Food and Chemical Toxicology 51 (2013) 259–266

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

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

Emerging *Fusarium* mycotoxins in organic and conventional pasta collected in Spain

A.B. Serrano, G. Font, J. Mañes, E. Ferrer *

Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Spain

ARTICLE INFO

Article history:
Received 26 April 2012
Accepted 27 September 2012
Available online 11 October 2012

Keywords:
Emerging *Fusarium* mycotoxins
Dietary exposure
Pasta
Liquid chromatography–tandem mass spectrometry

ABSTRACT

One of the main sources of emerging *Fusarium* mycotoxins in human nutrition is the cereals and cereal products. In this study, an analytical method to determine enniatins A, A1, B and B1 (ENs), beauvericin (BEA) and fusaproliferin (FUS) based on Ultra-Turrax extraction followed by liquid chromatography coupled to triple quadrupole mass spectrometer detector (MS/MS QqQ), was applied for the analysis of pasta. For this purpose, 114 commercial samples of pasta were acquired from supermarkets located in Valencia. The results showed higher frequencies of contamination in organic pasta than in conventional pasta, while the concentration levels were variable for both types of pasta. In positive samples, BEA levels varied from 0.10 to 20.96 μg/kg and FUS levels varied from 0.05 to 8.02 μg/kg. ENs levels ranged from 0.25 to 979.56 μg/kg, though the majority of the values were below 25 μg/kg. Besides, it was observed the simultaneous presence of two or more mycotoxins in a high percentage of the samples. Finally, an evaluation of the dietary exposure of the emerging *Fusarium* mycotoxins was performed in the Spanish population. The prevalence of ENs, BEA and FUS in cereal products suggests that the toxins may pose a health risk to Spanish population.

1. Introduction

Wheat is the most consumed cereal worldwide: global consumption of cereals (excluding beer) was 146.60 kg/*per capita* during 2007, and the global consumption for wheat reached about 66 kg/*per capita* (FAO, 2007). The Spanish population is one of the largest consumer of wheat in the world: the mean Spanish consumption of wheat (87.40 kg/*per capita*) was higher respect to the worldwide consumption in the year 2007 (FAO, 2007). Usually, wheat is ground to flour for the production of bread, pasta, biscuits and other products. Nowadays, pasta constitutes one of the most important wheat-based products of the Spanish economy: during the last years global consumption of pasta in Spain has increased around 6% between 2007 and 2011 (MAGRAMA, 2011).

Wheat-based products are one of the main sources of mycotoxins in both the human and animal diets. Surveillance studies have indicated that mycotoxin contamination is a world-wide problem (SCOOP, 2003), since it causes economic losses, both for the grain and for the marketing of foods and feeds, and is a potential threat to animal and human health. In 2006, the European Union proposed the maximum levels (MLs) for some mycotoxins in foodstuffs: aflatoxins, fumonisins and trichothecenes among other (Commission Regulation, 2006). For some *Fusarium* mycotoxins, MLs have been established mainly for cereals and cereal-based products, since usually *Fusarium* species are able to infect cereal crops. The contamination of mycotoxins in cereals is known to be affected by the local climate (rainfall, temperature or relative humidity), agricultural practices, harvest logistics, transport and storage conditions, and processing of products (Bakan et al., 2002). Specially, in the last few years the relationship between the influence of the agricultural practices (traditional or organic) and mycotoxin contamination have been discussed by many authors. In organic practices, chemical products (fungicides, pesticides, etc.) are no employed increasing the exposure of cereal grains to fungal colonization and to mycotoxin production. Some studies have supported this affirmation (ÓArco et al., 2009; Silva et al., 2009), but other studies have not observed significantly differences between organic and conventional products (González-Osnaya et al., 2007; Ariño et al., 2007). This fact can be due to the crop rotation used in organic and conventional agricultural practices, which prevents the transmission of plant diseases (Jestoi et al., 2004b).

Fusarium genus is probably the most prevalent toxin-producing fungi of the Northern temperate region. They are commonly found on cereals grown in the temperate regions of America, Europe and Asia (SCF, 2002). *Fusarium avenacum, Fusarium moniliforme, Fusarium proliferatum* and *Fusarium subglutinans* are the main producers of emerging *Fusarium* mycotoxins enniatins A, A1, B, B1 (ENA, ENA1, ENB and ENB1, respectively), beauvericin (BEA) and fusaproliferin (FUS) in various cereals, especially wheat, barley and maize (Jestoi, 2008). FUS is a bicyclic sesterterpene (Fig. 1) and enniatins (ENs) and BEA possess a cyclic hexadepsipeptide structure (Fig. 2). At the present, MLs have not been set for emerging *Fusarium*

* Corresponding author. Tel.: +34 963544950; fax: +34 963544954.
E-mail address: emilia.ferrer@uv.es (E. Ferrer).

0278-6915/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fct.2012.09.034

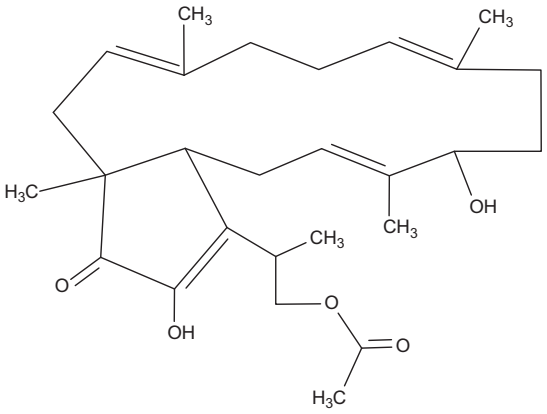
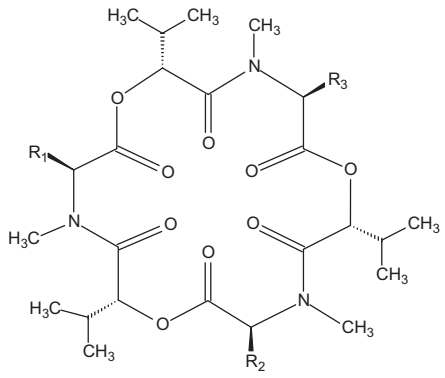


Fig. 1. Chemical structure of fusaproliferin (FUS).



	R_1	R_2	R_3
BEA	Phenilmethyl	Phenilmethyl	Phenilmethyl
ENA	*sec*-butyl	*sec*-butyl	*sec*-butyl
ENA1	*sec*-butyl	*sec*-butyl	*iso*-propyl
ENB	*iso*-propyl	*iso*-propyl	*iso*-propyl
ENB1	*iso*-propyl	*iso*-propyl	*sec*-butyl

Fig. 2. Chemical structures of beauvericin (BEA) and enniatins (ENA, ENA1, ENB and ENB1).

mycotoxins in spite that most of them represent an important risk for the public health. BEA and ENs have similar toxic actions including the induction of apoptosis, increasing the cytoplasmic calcium concentration and the DNA fragmentation in mammalian cell lines (Dombrink-Kurtzman, 2003; Jow et al., 2004; Lin et al., 2005). Besides, BEA and ENs have cytotoxic and insecticidal properties (Kamyar et al., 2004; Ivanova et al., 2006; Ferrer et al., 2009), and inhibitory effects on acyl-CoA: cholesterol acyltransferase activity (ACAT) (Tomoda et al., 1992). Fornelli et al. (2004) showed that on SF-9 cells were in line with earlier results for FUS with IC_{50} (50% inhibitory concentration) and CC_{50} (50% cytotoxic concentration) of >100 μM. FUS caused teratogenicity in a chicken embryotoxicity bioassay (Ritieni et al., 1997), and in the brine shrimp (*Artemia salina*) larvae bioassay FUS was toxic with a value (dosage leading to death of 50% larvae) of 53.4 μM, toxicity level similar to aflatoxin B1 and deoxynivalenol (Logrieco et al., 1996). Due to the described toxicity, emerging *Fusarium* mycotoxins may play a role in the health of the consumers. Monitoring studies have revealed the presence of ENs, BEA and FUS in some commodities and foodstuffs, mainly cereals in grain, cereal-based products and eggs (Jestoi, 2008; Garrido et al., 2011). Most of these studies have been performed through the application of the conventional extraction (Ultra-Turrax o el rotatory shaker extraction) (Uhlig et al., 2006; Meca et al., 2010), and posterior determination and quantification by liquid chromatography coupled to diode array ultraviolet–visible detector (Sifou et al., 2011) or triple quadrupole mass spectrometer detector (MS/MS QqQ) (Garrido et al., 2011). In MS/MS detection, the molecules are ionized and the ions are identified according their mass-to-charge (m/z) ratios. Therefore, high selectivity is achieved in the analysis employing LC–MS/MS, which increases confidence in the results of both qualitative and quantitative analyses (Zöllner and Mayer-Helm, 2006).

Monitoring studies for emerging *Fusarium* mycotoxins are necessary for legislative purposes, because in the near future an appropriate maximum contamination levels should be set for several mycotoxins by the authorities. Moreover, monitoring studies are required in order to obtain information about the real exposure of human population to mycotoxins (González-Osnaya et al., 2007; ÓArco et al., 2009). Risk assessment studies are of high interest since they permit the evaluation of the population exposure to toxic substances. Usually, risk assessment studies are carried out by comparison of the mycotoxin levels from the monitoring studies with the corresponding provisional maximum tolerable daily intake (PMTDI) established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (ÓArco et al., 2009). At the moment, studies of this type have not been carried out for emerging *Fusarium* mycotoxins in previous works. This is probably due to the absence of established PMTDIs for ENs, BEA and FUS. However, it is possible to perform an approach to the risk assessment comparing the levels of emerging *Fusarium* mycotoxins with the PMTDIs established for other *Fusarium* mycotoxins, such as T-2 toxin and HT-2 toxin or deoxynivalenol (JECFA, 2001; Serrano et al., 2012b).

The aim of this study was to assess the exposure of the Spanish population to emerging *Fusarium* mycotoxins present in pasta. Two objectives were proposed: (1) providing data on the natural occurrence of ENs, BEA and FUS in conventional and organic pasta from the Spanish market using LC–MS/MS QqQ determination, and (2) the approach to the risk assessment of ENs, BEA and FUS by evaluation of the dietary exposure.

2. Material and methods

2.1. Chemical and reagents

Acetonitrile (AcN) and methanol (MeOH) were provided by Merck (Darmstadt, Germany). Ammonium formate (99%) was supplied by Panreac Quimica S.A.U. (Barcelona, Spain) (Madrid, Spain). Deionized water (<18 MΩ cm^{-1} resistivity) was obtained in the laboratory using a Milli-Q SP® Reagent Water System (Millipore, Bedford, MA, USA). All solvents were passed through a 0.45 μm cellulose filter from Scharlau (Barcelona, Spain) before use.

The standards of ENA, ENA1, ENB, ENB1 and BEA were purchased from Sigma–Aldrich (St. Louis, MO, USA). The standard of FUS was kindly given by Professor A. Ritieni (Department of Food Science, University "Federico II" of Naples, Italy). Individual stock solutions of ENA1, ENB, ENB1, FUS and BEA with concentration of 1000 μg mL^{-1}, and the solution of ENA with concentration of 500 μg mL^{-1}, were prepared in methanol. They were stored in glass-stoppered bottles and darkness in security conditions at −20 °C. These stock solutions were then diluted with pure methanol in order to obtain the appropriate working solutions and were stored in darkness at −20 °C until the LC–MS/MS analysis.

2.2. Sample collection

Commercial samples of pasta (114 samples) were purchased during 2011 from different supermarkets located in Valencia (Spain). The method of sampling was accomplished according to the Commission Regulation (EC) No. 401, 2006a for the official control of the maximum levels established for aflatoxins, ochratoxin A and *Fusarium* toxins in cereals and cereal products. In this Regulation it has been established that for lots of cereals and cereal products less than 50 tons, the sampling plan shall the used with 10–100 incremental samples, depending on the lot weight, resulting in an aggregate sample of 1–10 kg. For very small lots (⩽0.5 tons) a lower number of incremental samples may be taken, but the aggregate sample combining all incremental samples shall be also in that case at least 1 kg.