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Emmanuel Kossi Tangni, Luc Pussemier, Jean Claude Motte, François Van Hove, Yves Jacques Schneider, Carlos Van Peteghem, Yvan Larondelle

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Complete List of Authors:	TANGNI, Emmanuel; Veterinary and Agrochemical Research Center, Quality and Safety Pussemier, Luc; Veterinary and Agrochemical Research Center, Quality and Safety Motte, Jean Claude; Veterinary and Agrochemical Research Center, Quality and Safety Van Hove, François; Université catholique de Louvain, Mycothèque de l'Université catholique de Louvain (BCCMTM/MUCL) Schneider, Yves Jacques; Université catholique de Louvain, Laboratoire de biochimie cellulaire, Institut des Sciences de la Vie Van Peteghem, Carlos; Ghent University, Laboratory of food analysis, Faculty of Pharmaceutical Sciences Larondelle, Yvan; Université catholique de Louvain, Unité de biochimie de la nutrition
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Comparison of ochratoxin A and deoxynivalenol in organically and conventionally produced beers sold on the Belgian market

ANSELME M.[1], TANGNI E.K[2,*], PUSSEMIER L[2], MOTTE J-C[2], VAN HOVE F[3], SCHNEIDER Y-J[4], VAN PETEGHEM C[5] & LARONDELLE Y[1]

[1]: Unité de biochimie de la nutrition, Institut des Sciences de la Vie, Université catholique de Louvain, Croix du Sud 2/8, 1348 Louvain-la-Neuve, Belgium

[2]: Department of quality and safety, Veterinary and Agrochemical Research Centre (CODA-CERVA), Leuvensesteenweg, 17, B-3080 Tervuren, Belgium

[3]: Mycothèque de l'Université catholique de Louvain (BCCM™/MUCL), Unité de Microbiologie, Croix du Sud 3/6, 1348 Louvain-La-Neuve, Belgium.

[4]: Laboratoire de biochimie cellulaire, Institut des Sciences de la Vie, Université catholique de Louvain, Croix du Sud 5/3, 1348 Louvain-la-Neuve, Belgium.

[5]: Laboratory of food analysis, Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, 9000 Ghent

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*: Correspondence: E. K. Tangni, E-mail: emtan@var.fgov.be

Abstract

Beer was chosen as a cereal-derived and homogeneous product which would allow a comparison to be made of organic and conventional production modes in terms of mycotoxin contamination levels. Ochratoxin A (OTA, a storage mycotoxin) and deoxynivalenol (DON, a field mycotoxin) were assessed by HPLC methods in organically and conventionally produced beers sold in Belgium. Immunoaffinity column (OchraTest® and DONPrep®) purification was used prior to HPLC analysis. For in-house validation, recovery experiments carried out with the spiked beers in the ranges of 50 - 200 ng OTA l^{-1} and 20 - 100 µg DON l^{-1} led to the overall averages of 91 % (RSD = 10 %, n =9) and 93 % (RSD = 5 %, n = 27), respectively. Organic beers collected during 2003-2004 tended to be more frequently OTA contaminated (95 %, n = 40) than their conventional counterparts (50 %, n = 40). Conventional beers were OTA contaminated at a mean concentration of 25 ng l^{-1} (range: 19 - 198 ng l^{-1}) whilst organic beers contained a mean level of 182 ng l^{-1}(range: 18 - 1134 ng l^{-1}). High OTA contamination above the limit of 200 ng l^{-1} (up to 1134 ng l^{-1}) occasionally occurred in organically produced beers. A complementary survey performed with the same brands in 2005 did not confirm this accidental presence of excessive OTA loads (range: 3 - 67 ng l^{-1} for 10 conventional beers and 19 - 158 ng l^{-1} for 10 organic beers). Establishing a maximum of 3 µg OTA kg^{-1} in malt, the application of the regulation EC 466/2001 (entered in force before the last sampling) may be related to the improvement observed. The overall incidence of DON was 67 and 80 % in conventional and organic beers, respectively. DON concentrations ranged from 2 to 22 µg DON l^{-1} (mean = 6 µg DON l^{-1}) in conventional beers whilst organic beers were contaminated from 2 to 14 µg DON l^{-1} (mean = 4 µg DON l^{-1}). DON in beers does thus not appear to be a major matter of concern. From the statistical tests, it could be concluded that the variation between different batches was significant ($p < 0.0001$) in contrast to that observed between different brands, showing thus a lack of homogeneity in the raw materials. This may occur either in organically or in conventionally produced materials. Considering these results, an optimized frequency of controls according to European Regulations EC No 466/2001 and EC No 856/2005 should be recommended in order to reject the irregular batches.

Keywords : Food safety, mycotoxin, ochratoxin A, deoxynivalenol, beer, organic, conventional, Belgium.

Introduction

Due to food crises in the recent past, organic food products have become more popular in Belgium and elsewhere (Pussemier *et al.* 2006). Indeed, the demand for organic foods is constantly increasing mainly due to the consumers' perception that they are healthier and safer than conventional foods (Magkos *et al.* 2003). The situation may however be more complex. Some authors indicate that organic food often contains relatively high amounts of natural toxic compounds produced by fungi or plants, whereas corresponding conventional food tends to contain more synthetic compounds such as pesticide residues (Finamore *et al.* 2004). By contrast, Pussemier *et al.* (2006) have concluded that the influence of organic cultivation methods on the presence of natural toxins is not yet clear. In terms of mycotoxins, even though the foodstuffs resulting from conventional production are not free of mycotoxins, the organic foodstuffs may present a greater risk of contamination. So, there is a need for information related to food safety to inform the consumers of the health benefits and/or hazards of food products of both origins, in order to optimize the impact on health and minimize the risks.

The mycotoxin ochratoxin A (OTA) has strong nephrotoxic, immunosuppressive and teratogenic properties. Its worldwide occurrence as a contaminant of raw agricultural products has been amply documented in a variety of plant products such as cereals, coffee, dried fruits, cocoa, grapes and spices (MacDonald *et al.* 1999, 2004, Otteneder & Majerus 2001, Serra *et al.* 2005, Thirumala-Devi *et al.* 2001). Mainly produced by toxigenic strains of the genera *Aspergillus* and *Penicillium* (Pitt 1987, Moss 1998, Samson *et al.* 2004), high OTA levels mainly occur in stored products. Before storage, cereals are prone to be contaminated in the field by fungi of different *Fusarium* species. These fungi may infect the plant and produce trichothecenes, with deoxynivalenol (DON) being the most abundant representative one in cereal grains (Boeira *et al*. 2000). Thus, malting barley, wheat and corn, which are used in brewing either as main ingredients or as adjuncts, can be contaminated by OTA and/or DON. So far, a handful studies have investigated the fate of these mycotoxins

during malting, brewing and fermentation and they have concluded that OTA and DON can at least partially withstand these complex food processing and, finally, may remain present in beer (Niessen & Donhauser 1993, Baxter 1996, Baxter *et al.* 2001, Papadopoulou-Bouraoui *et al.* 2004). Beer is thus a convenient cereal-derived foodstuff allowing to compare the organic and conventional production modes in terms of mycotoxin contamination levels. To our knowledge, the influence of the organic route of beer production on the presence of natural toxins, such as OTA and DON, has not been yet studied. Therefore, the present investigation was initiated to compare the OTA and DON contaminations in organic and conventional beers produced in Belgium, the European country with the greatest diversity of beers. The study includes the development of a method for the determination of DON in beer using immunoaffinity clean-up coupled with HPLC-UV detection.

Materials and methods

Sample collection and handling

Keeping, as far as possible, a balance between organic and conventional products, forty conventionally produced beers (representing 10 brands) and forty organically produced beers (representing 10 brands) were randomly purchased in the supermarkets and retail shops in Belgium from September 2003 to September 2004. All these brands were manufactured in Belgium. To ensure some variation in batches and brands of the sampled materials, two batches per brand and two samples per batch were tested. A complementary sampling was performed on the same brands (10 conventional and 10 organic beers) in March 2005 to check the temporal fluctuation in OTA contamination, especially for previously high OTA contaminated brands. Here, one bottle (one batch) of each brand was investigated. All samples were stored at 4°C till opening for analysis.

Reagents and materials

Pure crystalline forms of OTA and DON were obtained from Sigma Chemical Co. (St Louis, MO, USA). The stock solution of OTA was made in toluene:acetic acid (99:1) whereas that of DON was

made in acetonitrile far-UV (of HPLC grade). The concentrations were determined by spectrophotometry (Wood *et al.* 1996, Petterson 1998). The stock solutions were stored at -18°C until re-use (maximum storage duration of 12 months).

OTA and DON immunoaffinity columns (OchraTest® and DONPrep®) were obtained from Vicam (Watertown, MA, USA) and from R-Biopharm (Rhône diagnostics Ltd, Glasgow, Scotland), respectively. Microfilters type Millex®-HV, SLHV R04NL (0.45µm) and Durapore® membrane filters (type 0.22 µm GV, GVWP04700) were purchased from Millipore (Bedford, MA, USA).

Acetonitrile and toluene of HPLC grade were supplied by Lab Scan (Dublin, Ireland). Methanol of HPLC grade and acetic acid *pro analysis* were obtained from Acros Organics (New Jersey, NY, USA) or from Biosolve (Valkenswaard, The Netherlands) while hydrochloric acid (37 %) was purchased from Riedel-de Haën (Seelze, Germany). Phosphate-buffered saline (PBS) solution (NaCl 120 mM, KCl 2.7 mM, phosphate buffer 10 mM, pH 7.4) was supplied by Sigma. Sodium bicarbonate ($NaHCO_3$) was supplied by Merck (Darmstadt, Germany). All other chemicals were of analytical grade.

HPLC grade water was obtained using a twin system composed by Elix3 and Milli-Q system, both from Millipore (Molsheim, France).

Helium of 99.996 % purity and nitrogen of 99.995 % purity were provided by Air Liquide (Liège, Belgium).

OchraTest method for OTA determination in beer

OTA determination in beer was performed according to the method described by Tangni *et al.* (2002). First, bottles of beer were vigorously shaken and left for 10 min before opening. A sample of beer was taken and decarbonated by shaking again for 10 min. Degassed beer (40 ml) were then diluted with water (5 ml, containing 4 % (w/v) sodium bicarbonate) and with PBS solution (15 ml). Solid particles were separated by centrifugation of the mixture at 820x*g* during 10 min at 8 ± 2°C.

The supernatant (45 ml) were used for the next steps. Clean-up of the OTA extract was performed with an OchraTest column fitted with a 20-ml syringe reservoir and placed on a vacuum manifold. Before loading the extract, the OchraTest column was conditioned with 20 ml of PBS solution (pH 7.4). The OTA extract was passed through the column at about 1-2 ml min^{-1}. De-ionized water (20 ml) was used to wash the loaded immunoaffinity column and OTA was eluted with methanol (2 ml) and de-ionized water (2 ml). Atmospheric air (*ca* 20 ml) was passed through the column to collect all the eluate. The solution was stored at -20°C prior to HPLC analysis.

As for HPLC detection and quantification, the chromatographic system consisted of a Perkin-Elmer LC049 isocratic pump (Norwalk, CO, USA) equipped with a Rheodyne model 7125 NS injection valve (50 ml) (Rheodyne, Cotati, CA, USA), a RF-551 fluorescence spectrophotometeric detector (Shimadzu, Kyoto, Japan) equipped with a 150W xenon lamp ($\lambda_{excitation}$ = 332 nm, $\lambda_{emission}$ = 462 nm) and a Spectra- Physics SP4290 integrator (San Jose, CA, USA). The analytical column was a Hypersil™ BDS reversed phase C18 (15 cm×4.0 mm i.d., 3 mm particles) (Tracer Analytical, Barcelona, Spain). The column was used at room temperature. The mobile phase was made of a mixture of HPLC grade acetonitrile:water:acetic acid (45:54:1), filtered through a 0.22 µm filter membrane, degassed and eluted at a flow-rate of 1.0 ml min^{-1}. The eluate was filtered through a 0.45 µm microfilter and 50 µl were injected for HPLC analysis.

The identity of OTA was confirmed by methyl ester formation according to the method described by Zimmerli & Dick (1995). Contaminated eluates with OTA concentration > 200 ng l^{-1} beer were used for this preparation.

DONPrep method for DON determination in beer

Degassed beer (20 ml) were taken and mixed with acetonitrile (110 ml). This mixture was evaporated using a rotary evaporator at 45 ± 5°C in order to minimize the volume of water on the DONprep column. As DON is soluble in water, a bigger volume of water affect the DON recovery.

Water (4 ml) containing sodium bicarbonate (5 % w/v) was added to recover the extract, which was then submitted to the following purification step. Clean-up of DON extract was performed with a DONPrep column fitted with a 5-ml syringe reservoir. Column conditioning was not necessary and the solubilized extract was directly allowed to pass through the column by gravity. The column was washed with water (5 ml) and was then dried with atmospheric air. DON was eluted with methanol (2 ml) by back flush. The eluted extract was evaporated to dryness at 35 ± 5°C with a rotary evaporator, reconstituted in 1 ml of methanol:water (10:90) and stored at -20°C prior to HPLC analysis.

As for HPLC detection and quantification, the chromatographic system consisted of two Waters 515 pumps (Milford, MA, USA), an automatic 717 injector and a 2996 DAD UV detector. The chromatograms were processed using Millenium Empower. The Alltima (100 x 4.6 mm id, 3 µm particles) RP-C_{18} column (Alltech, Deerfield, IL, USA) preceded by an OptiGuard precolumn (10 × 1 mm i.d., 10 µm particles) obtained from Optimize Technology (Oregon City, OR, USA), was thermostatically maintained at 35°C by a temperature controller Model 7990 (Jones Chromatography, Hengoed, UK) during HPLC analysis.

The mobile phase was made of a mixture of HPLC grade acetonitrile:water (5:95), filtered through a 0.22 µm Durapore® membrane filter, degassed and eluted at a flow-rate of 1.0 ml min^{-1}. The eluate was filtered through a 0.45 µm microfilter and 50 µl were injected for HPLC analysis.

Validation

To evaluate the analytical procedures, recovery experiments were performed in triplicate with one type of beer spiked at 50, 150 and 200 ng OTA l^{-1}, while DON was added in triplicate samples of three types of beer at 20, 50 and 100 µg l^{-1}. The limit of detection (LOD) and limit of quantification (LOQ) of the chromatographic procedures were calculated by the signal-to-noise ratios of 3:1 and 10:1, respectively. The linearity of the detector responses was estimated in experiments with the

OTA and DON standards. The calibration curves were plotted and their linearity was assessed based on the correlation coefficient (r).

Data handling and statistical analyses

The calibration curves were calculated using the least-squares method. Experimental results are reported as mean ± standard deviation (SD). Non-detects were assumed to indicate that the sample contained half the LOD in terms of OTA and DON levels, whereas trace levels were assumed to indicate that the sample contained half the LOD + LOQ (Kroes *et al*. 2002). For both mycotoxins, the median and range values are given.

The statistical analysis attempted to first determine the presence of significant differences in contamination frequency between conventional and organic samples at levels over the limit of quantification (Chi-square test). Variance analyses were then performed on quantifiable concentrations in order to underline the effect of the mode of production, of the brand and of the batch on the level of contamination. Correlation analysis was used to study the consistency of the association between OTA and DON levels in the tested samples. Independent sample T-test was used to compare the mean OTA in DON positive and negative samples across the two groups of beers. Statement of significance was based on probability of $p < 0.05$.

Results and discussion

Analytical methods performances

Chromatograms did not show any impurity interfering with OTA or DON. Regarding OTA analysis, the analytical procedures showed a recovery rate of 91 % (RSD = 10 %, n = 9) and the respective LOD and LOQ values of 5 and 18 ng OTA l^{-1} of beer, within the tested range of spiked beer (50-200 ng OTA l^{-1}).

As for DON analysis, preliminary results (not published) have shown that the DONPrep column has a maximal capacity of about 1.5 µg DON. With the above described procedure, this amount

corresponds to a concentration of 75 µg DON l^{-1} beer. In order to be able to analyze concentrations above 75 µg DON l^{-1} beer, we adapted the first step of the method by decreasing the volume of 20 ml of beer. Thus, only 5 ml of degassed and centrifuged beer were mixed with 30 ml of acetonitrile. Assaying DON recovery experiments in triplicate on three types of beer spiked at 20, 50 and 100 µg l^{-1}, the overall average recovery was 93 ± 4 % (n = 27). The LOD and LOQ values were 2 and 6 µg DON l^{-1} of beer, respectively. Note that the recovery rate at 100 µg DON l^{-1} estimated at 94 % showed a good precision (RSD = 5 %, n = 9). Care must then be taken not to exceed the capacity of the immunoaffinity column.

Occurrence of OTA in organic and conventional beers marketed in Belgium

Table I presents the OTA occurrence in organic and conventional beers produced in Belgium during the 2003-2004 period.

[Insert table I about here]

OTA was detected in 78 % of conventional samples at a mean level of 25 ± 38 ng l^{-1}(range: 19 - 198 ng l^{-1}). All organic beers were OTA contaminated at a mean level of 182 ± 275 ng l^{-1} (range: 18 - 1134 ng l^{-1}). Nevertheless, the magnitude between these means was not statistically significant ($p = 0.1758$). OTA above 200 ng l^{-1} was quantified in 10 organically produced beers and in none of conventional samples. The statistical analysis showed that the frequencies of quantifiable levels of OTA were significantly higher ($p < 0.0001$) in contaminated organically produced beers (95 %) than in their conventional counterparts (50 %).

Twenty different brands of beers were tested and no significant brand effect ($p = 0.2093$) in the OTA content was observed for all samples combined. In contrast, the variation between different batches was significant ($p < 0.0001$), showing thus an heterogeneous quality in brewing raw materials (as far as OTA contamination is concerned) within the production of the same brand. This might indicate that different parts of the same stock of raw materials or stocks of various origins

were used for the preparation of different batches. One must be aware that in stored-grain bulks, “hot spots" of OTA may accidentally occur in a given stock, even if the optimal required conditions for mould development have not been reached. Exceptionally, high concentrations of OTA in a batch of beer may thus occur if highly contaminated raw materials were introduced in the brewing process. This finding corroborates with the temporal fluctuation in OTA contamination in Belgian beers observed by Tangni *et al.* (2002). One must realize that the mycotoxin contamination status covers a limited period and that year-to-year variations may often occur as specified by Scott & Kanhere (1995) and Jorgensen (2005).

Numerous surveys have been conducted on OTA contamination in conventionally produced beers across the world. Nakajima *et al.* (1999) analyzed OTA in 46 European beers and found an average concentration of 11.4 ng l^{-1} (range: 1.7 - 66.2 ng l^{-1}) in 43 contaminated samples (LOD = 1 ng l^{-1}). In Italy, Visconti *et al*. (2000) analysed the OTA content in 61 samples of 10 domestic and 51 imported beers and found OTA levels ranging from 10 to 135.2 ng l^{-1} (LOD = 10 ng l^{-1}). Beers purchased in Denmark were also investigated for OTA contamination by Jorgensen (1998). All samples (n = 21) contained traces of OTA (>1 ng l^{-1}) with a mean content of 49 ng l^{-1} (highest value = 160 ng l^{-1}). Similarly, Araguas *et al*. (2005) studied 21 Spanish beers and found in 67 % of the tested samples a mean of 38 ng OTA l^{-1} (highest value = 205 ng l^{-1}). One must note that these are in accordance with the current study for the conventional type of beer.

So far, a comparison between conventionally (49 samples) and organically (13 samples) produced beers in Belgium has been performed by Tangni *et al.* (2002). These authors found that the mean OTA content was significantly higher ($p = 0.007$) in organic (81 ng l^{-1}) than in conventional (22 ng l^{-1}) beers sampled in 1998-1999. In contrast, no significant difference ($p = 0.233$) was shown for beers collected in 2000-2001, although conventionally produced beers contained higher levels of OTA (49 ng l^{-1}) than organically produced counterparts (29 ng l^{-1}). The highest OTA content (185 ng l^{-1}) was found in an organically marketed beer. In the present study, attention was paid to the

sampling procedure by keeping a balanced sample size between organic and conventional groups. We observed that bad cases of contamination occasionally occur in organically produced beers, with concentrations that can be much higher (up to 1134 ng l^{-1}) than the limit of 200 ng l^{-1} (FAO 2004). Independently from the mode of its production, high OTA levels in beer have been seldom presented in the recent literature. In fact, Gumus *et al.* (2004) analysed 135 Turkish beers and 15 imported beers and found that 28 % of the samples were OTA contaminated at levels ranging from 100 to 8100 ng l^{-1}. It was found that OTA levels were above 3000 ng l^{-1} in 6 tested samples. So, the risk of highly contaminated batches occurring from time to time should not be underestimated.

Evidence of temporal fluctuation in OTA contamination in beers

Taking into account the high contamination levels obtained in the above sample, a complementary sampling was performed in March 2005. It was based on the same brands as those analysed in the 2003-2004 period. One bottle (one batch) of each brand was investigated.

Of 10 conventional beers, OTA was detected in 9 samples at mean concentration of 21 ng l^{-1} (median = 12; range: 3 - 67 ng l^{-1}) whereas all organically produced beers (10) were OTA contaminated at the mean level of 75 ng l^{-1} (median = 54, range:19 - 158 ng l^{-1}). So, none of these beers exceeded the limit of 200 ng OTA l^{-1}, showing thus the temporal fluctuation of OTA occurrence in beers belonging to the same brands. One should, however, pay attention to the fact that the samples included in this complementary survey were probably produced after the regulation EC No 466/2001 entered in force, which established a maximum level of 3 µg OTA kg^{-1} malt (European Commission Regulation 2001). The use of a good quality (as far as OTA contamination is concerned) malt within this legal limit may further avoid the high OTA levels sporadically found in some batches. So, regarding the legal limits, there is a need to continue the work of setting maximum levels for more mycotoxins in more foodstuffs.

Occurrence of DON in organic and conventional beers marketed in Belgium

Table II shows the results of DON contamination in organically (40) and conventionally (40) produced beers in Belgium.

[Insert table II about here]

The overall incidences of DON were of 67 and 80 % in conventionally and organically produced beers, respectively. Statistical analysis showed that the frequencies of quantifiable levels of DON were comparable ($p = 0.4586$) in organic (25 %) and conventional (32 %) beers. The respective averages were of 6 and 4 µg DON l^{-1} whilst the highest levels were of 22 and 14 µg DON l^{-1}. The magnitude between these means was not statistically significant ($p = 0.0768$). Analysis of variance revealed homogeneous DON contamination between different brands whatever the mode of production ($p = 0.8639$). In contrast, a statistically significant batch effect ($p < 0.0001$) was noticed within the contaminated samples belonging to the same brand.

As for all samples combined ($n = 80$), the evaluation of DON contamination showed that 74 % of the tested beers were contaminated at a mean level of 5 µg l^{-1}. So far, several reports are available on the extent of DON contamination in beer. Of 54 domestic and imported beers in Korea, Shim *et al.* (1997) reported an incidence of 26 % for DON contamination ranging from 1 to 23 µg l^{-1}. Molto *et al.* (2000) have found a mean of 14.3 µg l^{-1} (range: 5-221 µg l^{-1}) in 22 out of 50 Argentinean beers. The European evidence of DON occurrence in beer is backed up by a Canadian survey of 50 beers brewed in Canada or imported from Europe (Scott *et al.* 1993). This investigation revealed that 29 samples were DON contaminated with an average of 5.4 µg l^{-1} (range: 0.3 - 50.3 µg l^{-1}). More specifically, imported beers from Europe were contaminated at levels ranging from 1.1 to 15.8 µg DON l^{-1}. Weddeling *et al.* (1994) reported that all the 18 German beers tested were DON contaminated with an average of 5 µg l^{-1}. A much higher contamination level was found in another German study by Niessen *et al.* (1993). These authors found that 190 out of 196 German beers were contaminated at levels ranging from 148 to 569 µg l^{-1} (mean = 205 µg l^{-1}). These beers were, however, part of a study on gushing and were therefore not randomly selected. Recently,

contamination data have not shown high levels of DON in beer. Indeed, Schothorst & Jekel (2003) analysed 51 beer samples collected in the Netherlands and found that DON occurs at a concentration above 25 µg l^{-1} (LOQ value) in only 3 samples, with quantities ranging from 26 to 41 µg l^{-1}. Papadopoulou-Bouraoui *et al.* (2004) detected DON in 87 % of the analysed beers (n = 313, LOD = 3.7 µg l^{-1}) with concentrations ranging from 4.0 to 56.7 µg l^{-1}. Most samples (73 %) had contamination levels below 20 µg l^{-1}. Our results compare well with the low level of DON contamination obtained in these previous studies. We therefore conclude that DON contamination in Belgian beers should not be a major matter of concern. So far, no regulatory guideline for DON contamination in beer is available in terms of safe limit. Indeed, the recent European Commission Regulation No 856/2005 amending the EC regulation No 466/2001 as regarding the *Fusarium* toxins states only on cereals and cereal based product not including beer (European Commission Regulation 2005).

From an analytical point of view, the present HPLC analytical procedure allows to detect DON in beer at very low levels and can be routinely used to assay the concentrations of DON in beers. Previous studies of DON determination in beer commonly involved gas chromatography (Scott *et al.* 1993, Molto *et al.* 2000, Schothorst & Jekel 2003) coupled with ECD, FID, MS or MS/MS detectors after derivatisation. Enzyme-linked immunosorbent assay is also used (Niessen *et al.* 1993, Papadopoulou-Bouraoui *et al.* 2004), but accurate quantification with this method is often limited, owing to cross reactivities (Schneider *et al.* 2004).

Co-occurrence of OTA and DON contents in organically and conventionally produced beers

Based on the DON detection limit, two subgroups of DON-positive (levels ≥ LOD, n = 59) and DON-negative (level < LOD, n = 21) were considered.

The mean DON in the positive group (7 ± 4 µg l^{-1}) was obviously significantly higher ($p < 0001$) than the level of contamination in the negative group. The respective concentrations of 8 ± 6 and 5

± 2 µg DON l^{-1} were found in conventional and organic positive sub groups. In contrast, the mean OTA (107 ± 230 ng l^{-1}) in the DON-positive group was comparable (p = 0.085) to the mean OTA (48 ± 71 ng l^{-1}) found in the DON-negative group. The same trend was separately observed either for conventionally (30 ± 44 *versus* 14 ± 12 ng OTA l^{-1}) or organically (173 ± 296 *versus* 105 ± 92 ng OTA l^{-1}) produced beers, highlighting that a real significant co-occurrence of both toxins was not found for the tested beers, whatever their mode of production was. Moreover, non-significant correlation coefficients were derived between OTA and DON levels for either conventional (r = +0.125, p = 0.441) or organic (r = +0.091, p = 0.576) group of beers.

If contaminated raw materials are used during the brewing process, beer can be contaminated by all key cereal mycotoxins, except perhaps citrinin, which does not seem to survive the mashing step (Galvano *et al*. 2005). Zearalenone and OTA or aflatoxin B_1 and OTA coexisted in beers analysed in Japan (Nakajima *et al.* 1999) and in South-Africa (Odhav & Neckair 2002). Co-occurrence of trichothecenes such as DON, nivalenol, diacetoxyscirpenol, 3-acetyl deoxynivalenol, 15-acetyl deoxynivalenol and neosolaniol have been found in Canada (Scott *et al.* 1993) as well as in Argentina (Molto *et al.* 2000). Note that the simultaneous occurrence of two or more toxins in cereals has frequently been documented in the literature, e.g. barley, malt, maize and wheat contaminated with DON, nivalenol and zearalenone (Lee *et al.* 1986, Park *et al*. 1992, Tanaka *et al.* 2000), maize contaminated with fumonisin B1, DON, T-2 toxin, nivalenol and zearalenone (Ali *et al.* 1998), wheat contaminated with DON and OTA (Birzele *et al.* 2000) and barley contaminated with OTA and DON (Olsson *et al.* 2002). These findings highlight the problems associated with ingestion of multiple toxins that may have an interactive effect on the health of the consumers.

Assessment of the contribution of beer to the OTA and DON exposure for the consumers

In recent years, thorough risk assessments of the trichothecenes and of OTA established a provisional maximum tolerable daily intake of 1 µg DON kg^{-1} bw and a tolerable daily intake (TDI)

of 5 ng OTA kg^{-1} bw (FAO/WHO 2001). Considering a daily intake of 0.3 l beer per capita in Belgium (CBB 2004) and the mean levels of DON and OTA found in the present study, beer consumption contributes simultaneously to 3 % and 10 % of the TDI of DON and OTA for an adult of 60 kg. We conclude that moderate daily intakes of DON and OTA are provided through beer consumption in Belgium. Beer consumption should thus not lead to acute toxic reactions linked to DON and OTA. It should however be included in calculations of total exposure to DON and OTA, together with other sources of cereals and other foodstuffs, which undoubtedly provide these mycotoxins as well. So far, the effects of a chronic exposure to low levels of these mycotoxins (long-term exposure) are not yet known (Gudmestad *et al.* 1997) and the combined effects of mycotoxins may increase their toxic effects. As an example, Foster *et al.* (1986) showed that DON in naturally contaminated feed has a higher toxicity in comparison to pure DON, suggesting that co-contamination with some unknown toxic metabolite or co-occurrence with a non-toxic compound enhances the toxicity of DON. Moreover, it is noticed that DON belongs to the group of several trichothecenes with a common basic chemical structure, which are produced by *Fusarium* fungi (e.g. T-2 Toxin, HT-2 toxin, nivalenol). These mycotoxins may share common mechanisms of action, which may lead to significant interactions. For all these reasons, the TDI for DON is determined only temporarily (FAO/WHO 2001). Generally, the problem of multi-contamination should not be underestimated.

In conclusion, the present study has further warranted the occurrence of low levels of OTA in beer. OTA was detected in about 90 % of the samples, but the levels were often far below the limit of 200 ng OTA l^{-1}. A few batches from organic beers were found to be much more contaminated, at levels exceeding this limit. The situation appeared to have improved from 2003-2004 to 2005 since none of the sample collected in 2005 exceeded the limit of 200 ng OTA l^{-1}. This improvement may reflect the newly imposed controls on malt. Occurrence of DON in beers should not be a major cause for concern since this toxin was found at low levels in a limited number of beers.

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List of tables

Table I. Occurrence of OTA in organic and conventional beers sold on the Belgian market (period 2003 - 2004).

Occurrence	**Conventional**	**Organic**	**Total**
Number of samples	40	40	80
n.d.[a]	9 (22 %)	0	9 (11 %)
trace[b]	11 (28 %)	2 (5 %)	13 (16 %)
LOQ - 100 ng l^{-1}	18 (45 %)	22 (55 %)	40 (50 %)
101 - 200 ng l^{-1}	2 (5 %)	6 (15 %)	8 (10 %)
200.1 - 1200 ng l^{-1}	0	10 (25 %)	10 (13 %)
Incidence	**78 %**	**100%**	**89 %**
Levels of contamination (ng l^{-1})			
Mean[c] ± SD	25 ± 38	182 ± 275	103 ± 162
Median[c]	15	45	29
Range[d]	19 – 198	18 – 1134	18 - 1134

[a]: n.d., not detected (OTA concentration in sample < 5 ng l^{-1})
[b]: traces means that the concentration of OTA is between the limit of detection (LOD = 5 ng l^{-1}) and the limit of quantification (LOQ = 18 ng l^{-1})
[c]: mean and median are computed assuming that non-detected samples contain half the LOD while samples with traces levels of OTA contain half the LOD + LOQ
[d]: range of samples with OTA contents ≥ LOQ

Table II. Occurrence of DON in organic and conventional beers sold on the Belgian market (period 2003- 2004).

Occurrence	**Conventional**	**Organic**	**Total**
Number of samples	40	40	80
n.d.[a]	13 (33 %)	8 (20 %)	21 (26 %)
trace[b]	14 (35 %)	22 (55 %)	36 (45 %)
LOQ - 10 µg l^{-1}	5 (12 %)	8 (20 %)	13 (16 %)
10 - 20 µg l^{-1}	6 (15 %)	2 (5 %)	8 (10 %)
20 - 30 µg l^{-1}	2 (5 %)	0	2 (3 %)
> 30 µg l^{-1}	0	0	0
Incidence	**67 %**	**80 %**	**74 %**
Levels of contamination (µg l^{-1})			
Mean[c] ± SD	6 ± 6	4 ± 3	5 ± 4
Median[c]	4	4	4
Range[d]	6 – 22	6 – 14	6 – 22

[a]: n.d., not detected (DON concentration in sample < 2 µg l^{-1})

[b]: traces means that the concentration of DON is between the limit of detection (LOD = 2 µg l^{-1}) and the limit of quantification (LOQ = 6 µg l^{-1})

[c]: mean and median are computed assuming that non-detected samples contain half the LOD while samples with traces levels of DON contain half the LOD + LOQ

[d]: range of samples with DON contents ≥ LOQ