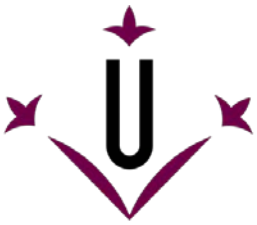

Stability and kinetics of leaching of deoxynivalenol, deoxynivalenol-3-glucoside and ochratoxin A during boiling of wheat spaghettis

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Highlights

- The stability and kinetics of some mycotoxins during boiling of pasta was studied.
- DON leaches to the broth during boiling but it is not degraded.
- A kinetic leaching model for DON was fitted.
- DON-3-glucoside is totally stable through the pasta making process.
- OTA is stable during pasta making, and scarcely transferred to broth during boiling.

Abstract:

The stability of deoxynivalenol (DON), deoxynivalenol-3-glucoside (DON-3-glucoside) and ochratoxin A (OTA) during spaghetti production and cooking was investigated. Initial mycotoxin concentration, boiling time and use of egg as ingredient were assayed as factors. DON was stable during kneading and drying, but a consistent reduction of DON (> 40 %) was observed in boiled spaghettis. According to our results, DON was transferred to broth, where it was not degraded, and boiling time determined the extend of the transfer. A DON leaching model was

fitted to data with a high goodness fit (r^2 = 0.99). This model can be used for prediction of final DON concentration in cooked pasta, and a useful tool in risk assessment models. DON-3-glucoside is totally stable through the pasta making process; moreover DON-3-glucoside is slightly released from pasta components and it is leached to broth. Similarly, OTA is also stable during pasta making, however, it is scarcely transferred to broth during boiling. The presence of egg as ingredient did not affect the final mycotoxin concentration in pasta in any case.

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. Moreover, cereal products represent one of the main sources of exposure to deoxynivalenol (DON) and ochratoxin A (OTA) (Marín, Ramos, Cano-Sancho, & Sanchis, 2013). Different studies show the high presence of mycotoxins in durum wheat (Brockmeyer & Thielert, 2004; Covarelli et al, 2014; Lippolis, Pascale, Cervellieri, Damascelli, & Visconti, 2014). In addition, it has been shown that durum wheat is generally more contaminated with DON than common wheat (Covarelli et al., 2014). The high presence of DON is of concern, because although DON is not classified as to its carcinogenicity to human by IARC (International Agency for Research on Cancer) (1993), but it is linked with human gastroenteritis. On the other hand, OTA is a nephrotoxic mycotoxin which possesses carcinogenic, teratogenic, immunotoxic and possibly neurotoxic properties. This mycotoxin has been classified, by the International Agency for Research on Cancer (IARC, 1993) in the group 2B, as a possible human carcinogen. 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 routinary mycotoxins analysis. The co-occurrence of conjugated DON forms has been documented in raw wheat, especially deoxynivalenol-3-glucoside (DON-3-glucoside) (Berthiller et al. 2009; Dall'Asta, Dall'Erta, Mantovani, Massi, & Galaverna, 2013; Rasmussen, Storm, Rasmussen, Smedsgaard, & Nielsen, 2010) and it is a plant metabolite of DON (Berthiller et al., 2009). Although DON-3-glucoside presence in durum wheat has been detected (Dall'Asta et al., 2013), few studies exist on its occurrence. Berthiller et al. (2011) showed that DON-3-glucoside can be hydrolysed to DON 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).

Processing of wheat at high temperatures might affect DON, DON-3-glucoside and OTA content. Up to now, few studies exist on the fate of DON during the cooking of durum wheat pasta (Table 1), but significant DON reductions have been reported. Such reduction levels may be affected by some factors like ingredients and boiling time. In this way, Visconti, Haidukowski,

Pascale, & Silvestri (2004) showed the importance of the pasta/water ratio: the lower the ratio the greater the reduction. Regarding boiling time, Cano-Sancho, Sanchis, Ramos, & Marín (2013) observed increasing reduction with longer times. Although important DON reductions are detected in cooked pasta, most authors confirm they are mainly attributed to the high water-solubility of DON, thermal degradation playing a minor role; thus, analysis of broth results in high DON concentrations after the boiling step (Cano-Sancho et al., 2013; Nowicki, Gaba, Dexter, Matsuo, & Clear, 1988; Visconti et al., 2004). Moreover, some enzymes can also affect DON stability (Vidal, Ambrosio, Sanchis, Ramos, & Marin, 2016) causing important increases (> 20 %) during the breadmaking process. Enzymes have not been studied in pasta making, however, eggs are a common ingredient in pasta and they contain abundant lysozyme (Alderton & Fevold, 1946), which was not studied in Vidal et al. (2016). Vidal et al. (2016) showed that DON and DON-3-glucoside could be bound to wheat components and enzymes may cleave them releasing DON and DON-3-glucoside. Moreover, egg contains some ovoinhibitors which are protease inhibitors (Liu, Means, & Feeney, 1971) and proteases, in their turn, can have an effect in DON and DON-3-glucoside stability during breadmaking process (Vidal et al., 2016). Although the thermo stability of DON-3-glucoside during baking of wheat products has been widely studied (Kostelanska et al., 2011; Vidal, Morales, Sanchis, Ramos, & Marín, 2014a; Vidal, Sanchis, Ramos, & Marín, 2015), few studies exist about DON-3-glucoside stability during boiling (Zhang & Wang, 2015). Concerning OTA, it showed higher thermo stability than DON during baking (Vidal et al., 2015). Looking at the few existing results, OTA, as well as DON, would be reduced in boiled pasta. For example, Sakuma et al. (2013) observed approximately a 34 % of OTA reduction after 6 min (10 g of pasta with 400 mL of water), and the authors also pointed out to the transfer of OTA to broth.

The existent literature about DON, DON-3-glucoside and OTA during boiling is scarce and more information is required, in particular for exposure assessments. The current study aims to investigate the stability of DON, DON-3-glucoside and OTA during boiling assaying different factors (boiling time, initial mycotoxin concentration and egg presence) in durum wheat pasta and modelling the kinetics of reduction of DON during boiling of pasta.

2. Materials and methods

2.1. DON and OTA contaminated semolina

In order to obtain DON or OTA contaminated semolina, one strain each of *Fusarium graminearum* (TA 3.234) and *Aspergillus ochraceus* (TA 3.201) were used, respectively. Both of them are kept in the Food Technology Dept. collection, University of Lleida, Spain. They were previously proven to be DON and OTA producers when cultured on wheat flour (Vidal et al., 2014a, 2014b, 2015). The concentration of DON and DON-3-glucoside in the initial uninoculated semolina (n=3) was 286.31 ± 21.91 and 72.15 ± 15.24 µg/kg, respectively, while OTA could not be detected.

The strains were inoculated and incubated in MEA (malt extract agar) at 25 ºC for 14 days until strong sporulation. For the inoculation of semolina we followed the method used by Jijakli & Lepoivre (1998). Briefly, a sterile inoculation loop was used to remove the conidia, suspending them in Tween 80 (0.005 %). A spore suspension of each strain was made. After homogenizing, five millilitres of either *F. graminearum* or *A. ochraceus* spore suspension were inoculated in glass flasks containing 250 g of semolina and 50 mL of water. In total, 3 kg of semolina 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. The incubation times were calculated based on our previous knowledge in recent similar studies (Vidal et al., 2015), to achieve the desired mycotoxin contamination in the semolina. Anyway, before ending the incubation period the semolina was sampled to check the concentration attained. Then, each kind of semolina (3 kg) was properly powdered and homogenized and underwent either DON or OTA analysis. The content of DON and OTA was of 3,212.32 ± 80.70 µg/kg and 10.5 ± 0.2 µg/kg respectively (n=3), in each contaminated semolina. DON-3-glucoside was not analysed in the semolina at this stage.

2.2 Spaghetti production

Spaghetti was prepared with 100 g of durum wheat semolina, and 50 g of egg or 40 mL of water. The semolina used was previously prepared by mixing uninoculated semolina with DON contaminated semolina and OTA contaminated semolina, depending on the desired initial mycotoxin concentration: high mycotoxin concentration (HMC) or low mycotoxin concentration (LMC). The analysed toxin levels in the initial mixed semolina (n=3) were: a) HMC, 1310.08 ± 51.63 µg/kg of DON, 60.74 ± 4.39 µg/kg of DON-3-glucoside and 3.52 ± 0.34 µg/kg of OTA; and b) LMC, 572.65 ± 21.51 µg/kg of DON, 70.08 ± 6.50 µg/kg of DON-3-glucoside and 1.58 ± 0.22 µg/kg of OTA. The levels were chosen to be close to real values in food samples (Juan, Covarelli, Beccari, Colasante, & Mañes, 2016). Moreover, the levels were around the maximum levels set by the European Union (European Comission 1881/2006) for processed cereals, such as semolina, which are 750 µg/kg and 3 µg/kg, for DON and OTA, respectively.The DON-3-glucoside concentration was not significantly different in both semolina batches.

The dough was manually mixed until held with a non-sticky, smooth and satiny appearance and optimum handling properties. Then, dough was transferred to a roller machine to get a thin dough sheet (approximately 5 mm), which was later cut into spaghetti (Imperia 650, Imperia & Monferrina SPA, Italy). The resulting spaghettis were hung on metal bars where they were allowed to dry for 12 hours. The water content of the final product was 12.6 ± 0.3 %. Spaghetti (100 g) were cooked for 9 different times (0, 1, 2, 3, 4, 6, 8, 10 and 12 minutes) in 500 mL of broth (2.5 g NaCl), so, the ratio pasta:water was 1:5. Thus 2 initial toxin concentrations x 9 boiling times x 3 replicates made 54 different runs. Additionally, egg pasta was made with the same two different toxin concentrations, however, egg spaghettis were tested only up to 10 minutes. From the 100 g cooked pasta, 25 g were used for OTA analysis, other 25 g for DON and DON-3-glucoside analysis, and the remaining 50 g were kept at - 20 ºC. All samples were

lyophilised for 72 h, and then stored at - 20 ºC until the analyses were performed. Moreover, for each run, 30 mL of broth was kept and stored at - 20 ºC until the mycotoxins analyses were performed.

2.3. Chemicals and reagents

Mycotoxin standard solution of OTA was supplied by Sigma (Sigma–Aldrich, Alcobendas, Spain). DON and DON-3-glucoside were supplied by Biopure (Tulln, Austria). Acetonitrile (≥ 99.9 %), methanol (≥ 99.9 %) and ethanol (≥ 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). Fresh eggs were purchased from La Receta (Madrid, Spain). Phosphate buffer saline (PBS) was prepared with potassium chloride (0.2 g) (Panreac, Castellar del Vallès, Spain), potassium dihydrogen phosphate (0.2 g) (98-100 %, Panreac, Castellar del Vallès, Spain), disodium phosphate anhydrous (1.16 g) (99 %, Panreac, Castellar del Vallès, Spain) and sodium chloride (8.0 g) (≥ 99.5 %, Fisher Bioreagents, New Jersey, USA) in 1 L of milli-Q water; the pH was brought to 7.4 with hydrochloric acid 1 M.

2.4. DON, DON-3-glucoside and OTA by HPLC

2.4.1. Preparation of standard solutions

The standard solution of OTA was dissolved in methanol at a concentration of 500 ng/mL and stored at 4 ºC in a sealed vial until use. From this, a stock solution was prepared and confirmed by UV spectroscopy according to AOAC Official methods of analysis (Horwitz & Latimer, 2006). Working standard solutions (5.0, 1.0, 0.5, 0.01 and 0.05 ng/mL) were prepared by appropriate dilution of known volumes of the stock solution with the mobile phase and were used to obtain calibration curves in the appropriated chromatographic system. The standard solutions of DON and DON-3-glucoside were dissolved in ethanol at a concentration of 10 µg/mL and stored at 4 ºC in a sealed vial until use. DON concentration in the stock solution was confirmed by UV spectroscopy according to AOAC Official methods of analysis (Horwitz & Latimer, 2006). Working standard solutions were 5.0, 1.0, 0.5, 0.1 and 0.05 µg/mL for DON and 1.0, 0.5, 0.1, 0.05 and 0.01 µg/mL for DON-3-glucoside. They were prepared as for OTA, as well as calibration curves.

2.4.2. Sample preparation and analysis with HPLC-UV and HPLC-FL.

For DON and DON-3-glucoside, 5 g of ground sample was extracted with 30 mL of distilled water by magnetically stirring for 10 min. Next, the sample was centrifuged for 8 min at 1780 x *g*. Supernatant was filtered through Whatman 1 filter. On the other hand, broth was centrifuged for 10 min at 1780 x *g* and then filtered through Whatman 1 filter. In both cases, five millilitres of

filtered sample was cleaned-up using a IAC DONPREP® column (R-Biopharm). Zachariasova, Vaclavikova, Lacina, Vaclavik, & Hajslova (2012) confirmed the robust cross-reactivity of DON-3-glucoside with the IAC DONPREP® columns (99-102 % recovery for DON and DON-3-glucoside when less than 500 ng of these toxins was loaded). The purified extracts were dried under a stream of nitrogen 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 quantified using a HPLC Waters 2695® system with an analytical column (Waters Spherisorb® 5 µm ODS2, 4.6 x 250 mm, coupled with a UV/Visible dual λ absorbance Detector Waters 2487). The absorption wavelength was set to 220 nm. The HPLC mobile phase flow rate was 0.6 mL/min. The injection volume was 100 µL. The column temperature was 40 ºC. The retention times for DON and DON-3-glucoside were 20 and 23 min, respectively.

Regarding OTA, 5 g of ground sample were extracted with 30 mL of extraction solution (60 % acetonitrile, 40 % water) by magnetically stirring for 10 min and filtered with Whatman 1 filter. On the other hand, the broth was centrifuged for 10 min at 1780 x *g* and then filtered through a Whatman 1 filter. In both cases, 4 mL of filtered solution was diluted with 44 mL of PBS solution and the resulting extract was cleaned-up using a IAC OCHRAPREP® column (R-Biopharm). The purified extract was dried under a stream of nitrogen. Each dried sample was resuspended with 0.5 mL of mobile phase (acetonitrile:water:acetic acid, 57:41:2). OTA was determined by HPLC (Waters 2695®) coupled with a Multi λ Fluorescence Detector Waters 2475®, and 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. The mobile phase flow rate was 1 mL/min, column temperature was 40 ºC, the injection volume was 100 µL, and the retention time was 15 minutes.

2.4.3. Methods performance for HPLC-UV and HPLC-FL

The analytical methods used were assessed for linearity, precision and recovery. Standard curves were generated by linear regression of peak areas against concentration (r^2 values were 0.99, 0.97 and 0.99 for DON, DON-3-glucoside and OTA, respectively). Precision was estimated by determining DON, DON-3-glucoside and OTA levels in broth and spaghettis, in triplicate, in fortified samples prepared to calculate recovery rates. The limit of detection (LOD) was considered to be three fold greater than the signal of blank noise, and the limit of quantification (LOQ) was calculated to be 3 x LOD. Characteristics of the method performance for DON, DON-3-glucoside and OTA are summarized in Table 2.

2.5. Statistical analysis

Multifactorial ANOVA was applied to assess the significance of sample traits in the observed mycotoxin levels; the software used for multifactorial ANOVA was Statistics 20.0 (IBM SPSS Statistics 20.0 Inc., Chicago, IL). Moreover, linear regression was applied to assess the rates of DON, DON-3-glucoside and OTA reduction during the boiling process.

2.6. Equations

2.6.1. Mass balance

A system of mass balance was developed for DON in the boiling process. The water mass balance was made with 4 products: uncooked pasta, water before boiling, pasta after boiling and broth. The water mass balance between pasta and broth resulted in:

$$H_0 + W_0 = H_t + W_t \qquad (1)$$

H_0 = Content of water in the uncooked pasta (g).

W_0 = Weight of water before to start the boiling step (g).

H_t = Content of water in the cooked pasta at time *t* (g).

W_t = Weight of broth at time *t* (g).

From eq. *1* the W_t is isolated and the weight of the broth at time *t* is known.

$$H_0 + W_0 - H_t = W_t \qquad (2)$$

Knowing W_t a DON mass balance can be made among uncooked pasta, initial water, and cooked pasta at time 12 minutes and broth at time 12 minutes. This balance was made under the assumption than no thermal degradation of DON occurred.

$$y_0 H_0 + x_0 W_0 = y_t H_t + x_t W_t \qquad (3)$$

y_0 = weight of DON in uncooked spaghettis (ng) / (weight of DON + weight of water in pasta in uncooked spaghetti) (g).

x_0 = weight of DON in initial boiling water (ng) / (weight of DON + weight of broth) (g).

y_t = weight of DON in pasta (ng) / (weight of DON + weight of water in pasta) at time t (g).

x_t = weight of DON in broth in balance conditions (ng) / (weight of DON in broth + weight of broth) at time t (g).

When equilibrium between DON in the spaghetti and DON in the broth is reached, y_t will equal x_t.

246 $y_t = x_t = b$ (4)

247 From eq. 3, the value *b* can be calculated as:

248 $$b = \frac{y_0 H_0}{H_t + W_t} = \frac{y_0 H_0}{H_0 + W_0} \quad (5)$$

249

250 *2.6.2. Kinetic calculations*

251 According to literature, several models can be used to explain the kinetics of sorption (e.g. first-
252 order, pseudo-first, pseudo-second-order reaction model) (Ho & McKay, 1999). The studies on
253 the kinetics of leaching of water-soluble compounds have revealed that the pseudo-second-
254 order model provides the best correlation (Ho, Harouna-Oumarou, Fauduet, & Porte, 2005).

255 $dp_t / dt = k \cdot (p_m - p_t)^2$ *(6)*

256 Where

257 p_t = percentage of DON leached at time t (%).

258 *t* = time (min).

259 p_m = maximum percentage of DON leached (%).

260 *k* = leaching rate constant (1/min %).

261 Accordingly, the pseudo-second-order reaction model was applied to our experimental data in
262 order to determine the leaching rate constant. The integrated linear form of the pseudo-second
263 order model is

$$\frac{t}{p_t} = \frac{t}{p_m} + \frac{1}{p_m^2 \cdot k} \quad (7)$$

264

265

266 The leaching rate constant (*k*) comes from the interception.

267

268 **3. Results and discussion**

269 *3.1. DON*

270 Kneading and drying did not cause any difference in DON concentration because DON
271 concentrations in semolina and in uncooked spaghettis were very similar (Table 3). However,
272 DON decreased along time in cooked spaghettis ($p < 0.05$) (Figure 1). Although DON content in
273 pasta dropped during boiling, no further significant DON reduction occurred from minute 2
274 (Figure 1). A similar trend was observed regardless of the initial toxin concentrations, with

percentages of reduction in spaghettis above 30 %. As a result, analysed broth showed a significant increase in DON through time till minute 3-6 (Figure 1), due to the leaching process from pasta to broth. The presence of egg did not affect DON content neither in the preparation nor in the boiling process.

Similar to what was observed here, the existing literature on DON fate during pasta making reported a high stability of DON during kneading. For example, Visconti et al. (2004) found a non-significant slight decrease of DON (10.8 %) after the kneading and drying process; they used a pasta extruder (40 °C at 80-100 bars) and dried the pasta at 80 and 90 °C for almost 5 hours, thus their process was harsher than ours. Also in boiling step, the levels of DON reduction in boiled pasta found in our study agreed with other studies (Brera et al., 2013; Visconti et al., 2004; Zhang et al., 2015). The results show boiling time is a crucial factor in the level of reduction. Cano-Sancho et al. (2013) tested three different times (2, 6 and 12 minutes), with higher reduction with longer time, although the levels after 6 and 12 minutes were very similar (Table 1). Alike, similar DON reduction after boiling for 12 minutes (48.54 %) and 22 minutes (54.30 %) were obtained by Nowicki et al. (1988). This suggests that transfer of DON from pasta to water occurs till equilibrium is reached. This equilibrium point depends on the initial DON concentration because there was more DON in the broth when the initial DON concentration was higher. That way, some authors suggested the ratio pasta/water was an important factor in DON reduction during boiling. Hence, Visconti et al. (2004) showed increasing DON reduction in pasta with decreasing ratio pasta:water (Table 1). The amount of DON retained by cooked spaghettis consistently decreased by increasing the pasta:water ratio during cooking. Different ratios were not tested in the present assay. This suggests that DON reduction in pasta is explained by leaching to water during the boiling process. Previous studies observed DON leaching to water but few information exists on the kinetics of such leaching process. The amount of DON in water plus that in pasta was nearly constant (Figure 1), thus DON thermal stability was confirmed. In fact, boiling conditions (100 °C) are mild and boiling time is short, thus this result was expectable. Baking of bread and bakery products has shown that harsh conditions are required for DON inactivation (e.g. 40 minutes at 160 °C or 20 minutes at 200 °C (Vidal et al., 2015). The high stability of DON in broth agrees with Mishra, Dixit, Dwivedi, Pandey, & Das (2014), who observed DON was only unstable at 125-250 °C showing 16-100 % degradation. Enzymes present in wheat or artificially added to doughs have shown to be important for DON fate (Simsek, Burgess, Whitney, Gu, & Qian, 2012; Vidal et al., 2016). The presence of egg did not cause any change in DON content during spaghetti making process. Water represents more than 75 % of total egg, the rest are mostly lipids and proteins. Regarding enzymes, lysozyme is the main enzyme found in egg and its effect on DON has not been tested. However, the short time involved in kneading and pasta production may not allow for significant enzymatic activity. To our knowledge, this is the first time different ingredients are tested to study DON stability during the boiling of pasta, although some studies exist regarding other food processes, mainly baking (Simsek, Burgess, Whitney, Gu, & Qian, 2012; Vidal et al., 2016).

315

316 3.1.1. Mass balance

317 Initially, DON concentration in pasta decreased quickly till a plateau was reached after six
318 minutes; a parallel increase occurred in the broth, suggesting that an equilibrium was reached
319 (Figure 1). A water mass balance between pasta and broth resulted in the application of the eq.
320 1 (see section 2.6.1), and in our experiment, H_0 is 12.6 because it is the average moisture find
321 in the uncooked spaghettis. W_0 is always 500 g because we always used 500 mL of water for
322 boiling. H_t is 253.4 g, it was the average moisture of our spaghettis cooked for 12 minutes. Eq. 2
323 results in a W_t = 259.2 g. Knowing W_t a DON mass balance (eq. 3) can be made among
324 uncooked pasta, initial water, and cooked pasta at time 12 minutes and broth at time 12
325 minutes. We used minute 12 but any time between 6 and 12 could have been used because all
326 of them are in equilibrium. From the eq. 3, only y_0 and x_0 are known, with y_0 = 9616.16 ng/g and
327 4097.24 ng/g for high and low initial DON concentration, respectively, and x_0 always 0. Then
328 from the eq. 3 we found the *b* values which are 236.84 ng/g and 101.91 ng/g, for high and low
329 initial DON concentration respectively. y_t found in the analysis are 276.77 ± 46.97 ng/g and
330 114.75 ± 22.86 ng/g. The high similarity between predicted and experimental y_t confirms that
331 the system was in equilibrium at minute 12. Experimental x_t were 189.76 ± 29.31 ng/g and 86.67
332 ± 11.19 ng/g for high and low initial DON concentration, so they are also similar to predicted x_t.
333 Thus, if equilibrium is reached at the end of the boiling time, the eq. 5 can be used directly to
334 find the final DON concentration in boiled pasta. It is only necessary to know the DON content in
335 uncooked pasta, the humidity of uncooked pasta, the volume of broth and the final humidity of
336 cooked pasta. The lack of thermal effects plus the equilibrium assumptions were also tested on
337 data from Visconti, et al. (2004), who described all information required for DON balance (Table
338 4). The obtained concentrations experimentally parallel predicted concentrations, so at the end
339 of boiling time the system is in balance and equations can be used to know the DON
340 concentration in boiling spaghettis. The agreement between observed and calculated data
341 confirms that there is not DON degradation during boiling, and that only a leaching process
342 takes place. The amount of DON detected in pasta plus that in the broth at the end of the boiling
343 process equals that in the pasta at the beginning.

344

345 3.1.2. Kinetics of DON leaching

346 As shown in section 3.1.1., DON leached from pasta to broth until an equilibrium point was
347 reached, with some DON still remaining in the pasta. In order to know the remaining DON
348 concentration in pasta at any time point the DON leaching process was studied. The equation
349 described in section 2.6.2 was followed and a pseudo-second-order reaction model was applied
350 to our experimental data in order to determine the leaching rate constant. When the eq. 6 was
351 applied to our data (Figure 2) the slope of the straight line led to a maximum percentage of DON
352 leached (p_m) at equilibrium of 45.45 %. The leaching rate constant (k) was 0.024 min (Table 5).

To our knowledge, there is no previous report on modelling DON leaching during boiling. However some differences in p_m and k could be found in other leaching situations because several factors can influence, mainly pasta:water ratio seems an important factor in DON reduction. It must be pointed out that modelling of mycotoxins behaviour during food processes is essential to provide an applied knowledge about mycotoxins intake by the population, but nowadays scarce works exist about this (Castells, Pardo, Ramos, Sanchis, & Marín, 2006; Ferraz et al., 2010; Numanoglu, Gökmen, Uygun, & Koksel, 2012; Vidal et al., 2015). In particular, exposure assessment studies could benefit from correction of the initial DON concentration in uncooked pasta.

3.2. DON-3-glucoside

The initial semolina contained also DON-3-glucoside (Table 3). DON-3-glucoside content was the same in the two assayed batches because it is a plant conjugate (Berthiller et al., 2009) and till now there is no evidence that it can be produced by fungi. The levels of DON-3-glucoside vary among wheat studies, however the ratio DON-3-glucoside/DON concentration is similar among the assays, from 10 to 30 % (Berthiller et al., 2009; Dall'Asta et al., 2013; Desmarchelier & Seefelder, 2010; Rasmussen et al., 2010). Hitherto, few studies exist about DON-3-glucoside in durum wheat but the ratio DON-3-glucoside/DON in durum wheat could well be similar. We got a ratio of 25 % and Dall'Asta et al. (2013) also obtained ratios between 20 and 30 %. Moreover, DON-3-glucoside is not only found in raw cereals, because some studies indicate the high presence of DON-3-glucoside in cereal based products (De Boevre et al., 2012; Malachova, Dzuman, Veprikova, Vaclavikova, Zachariasova, & Hajslova, 2011). Thus, although it seems it is important to study DON-3-glucoside stability during food processing, few investigations have been made about it and scarce knowledge exists for pasta making process.

The concentration of DON-3-glucoside did not change after kneading and drying pasta, thus the concentrations were similar in semolina and uncooked pasta (Table 3). Regarding boiling, DON-3-glucoside remained nearly constant in spaghettis (Figure 1) through the time. On the other hand, a slight and fast increase of DON-3-glucoside in broth was detected ($p < 0.05$) (Figure 1). This increase suggests that an increase in the total amount of DON-3-glucoside occurred during boiling (Figure 1). The DON-3-glucoside concentration in broth was the same regardless of the initial DON concentration. The presence of egg in formulation instead of water did not cause any change in DON-3-glucoside content (Table 3).

By contrast, Zhang et al. (2015), who studied DON-3-glucoside stability in noodles production detected a significant increase of DON-3-glucoside (69 %) in uncooked pasta. However, they used fermentation (30 minutes at room temperature) after mixing of the ingredients. Fermentation showed to cause an increase in DON-3-glucoside in breadmaking studies (Kostelanska et al., 2011; Vidal et al., 2014a; Vidal, Marín, Morales, Ramos, & Sanchis, 2014b). The high stability of DON-3-glucoside found after boiling of pasta agrees with the results found by Zhang et al. (2015). They did not find any DON-3-glucoside reduction after boiling noodles

for 5 minutes. Similarly, increases of DON-3-glucoside have been observed during baking (Vaclavikova, Malachova, Veprikova, Dzuman, Zachariasova, & Hajslova, 2013; Vidal et al., 2014b), although some studies showed important reductions after baking (De Angelis, Monaci, Pascale, & Visconti, 2013; Kostelanska et al., 2011; Simsek et al., 2012). Vidal et al. (2015) revealed that DON-3-glucoside could either increase under mild baking conditions (for instance 140 º for 35 minutes or 200 ºC for less than 10 minutes), or decrease under harsher temperature/time conditions. The mild conditions involved in boiling (100 °C and short times) may lead to DON-3-glucoside release instead of thermal degradation as in baking. The detected increase of DON-3-glucoside content could be caused by the release of DON-3-glucoside from the matrix due to the thermal treatment. DON-3-glucoside found in broth was not be linked to DON presence, because in one hand no change in the total amount of DON was detected and, in the other hand, DON-3-glucoside content found in broth was independent of the initial DON content. Other baking studies did not find any relation between both toxins (Kostelanska et al. 2011; Vidal et al., 2015); they pointed out to a possible splitting of glycosidic bonds between DON-3-glucoside and cell polysaccharides. However, to our knowledge, their possible relation has not been studied in depth yet.

DON-3-glucoside presence in the broth confirms that leaching from pasta took place (Figure 1). The high solubility of DON-3-glucoside and other DON conjugates has been observed in malting and brewing process (Lancova et al., 2008). Thus during boiling, an increase of DON-3-glucoside content occurs in the pasta due to a release from its components, which is subsequently transferred to broth. Finally, the stability of DON-3-glucoside in spaghettis during boiling is of concern because, although DON-3-glucoside is far less active as protein biosynthesis inhibitor than DON (Poppenberger et al., 2003), DON-3-glucoside will likely be cleaved in the gastrointestinal tract due to chemical hydrolases or, more important, to microbial activity in the intestine as shown *in vivo* in swine and *in vitro* using human intestinal microbiota (Berthiller et al., 2011), thus its presence is important for food safety.

3.3. OTA

Although our semolina batch did not contain OTA, durum wheat has been shown to contain OTA in previous studies (Winnie, Mankotia, Pantazopoulos, Neil, Scott, & Lau, 2009), and some authors pointed out that durum wheat may be more contaminated by OTA than other types of wheat (Kuruc, Manthey, Simsek, & Wolf-Hall, 2014). So, it is important to study the fate of OTA during durum wheat processing to food products.

OTA showed a high stability during the entire studied process. Kneading, drying and boiling of spaghettis did not cause any significant change in OTA concentration. Thus, OTA concentration in semolina and cooked spaghetti was similar regardless of the two initial assayed concentrations (Table 3). However, slight increases of OTA through time were detected in broth (Figure 1) ($p < 0.05$). Furthermore, the level of OTA in the broth depended on the initial OTA

concentration in spaghettis. So, transfers of OTA from spaghetti to water obviously occurred during boiling, although no significant changes in OTA concentration of cooked spaghettis were detected. On the other hand, no variations were detected when egg was used (Table 3).

There is limited information about food processing effects on OTA. OTA stability has been confirmed in the breadmaking process where kneading and fermentation of flour wheat did not cause differences in OTA content (Vidal et al., 2014a). An existing study on OTA fate after boiling of spaghetti showed, by contrast, a 35 % of OTA reduction after boiling 10 g during 6 minutes in 400 mL of water (Sakuma et al., 2013). However they worked with OTA spiked spaghettis, which may easily loose the toxin. In addition they used a high ratio water:pasta which may favour OTA leaching, nevertheless this factor has not yet been studied during boiling process in OTA. The transfer of OTA to water was suggested by Sakuma et al. (2013) because their OTA content in broth paralleled OTA losses in spaghettis. Thus a transfer of OTA to water is possible but not clearly observed in our study. An increase of OTA content in broth was observed when boiling time increased, and the transfer of OTA reached over 15 % in the last minutes of boiling. On the other hand, a 47 % of OTA transfer was reached after boiling for 3 hours in decoctions of herbal medicines (Shim, Ha, Kim, Kim, & Chung, 2014) and 1 % of OTA transfer occurred after 5 minutes of boiling infusion tea (Ariño, Herrera, Estopañan, & Juan, 2007). So, boiling time has an importance in the level of OTA transfer. Finally, the sum of OTA content in broth and boiled spaghetti showed no loss of OTA during the process, so the temperature used in boiling does not cause OTA degradation. OTA is thermo stable; baking studies only showed some reduction under high temperatures (> 140 ºC) and long times (Vidal et al., 2015). The higher transfer of DON to broth could be caused by its higher solubility in water than OTA. DON is one of the more polar trichothecenes with a solubility of 11 g/L at 25 °C in water (Chemicaldictionary, 2009), whereas OTA is hardly soluble in water (1.31 mg/L at 25 °C) (SCR, 2010).

4. Conclusion

DON is stable during kneading and drying, but a high DON reduction (> 40 %) was observed in boiled spaghettis. DON is transferred to broth, where it is not degraded and boiling time determines the extent of the transfer. The use of the DON leaching model developed in the work can be a useful tool in risk assessment under different scenarios of pasta cooking when the initial mycotoxin concentrations in the raw materials are known. By contrast, DON-3-glucoside is totally stable through the pasta making process; moreover DON-3-glucoside is released from pasta components and it is leached to broth. OTA is also stable during pasta making, however it is scarcely transferred to broth during boiling.

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References

Alderton, G, & Fevold, H.L. (1946). Direct crystallization of lysozyme from egg white and some crystalline salts of lysozyme. *The Journal of Biological Chemistry, 164,* 1-5.

Ariño, A., Herrera, M., Estopañan, G., & Juan, T. (2007). High levels of ochratoxin A in licorice and derived products. *International Journal of Food Microbiology, 114,* 366-369.

Berthiller, F., Dall'Asta, C., Corradini, R., Marchelli, R., Sulyok, M., Krska, R., Adam, G., & Schuhmacher, R. (2009). Occurrence of deoxynivalenol and its 3-β-D-glucoside in wheat and maize. *Food Additives and Contaminants – Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 26,* 507–511.

Berthiller, F., Krska, R., Domig, K. J., Kneifel, W., Juge, N., Schuhmacher, R., & Adam, G. (2011). Hydrolytic fate of deoxynivalenol-3-glucoside during digestion. *Toxicology Letters, 206,* 264–267.

Brera, C., Peduto, A., Debegnach, F., Pannunzi, E., Prantera, E., Gregori, E., De Giacomo, M., & De Santis, B. (2013). Study of the influence of the milling process on the distribution of deoxynivalenol content from the caryopsis to cooked pasta. *Food Control, 31,* 309-312.

Brockmeyer, A., & Thielert, G. (2004). Deoxynivalenol (DON) in Durum wheat. *Mycotoxin Research, 20,* 37-41.

Cano-Sancho, G., Sanchis, V., Ramos, A.J., & Marín, S. (2013). Effect of food processing on exposure assessment studies with mycotoxins. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 30,* 867-875.

Castells, M., Pardo, E., Ramos, A. J., Sanchis, V., & Marín, S. (2006). Reduction of ochratoxin A in extruded barley meal. *Journal of Food Protection, 69,* 1139–1143.

Chemicaldictionary, 2009. <http://www.chemicaldictionary.org/dic/D/Deoxynivalenol_842.html> (accessed: 07.01.2015).

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. <ftp://ftp.fao.org/codex/meetings/cccf/cccf5/cf05_06e.pdf> (accessed 07.01.13).

Covarelli, L., Beccari, G., Prodi, A., Generotti, S., Etruschi, F., Juan, C., Ferrer, E., & Mañes, J. (2014). *Fusarium* species, chemotype characterisation and trichothecene contamination of

498 durum and soft wheat in an area of central Italy. *Journal of the Science of Food and Agriculture,*
499 *95,* 540-551.

500 Dall'Asta, C., Dall'Erta, A., Mantovani, P., Massi, A., & Galaverna, G. (2013). Occurrence of
501 deoxynivalenol and deoxynivalenol-3-glucoside in durum wheat. *World Mycotoxin Journal, 6,*
502 83-91.

503 De Angelis, E., Monaci, L., Pascale, M., & Visconti, A. (2013). Fate of deoxynivalenol, T-2 and
504 HT-2 toxins and their glucoside conjugates from flour to bread: An investigation by high-
505 performance liquid chromatography high-resolution mass spectrometry. *Food Additives and*
506 *Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 30,* 345-
507 355.

508 De Boevre, M., Di Mavungu, J.D., Landschoot, S., Audenaert, K., Eeckhout, M., Maene, P.,
509 Haesaert, G., & De Saeger, S. (2012). Natural occurrence of mycotoxins and their masked
510 forms in food and feed products. *World Mycotoxin Journal, 5,* 207-219.

511 European Commission, 2006. Commission Regulation (EC) No. 1881/2006 of 19 December
512 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of European*
513 *Union, L364*, 5–24.

514 Ferraz, M. B. M., Farah, A., Iamanaka, B. T., Perrone, D., Copetti, M. V., Marques, V. X., Vitali,
515 A.A., & Taniwaki, M.H. (2010). Kinetics of ochratoxin A destruction during coffee roasting. *Food*
516 *Control, 21,* 872–877.

517 Ho, Y.S., & McKay, G. (1999). Pseudo-second order model for sorption processes. *Process*
518 *Biochemistry, 34,* 451-465.

519 Ho, Y.S., Harouna-Oumarou, H.A., Fauduet, H., & Porte, C. (2005). Kinetics and model building
520 of leaching of water-soluble compounds of *Tilia sapwood*. *Separation and Purification*
521 *Technology, 45,* 169-173.

522 Horwitz, W., & Latimer, G. W. (2006). Official methods of analysis of AOAC International (18th
523 ed.). Gaithersburg, Maryland: AOAC.

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

527 JEFCA, 2010. WHO Technical Report Series 959: JEFCA, 2010: Seventy-second Report of the
528 Joint FAO/WHO Expert Committee on Food Additives: Evaluation of Certain Contaminants in
529 Food. WHO Technical Report Series. 959.

530 Jijakli, M.H., & Lepoivre, P. (1998). Characterization of an exo-β-1,3-glucanase produced by
531 Pichia anomala strain K, antagonist of Botrytis cinerea on apples. *Phytopathology, 88*, 335-343.

Juan, C., Covarelli, L., Beccari, G., Colasante, V., & Mañes, J. (2016). Simultaneous analysis of twenty-six mycotoxins in durum wheat grain from Italy. *Food Control, 62,* 322-329.

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. *Journal of Agricultural and Food Chemistry, 59,* 9303–9312.

Kuruc, J.A., Manthey, F., Simsek, S., & Wolf-Hall, C. (2014). Survey of ochratoxin A in freshly harvested durum and hard red spring wheat in the United States, 2011 and 2012. *Journal of Food Protection, 77,* 1005-1009.

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 Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 25,* 732-744.

Lippolis, V., Pascale, M., Cervellieri, S., Damascelli, A., & Visconti, A. (2014). Screening of deoxynivalenol contaminantion in durum wheat by MOS-based electronic nose and identification of the relevant pattern of volatile compounds. *Food Control, 37,* 263-271.

Liu, W. H., Means, G. E., & Feeney, R. E. (1971) The inhibitory properties of avian ovoinhibitors against proteolytic enzymes. *Biochimica et Biophysica Acta, 229,* 176–185.

Malachova, A., Dzuman, Z., Veprikova, Z., Vaclavikova, M., Zachariasova, M., & Hajslova, J. (2011). Deoxynivalenol, deoxynivalenol-3-glucoside, and enniatins: The major mycotoxins found in cereal-based products on the Czech market. *Journal of Agricultural and Food Chemistry, 59,* 12990–12997.

Marin, S., Ramos, A.J., Cano-Sancho, G., & Sanchis, V. (2013). Mycotoxins: Occurence, toxicology, and exposure assessment. *Food and Chemical Toxicology, 60,* 218-237.

Mishra, S., Dixit, S., Dwivedi, P. D., Pandey, H. P., & Das, M. (2014). Influence of temperature and pH on the degradation of deoxynivalenol (DON) in aqueous medium: Comparative cytotoxicity of DON and degraded product. *Food Additives and Contaminants – Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 31,* 121–131.

Neira, M.S., Pacin, A.M., Martínez, E.J., Moltó, G., & Resnik, S.L. (1997). The effects of bakery processing on natural deoxynivalenol contamination. *International Journal of Food Microbiology, 37,* 21-25.

Nowicki, T.W., Gaba, D. G., Dexter, J. E., Matsuo, R. R., & Clear, R. M. (1988). Retention of the *Fusarium* mycotoxin deoxynivalenol in wheat during processing and cooking of spaghetti and noodles. *Journal of Cereal Chemistry, 8,* 189-202.

567 Numanoglu, E., Gökmen, V., Uygun, U., & Koksel, H. (2012). Thermal degradation of
568 deoxynivalenol during maize bread baking. *Food Additives and Contaminants – Part A*
569 *Chemistry, Analysis, Control, Exposure and Risk Assessment, 29,* 423–430.

570 Poppenberger, B., Berthiller, F., Lucyshyn, D., Siebrer, T., Schumacher, R., Krska, R., Kuchler,
571 K., Glössl, J., Luschnig, C., & Adam, G. (2003). Detoxification of the *Fusarium* mycotoxin
572 deoxynivalenol by aUDP-glucosyltransferase from *Arabidopsis thaliana*. *Journal of Biological*
573 *Chemistry, 278,* 47905–47914.

574 Rasmussen, R.R., Storm, I.M.L.D., Rasmussen, P.H., Smedsgaard, J., & Nielsen, K.F. (2010).
575 Multi-mycotoxin analysis of maize silage by LC-MS/MS. *Analytical and Bioanalytical Chemistry,*
576 *397,* 765-776.

577 Sakuma, H., Watanabe, Y., Furusawa, H., Yoshinari, T., Akashi, H., Kawakami, H., Saito, S., &
578 Sugita-Konishi, Y. (2013). Estimated dietary exposure to mycotoxins after taking into account
579 the cooking of staple foods in Japan. *Toxins, 5,* 1032-1042.

580 Shim, W.-B., Ha, K.-S., Kim, M.-G., Kim, J.-S., & Chung, D.-H. (2014). Evaluation of the transfer
581 rate of ochratoxin A to decoctions of herbal medicines. *Food Science and Biotechnology, 23,*
582 2103-2108.

583 Simsek, S., Burgess, K., Whitney, K. L., Gu, Y., & Qian, S. Y. (2012). Analysis of deoxynivalenol
584 and deoxynivalenol-3-glucoside in wheat. *Food Control, 26,* 287–292.

585 SRC. 2010. Interactive PhysProp Database Demo. Syracuse Research Corporation <
586 https://esc.syrres.com/fatepointer/webprop.asp?CAS=303479> (accessed: 07.01.2015).

587 Vaclavikova, M., Malachova, A., Veprikova, Z., Dzuman, Z., Zachariasova, M., & Hajslova, J.
588 (2013). 'Emerging' mycotoxins in cereals processing chains: Changes of enniatins during beer
589 and bread making. *Food Chemistry, 136,* 750-757.

590 Vidal, A., Morales, H., Sanchis, V., Ramos, A. J., & Marín, S. (2014a). Stability of DON and OTA
591 during the breadmaking process and determination of process and performance criteria. *Food*
592 *Control, 40,* 234–242.

593 Vidal, A., Marín, S., Morales, H., Ramos, A.J., & Sanchis, V. (2014b). The fate of deoxynivalenol
594 and ochratoxin A during the breadmaking process, effects of sourdough use and bran content.
595 *Food and Chemical Toxicology, 68,* 53-60.

596 Vidal, A., Sanchis, V., Ramos, A.J., & Marín, S. (2015). Thermal stability and kinetics of
597 degradation of deoxynivalenol, deoxynivalenol conjugates and ochratoxin A during baking of
598 wheat bakery products. *Food Chemistry, 178,* 276-286.

Vidal, A., Ambrosio, A., Sanchis V., Ramos, A.J., & Marin, S. (2016). Enzyme bread improvers affect the stability of deoxynivalenol and deoxynivalenol-3-glucoside during breadmaking. *Food Chemistry, Article in press.*

Visconti, A., Haidukowski, E.M., Pascale, M., & Silvestri, M. (2004). Reduction of deoxynivalenol during durum wheat processing and spaghetti cooking. *Toxicology Letters, 153,* 181-189.

Winnie, N.G., Mankotia, M., Pantazopoulos, P., Neil, R.J., Scott, P.M., & Lau, B. P. Y. (2009). Survey of dry pasta for Ochratoxin A in Canada. *Journal of Food Protection, 72,* 890-893.

Zachariasova, M., Vaclavikova, M., Lacina, O., Vaclavik, L., & Hajslova, J. (2012). Deoxynivalenol oligoglycosides: New ‘‘masked’’ *Fusarium* toxins occurring in malt, beer, and breadstuff. *Journal of Agricultural and Food Chemistry, 60,* 9280–9291.

Zhang, H., & Wang, B. (2015). Fates of deoxynivalenol and deoxynivalenol-3-glucoside during bread and noodle processing. *Food Control, 50,* 754-757.

625 Table 1. Effect of boiling in DON content in pasta.

Reference	Cereal	Product	Mycotoxin	Initial mycotoxin concentration (µg/g)	Cooked spaghetti quantity (g)	Pasta/water ratio	Boiling time (min)	NaCl in water (%)	% of mycotoxin reduction	% of mycotoxin in water	Recovered toxin in pasta+water (%)
Nowicki et al., 1988	Durum wheat semolina	Spaghettis	DON	3400-4330 (Natural)	75	1:10	12	0	49.5	39.8	90.3
					75	1:10	22	0	53.4	48.1	94.8
Visconti et al., 2004	Durum wheat semolina	Spaghettis	DON	190-6370 (Natural)	25	1:5	7	0.4	79.6	58.4	78.8
					25	1:4	7	0.5	50.4	55.3	91.56
Sugita-Konishi et al., 2006	Soft wheat flour	Noodles	DON	850 (Natural)	50	1:20	10	0.2	69.4	50.58	81.2
Brera et al., 2013	Durum wheat semolina	Spaghettis	DON	140-190 (Natural)	100	1:10	-	1.0	36.1	-	-
Cano-Sancho et al., 2013	Durum wheat flour	Spaghettis	DON	620 (Natural)	-	-	2	0	38.9	22.1	83.2
							6	0	56.5	58.5	102
							10	0	74.6	73.9	99.3
Sakuma et al., 2013	Soft wheat semolina	Noodles	OTA	5-10 (Spiked)	10	1:40	6	0.1	34.1	34.3	100.2
Zhang et al., 2015	Soft wheat flour	Noodles	DON	900-6870 (Natural)	100	1:10	5	0	52.0	-	-

626 - = data not provided.

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636 **Table 2.** Performances of the DON, DON-3-glucoside and OTA determination in spaghetti and broth.

Mycotoxin	Product	LOD[a] (µg·kg^{-1})	LOQ[b] (µg·kg^{-1})	n	Spiking level (µg·kg^{-1})	Recovery[c] (%)	RSDr[d](%)
DON	Spaghetti	50.0	150.0	3	100	93±6	5.9
				5	500	81±3	3.2
				3	1000	92±7	7.2
	Broth	2.5	7.5	3	20	91±2	14.2
				5	100	87±2	1.9
				3	500	92±6	7.2
DON-3-glucoside	Spaghetti	25.0	75.0	3	50	93±6	5.9
				5	150	82±3	3.2
				3	500	92±7	7.2
	Broth	2.0	6.0	3	5	82±4	4.3
				5	15	84±5	6.5
				3	30	84±4	4.2
OTA	Spaghetti	0.02	0.06	3	0.1	87±13	15.4
				5	1.0	81±9	11.8
				3	5.0	96±1	1.4
	Broth	0.005	0.015	3	0.05	86±4	4.3
				5	0.5	108±2	1.3
				3	1.0	102±3	3.3

637 [a] LOD = Limit of detection.
638 [b] LOQ = Limit of quantification.
639 [c] Mean value ± standard deviation.
640 [d] RSDr = relative standard deviation.
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647 **Table 3.** Evolution of mycotoxin concentration (mean ± standard deviation) in the different steps of pasta making process: semolina (ng/g), uncooked
648 spaghetti (ng/g), cooked spaghetti for 10 min (ng/g) and in broth (ng/mL).

		High Initial Concentration				Low Initial Concentration			
Mycotoxin		Semolina	Uncooked spaghetti	Cooked spaghetti	Broth	Semolina	Uncooked Spaghetti	Cooked spaghetti	Broth
DON	Egg	1310.08±51.63	1323.66±98.96	640.20±18.19*	172.32±15.22	572.65±21.51	562.33±32.23	331.00±45.58*	58.76±5.13
	Without egg		1389.14±18.05	772.82±140.34*	181.60±21.52		591.88±15.68	372.40±28.63*	75.18±14.41
DON-3-glucoside	Egg	60.74±4.39	59.18±11.02	103.65±31.32	8.26±2.37	70.08±6.50	75.03±3.78	73.28±2.77	7.28±1.68
	Without egg		62.99±15.97	85.06±27.56	8.66±3.72		73.45±1.04	82.65±12.47	9.46±2.04
OTA	Egg	3.52±0.34	3.69±0.47	3.51±0.23	0.23±0.00	1.58±0.22	1.47±0.15	1.61±0.27	0.09±0.00
	Without egg		4.26±0.42	4.47±0.22	0.33±0.04		1.69±0.10	1.97±0.53	0.14±0.01

649 * There are significant differences compared to the previous step ($p < 0.05$).

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655 Table 4. Comparison of DON concentration (ng/g) remaining in pasta boiled for 12 minutes without egg from mass balance equation (eq. 5) and
656 experimental values at the end of boiling process for our experiments (high and low initial concentration) and Visconti et al. (2004) results with the DON
657 concentration (ng/g) in the uncooked spaghettis.

		DON content remaining in pasta (ng/g)	
	Initial DON content (ng/g)	Calculated	Observed
High initial concentration	1389.14±18.05	698.91	820.28±180.93

Low initial concentration		591.88±15.68	297.15	325.31±51.32
Visconti et al., 2004	Sample 1	170±30	42.14	37.51±6.78
	Sample 2	230±0.00	58.46	47.00±22.14
	Sample 3	260±20	61.00	48.80±29.82
	Sample 4	500±30	205.51	280.61±35.25
	Sample 5	420±10	175.56	203.34±27.11
	Sample 6	790±70	339.44	389.06±27.11
	Sample 7	1850±60	873.99	993.67±93.09
	Sample 8	3280±410	1281.74	1619.97±150.93
	Sample 9	6970±100	3062.25	2816.99±311.79

Table 5. Comparison of observed and predicted DON concentration in spaghetti without egg during boiling process using the kinetic model.

		High Concentration			Low concentration		
Time (minutes)	Predicted reduction (%)	Observed reduction (%)	Predicted concentration (ng/g)	Observed concentration (ng/g)	Observed reduction (%)	Predicted concentration (ng/g)	Observed concentration (ng/g)
0	0	-0.21±6.11	1389.14	1392.12±84.90	12.27±7.73	591.88	504.71±27.32
1	23.69	20.01±5.02	1060.05	1111.25±69.69	21.12±2.50	451.66	467.05±14.81
2	31.15	29.58±5.40	956.42	978.22±74.97	31.90±4.19	407.51	403.24±24.82
3	34.80	31.28±6.02	905.72	954.66±83.66	30.41±3.85	385.91	412.03±22.88
4	36.97	30.63±9.23	875.57	963.61±128.22	33.89±4.27	373.06	391.44±25.31
6	39.42	44.56±8.98	841.54	770.23±124.79	39.03±1.64	358.56	361.19±9.79
8	40.77	42.12±10.48	822.79	804.07±145.55	41.53±8.17	350.57	346.23±48.39
10	41.63	44.43±10.10	810.84	772.82±140.34	37.10±4.84	345.48	372.40±28.63
12	42.22	40.97±13.02	802.65	820.28±180.93	45.07±8.67	341.99	325.25±51.32

Figure 1. Content of DON (µg) in spaghettis (◆), broth (■) and sum of DON content in spaghettis and broth (▲) over time at high initial DON concentration (a) and low initial DON concentration (b), content of DON-3-glucoside (µg) in spaghettis (◆), broth (■)and sum of DON-3-glucoside content in spaghettis and broth (▲) over time at high initial DON-3-glucoside concentration (c) and low initial DON-3-glucoside concentration (d)and content of OTA (ng) in spaghettis (◆) and broth (■) and sum of OTA content in spaghettis and broth (▲) over time at high initial OTA concentration (e) and low initial OTA concentration (f) (bars indicate standard deviation).

Figure 2. Linear model of DON leaching model through the time.

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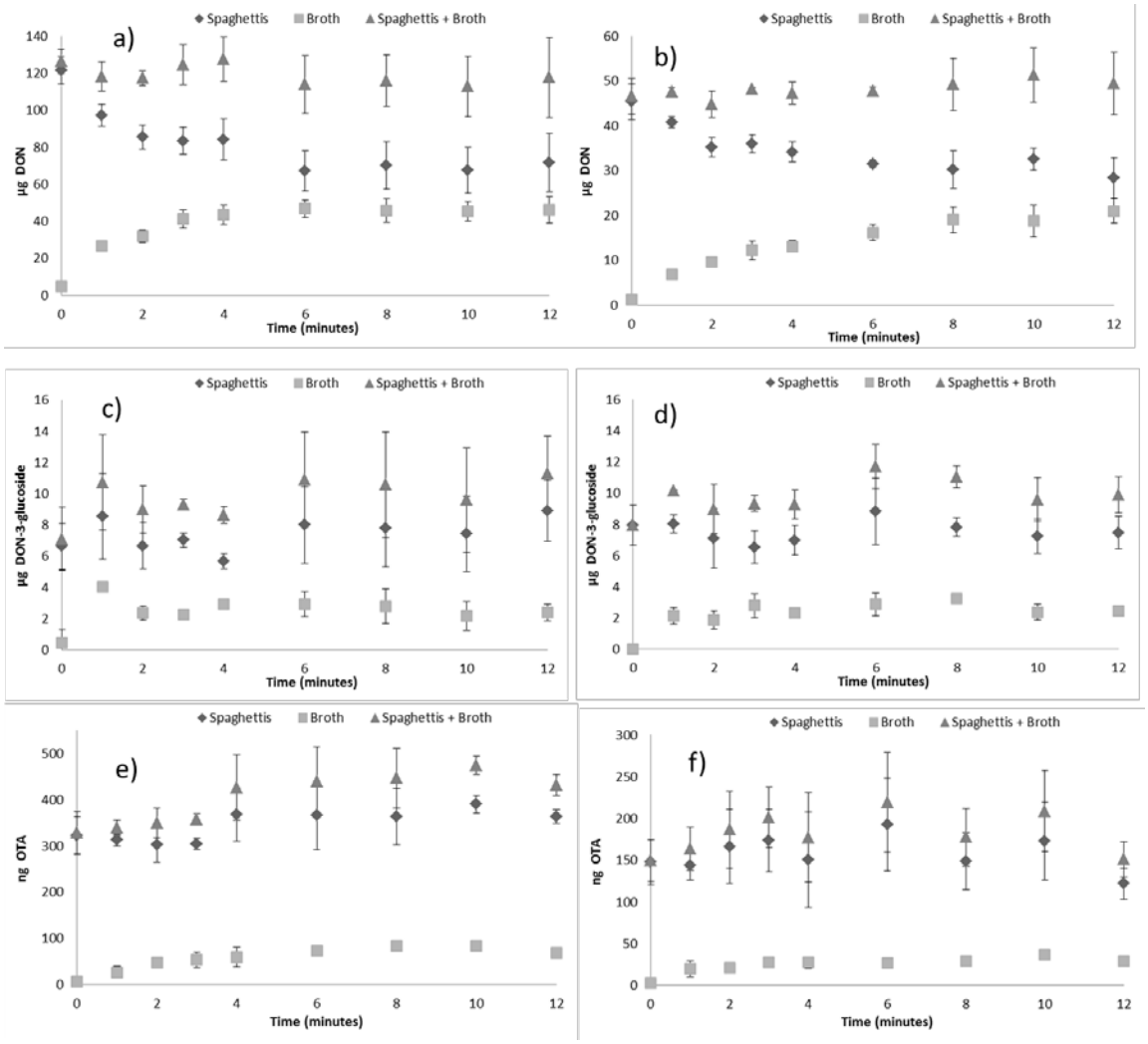

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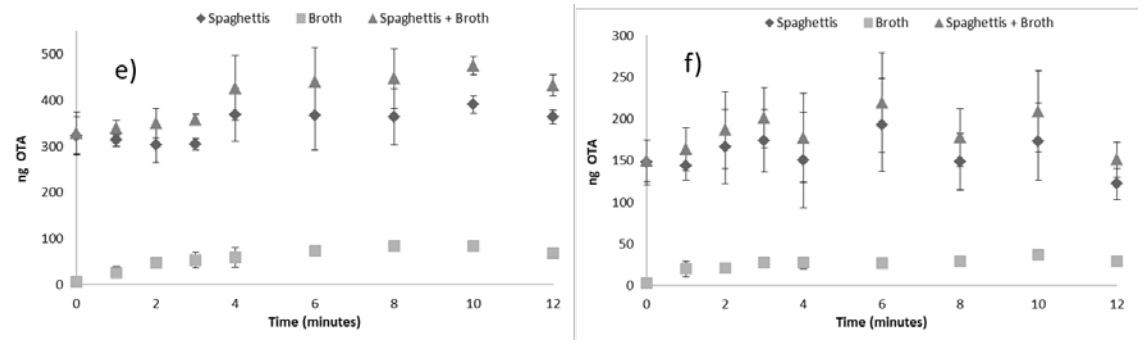

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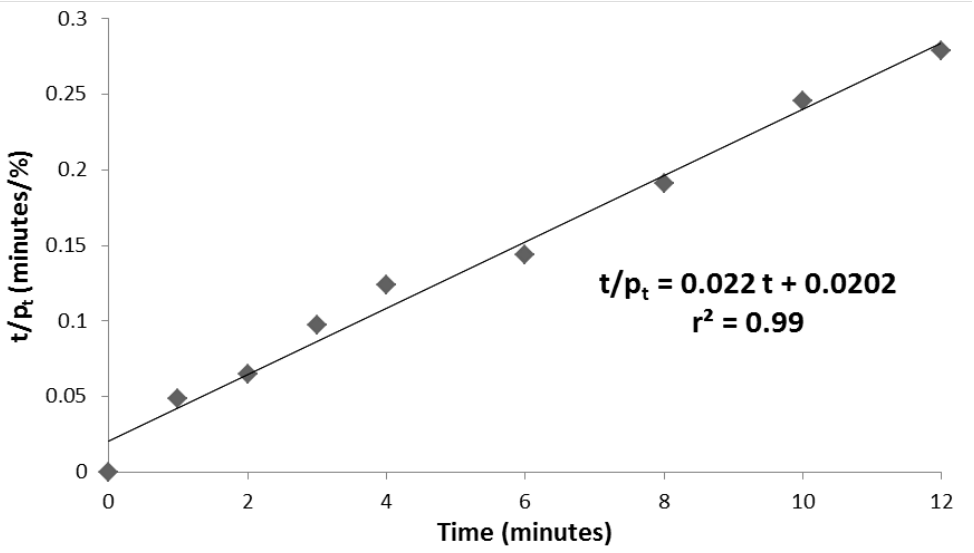
0.3
0.25
0.2
0.15
0.1
0.05
0
0
2
4
6
8
10
12
t/pt (minutes/%)
Time (minutes)
t/pt = 0.022 t + 0.0202
r² = 0.99

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