Masked Mycotoxins: an Emerging Issue for Food Safety

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Abstract: The presence of masked or hidden forms of *Fusarium* mycotoxins (deoxynivalenol, DON, zearalenone, ZEN and fumonisins B1, B2 and B3) were studied in wheat and maize derived products. Significant amounts of these forms were found both in raw and in processed food commodities. Deoxynivalenol-3-glucoside was found in wheat products up to 30% of DON concentration. Bound forms of fumonisins often account for an equal or even higher amount in comparison with the free forms.

Keywords: masked mycotoxins; *Fusarium* toxins; maize; wheat; malting

INTRODUCTION

In the last years it has clearly emerged that, in mycotoxin-contaminated commodities, many structurally related compounds generated by plant metabolism or by food processing can co-exist together with the native toxins. These mycotoxin derivatives (conjugated or "masked" mycotoxins) may have a very different chemical behaviour, thus they can easily escape routine analyses. Nevertheless, these forms could be hydrolysed to their precursors in the digestive tracts of animals or could exert toxic effects comparable to those imputable to free mycotoxins. In particular, this phenomenon is related to *Fusarium* toxins (trichothecenes, zearalenone, fumonisins) (Gareis *et al*. 1990; Böswald *et al.* 1995). As detoxifying mechanism, plants are able to convert the relatively apolar trichothecenes and zearalenone in more polar derivatives via conjugation with sugars, amino acids or sulphate groups, in order to compartmentalise them in vacuoles: zearalenone-4-glucoside and deoxynivalenol-3-glucoside were detected in wheat naturally contaminated by *F. graminearum* (Berthiller *et al*. 2005). Also the technological process has an important role in the masking mechanism, in particular in cereal derived products. Indeed, mechanical or thermal energy during the transformation process may cause significant modification, inducing reactions with macromolecular components such as sugars, proteins or lipids or release of the native forms upon decomposition of the masked derivatives. In the case of fumonisins, a more intriguing phenomenon has been described as "the fumonisin paradox" as also apparently low contaminated commodities have been found to induce toxic effects, highlighting the problem of "bound" or "hidden" fumonisins, which may be released upon alkaline hydrolysis and have been found to contribute quantitatively to the total amount of mycotoxins in several maize derived products (Dall'Asta *et al*. 2009). The occurrence of these masked mycotoxins in several wheat and maize products will be reported in this communication.

MATERIALS AND METHODS

Deoxynivalenol (DON), deoxynivalenol-3-glucoside (DON-3-Glu), zearalenone (ZEN), fumonisins B1, B2 and B3 standard solutions were purchased from Biopure (Tulln, Austria). Zearalenone-4-glucoside was synthesised in our laboratory according to the literature (Grabley *et al*. 1992). All solvents used (LC grade) were obtained from Carlo Erba (Milan, Italy); bidistilled water was produced in our laboratory utilising an Alpha-Q system (Millipore, Marlborough, MA, USA). HPLC-MS/MS

analysis were performed with a 2695 Alliance (Waters Co., Milford, MA, USA) equipped with a Quattro^{TM} triple quadrupole mass spectrometer with an electrospray source (Micromass, Waters, Manchester, UK). Analysis of trichotecenes and zearalenone were performed with a Synergy 2.5 µm Fusion-RP 100Å (50 × 2 mm) with 5mM CH_3COOH water solution (eluent A) and CH_3OH (eluent B), applying the following gradient: 0–9 min, from 95% A to 30% A, 9–20 min isocratic step 30% A, 20–21 min to 95% A, 21–30 re-equilibration step at 95% A (initial conditions). Flow rate 0.25 ml/min, temperature 30°C, inj. vol. 5 µl. MS parameters: capillary 2.5 kV, cone 10 V, collision gas Argon, source temperature 150°C, desolvation temperature 160°C, LM1 5.0, HM1 2.0, Ion energy 10.6, Entrance 3, Collision 15 eV, Exit 2, LM2 15.0, HM2 15.0, Multiplier 650 V, Gas cell pirani 2.93 × 10^{-23} mbar, dwell time 0.05 s. Sample preparation: 10 g of wheat (milled) and wheat flour sample were extracted with 40 ml H_2O:CH_3CN = 20:80 by Ultraturrax homogenisation (3 min at 6400 rpm). 5 ml of the extract, filtered on paper filter, were applied to Mycosep^{TM} columns: 2 ml of the purified extract were dried under N_2 and the residue redissolved in MeOH for HPLC analysis. Analyses of fumonisins and bound fumonisins were performed according to an original method developed in our laboratory (Dall'Asta *et al*. 2009).

RESULTS AND DISCUSSION

Owing to the climatic and environmental conditions, in Italy wheat is often contaminated by DON and ZEN, whereas fumonisins are major contaminants of maize. We analysed samples of wheat flour (n = 6) from the Italian market (year 2007) for the presence of trichothecenes, zearalenone and their glycosylated derivatives: for the latter, standards are usually not commercially available, making their identification and quantification a hard task. In particular, ZEN-4-glucoside was synthesised and purified in our laboratory (see Materials and Methods). In the analysed samples a generally low contamination from DON was observed (< 400 ppb), whereas nor zearalenone neither glycosylated derivatives were found. Instead, the analysis of wheat samples artificially inoculated with *Fusarium* strains afforded different results: in all the samples, a high content of DON was detected along with acetyl-DON and, interestingly, DON-3-glucoside ranging from 2 to 30% of the DON concentration (Table 1). These results are in agreement with observations by other groups and underline the significant contribution of the glycosylated form to the overall contamination. Although produced via a plant detoxification mechanism, the toxicity of these bound forms for humans is not defined.

Table 1. Mycotoxins in wheat samples artificially inoculated with *Fusarium*

Sample	DON	DON-3-GLU	Ac-DON	ZEN
1	6541 ± 272	2009 ± 203	575 ± 72	112 ± 15
2	694 ± 73	77 ± 19	n.d.	n.d.
3	405 ± 6	8 ± 1	n.d.	n.d.
4	5149 ± 196	841 ± 3	76 ± 5	n.d.

n.d.: not detected

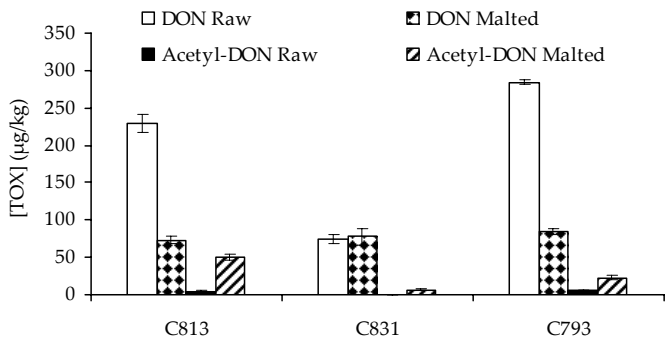

Figure 1. Variation of DON and derivatives upon malting of wheat

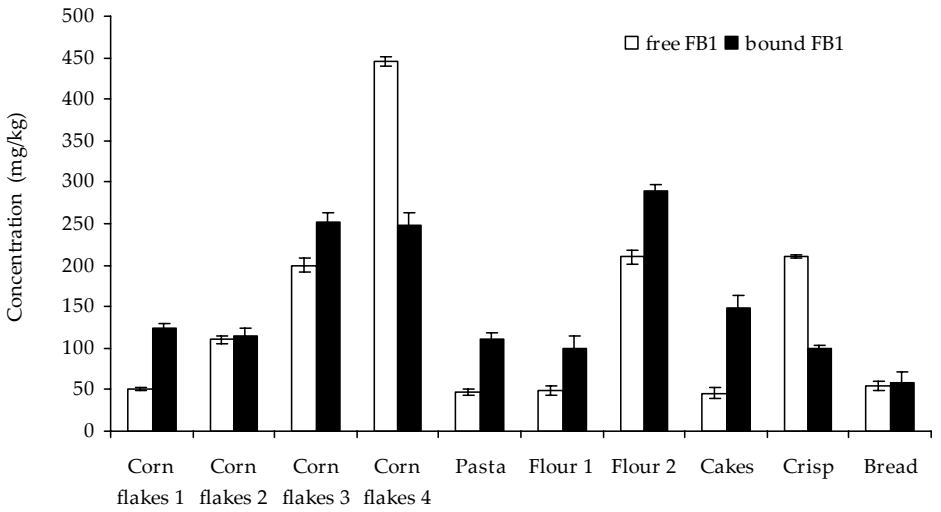

Figure 2. Free and "bound" fumonisins (expressed as FB1) in maize products

Moreover, most interesting is their fate during the technological processes, as eventually the native forms may be released. It has been demonstrated that ZEN-4-Glu may be hydrolysed in gastric conditions in swine (Gareis *et al*. 1990). More recently, it was found (Scudamore *et al*. 2008) that in wheat extruded products DON and NIV content increases after processing, probably on account of a higher extractability of the toxin or a release of bound forms, or a combination of both. In the present study, we investigated the effect of malting process on the content of DON and ZEN in wheat samples. Indeed, malting had a strong effect on mycotoxin content: in particular a large decrease in the content of DON was observed, a phenomenon explained by the dissolution in the malting water. Simultaneously, a significant conversion of DON into acetylated forms was also observed, probably as a result of the enzymatic activities elicited by the malting process (Figure 1). Instead, a high increase of zearalenone content was observed in the malted wheat in comparison with the raw material (up to 20 times the initial value). It is currently under study if the malting process induced the hydrolysis of glycosylated forms or the degradation of other complexes between zearalenone and macromolecular components. These preliminary results highlight the importance of investigating this phenomenon in deep details, as in these cases the control of the contamination of the raw materials is not sufficient to assure the safety of the food products derived from it.

As far as fumonisins are concerned, we analysed several maize products for the presence of free and "bound" fumonisins: the latter components may be determined after alkaline hydrolysis, which, cleaving the ester bonds between the tricarballylic acid groups and the polyhydroxyamino backbone, release hydrolysed fumonisins (HFBs). This phenomenon has been attributed to the formation of covalent bond between fumonisins and starch or proteins, but also to other non covalent interactions (complexation, intercalation, etc.).

In any case, the problem is particularly significant as in many products the amount of bound forms is equal or overpass the content of free forms. This phenomenon is particularly evident in several samples of maize products from the market (Figure 2).

On the base of the data obtained by our group and others, this problem has to be furtherly investigated in order to understand its real impact and its implications for consumer safety.

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