	38	210	841
2005	Oat flakes	30	0	19	11	0	0	0	48	46	83	96
2006	Oat flakes	70	3	55	12	0	0	0	25	17	54	74
2006/2007	Oat flakes	57	15	42	0	0	0	0	12	10	22	31
2007	Oat flakes	66	1	59	6	0	0	0	24	16	36	197
2008	Oat flakes	177	67	110	0	0	0	0	11	9	21	42
2009	Oat flakes	99	33	66	0	0	0	0	12	11	20	32
2005–2009	Oat flakes	529	122	377	30	0	0	0	17	12	35	197
2006	Oat by-product	10	0	0	0	1	1	8	1144	1280	1462	1558
2007	Oat by-product	22	0	0	7	13	2	0	313	161	491	659
2008	Oat by-product	90	3	23	48	16	0	0	111	84	228	487
2009	Oat by-product	80	0	5	27	29	7	12	370	272	870	1711
2006–2009	Oat by-product	209	3	28	82	64	12	20	293	152	662	1711

Table 4. Occurrence by country of harvest of T-2 and HT-2 toxins, as the sum of their concentrations in oats and oat flakes in 2005–2009.

Country of origin	Product	Number of samples	Number of samples in the range ($\mu g\,kg^{-1}$)						Mean ($\mu g\,kg^{-1}$)	Median ($\mu g\,kg^{-1}$)	90th %ile ($\mu g\,kg^{-1}$)	Max ($\mu g\,kg^{-1}$)
			<5	5–50	50–200	200–500	500–750	>750				
UK	Oats raw	180	15	11	136	14	3	1	83	27	190	758
Finland	Oats raw	25	0	13	10	2	0	0	67	41	124	283
Ireland	Oats raw	28	0	4	17	6	0	1	190	162	341	841
Sweden	Oats raw	8	0	3	4	1	0	0	103	98	177	302
UK	Oat flakes	261	85	153	23	0	0	0	19	12	46	197
Finland	Oat flakes	141	9	116	6	0	0	0	18	13	34	96
Ireland	Oat flakes	6	3	3	0	0	0	0	9	8	15	16
Sweden	Oat flakes	11	0	10	1	0	0	0	28	20	40	87

under unfavourable conditions. More research is required on the issue.

The survey showed that the incidence of T-2 and HT-2 toxins in European oats was high and the mean concentrations varied considerably by year. Processing of oats to oat flakes and oat meal in the mills reduced mean toxin levels by 82 and 88%, respectively. Mean and median concentrations in the food products were low and below $20\,\mu g\,kg^{-1}$. On the other hand, toxin levels in oat by-products increased 3–4-fold and their use as feed ingredients merits further investigation.

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