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Food Chemistry 92 (2005) 459–464
Food Chemistry
www.elsevier.com/locate/foodchem

Study on ochratoxin A in cereal-derived products from Spain

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Received 23 February 2004; received in revised form 2 August 2004; accepted 2 August 2004

Abstract

A study on ochratoxin A (OTA) in cereal-derived products was carried out. Cereal-based baby foods, breakfast cereals and beers were analyzed for mycotoxin OTA using an in-house developed high-performance liquid-chromatographic method.

OTA was detected in 19 of the 21 samples of breakfast cereals (limit of detection 0.066 μg/kg), in 14 of the 20 samples of cereal-based baby foods (limit of detection 0.035 μg/kg) and in 24 of the 31 samples of beer (limit of detection 0.012 μg/l). The mean concentrations of OTA found were the following: 0.265 μg/kg in breakfast cereals, 0.187 μg/kg in cereal-based baby food and 0.044 μg/l in beer. The influence of different factors, such as the fibre content in breakfast cereals, type of cereals used in cereal-based baby food and alcohol content in beer, on the OTA levels was studied.

Keywords: Mycotoxins; Ochratoxin A; OTA; HPLC; Cereals; Cereal-based baby food; Breakfast cereals; Beer

1. Introduction

Ochratoxin A (OTA) is a mycotoxin produced by certain toxigenic species of *Aspergillus* and *Penicillium*. It has been shown to be hepatotoxic, nephrotoxic, teratogenic and carcinogenic to animals and has been classified as a possible human carcinogen (category 2B) by the International Agency for Research on Cancer (IARC, 1993). OTA could be a risk factor for Balkan Endemic Nephropathy (Petkova-Bocharova & Castegnaro, 1991).

The natural presence of OTA in food and foodstuffs is widespread, especially in temperate climates (Jørgensen, Rasmussen, & Thorup, 1996), and it is generally associated with a variety of products, such as cereals, coffee beans, cocoa beans, and dried fruit.

OTA is a moderately stable molecule that can survive most food processing operations (Harwig, Kuiper-Goodman, & Scott, 1983; Scott, 1991) and, therefore, it appears in derived products such as cereal products, coffee, wine, beer and grape juice; OTA is also found in products of animal origin.

The main contributors to OTA intake in humans are cereals and cereal-derived products because of the resistance to technological processes by this mycotoxin (Alldrick, 1996). Many countries have regulatory or guideline limits for the presence of OTA in foods and, in the majority of theses countries, OTA content limits have been established for cereals (FAO, 1997). In the EU, regulations list the maximum tolerable levels of OTA in cereals (5 μg/kg), cereal-derived products (3 μg/kg), dried vine fruits (10 μg/kg) (European Commission, 2002a) and in food for infants and young children (0.5 μg/kg) (European Commission, 2004). There is some discussion regarding the limit that should be established for ochratoxin A in some cereal-derived foods. The appropriate level proposed for beer was 0.2 μg/l (Burdaspal, Legarda, & Gilbert, 2001).

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doi:10.1016/j.foodchem.2004.08.012

Many studies on the presence and quantity of OTA in cereals have been carried out, worldwide. In spite of the fact that OTA is present in human plasma among the Spanish population (Burdaspal & Legarda, 1998; Jiménez, López de Cerain, González-Peñas, & Bello, 1999; Pérez de Obanos, López de Cerain, Jiménez, González-Peñas, & Bello, 2001), few studies have been carried out in Spain with regard to the presence of OTA in cereals. In a recent study, 115 samples of cereals (wheat, barley and corn) from a northern region of Spain (Navarra) were analyzed for OTA contamination (Araguas, González-Peñas, López de Cerain, & Bello, 2003). OTA was detected in 58 of the 115 samples (limit of detection 0.066 μ g/kg), with a mean concentration of ochratoxin A of 0.219 μg/kg. One sample of corn contained a quantity of this mycotoxin that surpassed the EU legal limit (5 μg/kg). This result, along with the moderate stability of OTA during food processing, adds special interest to the study of OTA in cereal-derived products, especially if they are consumed in large quantities and, as in the case of breakfast cereals and cereal baby food, principally consumed by children.

The technique most often used in the determination of OTA in food is HPLC with fluorescence detection (Valenta, 1998). In the development of new analytical methods for OTA determination there is a tendency toward the use of immunoaffinity columns (IAC), the reduction or elimination of organic solvents in the extraction process (especially chloroform due to its toxicity and environmental impact), and to obtain sensitive methods that permit the detection and quantification of OTA at the levels established by legislation. Moreover, analytical methods for the determination of ochratoxin A must be fully validated if they are to be used for control, monitoring and risk assessment studies.

In this paper, we describe an HPLC method for quantitative analysis of OTA in breakfast cereals, cereal-based baby food and beer, which is versatile, validated and sensitive enough to comply with the regulatory limits; in addition, it is a contribution to the knowledge of OTA contamination in certain cereal-derived products in Spain.

2. Materials and methods

2.1. Samples

Twenty baby food samples, 21 breakfast cereal samples and 31 beer samples of different brands were purchased from supermarkets and pharmacies in Navarra (Spain). The cereal contents in the baby food samples ranged from 56% to 94%. The sample quantities were 600 g for baby food and 375 g for breakfasts cereals. Both, bottled and canned beer were analysed. Ten samples were advertized as being non-alcoholic (<1% of alcohol), whereas twenty-one were advertized as containing alcohol. All of the samples were stored at 4 °C until their analysis.

2.2. Reagents

Acetonitrile and methanol (both of HPLC grade) were purchased from Riedel de Haën (Seelze, Germany). Phosphoric acid, sodium acetate, sodium hydrogen carbonate and hydrochloric acid (37%) were obtained from Merck (Darmstadt, Germany). Water was obtained daily from a Milli-Q system. OTA was supplied by Sigma (Madrid, Spain). Phosphate buffered saline (PBS) was prepared by adding the following to one litre of water: anhydrous dibasic sodium phosphate (2.04 g) and sodium chloride (87.9 g), both from Merck (Darmstadt, Germany), along with sodium dihydrogen phosphate monohydrate (12.62 g) from Panreac (Barcelona, Spain). One hundred millilitres of this solution were diluted to 1 litre and the pH was adjusted to 7.4 with NaOH; 100 μl of Tween 20 from Sigma (Madrid, Spain) were then added.

2.3. Apparatus and chromatographic conditions

The instrument used was an Agilent Technologies 1100 liquid chromatographic system equipped with a fluorescence detector (model G1321A), controlled by the ChemStation 3D software. The chromatographic conditions were those employed in the OTA determination in cereals (Araguas et al., 2003). Briefly, OTA was separated on a 5 μm (25 cm × 0.4 cm) Tracer Extrasil ODS-2 column with a Tracer Extrasil ODS-2 precolumn, both from Teknokroma (Barcelona, Spain). The injection volume was 100 μl and the flow rate was 1.5 ml/min, with a mobile phase of 29:29:42 (v/v/v) methanol–acetonitrile–5 mM sodium acetate acidified to pH 2.2 with phosphoric acid. The aqueous phase was filtered through a 0.45 μm membrane filter (Millipore, Ibérica S. A. Spain). Chromatography was performed at 40 °C and the fluorescence conditions, selected from the OTA excitation spectrum that was obtained when an OTA calibration sample was chromatographied, were as follows: Ex = 225 nm; Em = 461 nm.

2.4. Standard solutions

A standard stock solution of 100 μg/ml was prepared by dissolving 1 mg of OTA in 10 ml of methanol and storing it at −20 °C. The concentration of OTA was determined by UV at 333 nm (Mr: 403.8; ε 5500 M^{-1} cm^{-1}) (Bacha et al., 1988). Standard working solutions and calibration samples were prepared by dilution of the stock solution with methanol.

Qualitative confirmation of positive samples was performed by re-analyzing them after derivatization of