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Occurrence of deoxynivalenol in durum wheat from Morocco

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ABSTRACT

Eighty one (81) samples of durum wheat collected from seven areas from Morocco (Khmiset, Beni Mellal, Settat, Fès–Meknès, Skhirat–Témara, Tansift-El Haouz and Rabat–Salé) were surveyed for the presence of the mycotoxin deoxynivalenol (DON). Samples were extracted with water, the extracts were purified by immunoaffinity chromatography (IAC) columns and DON was then identified and quantified with liquid chromatography (LC) coupled to diode array detection (DAD). Analytical results showed that 9 out of 81 total samples (11.1%) were contaminated with DON. DON levels in positive samples ranged between 65 and 1310 μg/kg. The maximum contamination level of DON (1310 μg/kg) was found in a durum wheat sample from Rabat–Salé area, while the minimum value (65 μg/kg) was registered in a sample from Beni Mellal area. The mean DON level in positive samples was 502.1 ± 40.4 μg/kg. Only one previous work exists on DON contamination in Moroccan wheat samples.

1. Introduction

Cereals and derivatives are the basis of the food and feed in Morocco. However, the development of fungi can affect their sanitary quality and cause economic losses. Mycotoxins are natural contaminants produced by a range of fungal species. The Food and Agriculture Organisation (FAO) estimates that 25% of the World food crops, including many basic foods, are affected by mycotoxin producing fungi (Köppen et al., 2010). Indeed, a major cause of impaired health of agricultural crops is contamination by mycotoxins such as trichothecenes. These secondary metabolites are produced by species of the genera *Fusarium*, *Trichothecium*, and *Stachyobotris mycothecium* (Jarvis, 1992; Sharma & Kim, 1991).

Forty trichothecenes were isolated and separated into 4 groups (A, B, C, D) in 1977 (Ueno, 1980). The group B trichothecenes includes the toxin deoxynivalenol. Deoxynivalenol (DON) is a secondary metabolite produced by several *Fusarium* species in various types of grain. It is the most frequently occurring type-B trichothecene and can be found worldwide. DON is also known under its synonym "vomitoxin" and its presence in foods can cause clinical or subclinical manifestations to humans and animals (reviewed by Pestka, 2010). DON occurs in wheat and barley cultivars infected with *Fusarium* head blight (Kokkonen, Ojala, Parikka, & Jestoi, 2010).

A direct relationship between the incidence of FHB and contamination of wheat with DON has been established. The incidence of FHB is strongly associated with moisture at the time of flowering (anthesis). The timing of rainfall, rather than the amount, is the most critical factor in *Fusarium* infection. DON contents are significantly affected by the susceptibility of cultivars toward *Fusarium* species, previous crop, tillage practices, and fungicide use. *Fusarium graminearum* grows optimally at 25 °C and at a water activity above 0.88, while *Fusarium culmorum* grows optimally at 21 °C and at a water activity above 0.87 (Moss, 2002). Sanchis and Magan (2004) reported that the optimal temperatures for in vitro growth were (20–22 °C) and (20–25 °C) for *F. graminearum* and *F. culmorum*, respectively. However, the temperature values for the synthesis of DON in vitro for the same species were 30 °C and 26 °C respectively. The geographical distribution of *Fusarium* species is related to temperature and humidity requirements (Bhatnagar, Payne, Cleveland, & Robens, 2004).

The toxin DON (vomitoxin) is known for its toxicological effects mainly on the immune system and gastrointestinal tract. Indeed, chronic ingestion of DON in low doses causes anorexia, reduced growth, alters nutritional efficiency, while at high doses causes more acute effects such as vomiting, rectal bleeding and diarrhea (Pestka & Smolinski, 2005; Rotter, Prelusky, & Pestka, 1996; SCF 1999).

DON has been described as teratogen, neurotoxin, and immunosuppressant and trichothecenes in general have been associated with chronic and fatal intoxication of humans and animals through

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consumption of contaminated food and feed (JECFA, 2001a, 2001b; Rotter et al., 1996). The acute effects of DON (nausea, vomiting, diarrhea, abdominal pain, etc.) are difficult to distinguish from the gastrointestinal effects of microbes and have been attributed to the presence of DON at concentrations of 3–93 mg/kg in grain for human consumption. It was also demonstrated that DON has long-term toxic effects on the immune system, growth, and reproduction (Bhatnagar et al., 2004).

Given their toxicity, the international legislations have been set to limit and restrict the contamination of food by these toxins and therefore protect consumers health. According to the regulations of the European Commission, the maximum allowable level of DON in durum wheat for human consumption is 1750 μg/kg (European commission (EC), 2007).

In Africa, the presence of mycotoxins in food is often overlooked due to public ignorance about their existence. Morocco has a climate characterized by high humidity and high temperature which favors growth of molds. The presence of mycotoxins (aflatoxins, ochratoxin A, fumonisins, zearalenone, etc.) in foods has been reported in Morocco (Zinedine, Juan, et al., 2007; Zinedine & Mañes, 2009; Zinedine, Soriano, Juan, Mojemmi, et al., 2007; Zinedine, Soriano, Juan, Molto, et al., 2007). However limited information is available on the presence of DON and its associated fungi, in cereals.

The aim of this study was to analyze for the first time, samples of durum wheat from seven areas from the kingdom of Morocco for determination of DON by using liquid chromatography with DAD detection.

2. Materials and methods

2.1. Chemical and reagents

DON standard was supplied by Sigma (Sigma-Aldrich, Alcobendas, Spain). Acetonitrile and methanol were purchased from J.T. Baker (Deventer, The Netherlands).

All solvents were LC grade. Pure water was obtained from a Milli-Q apparatus (Millipore, Billerica, MA, USA) and was used when water was required.

2.2. Sampling

Eighty one (81) samples of durum wheat were randomly collected from different traditional markets, farms or in establishments of the Ministry of Agriculture ("Office National Interprofessionnel des Céréales et des Légumineuses", ONICL and The "Office National de Sécurité Sanitaire des Produits Alimentaires" ONSSA) in seven areas of Morocco Khmiset (12), Beni Mellal (10), Settat (11), Fès–Meknès (13), Skhirat–Témara (10), Tansift-El Haouz (12) and Rabat–Salé(13). The samples were packed in plastic bags, then stored at 4 °C prior to analyses.

2.3. DON analysis

The extraction of DON was carried out with an in-house validated method at the Laboratory of Applied Mycology of the Food Technology Department of the University of Lleida. Briefly, samples were first ground and 10 g were extracted with 40 mL of MilliQ water by shaking on a magnetic stirrer (SBS multipoint) at 700 rpm for 10 min. Samples were centrifuged (hettich zentrifugen EBA12) at 6000 rpm for 5 min. Four (4) mL of the supernatant were collected and passed through an immuno-affinity column (DONPREP®, R-Biopharm, Rhone Ltd). The column was then washed with 10 mL of MilliQ water, the toxin was finally eluted with 3 mL of methanol. The collected eluate was evaporated with a stream of nitrogen at 40 °C until dry, and then dissolved with 500 μL of the mobile phase for LC analysis.

2.4. Chromatographic conditions

The DON was detected and quantified by using an LC system (Waters 2695, separations module, Waters, Milford, USA) and a column (Waters Spherisorb 5 μm 4.6 × 150 mm analytical column). The mobile phase (water/acetonitrile/methanol; 94/3/3, v/v/v) was pumped at 1.2 mL/min. The temperature of the column oven was 35°. The absorbance detector 2487 (Waters, Milford, USA) was set at 220 nm. The retention time of DON under these conditions was 16 min. The detection limit was 62.5 μg/kg. Quantification was always achieved with a software integrator (Empower, Milford, MA, USA). The toxin DON was quantified on the basis of the HPLC absorbance response compared with that of a range of analytical standards.

2.5. Validation of analytical method

The analytical method used for DON was assessed for selectivity, linearity (Fig. 1), and precision. Selectivity was checked by injecting three times 50 mL of DON standard solutions before injecting extracted samples and comparing the peak retention times and the UV spectra of the substances that produce these peaks. Linearity was assessed by performing triplicate injections of standard solutions at concentrations of 0.02, 0.05, 0.1, 0.25, 0.5 and 1.0 μg DON/ml. Standard curves were generated by linear regression of peak areas against concentrations. Calibration curves showed good linearity with correlations coefficients R^2 ranging from 0.9799 to 0.9994.

Accuracy and recovery were established by determination of DON in wheat spiked samples at 0.05, 0.2 and 1.0 μg/g ($n = 3$). Recovery values ranged from 76 to 110%, while RSDr ranged from 6 to 18%. The limit of detection (LOD) was considered as the DON concentration that provides a signal equal to $b + 3S_b$, where b is the intercept of the calibration curve and S_b is the standard error of the estimate assuming to be the blank, and the limit of quantification (LOQ) was considered equal to 3 × LOD. Quantification limit for DON was 0.04 μg/g.

3. Results and discussion

The presence of DON in durum wheat samples is summarized in Table 1. As shown, there were 11.1% of samples contaminated with DON. The toxin levels in positive samples ranged from 65 to 1310 μg/kg. The maximum contamination level of DON (1310 μg/kg) was found in a durum wheat sample from Rabat–Salé area, while

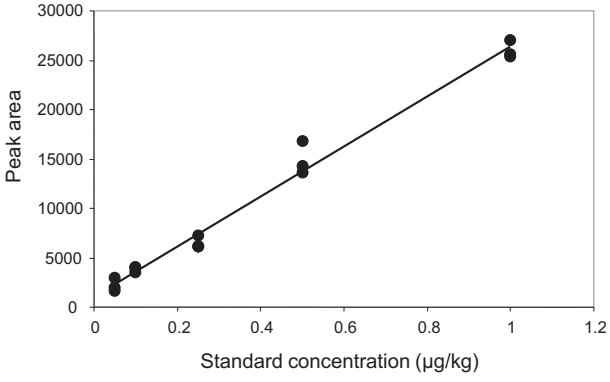

Fig. 1. DON calibration curve for HPLC analysis.