Food Control 37 (2014) 56–61

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Fumonisin occurrence in naturally contaminated wheat grain harvested in Argentina

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ARTICLE INFO

Article history:
Received 2 July 2013
Received in revised form
2 September 2013
Accepted 14 September 2013

Keywords:
Fumonisin
Durum wheat
Common wheat
Deoxynivalenol

ABSTRACT

A survey was carried out to determine fumonisin contamination in 135 common and 40 durum wheat samples collected during 2011 harvest season (non-FHB epidemic year) in the main wheat production area of Argentina using LC–MS/MS. A 93% of total samples showed fumonisin contamination, with levels ranging from 0.16 to 680.44 ng/g in common and from 0.15 to 1304.39 ng/g in durum wheat samples, respectively. FB_1 was the fumonisin most frequently found during the evaluated year. Twenty five wheat samples (15 common and 10 durum) were selected for a deoxynivalenol (DON) analysis among all the samples analyzed for fumonisin content using different contamination levels as selection criteria. DON contamination was present in 24 out of 25 wheat samples, the levels ranging from 50.60 to 28650 ng/g. Nine out of 25 wheat samples reached values higher than 1000 ng/g. However there was no correlation between fumonisin and DON contamination. This is the first report of natural fumonisin presence in common wheat grains in Argentina, as well as of DON co-occurrence in both types of wheat.

1. Introduction

Fumonisins are toxic fungal metabolites produced mainly by *Fusarium* species. Fumonisin B_1 (FB_1) is the most significant in terms of occurrence and toxicity. FB_1 can cause severe disorders in animals such as leukoencephalomalacia in horses (Marasas et al., 1988), as well as pulmonary edema syndrome and hydrothorax in pigs (Haschek et al., 1992); this toxin has also shown nephrotoxic, hepatotoxic and hepatocarcinogenic activities in rats (Wan Norhasima, Abdulamir, Abu Bakar, Son, & Norhafniza, 2009). Further, consumption of fumonisin-contaminated maize has been epidemiologically associated with esophageal cancer (Marasas, 2001) and neural tube defects in some human populations (Missmer et al., 2006). The International Agency for Research on Cancer (IARC) designated FB_1 in Group 2B as "a possible carcinogenic to humans" (IARC, 2002).

Several *Fusarium* species are able to produce fumonisins, but the two most important ones are *Fusarium verticillioides* and *Fusarium proliferatum*, which are common fungi associated with maize, but can also be isolated from other substrates such as wheat. Fumonisins are geographically widely distributed and their natural occurrence has been reported mostly in maize, but also in other grains and grain-based products such as wheat, wheat based foods, semolina, farro, bread and others (Scott, 2012).

Wheat is the most important cereal consumed by the Argentine population. In this country human consumption of products manufactured with wheat, either semolina (*Triticum turgidum* L. var. *durum*) or bread (*Triticum aestivum*), is much greater than for products made from other cereals (Food Balance Sheet, 2007; Pacin, Ciancio Bovier, Canoa, Taglieri, & Hernandez Pezzani, 2012). Durum wheat in Argentina is mainly used for pasta elaboration, with its production reaching 604,651 tons in 2011. Pasta national production reached almost 183,000 tons in 2011, and the consumption per capita was estimated at 7.9 kg/year. On the other hand, common wheat is mostly used for bread elaboration, breakfast cereals, cookies and cupcakes, its production was of 15,271,000 tons in 2011. It is remarkable that wheat flour consumption per capita in Argentina was estimated at 7.4 kg/habitant/month. Sixty three percent of the total wheat cultivation area is concentrated in Buenos Aires province (MAGyP, 2013).

The main pathogen associated with *Fusarium* Head Blight (FHB) wheat disease in common and durum wheat in Argentina is

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http://dx.doi.org/10.1016/j.foodcont.2013.09.031

Table 1
Geographic locations and climatic conditions of the sampled grain storage companies and commercial fields.

Locations		Elevation (m)	Annual precipitation (mm)	Average annual temperature (°C)
Common wheat				
Junin	34° 31′ S, 60° 52′ W	81	953.52[a]	16[a]
Baigorrita	34° 45′ S, 60° 59′ W	80		
Alberti	35° 1′ S, 60° 15′ W	38		
Bragado	35° 7′ S, 60° 30′ W	50		
9 de Julio	35° 27′ S, 60° 52′ W	78		
Casares	35° 37′ S, 61° 22′ W	72		
Durum wheat				
La Dulce	38° 20′ S, 59° 0′ W	72	685.4	14.1
Balcarce	37° 45′ S, 58° 18′ W	130	758.9	13.3
Miramar	38° 10′ S, 58° 0′ W	50	776.5	14,3
Barrow	38° 20′ S, 60° 13′ W	120	688.5	14.7
Bordenave	37° 50′ S, 63° 1′ W	212	711.4	15.1

[a] Conditions for all the whole area between latitude 34°–37°S and longitude 58°–62°W.

Fusarium graminearum Schw, perfect stage *Gibberella zeae* (Schw) Petch (Lori, Sisterna, Haidukowsky, & Rizzo, 2003; Ramirez, Reynoso, Farnochi, & Chulze, 2006; Ramirez et al., 2007). Also deoxynivalenol (DON) has been reported in both types of wheat (Dalcero, Torres, Etcheverry, Chulze, & Varsavsky, 1997; González, Pacin, Resnik, & Martinez, 1997; Lori et al., 2003). However Ramirez, Oviedo, Farnochi, and Chulze (2006) carried out a mycological survey during a non-FHB epidemic year in common wheat, and found that the predominant *Fusarium* species were *F. proliferatum*, *Fusarium subglutinans* and *F. verticillioides*. Also, Palacios et al. (2011) found that the predominant *Fusarium* species isolated from durum wheat during a non-FHB epidemic year were *F. proliferatum*, *F. subglutinans*, *F. verticillioides* and *Fusarium equiseti*. Moreover, fumonisin contamination has been reported in both kinds of wheat and sub-products in many countries (Busman, Desjardins, & Proctor, 2012; Chehri, Jahromi, Reddy, Abbasi, & Salleh, 2010; Cirillo, Ritieni, Galvano, & Amodio Cocchieri, 2003; Jakšić et al., 2012; Roscoe et al., 2008) but not in Argentina, where fumonisins were reported only in durum wheat (http://www.ncbi.nlm.nih.gov/pubmed?term=Palacios%20SA%5BAuthor%5D&cauthor=true&cauthor_uid=21999326 Palacios et al., 2011).

Due to the importance of wheat in Argentinean population diet, and because it has been proposed, in a study in the Netherlands, that fumonisin intake occurs mainly via the intake of wheat and wheat-products (Bakker, Speijers, & Paulsch, 2003), the aim of this work was to determine the natural occurrence of fumonisins in common and durum wheat during a non-FHB epidemic year, and its possible co-occurrence with DON.

2. Materials and methods

2.1. Sample collection

One hundred thirty-five common wheat samples (harvest 2011) were obtained upon arrival from 6 local grain storage companies located in Junin, Bragado, Casares, Baigorrita, 9 de Julio and Alberti (Table 1), in Buenos Aires Province, the main wheat production area in Argentina. Wheat samples were taken from trucks, with a load capacity between 25 and 30 tons. Wheat was taken in six different truck positions using a vacuum sampling device in order to obtain an aggregate sample of 10 kg of randomized seeds. This sample was homogenized and sub-samples of 1 kg were taken, finely milled using a Romer mill (Romer, Union, MO, USA), thoroughly mixed and stored in bags in the dark at 4 °C until analysis. Also, forty durum wheat samples (500 g) were randomly collected during the 2011 harvest season in 5 different commercial fields located in the major durum wheat production area in Argentina, south of Buenos Aires province (Table 1). Samples were collected from each field and pooled; from this pool, a subsample of 500 g was taken. These subsamples were immediately stored at 4 °C until mycotoxin analyses. All wheat samples (common and durum) were asymptomatic i.e. without evident kernel damage.

2.2. Analytical reagents

The standard of DON was purchased from Sigma–Aldrich (Buenos Aires, Argentina). FB_1 and FB_2 stock solutions in acetonitrile:water (1:1) were provided by Biopure (Tull, Austria). Analytical grade reagents, HPLC grade solvents and HPLC grade water were purchased from Panreac Quimica S.A.U. (Barcelona Spain). MycoSep®227 Trich+ cleanup columns were obtained from Romer (Romer Labs Inc., Union, MO). Bond-Elut strong anion-exchange (SAX) cartridges were obtained from Agilent Technologies Inc. (Agilent Technologies Inc., Santa Clara, CA).

DON stock I solution of 500 μg/mL was prepared in methanol, a second stock was obtained diluting the stock I in methanol to achieve a final concentration of 100 μg/mL. They were stored in the darkness in glass-stoppered bottles under secure conditions at −20 °C. DON working standard solutions for HPLC calibration curve were prepared by dissolving diluting adequate amounts previously evaporated to dryness under nitrogen stream of the stock II solution in water:methanol (95:5), previously evaporated to dryness under nitrogen stream. Stock solution of FB_1 and FB_2 (50 μg/g) was diluted with acetonitrile:water (1:1) in order to obtain the appropriate working solutions, and were stored in darkness at −20 °C until LC–MS/MS analysis.

2.3. Wheat fumonisin extraction

The fumonisin analysis performed was based mainly on the method of Shephard, Sydenham, Thiel, and Gelderblom (1990) as described by Doko, Rapior, Visconti, and Schjoth (1995). Subsamples of about 100 g were finely ground in a Buehler laboratory mill and thoroughly mixed. Aliquots of the ground subsamples (25 g) were shaken with 50 mL of methanol:water (3:1) for 30 min and filtered through Whatman N° 4 filter paper. While the flow rate was maintained below 2 mL/min, 10 mL of the filtered extract was applied to a Bond-Elut strong anion-exchange (SAX) cartridge (Agilent Tecnologies Inc., Santa Clara, CA) fitted to a Supelco solid-phase extraction (SPE) manifold (Supelco, Bellefonte, PA), previously conditioned by the successive passage of methanol (5 mL) and methanol:water (3:1, 5 mL). The cartridge was then washed with methanol:water (3:1, 8 mL) followed by methanol (3 mL), and fumonisins were eluted with 0.5% acetic acid in methanol (14 mL). The elute was evaporated to dryness at 40 °C, under a moderate stream of nitrogen, and stored dry at 4 °C until HPLC LC–MS/MS analysis.

2.4. Fumonisin LC–MS/MS analysis

Fumonisin detection was performed using a Waters 2695 Alliance HPLC (Waters Corporation, Milford, MA, USA) equipped with a Waters Alliance 2685 pump, a Waters Alliance 2695 autosampler, a diode array detector Waters 2996 PDA interfaced to a Quattro Ultima Platinum tandem quadrupole mass spectrometer with electrospray ionization (ESI) source. An XBridge™ C18 column (3.5 μm, 2.1 × 150 mm) with a guard column of the same material (Waters, Milford, MA) was used. An isocratic chromatographic procedure was performed with 5 mM ammonium acetate in