



Thermal stability and kinetics of degradation of deoxynivalenol, deoxynivalenol conjugates and ochratoxin A during baking of wheat bakery products



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ABSTRACT

The stability of deoxynivalenol (DON), deoxynivalenol-3-glucoside (DON-3-glucoside), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), de-epoxy-deoxynivalenol (DOM-1) and ochratoxin A (OTA) during thermal processing has been studied. Baking temperature, time and initial mycotoxin concentration in the raw materials were assayed as factors. An improved UPLC–MS/MS method to detect DON, DON-3-glucoside, 3-ADON, 15-ADON and DOM-1 in wheat baked products was developed in the present assay. The results highlighted the importance of temperature and time in mycotoxin stability in heat treatments. OTA is more stable than DON in a baking treatment. Interestingly, the DON-3-glucoside concentrations increased (>300%) under mild baking conditions. On the other hand, it was rapidly reduced under harsh conditions. The 3-ADON decreased during the heat treatment; while DOM-1 increased after the heating process. Finally, the data followed first order kinetics for analysed mycotoxins and thermal constant rates (k) were calculated. This parameter can be a useful tool for prediction of mycotoxin levels.

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1. Introduction

Mycotoxins are produced by fungi and can contaminate various agricultural commodities either before harvest or under post-harvest conditions. The main mycotoxin-producing fungi in food commodities belong to the genera *Aspergillus*, *Penicillium* and *Fusarium*. Wheat, such as the majority of cereals, is susceptible to be contaminated with mycotoxins. Different studies show the high presence of mycotoxins, mainly deoxynivalenol (DON), in wheat products

(Pacin, Resnik, Neira, Moltó, & Martínez, 1997; Vidal, Marín, Ramos, Cano-Sancho, & Sanchis, 2013). Moreover, cereal products represent one of the main sources of exposure to DON and ochratoxin A (OTA) (Marín et al., 2013).

DON is not classified as to its carcinogenicity to human by IARC (International Agency for Research on Cancer) (1993), and it is linked with human gastroenteritis. OTA is a nephrotoxic mycotoxin which possesses carcinogenic, teratogenic, immunotoxic and possibly neurotoxic properties. This mycotoxin has been classified, as a possible human carcinogen, in the group 2B, by the International Agency for Research on Cancer (IARC (International Agency for Research on Cancer), 1993).

Processing of cereals at high temperatures may affect DON and OTA content. However, the extent of DON and OTA reduction during

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thermal food processing seems to be quite variable and dependent on the processing conditions applied: temperature, time, type of mycotoxin, and size of cereal product. For bakery products, some studies reported a significant decrease in DON levels during baking (Numanoglu, Gökmen, Uygün, & Koksels, 2012; Valle-Algarra et al., 2009). By contrast, the studies of De Angelis, Monaci, Pascale, and Visconti (2013) and Zachariasova, Vaclavikova, Lacina, Vaclavik, and Hajslova (2012) reported that DON is stable in processing steps involving high temperatures. Similarly, OTA seems to be quite more stable at high temperature than DON through baking (Vidal, Morales, et al., 2014; Vidal, Marín, et al., 2014). Only results on coffee roasting show a clear reduction of OTA, as the temperature achieved in the product is higher than in bakeries (Castellanos-Onorio et al., 2011; Scudamore, Banks, & MacDonald, 2003; Valle-Algarra et al., 2009). Sometimes, the contradictory published results may be due to the different size of assayed products, which limits the temperature levels attained inside them. Some studies have been carried out in aqueous systems in order to avoid the temperature gradient (Jackson, Hlywka, Senthil, Bullerman, & Musser, 1996). All of them showed effective mycotoxin reductions over 150 °C; however their results cannot be extrapolated to solid food products.

On the other hand, unaltered mycotoxins might not be the only source of health hazard for consumers, because there is a group of metabolites called conjugated mycotoxins which cannot be detected in the routine mycotoxins analysis. The mycotoxin conjugates are mycotoxins attached to functional groups (masked mycotoxins) such as glycosyl residues or sulfates or attached to polymeric carbohydrate or protein matrices (bound mycotoxins) (Berthiller, Schuhmacher, et al., 2009). The conjugated mycotoxins may have plant, fungal, mammalian and food processing origins. The co-occurrence of conjugated DON forms has been documented in wheat, especially deoxynivalenol-3-glucoside (DON-3-glucoside) (Simsek, Burgess, Whitney, Gu, & Qian, 2012), 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON) (Yang et al., 2013). The fate of DON-3-glucoside through breadmaking has been hardly studied. While some authors point out to some reduction (De Angelis et al., 2013), others have seen marked increases (Vidal, Morales, et al., 2014). On the other hand, 3-ADON, 15-ADON and de-epoxydeoxynivalenol (DOM-1) behaviour has not been studied before.

HPLC–MS is usually applied for simultaneous detection of DON and its conjugates (Vendl, Berthiller, Crews, & Krska, 2009), specially DON-3-glucoside (Berthiller, Dall'asta, et al., 2009). Due to the low concentration of DON conjugates found in wheat products, the methods of analysis require low limits of detection. Lately, the ultra-high performance liquid chromatography (UPLC) has demonstrated to be highly effective for the quantification of DON conjugates in cereal products, such as malt and beer (Zachariasova et al., 2012). No previous studies exist on simultaneous analysis of DON, DON-3-glucoside, 3-ADON, 15-ADON, and DOM-1 in bakery products.

The current study aimed to investigate DON and DON conjugates (DON-3-glucoside, 3-ADON, 15-ADON and DOM-1) and OTA kinetics during baking in a short size model bakery product, small enough to avoid temperature gradients in it. Temperature, time and initial mycotoxin concentration were assayed as factors. Moreover an optimized method to quantify DON conjugates in bakery products is presented.

2. Materials and methods

2.1. DON and OTA contaminated flours

In order to obtain DON or OTA contaminated flour, one strain each of *Fusarium graminearum* (TA 3.234) and *Aspergillus ochraceus*

(TA 3.201) were used. Both of them are kept in the Food Technology Dept. collection, University of Lleida, Spain. They were previously proved to be DON and OTA producers when cultured on wheat flour. The concentration of DON and DON-3-glucoside in the initial uninoculated flour ($n = 5$) was 250 ± 44.78 and 45.1 ± 15.3 µg/kg, respectively, while OTA could not be detected. The remaining DON conjugates were not analysed in the initial flour.

The strains were inoculated and incubated in MEA (malt extract agar) at 25 °C until strong sporulation. A spore suspension of each strain was made in water and Tween 80 (0.005% v/v). Five millilitres of either *F. graminearum* or *A. ochraceus* spore suspension were inoculated in glass flasks containing 250 g of flour and 50 mL of water. In total, 3 kg of flour were inoculated with one of the two strains. The flasks were stored at 25 °C for 19 days in the case of *F. graminearum* and 8 days in the case of *A. ochraceus*, with periodic shaking. Then, each kind of flour (3 kg) was properly powdered and homogenized and underwent either DON or OTA analysis. The content of DON and OTA was of $12,500 \pm 1235$ µg/kg and 75.5 ± 15.2 µg/kg respectively ($n = 3$), in each contaminated flour. DON conjugates were not analysed in the flour at this stage.

2.2. Bakery analogue preparation

The bakery analogue was prepared for each 100 g of mix with 27 g of wheat flour, 26 g of sugar, 26 g of eggs, 21 g of sunflower oil and adding to the 100 g of mix 0.5 g of baking powder (maize starch, sodium bicarbonate and disodium diphosphate). The flour used was previously prepared by mixing the uninoculated flour with the DON contaminated flour and the OTA contaminated flour depending on the desired initial mycotoxin concentration: high mycotoxin concentration (HMC) or low mycotoxin concentration (LMC). The analysed toxin levels in the initial mixed flours ($n = 3$) were: (a) HMC, 1042 ± 170 µg/kg of DON and 3.01 ± 0.24 µg/kg of OTA; and (b) LMC, 550 ± 98 µg/kg of DON and 2.11 ± 0.30 µg/kg of OTA. The levels were chosen to be close to real values in food samples.

The mix was manually mixed and 3 g aliquots were poured in small paper moulds. From this point, thermoprobes (Proges Plus, Pluck&Track, Thermo bouton) were always used in some of them to register the baking temperatures; probes were placed in the centre of the moulds. Four oven temperature levels (200, 180, 160 and 140 °C) and 8 baking times (every five minutes starting at minute 5 and finishing at minute 40) were assayed in a full factorial design. These conditions were established on the basis of previous experiments. Thus 2 initial toxin concentrations $\times$ 4 baking temperatures $\times$ 8 baking times $\times$ 3 replicates made 192 different runs (9 equal cakes weighing 3 g each conformed each of the 192 runs). From the 9 cakes, 3 were pooled and used for OTA analysis, other 3 for DON analysis, and the remaining 3 were kept at –20 °C. All samples were lyophilised for 72 h, and then the samples were stored at –20 °C until analysis.

2.3. Chemicals and reagents

Mycotoxin standard solution of OTA was supplied by Sigma (Sigma–Aldrich, Alcobendas, Spain). DON, DON-3-glucoside, 3-ADON, 15-ADON, DOM-1 and isotolabeled ($^{13}\text{C}_{15}$) DON were supplied by Biopure (Tulln, Austria). ($^{13}\text{C}_{15}$) DON was used as internal standard for UPLC–MS/MS. Acetonitrile (99.9%), methanol (99.9%) and ethanol (99.5%) were purchased from J.T. Baker (Deventer, The Netherlands). Dichloromethane ($\geq 99.8\%$) and ammonium acetate ($\geq 98\%$) were purchased from Sigma (Sigma–Aldrich, Alcobendas, Spain). All solvents were LC grade. Filter paper (Whatman No. 1) was purchased from Whatman (Maidstone, UK). Immunoaffinity chromatography columns (IAC) for DON (DONPREP®) and OTA

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