## 6.5. Study V. The fate of deoxynivalenol and ochratoxin A during the breadmaking process, effects of sourdough use and bran content.

*Food and Chemical Toxicology, 68*, 53-60.

**Authors:** Vidal, A., Marín, S., Morales, H., Ramos, A.J., & Sanchis, V.

# The fate of deoxynivalenol and ochratoxin A during the breadmaking process, effects of sourdough use and bran content.

**Arnau Vidal[1], Sonia Marín[1]*, Hector Morales[2], Antonio J. Ramos[1], Vicente Sanchis[1]**

[1]Applied Mycology Unit, Food Technology Department, University of Lleida, UTPV-XaRTA, Agrotecnio Center, 25198 Lleida, Spain.

[2]IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Braga, Portugal.

*Corresponding author Sonia Marín, Food Technology Dept, XaRTA-UTPV, Agrotecnio Center, University of Lleida, Rovira Roure 191, 25198 Lleida, Spain. smarin@tecal.udl.cat

**Abstract**

Deoxynivalenol (DON) and ochratoxin A (OTA) are mycotoxins produced by fungal species which can contaminate, alone or simultaneously, cereal-based products such as bread. Due to the increasing interest in the beneficial effects of dietary bran, bran bread has attained high consumption. Usually, the higher mycotoxin concentrations in cereals are found in the external layers of the grain (bran), leading to higher concentration of DON and OTA in breads with added bran. Moreover, the use of sourdough in breadmaking is increasing, but no studies

about its effect in the mycotoxins content exist. The objective of this study was to determine the variation of concentration of these mycotoxins during the breadmaking process including the following factors: two initial mycotoxin concentrations in the initial mix of ingredients, four different bran contents, and use of sourdough. OTA was confirmed to be quite stable during the breadmaking process, regardless of the assayed factors. DON concentration during breadmaking was not significantly affected by bran content of bread. However, it was significantly affected by kneading and fermentation steps in different way depending on sourdough use and flour contamination level: if DON reduction occurs during fermentation, this leads to a safer situation, but the possible increase in DON should be considered with care, as it can compensate the expected dilution effect by recipe. Finally, the results on deoxynivalenol-3-glucoside (DON-3-glucoside), although preliminar, suggest an increase of this toxin during fermentation, but mainly during baking.

**Keywords:** deoxynivalenol, ochratoixn A, masked mycotoxins, baking process.

## 1. Introduction

Wheat bread provides more nutrients to the world population than any other single food source (Peña, 2002). Bread is particularly important as a source of carbohydrates, proteins and vitamins B and E (Pomeranz, 1987). Consumption of bread prepared with whole grain flours is currently increasing in developed countries, due to the increasing interest in the beneficial effects of dietary fiber (Faridi and Faubion, 1995). Dietary fiber is defined as carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence (European Comission, 2011). The benefits of dietary fiber intake are numerous, ranging from improved large bowel function to slowed digestion and absorption of carbohydrate and fat and reduced risk for certain diseases. Based on the effects on bowel function, the EFSA Panel considered bran intake of 25 $g \cdot d^{-1}$ to be adequate for normal laxation in adults (EFSA, 2010).

Cereals and cereal based products like bread may contain mycotoxins, particularly deoxynivalenol (DON) and ochratoxin A (OTA). Wheat and wheat containing products (like bread and pasta) represent the major source of intake for DON (Cano-Sancho et al., 2011a; SCOOP, 2003). 55% of samples (raw cereals and derived products) were reported to be OTA positive (SCOOP, 2002) and cereals are considered one of the major sources of intake for OTA (Coronel et al., 2012).

DON, also known as vomitoxin, is one of the most regular contaminants in cereals (Alexa et al., 2013; Cano-Sancho et al., 2011b; EFSA, 2006a; Jelinek et al., 1989; Li et al., 2014). Although DON is not classifiable as to its carcinogenicity to humans by the International Agency for Research on Cancer (IARC, 1993), it has been linked with human gastroenteritis (Pestka, 2010a,b). At the molecular level, DON disrupts normal cell function by inhibiting protein synthesis, affecting cell signaling, differentiation, and proliferation. An acute and high dose of DON can induce vomiting, whereas chronic dietary exposure to DON causes reduced food intake, decreased nutritional efficiency, reduced weight gain, and immune dysregulation. OTA is a nephrotoxic mycotoxin which possesses carcinogenic, teratogenic, immunotoxic and possibly neurotoxic properties (EFSA, 2006b). This mycotoxin has been classified as a possible human carcinogen in the group 2B by the IARC (IARC, 2002). OTA has been reported in cereals, coffee, grape juice, wine, beer and meat based foodstuffs (Coronel et al., 2012).

The European Commission has set a maximum permitted level for OTA in processed cereal products for direct human consumption of 3 $\mu g \cdot kg^{-1}$, and a maximum concentration of 500 µg/kg for DON in bread (European Commission, 2006).

Deoxynivalenol-3-glucoside (DON-3-glucoside) is a plant metabolite from DON, and then considered a masked mycotoxin. Berthiller et al. (2005) showed its high presence in wheat. Some studies pointed out a possible conversion of DON-3-glucoside to DON during baking (De Angelis et al., 2013). Moreover, Berthiller et al. (2011) showed that DON-3-glucoside can be hydrolised by several lactic acid bacteria. Thus the Joint European Commission FAO/WHO Expert Committee (JEFCA) considered DON-3-glucoside as an additional contributing factor of the total dietary exposure to DON (Codex, 2011; JEFCA, 2010).

Nishio et al. (2010) studied the level of *Fusarium graminearum* infection in wheat and observed that the level of DON production was directly related to the incidence of fungal growth, and that fungal infection was greatest at or near the kernel surface. Generally, cereal bran is the part of the grain with the highest concentration of mycotoxins (Thammawong et al., 2010). L'Vova et al. (1998) reported that up to 60% of the initial DON content in grain passed into bran and that mycotoxin content in the bran exceeded that in the grain. Scudamore (2005) reported that by-products such as 'cleanings' or bran may contain high concentrations of OTA. Vidal et al. (2012) assessed the content of several mycotoxins in wheat and oat based bran supplements sold in the Spanish market; DON was present in 42% of the samples, with levels above the EU legislation in 19% of samples, whereas OTA was present in 25% of the samples.

Thermal treatment of contaminated cereals, flour and bran may decrease the levels of mycotoxins. However, some studies with OTA contaminated flour reported that this mycotoxin is stable at high temperatures, and that its concentration does not decrease during baking (Scudamore, 2005; Vidal et al., 2014). Also, the studies of Bullerman and Bianchini (2007) and Kabak (2009) reported that DON is stable in processing steps involving high temperatures. By contrast, Scott et al. (1984) reported a reduction of DON up to 74% in white flour bread, although

other studies reported lower reduction of DON during baking (Lancova et al., 2008; Pacin et al., 2012; Vidal et al., 2014). DON stability during the baking process can be influenced by temperature, time and loaf size (Vidal et al., 2014). Finally, DON degradation may take place mostly in the bread crust, which reaches the highest temperature, whereas the content of DON in the breadcrumbs scarcely decreases (Kostelanka et al., 2011). Thus, the inactivation of DON and OTA during baking and the influence of bran content are not clear yet. It is obvious that the initial content of bran influences on the final content of mycotoxins in bread. Moreover, the effects of bran content of bread in toxin thermal stability may lead to different dynamics of the mycotoxin during baking.

The interest in the use of sourdough in the manufacture of bakery products is increasing as a traditional added value; sourdough is a very complex biological ecosystem where yeasts and lactic acid bacteria contribute to the dough fermentation (Gobbetti, 1998).

The objective of the assay presented herein was to assess the effect of dough fermentation and baking on DON and OTA content in bran bread combined with sourdough addition. For an additional experiment, OTA in the initial mixture came either from contaminated bran or contaminated flour, in order to study whether the source of the mycotoxin influences on its dynamics during dough fermentation and baking. Finally, DON-3-glucoside natural contamination was followed through the breadmaking process.

## 2. Materials and methods

### *2.1. DON and OTA contaminated flour and bran*

In order to obtain DON or OTA contaminated flour, two strains of either *Fusarium graminearum* (TA 3.234) or *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 initial flour did not contain OTA, and DON and DON-3-glucoside levels (n=3) were 250±76 and 45.1±15.3 $\mu g \cdot kg^{-1}$, respectively.

The strains were inoculated and incubated in MEA (malt extract agar) at 25 ºC until strong sporulation. A spore suspension of each strain was made in water and Tween 80 (0.005% v/v). Five milliliters of either *F. graminearum* or *A. ochraceus* spore suspension were inoculated in glass flasks containing 250 g of wheat flour and 50 mL of water. In total, 3 kg of flour were inoculated with each strain. The flasks were incubated at 25 ºC for 19 days in the case of *F. graminearum* and 8 days in the case of *A. ochraceus*, with periodic shaking. Then, each contaminated flour (3 kg) was properly homogenized and underwent either DON or OTA analysis. The content of DON and OTA was of 12,500±1,235 µg/kg and 75.5±15.2 µg/kg respectively (n=3), in each contaminated flour, while DON-3-glucoside level remained unchanged.

Commercial wheat bran was used to obtain mycotoxin contaminated bran. The initial analysis of this bran showed that it was naturally contaminated (n=3) with DON (2,070 ±49 µg/kg). Analogously to the flour contamination procedure described above, a sublot of DON naturally contaminated bran was further contaminated with OTA by inoculation with *A. ochraceus* and subsequent incubation. Thus bran contaminated with both OTA and DON was obtained. The concentration of OTA (n=3) reached in such bran was 85.7±19.6 µg/kg.

*2.2. Dough preparation and baking*

Pieces of dough (260 g) were made with different contents of bran (0, 100, 200 and 300 g/kg flour) and either with addition of sourdough or not. The desired DON and OTA concentration was achieved by adding either DON/OTA contaminated bran as well as DON/OTA contaminated flour and uninoculated flour.

Different flour/bran mixes were prepared, depending on the bran content and toxin levels to be achieved: a) dough without bran, DON and OTA was added through contaminated flour, b) dough with 100 or 200 g bran/kg flour, which source of OTA was contaminated flour, while DON contamination came from both DON contaminated flour and commercial bran, c) dough with 200 g bran/kg flour, which OTA contamination source was contaminated bran, while DON contamination came from both DON contaminated flour and commercial bran; d) dough with 300 g bran/kg flour, where DON contamination source was commercial bran whereas OTA contamination came from flour. Table 1 summarizes all the initial mixtures prepared. In all cases, two levels of mycotoxin contamination were assayed: High mycotoxin contaminated (HMC) dough (n=30), with 1,197±319 µg/kg of DON, 20.6±14.8 µg/kg of DON-3-glucoside and 9.7±1.8 µg/kg of OTA, and low mycotoxin contaminated (LMC) dough (n=30), with 565±247 µg/kg of DON, 23.9±16.9 µg/kg DON-3-glucoside and 0.9±0.2 µg/kg of OTA. These results come from the initial mixes prepared for the breadmaking processes of the assay.

To each flour/bran mix (156 g), 2.3 g of salt, 4.7 g of sucrose, 4.7 g of lard, 6.2 g of commercial compressed yeast (*Saccharomyces cerevisiae*), and 3.1 g of flour improver (containing calcium carbonate, wheat flour, soya flour, lecithin, ascorbic acid and enzymes) were added. The dough was obtained by adding ca. 83 mL of water to the mixture.

| Level of DON and OTA concentration | Source of DON | Source of OTA | g of bran/1000 g of flour | Sourdough use |
|---|---|---|---|---|
| High concentration | Flour | Flour | 0 | Yes |
| | | | | No |
| | Flour and bran | Flour | 100 | Yes |
| | | | | No |
| | Flour and bran | Flour | 200 | Yes |
| | | | | No |
| | Flour and bran | Bran | 200 | Yes |
| | | | | No |
| | Bran | Flour | 300 | Yes |
| | | | | No |
| Low concentration | Flour | Flour | 0 | Yes |
| | | | | No |
| | Flour and bran | Flour | 100 | Yes |
| | | | | No |
| | Flour and bran | Flour | 200 | Yes |
| | | | | No |
| | Flour and bran | Bran | 200 | Yes |
| | | | | No |
| | Bran | Flour | 300 | Yes |

The sourdough was obtained from a bakery, and was refreshed every 8 days and stored at 4 ºC. For refreshment, one half of the sourdough was removed and the other half was mixed with water and wheat flour (50:50). Sourdough was added in 10% w/w total dough. Initial flour/bran mixtures were recalculated taking into account the amount of flour/water contained in the sourdough in order to make results as much comparable as possible. The addition of lactic acid bacteria (LAB) in the form of sourdough has been reported to have positive effects on wheat bread quality and shelf-life (Corsetti et al., 2000; Crowley et al., 2002).

**Table 1. Initial prepared flour/bran mixes to assay the factors: initial toxin concentration, bran concentrations, sourdough use and source of OTA, on DON and OTA fate during breadmaking.**

Dough was manually kneaded until held together with a non-sticky, smooth and satiny appearance and optimum handling properties. Rounded pieces weighing 260 g each were prepared. From this point, thermoprobes were always used in the dough to record fermentation and baking temperatures; in particular, probes were placed in the centre of the loaf and close to the surface. Doughs were covered with a damp cloth and fermentation was carried out at 30 ºC for 15 minutes. Then the pieces were placed in moulds, where the dough further fermented for 1 hour at 30 ºC. After the fermentation a sample of 25 g was taken from every proofed dough and stored at -20 ºC until mycotoxin analysis (results for the finished bread were corrected accordingly). The proofed dough was then baked in an oven (Eurofred PE46SVR, Eurofred, Spain). Baking conditions were 180 ºC and 105 min. Such conditions were established on the basis of previous experiments to obtain suitable bread. After baking, a representative sample was taken and stored at -20 ºC until analysis.

A full factorial design with mycotoxin level, bran content, and use of sourdough as assayed factors with three replicates was performed.

*2.3. Chemicals and reagents*

Mycotoxin standards were supplied by Sigma (Sigma-Aldrich, Alcobendas, Spain). Acetonitrile (purity 99.99%), methanol (purity 99.99%) and ethanol (purity 99.5%) were purchased from J.T. Baker (Deventer, The Netherlands). 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 (OCHRAPREP®) extracts clean-up were purchased from R-Biopharm (Rhone LTD Glasgow, UK). Pure water was obtained from a milli-Q apparatus (Millipore, Billerica, MA, USA). Phosphate buffer saline (PBS) was prepared with potassium chloride (0.2 g) (Panreac, Castellar del Vallès, Spain), potassium dihydrogen phosphate (0.2 g) (purity 98-100%, Panreac, Castellar del Vallès, Spain), disodium phosphate anhydrous (1.16 g) (purity 99%, Panreac, Castellar del Vallès, Spain) and sodium chloride (8.0 g) (purity >99,5%,

Fisher Bioreagents, New Jersey, USA) in 1 L of pure water; the pH was brought to 7.4 with hydrochloric acid 1 M.

### *2.4. Preparation of standard solutions*

OTA standard solution was dissolved in methanol at a concentration of 5.0 mg/mL and stored at 4 ºC in a sealed vial until use. The concentration in the stock solution was checked by UV spectroscopy according to AOAC Official methods of analysis (Horwitz and Latimer, 2006). Working standard solution (0.5, 0.01, 0.005, 0.001 and 0.0005 µg/mL) were prepared by appropriate dilution of known volumes of the stock solution with mobile phase and used to obtain calibration curves in the appropriated chromatographic system.

DON standard solution was dissolved in ethanol at a concentration of 10.0 mg/mL and stored at 4 ºC. The concentration in the stock solution was checked by UV spectroscopy according to AOAC Official methods of analysis (Horwitz and Latimer, 2006). Working standards (50.0, 10.0, 5.0, 1.0, 0.5, 0.1 and 0.05 µg/mL) were prepared as for OTA, as well as calibration curves.

DON was dissolved in acetonitrile at a concentration of 10.0 mg/mL and stored at 4 ºC in a sealed vial until use. Working standards (1.0, 0.5, 0.1, 0.05 and 0.01 µg/mL) were prepared as for OTA, as well as calibration curves.

### *2.5. Mycotoxins extraction, detection and quantification.*

Prior to extraction, all samples were dried at 40 ºC for 24 hours and weight loss recorded in order to present the results in an ‘as is’ basis (µg/kg) or in total content of toxin (µg or ng).

Briefly, for OTA analysis, 5 g of ground sample (Ika, A11B) were extracted with 30 mL of extractant solution (60% acetonitrile, 40% water) by magnetically stirring for 10 min and filtered with filter Whatman number 1. 4 mL of filtered solution was diluted with 44 mL of PBS solution and loaded on the IAC column. After this, the column was washed with 20 mL of PBS and OTA was eluted by applying 1.5 mL of methanol grade HPLC (three times back flushing) and 1.5 mL of milli-Q water, consecutively. The purified extract was dried under nitrogen stream at 40 ºC. Each dried sample was resuspended with 0.5 mL of acetonitrile:water:acetic acid (57:41:2). OTA was determined by HPLC (Waters 2695®) coupled with a Multi λ Fluorescence Detector Waters 2475®, an analytical column Waters Spherisorb® 5 µm ODS2, 4.6 x 250 mm. Excitation and emission wavelengths were set, respectively, at 330 and 463 nm. HPLC mobile phase consisted of acetonitrile, water and acetic acid (57:41:2). The mobile phase flow rate was 1 $mL \cdot min^{-1}$, the injection volume was 100 µL, and the retention time was 15 minutes.

Regarding DON and DON-3-G, 5 g of ground sample (Ika, A11B) were extracted with 30 mL of distilled water by magnetically stirring for 10 min. Then the sample was centrifuged for 8 min at 1780 g. Supernatant was filtered through a glass microfiber filter. Five milliliters of filtered

sample were loaded on the IAC column and the column washed with 10 mL of distilled water. DON and DON-3-glucoside were eluted by applying 1.5 mL of methanol grade HPLC (with three backflushing steps) and 1.5 mL of milli-Q water, consecutively. Zachariasova et al. (2012) confirmed the good cross-reactivity of DON-3-glucoside with the IAC DONPREP® columns. The purified extracts were dried under nitrogen stream at 40 ºC. Each dried sample was resuspended with 0.5 mL of the mobile phase solution (water:acetonitrile:methanol, 92:4:4). DON and DON-3-glucoside were determined by HPLC (Waters 2695®) coupled with a UV/Visible dual λ absorbance Detector Waters 2487. Absorption wavelength was set at 220 nm. The HPLC mobile phase flow rate was 1.2 $mL \cdot min^{-1}$, the injection volume was 100 µL, and the retention time for DON and DON-3-glucoside were 20 and 23 min, respectively.

*2.6. Methods performance*

The analytical methods used were assessed for linearity, precision and recovery. Standard curves were generated by linear regression of peak areas against concentrations ($r^2$ were 0.97, 0.96 and 0.99 for DON, DON-3-glucoside and OTA, respectively). Precision was established by determining OTA and DON levels in bread and DON and DON-3-glucoside in flour samples at least by triplicate, in those samples fortified in order to calculate the recovery rates. The limit of detection (LOD) was considered to be three fold the signal of blank noise, and the limit of quantification (LOQ) was calculated as 3 x LOD. Method performance characteristics for DON and OTA are summarized in Table 2.

**Table 2. Method performances for ochratoxin A (OTA). deoxynivalenol-3-glucoside (DON-3-glucoside) and deoxynivalenol (DON) determination in some analyzed substrates.**

| Mycotoxin | Product | LOD[a] (µg/kg) | LOQ[b] (µg/kg) | n | Spiking level (µg/kg) | Recovery (%)[c] | RSDr[d](%) |
|---|---|---|---|---|---|---|---|
| OTA | Bread | 0.14 | 0.42 | 5 | 0.5 | 103.9±15.6 | 4 |
| | | | | 5 | 1.5 | 110.1±13.0 | 2 |
| | | | | 5 | 3.0 | 99.6±12.6 | 1 |
| DON | Bread | 60 | 180 | 5 | 100 | 100.0±16.3 | 16 |
| | | | | 5 | 500 | 98.8±9.0 | 9 |
| | | | | 5 | 1000 | 102.3±5.3 | 5 |
| | Flour | 60 | 180 | 3 | 300 | 123.3±30.3 | 41 |
| | | | | 3 | 500 | 87.4±8.6 | 7 |
| DON-3-G | Flour | 14 | 42 | 5 | 50 | 80.0±9.6 | 12 |
| | | | | 5 | 250 | 79.7±4.8 | 6 |
| | | | | 5 | 500 | 66.7±11.2 | 18 |

[a] LOD = Limit of detection.
[b] LOQ = Limit of quantification.
[c] Mean value ± standard deviation.
[d] RSDr = relative standard deviation.

*2.7. Statistics*

Multifactorial ANOVA was applied to assess the significance of sample traits in the observed mycotoxin concentration levels as well as in the calculated percentages of increase/reduction at a $p<0.05$. Moreover, HSD-Tukey tests were applied to establish significant differences among levels of factors (the significance level was set at 5%). The results obtained were processed in two different ways: a) DON, DON-3-glucoside or OTA content in each step (µg of DON and DON-3-glucoside or ng of OTA) and, b) concentration 'as is' (µg/kg of DON, DON-3-glucoside and OTA in wet basis). In the first case it was possible to assess the real impact of the processing steps in the mycotoxins, while in the second one it is possible to draw conclusions on compliance of maximum permitted levels. For the 'no kneaded mix' results obtained for dry ingredients from the mycotoxin analysis were corrected for the amount of water to be added.

**3. Results and discussion**

*3.1. Recorded temperatures*

The temperature probes placed in the inner part of the dough and near the surface recorded higher temperatures near the surface (Fig. 1). In the fermentation step, the temperature reached a maximum of 31 ºC and no differences existed between the internal and external part. During the baking process, the dough reached in the centre the maximum temperature (97 ºC) within the first 30 minutes of baking and slightly decreased afterwards; this decrease was probably due to the formation of the crust, which may hinder the penetration of heat. Near the surface the temperature kept on increasing and reached almost 120 ºC at the end of baking (far from the 180 ºC oven baking temperature).

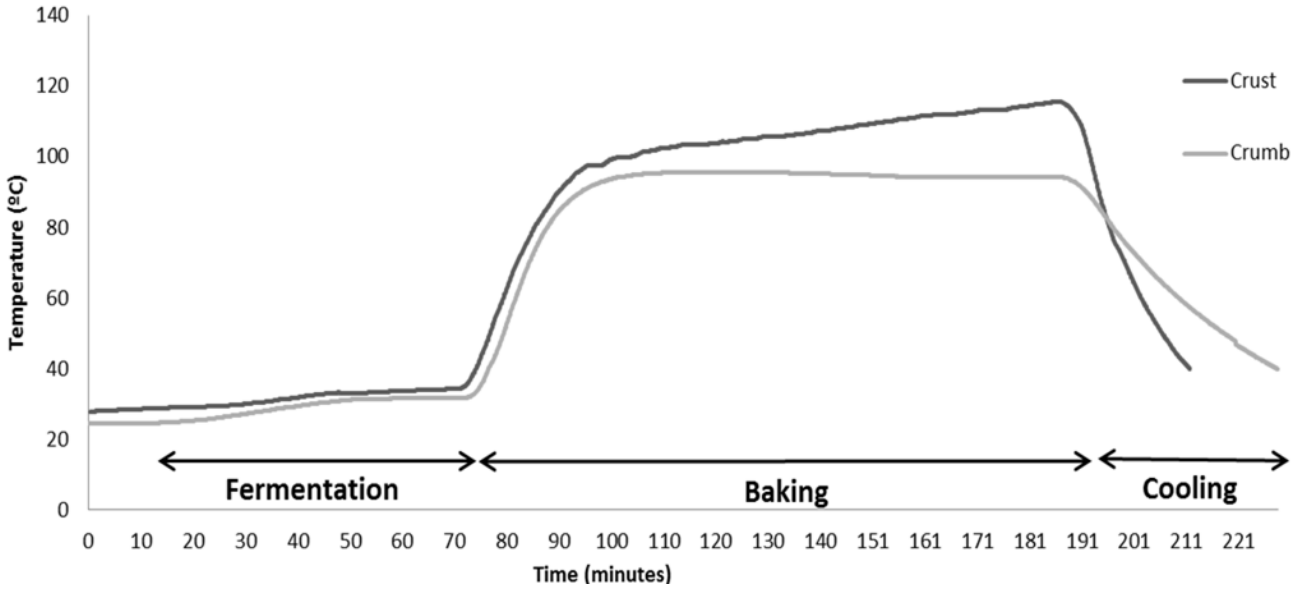
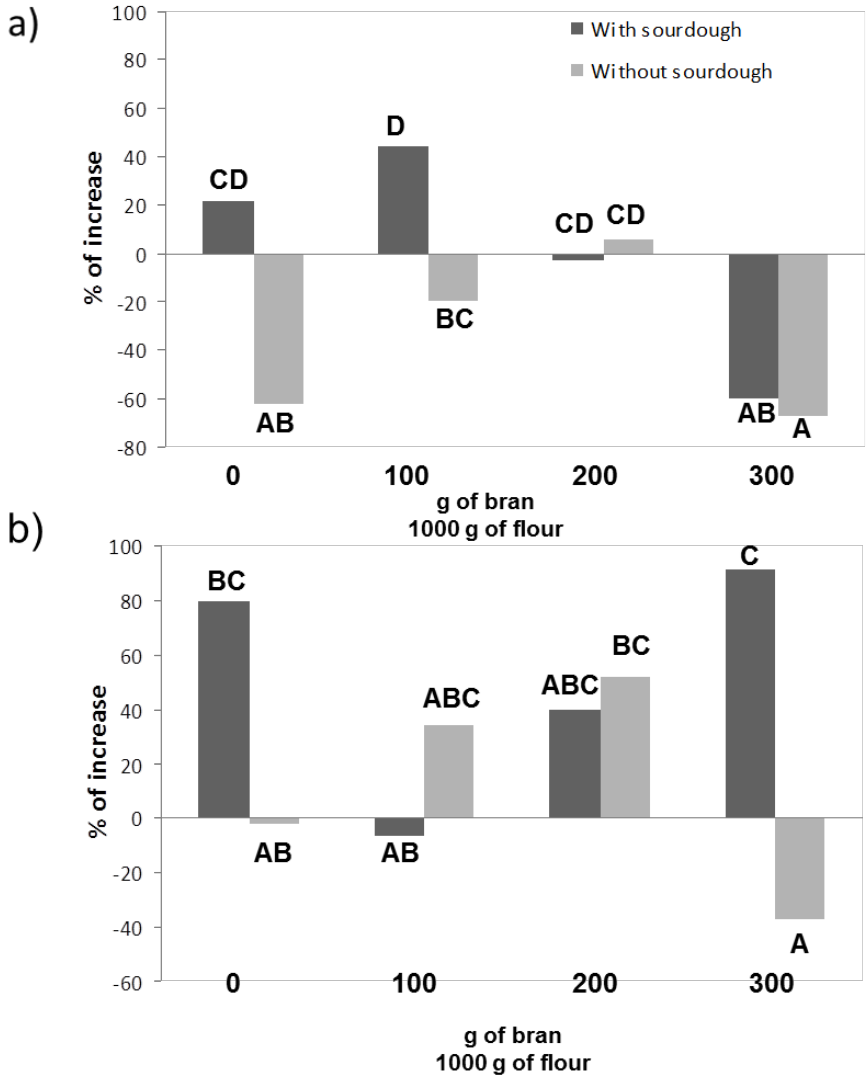


**Figure 1. Mean temperatures recorded during the bread making process in the center and near the crust.**

*3.2. Effect of dough fermentation and baking on DON and DON-3-*glucoside *content*

Average DON content in the initial mixture and proofed dough was not significantly different (142.9 µg vs 122.7 µg for the overall mean, respectively) ($p>0.05$), although this difference depended on the other assayed factors. However, when percentages of reduction were calculated, and their variance analysed, the contribution of the sourdough and the initial concentration was significant ($p<0.05$), as well as their interactions. When sourdough was added, a mean increase of DON of 24% was observed, with lower values with increasing bran content and initial DON concentration. On the other hand, doughs prepared without sourdough suffered a mean reduction of DON of 7%, such reduction was higher with 300 g/kg bran and HMC (Fig. 2a, 2b). Overall, doughs prepared with HMC suffered a mean 17% decrease in DON




concentration, while those with LMC an increase of 33%.

**Figure 2. Percentage of increase in mean DON content (µg) ± SD due to dough kneading, fermentation and proofing as a function of bran level and presence of sourdough at the high (a) and low (b) initial DON concentration.**

$^{A,B,C,D}$ Different groups of letters next to the bars indicate significant differences among treatments, same groups of letters mean no significant difference (Tukey's test $p < 0.05$).

As the experiment was not designed for different levels of DON-3-glucoside in the initial flour mix, the natural levels detected in the mix of solid ingredients were not different in those treatments with high/low DON content, and the data were pooled, with a mean value before kneading of 3.5 µg, with most of the data under the limit of detection (the LOD value was used to calculate the mean). After fermentation, DON-3-glucoside was still undetectable in most of the treatments, thus although a mean 79% increase in DON-3-glucoside occurred, the significance of the different factors was not determined for this step.

Published studies on the effect of fermentation on DON content generally showed an increase in DON content when fermented dough was compared to the kneaded dough and enzymes were used as flour improvers (Simsek et al., 2012; Suman et al., 2012; Vidal et al., 2014). By contrast, when no improvers were used, no changes or reduction in DON concentration were observed (Konstelanska et al., 2011; Neira et al., 1997; Samar et al., 2001). In our case, although improvers were used and DON release was observed when sourdough was included in the recipe, reduction could be observed at HMC and high added bran. Some authors (Kostelanska et al., 2011; Zachariasova et al., 2012) concluded that the use of bakery improvers containing enzymes may lead to a higher relative increase in DON-3-glucoside (consistent with our results), than in DON. When proofed/fermented dough was compared to initial flour, no change or increase in DON was reported (Bergamini et al., 2010; Valle-Algarra et al., 2009), this fact may be explained by the results of Lancova et al. (2008) who suggested that a release of DON took place during kneading, it decreased during fermentation and further increased during proofing; in those studies in which proofed dough was compared directly to flour, these sub-steps were not considered and then the averaged results may be contradictory.

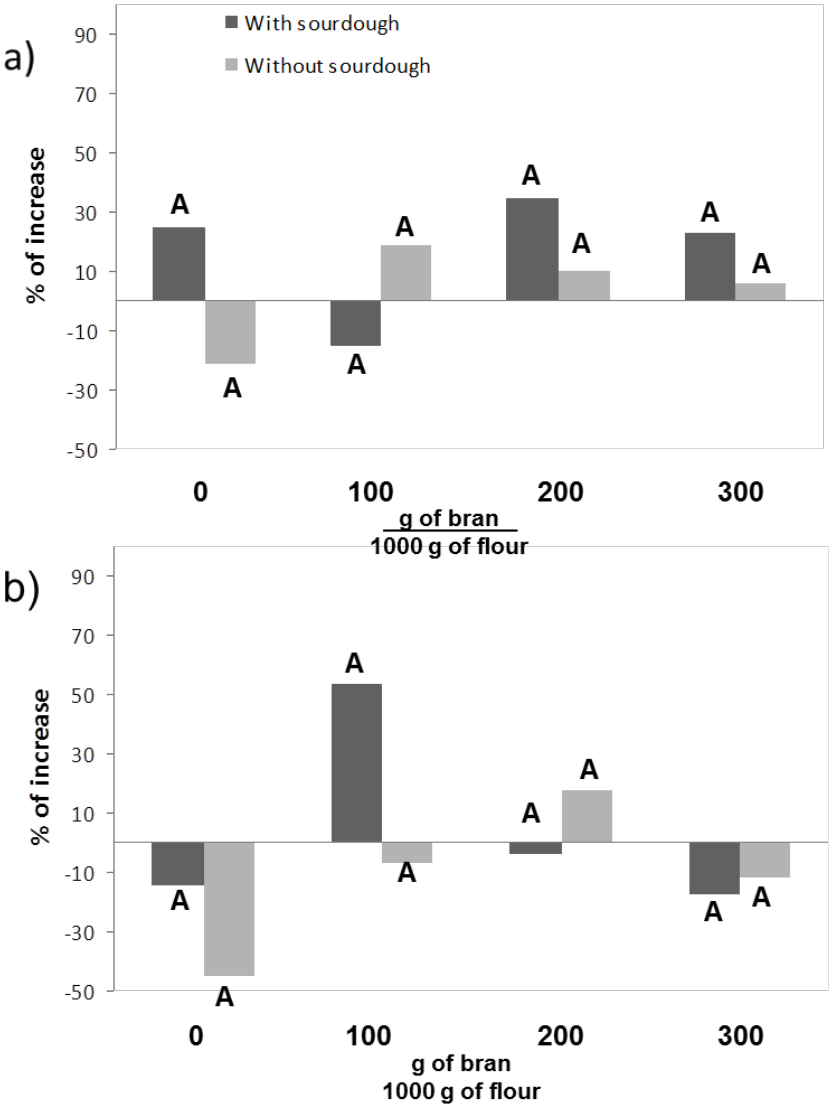
In our assay an increase of DON content was observed in sourdough fermented samples ($p<0.05$). Bergamini et al. (2010) studied the fate of DON during the fermentation step using sourdough powder as well as bread making promoting agents. The authors reported a significant increase in DON level after fermentation (30-40 ºC for 45 to 85 minutes) and suggested that such increase could be due to the release of bound DON. Such release might be attributed to both enzymes contained in the improver and to the metabolism of sourdough bacteria which may be able to either transform DON precursors into DON or to release bound DON. In addition, Suman et al. (2012) prepared crackers with the sponge technique (fermentation for 2-4min at 20-30 ºC) and reported from 10% decrease to 14% increase in DON concentration compared to kneaded dough, depending on fermentation time and temperature and $NaHCO_3$ concentration.

Bran content did only affect the fermentation effect when added at 300 g·kg$^{-1}$, leading to a more marked DON reduction; no studies exist in this point, only Scudamore et al. (2009) found similar global percentages of reduction for the whole breadmaking process from flour to bread of 35% for white bread and of 39% for wholemeal bread.

Average DON content in the fermented dough and bread was not significantly different (122.7 µg vs 126.2 µg, overall mean). Regarding the percentage of reduction/increase in DON content, it was independent of the factors assayed (DON concentration in flour, levels of bran and use of sourdough) and accounted for a mean value of 3% increase, although a trend existed for a certain release in sourdough containing samples and DON reduction without sourdough (Fig. 3a, 3b).

**Figure 3. Percentage of increase in mean DON content (µg) ± SD due to baking as a function of bran level and presence of sourdough at the high (a) and low (b) initial DON concentration.**

**[A] Same letter next to the bars indicate no significant differences among the treatments (Tukey's test p < 0.05).**



As described before, DON-3-glucoside results for HMC and LMC samples were pooled, with most of the data under the limit of detection (the LOD value was used to calculate the mean) for kneaded+fermented dough. A significant increase of 229% (this value should be taken with care as only a few data for the fermented dough showed detectable values; and calculated values close the LOD may be inaccurate) was observed during baking (Fig. 4), which was independent of the assayed factors: bran concentration and sourdough addition. Similarly, Vidal et al. (2014) observed an increase of DON-3-glucoside after baking; however it could not be related to DON reduction. By contrast, previous studies reported DON-3-glucoside reduction during baking (Kostelanska et al., 2011; Simsek et al., 2012). The possible increase of DON-3-glucoside during baking is of concern; although little knowledge exists in the toxicological effects of DON-3-glucoside, an important cleavage of DON-3-glucoside has been observed in the intestinal tract (Berthiller et al., 2011; Gratz et al., 2013). Thus DON-3-glucoside should be considered as an additional contributing factor of the total dietary exposure to DON (Codex, 2011; JEFCA, 2010).

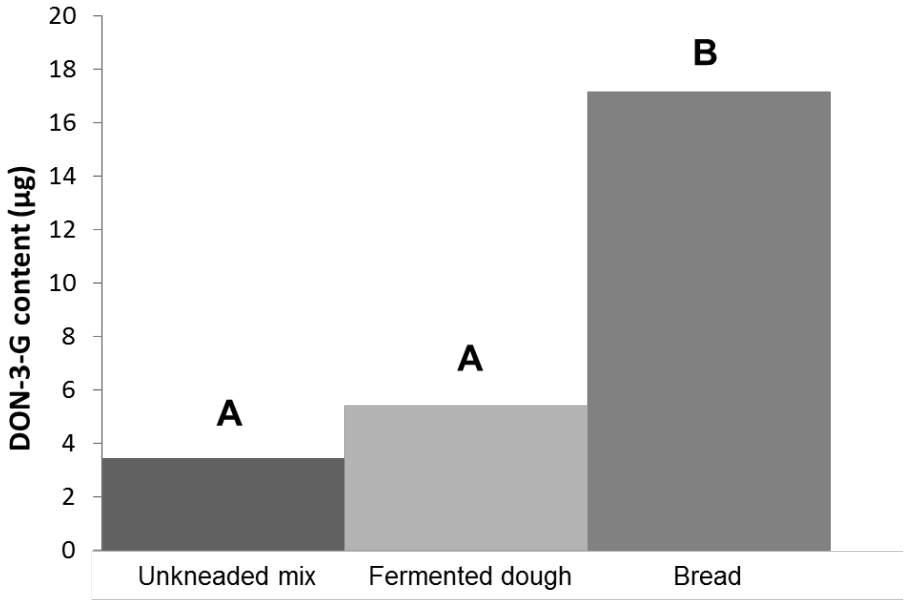



**Figure 4. DON-3-glucoside content (µg) through the breadmaking process (Unkneaded mix, fermented dough and bread).**

**[A,B] Different letters next to the bars indicate significant differences among the treatments, same letter no significant difference (Tukey's test $p < 0.05$).**

Contradictory reports exist regarding the fate of DON during the baking step. Most studies reported DON reduction during baking (El-Banna et al., 1983; Scott et al., 1984; Valle-Algarra et al., 2009), while others (Bergamini et al., 2010; Simsek et al., 2012; Zachariasova et al., 2012) reported an increase in DON concentration after baking; two reasons could lead to such increase: i) shorter baking times together with lower temperatures and ii) bigger bread sizes, as in general those studies reporting reduction were usually at laboratory level with small size loaves (40-80 g). In our case, loaf size was bigger than in most published studies, and according to our temperature

records the inner part of the dough did not reach more than 97 ºC. Probably, DON degradation took place exclusively in the crust but such degradation is not enough to detect a significant decrease in DON content in the whole sample. Finally, baking temperature in our study was lower than average studies and baking time longer, thus the absence of effect of baking does not contradict the general trend of the existing studies.

*3.3. Effect of dough fermentation and baking on OTA*

Due to the high OTA stability observed in preliminary studies, only those samples with 200 g bran/kg flour were analysed. First of all, no significant differences were observed in the results when OTA was added either as contaminated flour or as contaminated bran. The total content of OTA (ng) did not significantly change during fermentation ($p<0.05$, mean of 1518 vs 1581 ng at HMC, and mean of 143 vs 129 ng at LMC). No significant effect of sourdough addition was observed.

The analysis of the sourdough used in this investigation resulted in a high concentration of lactic acid bacteria ($1 \cdot 10^7$ CFU/g) which have been reported to be able to degrade OTA to ochratoxin α (OTα) (Varga et al., 2000). However, the time required to cause OTA degradation should be probably longer than that assayed herein.

Although plant metabolism of OTA has been described in wheat, and OTα, (4R)- and (4S)-4-hydroxy-ochratoxin A and glucosides of both isomers have been found in large amounts, no studies exist either on their fate or their conversion from or to the parent molecule during processing of wheat derivatives.

The total content of OTA (ng) tended to increase during baking although the trend was not significant ($p=0.07$, mean of 1581 vs 2291 ng at the HMC, and mean of 129 vs 245 ng at the LMC). Such increase (mean of 75%) did not depend on any of the assayed factors (initial OTA concentration in flour/bran and sourdough addition).

The high OTA stability through baking observed in the present study is in accordance with Scudamore et al. (2003) and Vidal et al. (2014), under similar conditions. Some studies, however, reported OTA reduction during baking. Valle-Algarra et al. (2009) reached over 20% reduction in the baking bread process. Subirade (1996) also reported OTA reduction (66%) during baking process of wheat biscuits. However, due to the size and shape of such product the penetration of the heat may be higher. Moreover, studies on OTA stability in coffee showed very important reductions (over 90% in some assays) (Pérez de Obano et al., 2005; Van der Stegen et al., 2001), but the temperatures used are much higher than those used in cereal processing (approximately 400 ºC). In summary, OTA in bread baking process seems to be stable and no reduction is observed.

*3.4. DON and DON-3-*glucoside *concentration in flour and bread 'as is'*

DON concentration in bread produced from a given flour batch will depend on the 'dilution' of the toxin with the addition of other ingredients, which depends on the recipe, to a minor extent on water, carbon dioxide and ethanol balance, and finally on the stability of the toxin through the process, which was explained in the previous sections.

The results suggest that from flour with a contamination of 1197 µg/kg it is possible to produce bread with a significantly lower contamination (44-63%) depending on whether sourdough is used or not (Table 3), while the theoretical calculation taking into account the dilution effects of the process leads to a 40% reduction. On the other hand, from a lot of flour containing 565 $\mu g \cdot kg^{-1}$, the final bread would contain DON levels 31% lower. This suggests that the diluting effect of the recipe may be either increased or limited, and even higher concentration of DON than expected thorough ingredients mixing can be encountered depending on initial DON concentration in the flour. This limited reduction is attributed to a certain increase in DON concentration during kneading and fermentation. Moreover, increase in DON concentration from proofed dough to bread was not significant, thus similar concentration can be observed in Table 3.

While DON-3-glucoside level in the fermented dough was not significantly different to that in the flour suggesting that the dilution effect was balanced by DON-3-glucoside increase during kneading and fermentation, a significant increase was observed from fermented dough to bread (Table 3). The resulting bread from flour containing 19.6 µg/kg might contain a concentration more than three times higher. Further studies are required to assess the significance of this fact, and the consequences it might have.

Looking at DON results and literature, it seems that standard fermentation temperature, plus the widespread use of improvers might lead to a minimising of the 'dilution' effect due to recipe. According to Samar et al. (2001) higher fermentation temperatures, by contrast, may lead to DON degradation. The baking stage led to no significant reduction and, overall, a minimum 17% reduction (mean 35%) can be expected for the breadmaking process from flour to bread (in a 260 g bread loaf, with improvers added), which is lower than the 33% assumed by Commission Regulation 1881/2006 setting a maximum level of 750 µg/kg for flour and 500 µg/kg for bread. In our case the only dilution effect would account for 40% reduction, but different recipes for bakery products could lead to much lower reduction, as suggested by Scudamore et al. (2003). On the other hand, DON-3-glucoside concentration greatly increased with baking; the increase could be the glycosidation of DON during the initial stages of baking, before enzyme inactivation (Vidal et al., 2014). This hypothesis is reinforced by the fact that DON-3-glucoside also increased during fermentation, and was previously suggested by Zachariasova et al. (2012). As a consequence, higher DON-3-glucoside concentration could be found in bread than in the initial flour (opposite of what reported by De Angelis et al., 2013). This point is of outmost importance as part of DON can be *in vivo* released from its conjugate (Berthiller et al., 2013) and should be further

investigated. Moreover, a joint regulation of DON+DON-3-glucoside maximum levels might be proposed.

**Table 3. Evolution of mycotoxin concentration (µg/kg) in the different steps of breadmaking process (flour, fermented dough and bread).**

| | Sourdough use | Flour (µg/kg) | Fermented dough 'as is' (µg/kg)* | Bread 'as is' (µg/kg) | % Reduction (kneading +fermentation+proofing)* 30ºC 75 min | % reduction baking |
|---|---|---|---|---|---|---|
| DON | Yes | 1101 | 613 (661) | 747 | 44 % (40%) | -22 % |
| | | 563 | 389 (338) | 469 | 31 % (40%) | -21 % |
| | No | 1293 | 474 (776) | 588 | 63 % (40%) | -24 % |
| | | 566 | 390 (340) | 363 | 31 % (40%) | 7% |
| DON-3-glucoside | | 19.6 | 20.8 (11.8) | 73.8 | -6 % (40%) | -255 % |
| OTA | | 8.6 | 6.1 (5.2) | 9.9 | 29 % (40%) | -62 % |
| | | 0.8 | 0.5 (0.5) | 1.1 | 38 % (40%) | -54 % |

*values in parentheses are calculated taking into account only dilution by recipe

*3.5. OTA concentration in flour and bread 'as is'*

OTA concentration in bread produced from a given flour will depend only on the 'dilution' of the toxin with the addition of ingredients, and on a minor extent to water, carbon dioxide and ethanol balance due to OTA stability during the breadmaking process, as explained in the previous sections, although a trend to increase during baking was observed. Thus OTA concentration (wet basis) was reduced significantly by 29-38% from flour to fermented dough (theoretical decrease, assuming complete stability would be 40%). On the other hand, the average increase from fermented dough to bread was 58% (wet basis), although not significant (theoretical increase, assuming complete thermostability of OTA and 8% weight loss in the bread would be 9%). The whole breadmaking process could end in a concentration 1.4 times higher in bread than in the initial flour, which is in the line of an equal maximum level for both flour and bread (3 µg/kg), as set in the EC (2006).

## 4. Conclusions

As conclusion, OTA was confirmed to be quite stable during the breadmaking process. DON concentration was significantly affected by kneading+fermentation steps in different way

depending on sourdough use and flour contamination level; while DON reduction by fermentation leads to a safer situation, the possible increase in DON should be considered with care, as it can compensate the expected dilution effect by recipe. Finally, the results on DON-3-G, although preliminary because a high percentage of samples were <LOD, suggest a marked release of this toxin during fermentation, but mainly during baking. This point, in particular, requires further investigation.

**Acknowledgements**

The authors are grateful to the Spanish government (projects AGL2010-22182-C04-04 and AGL2011-24862) for the financial support. A. Vidal thanks the Spanish Government (Ministry of Education) for the pre-doctoral grant. H. Morales is grateful to the Portuguese Government (Ministério da Ciência, Tecnologia e Ensino Superior; FCT Fundação para a Ciência e a Tecnologia) Grant ref. SFRH/BPD/38011/2007.

**References**

Abrunhosa, L., Paterson, R.R.M. , Venâncio, A. Biodegradation of ochratoxin a for food and feed decontamination. Toxins 2010, 2, 1078-1099.

Alexa, E., Dehelean, C.A., Poiana, M.-A., Radulov, I., Cimpean, A.-M., Bordean, D.-M., Tulcan, C., Pop, G., 2013. The occurrence of mycotoxins in wheat from western Romania and histopathological impact as effect of feed intake. Chem. Cent. J. 7, 99.

Bergamini, E., Catellani, D., Dall'Asta, C., Galaverna, G., Dossena, A., Marchelli, R., Suman, M., 2010. Fate of F*usarium* mycotoxins in the cereal product supply chain: The deoxynivalenol (DON) case within industrial bread-making technology. Food Addit. Contam. 27, 677-687.

Berthiller, F., Dall'Asta, C., Schuhmacher, R., Lemmens, M., Adam, G., Krska, A.R., 2005. Masked mycotoxins: Determination of a deoxynivalenol glucoside in artificially and naturally contaminated wheat by liquid chromatography-tandem mass spectrometry. J. Agric. Food Chem. 53, 3421-3425.

Berthiller F., Krska R., Domig K.J., Kneifel W., Juge N., Schuhmacher R., Adam G., 2011. Hydrolytic fate of deoxynivalenol-3-glucoside during digestion . Toxicol. Lett. 206, 264-267.

Berthiller, F., Crews, C., Dall'Asta, C., Saeger, S.D., Haesaert, G., Karlovsky, P., Oswald, I.P., Seefelder, W.h, Speijers, G., Stroka, J., 2013. Masked mycotoxins: A review. Mol. Nutr. Food Res. 57, 165-186.

Bullerman, L.B., Bianchini, A., 2007. Stability of mycotoxins during food processing. Int. J. Food Microbiol.119, 140-146.

Cano-Sancho, G., Gauchi, J.-P., Sanchis, V., Marín, S., Ramos, A.J., 2011a. Quantitative dietary exposure assessment of the catalonian population (Spain) to the mycotoxin deoxynivalenol. Food Addit. Contam. 28, 1098-1109.

Cano-Sancho, G., Valle-Algarra, F.M., Jiménez, M., Burdaspal, P., Legarda, T.M., Ramos, A.J., Sanchis, V., Marín, S., 2011b. Presence of trichothecenes and co-occurrence in cereal-based food from Catalonia (Spain). Food Control 22, 490-495.

Codex Alimentarius Comission. 2011. Proposed draft maximum levels for deoxynivalenol (DON) and its acetylated derivatives in cereals and cereal-based products. The Hague, The Netherlands. Available at: ftp://ftp.fao.org/codex/meetings/cccf/cccf5/cf05_06e.pdf (accessed 07.01.13).

Coronel, M.B., Marín, S., Cano-Sancho, G., Ramos, A.J., Sanchis, V., 2012. Exposure assessment to ochratoxin A in Catalonia (Spain) based on the consumption of cereals, nuts, coffee, wine, and beer. Food Addit. Contam. 29, 979-993.

Corsetti, A., Gobbetti, M., De Marco, B., Balestrieri, F., Paoletti, F., Russi, L., Rossi, J., 2000. Combined effect of sourdough lactic acid bacteria and additives bread firmness and staling. J. Agric. Food. Chem. 48, 3044-3051.

Crowley, P., Schober, T.J., Clarke, C.I., Arendt, E.K., 2002. The effect of storage time on textural and crumb grain characteristics of sourdough wheat bread. Eur. Food Res. Technol. 214, 489-496.

De Angelis, E., Monaci, L., Pascale, M., Visconti, A., 2013. Fate of deoxynivalenol, T-2 and HT-2 toxins and their glucoside conjugates from flour to bread: An investigation by high-performance liquid chromatography high-resolution mass spectrometry. Food Addit. Contam. 30, 345-355.

El-Banna, A.A., Lau, P.Y., Scott, P.M., 1983. Fate of mycotoxins during processing of foodstuffs II. Deoxynivalenol (vomitoxin) during making of Egyptian bread. J. Food Prot. 46, 484-486.

European Commission (EC), 2006. Commission Regulation (EC) No. 1881/2006 of 19/12/2006, setting maximum levels for certain contaminants in foodstuffs. Official J. Eur. Union L364, 5-24.

European Commission (EC), 2011. Commission Regulation (EC) No. 1169/2011 of 25/10/2011, on the provision of food information to consumers. Official J. Eur. Union L304, 18-63.

European Food Safety Authority (EFSA). 2006. Deoxynivalenol in food and feed: occurrence and exposure. EFSA J. 11, 3379.

European Food Safety Authority (EFSA). 2006. Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to ochratoxin A in food. The EFSA J., 365, 1-56. Available at: <http://www.efsa.europa.eu/> (accessed 04.04.13).

European Food Safety Authority (EFSA). 2010. Scientific opinion on dietary reference values for carbohydrates and dietary fibre. EFSA J., 8, 1462. Available at: <http://www.efsa.europa.eu/> (accessed 21.01.13).

Faridi, H., Faubion, J.M., 1995. Wheat usage in North America. In H. Faridi and J.M. Faubion, (Eds), Wheat end uses around the world (1-41). St Paul, MN, USA: American Association of Cereal Chemists.

Gobbetti, M., 1998. The sourdough microflora: Interactions of lactic acid bacteria and yeasts. Trends Food Sci. Tech. 9, 267-274.

Gratz, S.W., Duncan, G., Richardson, A.J., 2013. The human fecal microbiota metabolizes deoxynivalenol and deoxynivalenol-3-glucoside and may be responsible for urinary deepoxy-deoxynivalenol. Appl. Environ. Microbiol. 79, 1821-1825.

Horwitz, W., Latimer, G.W., 2006. Official methods of analysis of AOAC International. (18th ed.). AOAC Gaithersburg, Maryland. Chapter 49, 1-49.

IARC (International Agency for Research on Cancer). 1993. Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins In IARC monographs on the evaluation of carcinogenic risks to humans, Vol. 56. Lyon, France.

IARC (International Agency for Research on Cancer). 2002. Monograph on the evaluation of carcinogenic risk to humans, vol. 82. Lyon, France.

Jelinek, C.F., Pohland, A.E., Wood, G.E., 1989. Worldwide occurrence of mycotoxins in foods and feeds an update. Journal AOAC International 72, 223–230.

JEFCA, 2010. WHO Technical Report Series 959: JEFCA, 2010: Seventy-second report of the Joint FAO/WHO Expert Committee on Food Additives: evaluation of certain contaminants in food. WHO Technical Report Series. 959.

Kabak, B., 2009. The fate of mycotoxins during thermal food processing. J. Sci. Food Agric. 89, 549-554.

Kostelanska, M., Dzuman, Z., Malachova, A., Capouchova, I., Prokinova, E., Skerikova, A., Hajslova, J., 2011. Effects of milling and baking technologies on levels of deoxynivalenol and its masked form deoxynivalenol-3-glucoside. J. Agric. Food. Chem. 59, 9303-9312.

Lancova, K., Hajslova, J., Poustka, J., Krplova, A., Zachariasova, M., Dostalek, P., Sachambula, L., 2008. Transfer of *Fusarium* mycotoxins and 'masked' deoxynivalenol (deoxynivalenol-3-glucoside) from field barley through malt to beer. Food Addit. Contam. 25, 732-744.

Li, R., Wang, X., Zhou, T., Yang, D., Wang, Q., Zhou, Y., 2014. Occurrence of four mycotoxins in cereal and oil products in Yangtze Delta region of China and their food safety risks. Food Control 35, 117-122.

L'vova, L.S., Kizlenko, O.I., Shul'gina, A.P., Omel'chenko, M.D., Bystryakova, Z.K., 1998. Distribution of deoxynivalenol in products of processing *Fusarium*-affected soft and hard wheats and barley. Appl. Biochem. Biotechnol. 34, 404-408.

Neira, M.S., Pacin, A.M., Martínez, E.J., Moltó, G., Resnik, S.L., 1997. The effects of bakery processing on natural deoxynivalenol contamination. Int. J. Food Microbiol. 37, 21-25.

Nishio, Z., Kanenori Takata, Ito, M., Tanio, M., Yamauchi, H., Tabiki, T., Ban, T., 2010. Deoxynivalenol distribution in flour and bran of spring wheat lines with different levels of *Fusarium* head blight resistance. Plant Dis. 94, 335-338.

Peña, R.J. Wheat for bread and other foods. In Curtis, B.C., Sajaram, S., Gómez Macpherson, H. (Eds.), *FAO Plant Production and Protection Series: Wheat Bread*. FAO, Rome, 2002. Available at: <http://www.fao.org/DOCREP/006/Y4011E/Y4011E00.htm> (accessed 21.01.13.)

Pacin, A., Ciancio Bovier, E., Cano, G., Taglieri, D., Hernandez Pezzani, C., 2010. Effect of the bread making process on wheat flour contaminated by deoxynivalenol and exposure estimate. Food Control 21, 492-495.

Pérez de Obanos, A., González-Peñas, E., López de Cerain, A., 2005. Influence of roasting and brew preparation on the ochratoxin A content in coffee infusion. Food Addit. Contam. 22, 463-471.

Pestka, J.J., 2010a. Toxicological mechanisms and potencial health effects of deoxynivalenol and nivalenol. World Mycotoxin J. 3, 323–347.

Pestka, J.J., 2010b. Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. Arch. Toxicol. 84, 663–679.

Pomeranz, Y., 1987. Bread around the world. In Y. Pomeranz (Eds.), *Modern cereal science and technology* (pp. 258-333). New York, NY, USA: VCH Publishers.

Samar, M.M., Neira, M.S., Resnik, S.L., Pacin, A., 2001. Effect of fermentation on naturally occurring deoxynivalenol (DON) in Argentinean bread processing technology. Food Addit. Contam.18, 1004-1010.

Scientific Committee on Food (SCF), 1998. Commission of the European Communities, Outcome of discussions 14. Directorate General XXIV. Expressed 17.09.88.

SCOOP, 2002. *Assessment of dietary intake of ochratoxin A by the population of EU member states.* Reports on Tasks for Scientific Cooperation, task 3.2.7. http://ec.europa.eu/food/fs/scoop/3.2.7.pdf

SCOOP, 2003. *Collection of occurrence data of Fusarium toxins in food and assessment of dietary intake by the population of EU member states*. Report on Tasks for Scientific Cooperation task 3.2.10. http://europa.eu.int/comm/food/fs/scoop/task3210.pdf. Accessed 13.10.13

Scott, P.M., Kanhere, S.R., Dexter, J.E., Brennan, P.W., Trenholm, H.L., 1984. Distribution of the trichothecene mycotoxin deoxynivalenol (vomitoxin) during the milling of naturally contaminated hard red spring wheat and its fate in baked products. Food Addit. Contam. 1, 313-323.

Scudamore, K.A., Banks, J., MacDonald, S.J., 2003. Fate of ochratoxin A in the processing of whole wheat grains during milling and bread production. Food Addit. Contam. 20, 1153-1163.

Scudamore, K.A., 2005. Prevention of ochratoxin A in commodities and likely effects of processing fractionation and animal feeds. Food Addit. Contam. 22, 17-25.

Scudamore, K.A., Hazel, C.M., Patel, S., Scriven, F., 2009. Deoxynivalenol and other *Fusarium* mycotoxins in bread, cake, and biscuits produced from UK-grown wheat under commercial and pilot scale conditions. Food Addit. Contam. 26, 1191-1198.

Simsek, S., Burgess, K., Whitney, K.L., Gu, Y., Qian, S.Y., 2012. Analysis of Deoxynivalenol and Deoxynivalenol-3-glucoside in wheat. Food Control 26, 287-292.

Subirade, I., 1996. Fate of ochratoxin A during breadmaking. Food Addit. Contam. 13, 25-26.

Suman, M., Manzitti, A., Catellani, D., 2012. A design of experiments approach to studying deoxynivalenol and deoxynivalenol-3-glucoside evolution throughout industrial production of whole grain crackers exploiting LC-MS/MS techniques. World Mycotoxin J. 5, 241-249.

Thammawong, M., Okabe, M., Kawasaki, T., Nakagawa, H., Nagashima, H., Okadome, H., Nakajima, T., Kushiro, M., 2010. Distribution of deoxynivalenol and nivalenol in milling fractions from *Fusarium*-infected Japanese wheat cultivars. J. Food Prot. 73, 1817-1823.

Valle-Algarra, F.M., Mateo, E.M., Medina, Á., Mateo, F., Gimeno-Adelantado, J.V., Jiménez, M., 2009. Changes in ochratoxin A and type B trichothecenes contained in wheat flour during dough fermentation and bread-baking. Food Addit. Contam. 26, 896-906.

Van der Stegen, G.H.D., Essens, P.J.M., Van der Lijn, J., 2001. Effect of roasting conditions on reduction of ochratoxin A in coffee. J. Agric. Food. Chem. 49, 4713-4715.

Varga, J., Péteri, Z., Tábori, K., Téren, J., Vágvölgyi, C., 2005. Degradation of ochratoxin A and other mycotoxins by *Rhizopus* isolates. . Int. J. Food Microbiol. 99, 321-328.

Vidal, A., Marín, S., Ramos, A.J., Cano-Sancho, G., Sanchis, V., 2013. Determination of aflatoxins, deoxynivalenol, ochratoxin A and zearalenone in wheat and oat based bran supplements sold in the Spanish market. Food Chem. Toxicol. 53, 133-138.

Vidal, A., Morales, H., Sanchis, V., Ramos, A.J. Marín, S. 2014. Stability of DON and OTA during the breadmaking process and determination of process and performance criteria.Food Control 40, 234-242.

Zachariasova, M., Vaclavikova, M., Lacina, O., Vaclavik, L., Hajslova, J., 2012. Deoxynivalenol oligoglycosides: New "masked" *Fusarium* toxins occurring in malt, beer, and breadstuff. J. Agric. Food. Chem. 60, 9280-9291.