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

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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 extent of the transfer. A DON leaching model was fitted to data with a high goodness of 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) (Marin, 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,

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**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, Gaba, Dexter, Matsuo, and Clear (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, Haidukowski, Pascale, and Silvestri (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 | 91.6 |
| Sugita-Konishi et al. (2006) | Soft wheat flour | Noodles | DON | 850 (Natural) | 50 | 1:20 | 10 | 0.2 | 69.4 | 50.6 | 81.2 |
| Brera et al. (2013) | Durum wheat semolina | Spaghettis | DON | 140–190 (Natural) | 100 | 1:10 | – | 1.0 | 36.1 | – | – |
| Cano-Sancho, Sanchis, Ramos, and Marín (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 and Wang, (2015) | Soft wheat flour | Noodles | DON | 900–6870 (Natural) | 100 | 1:10 | 5 | 0 | 52.0 | – | – |

– = data not provided.

Visconti et al. (2004) showed the importance of the pasta/water ratio: the lower the ratio the greater the reduction. Regarding boiling time, Cano-Sancho et al. (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 et al., 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, Morales, et al., 2014a; Vidal, Marín, Morales, Ramos, & Sanchis, 2014b; Vidal et al., 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 °C for 14 days until strong sporulation. For the inoculation of semolina we followed the method used by Jijakli and 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, 5 mL 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 °C 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 contents of DON and OTA were of 3212.32 ± 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