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Occurrence of patulin in apple-based-foods largely consumed in Tunisia

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ABSTRACT

Patulin (PAT) is a toxic metabolite produced by several filamentous fungi of the genera of *Penicillium*, *Aspergillus* and *Byssochlamys*, principally by *Penicillium expansum*. A total of 85 samples of apple products largely consumed by Tunisian population were analyzed for PAT content, including apple juice, baby food and mixed juice collected during 2011 from the major supermarkets and stores located in Tunisia. The aim of this study was to investigate the presence of PAT in the widely-consumed apple products in Tunisia, to compare the levels of PAT contamination with the European norms and to suggest some factors that can promote the production of this mycotoxin in our country. To perform this study, we developed and validated, in our laboratory conditions, an HPLC method for a quantitative analysis of PAT in apple products. Our results showed that the incidence of PAT contamination was 35%. The levels of contamination determined in the total samples ranged between 0 and 167 μg/l with a mean value of 20 μg/l and a median of 13 μg/l. Eighteen percent (18%) of the total juice samples (apple juices and mixed juices) and twenty-eight percent (28%) of the baby food samples exceeded the tolerable limit recommended by the European Union, which are respectively 50 μg/l and 10 μg/l.

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1. Introduction

Nowadays, food safety is a great concern since more and more chemicals are present in our environment. Food is an important route of exposure to contaminants as dioxins, mycotoxins, heavy metals, pesticides, polycyclic aromatic hydrocarbons, drugs and hormones. Mycotoxins are a class of highly toxic compounds, secondary metabolites that are produced under particular environmental conditions by certain fungi or molds developing in many foodstuffs (Barreira, Alvito, & Almeida, 2010).

Patulin (PAT), a polyketide lactone (4-hydroxy-4H-furo[3,2-c] pyran-2(6H)-one) is a polyketide lactone produced by a wide range of fungal species of the *Penicillium*, *Aspergillus* and *Byssochlamys* species, principally by *Penicillium expansum* (Baert et al., 2007) growing on fruits such as apples and apple products (Leggott & Shephard, 2001). It has also been identified in oranges, peaches, apricots, tomatoes and their by-products (Gokmen & Acar, 1998).

The occurrence of PAT as a natural contaminant of fruit products is an indicative of the quality of the fruit used in the production and it is a worldwide problem. This mycotoxin is easily transferred into the fruit products during the processing owing to its solubility in water. It is very stable to heat in acidic medium as in fruit juice (Anderson de Souza, Rosenthal, & Rodriguez de Massaguer, 2008; Gokmen, Artik, Acar, Kahraman, & Poyrazoglu, 2001).

Acute symptoms of PAT consumption can include agitation, convulsions, edema, ulceration, intestinal inflammation and vomiting (Speijers, 2004). The chronic health effects of patulin include genotoxicity, immunotoxicity, embryotoxicity and neurotoxicity (Wouters & Speijers, 1996). However, no adequate evidence exists for carcinogenicity in experimental animals and humans. It is not classifiable as to its carcinogenicity to humans, and it is included in Group 3 of the International Agency for Research on Cancer (IARC, 1993).

Due to the high consumption of apple products during the first years of life, children are more exposed to patulin toxicity when compared to adults (Moake, Padilla-Zakour, & Worobo, 2005). The European Commission (EC) regulation introduces a separate limit of 10 μg/kg for PAT in apple juice, solid apple products, including apple compote, apple puree and baby-foods (Commission Regulation, EC No. 1881/2006). This limit is lower than the maximum permitted levels of PAT in the different apple-based-foods established by the European Union, like 50 μg/kg for fruit juices, reconstituted concentrated fruit juices, fruit nectars, spirit drinks, cider and other fermented drinks derived from apples or containing apple juice and 25 μg/kg for solid apple products, including apple compote and apple puree intended for direct consumption. A PAT maximum intake is estimated to be 0.2 μg/kg bw/day for children and 0.4 μg/kg bw/day for adults, greatly below the tolerable intake

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established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (World Health Organisation, 1995).

Liquid–Liquid extraction and Solid-phase extraction (SPE) are the traditional methods of sample preparation in the analysis of PAT in food samples prior to the analysis by Thin-Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC), Gas-Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS) (Elvira, Gaspar, & Lucena, 2009; Iha, de Souza, & Sabino, 2009; Kataoka, Itano, Ishizaki, & Saito, 2009; Moukas, Panagiotopoulou, & Markaki, 2008; Songsermsakul & Razzazi-Fazeli, 2008). Among them, HPLC coupled with UV detection, have been found to be the most suitable in PAT trace analysis as it exhibits strong UV absorption at 276 nm (Al-Hazmi, 2010; Funes & Resnik, 2009; Li, Wu, Hu, & Wang, 2007).

Tunisia provides favorable climatic, geographic, social and economic conditions for toxigenic fungi proliferation and mycotoxins production (Bacha et al., 1988; Maaroufi et al., 1999; Zaied et al., 2009; 2010; Zaied, Zouaoui, Bacha, & Abid, 2012a, 2012b). Tunisian population daily consumes great amounts of apple-based-foods mainly in holidays and festival days. Indeed, large amounts of apple products commercialized in Tunisia are imported and little is known about their eventual mycotoxin contamination. In addition, there are no applicable norms concerning these products contamination, particularly by PAT in our country.

The aim of this study was to determine the PAT levels in some apple-based-products, largely consumed by Tunisian population and to compare the levels of PAT contamination to the European norms. To perform this study, we validated a method for a quantitative analysis of PAT by using High-Performance Liquid Chromatography (HPLC) method coupled with UV detection. This method has better precision and sensitivity than the previously reported methods and it focused mainly on extraction and clean-up.

2. Materials and methods

2.1. Sample collection

Eighty five (85) apple products obtained randomly from the major supermarkets and stores located in Tunisia were analyzed during 2011. Thirty (30) of them belonged to apple juice, thirty (30) samples of fruit juices and twenty-five (25) samples of compotes. Different brand names were selected in order to have a market-representative sampling. The volume of the samples was between 130 ml and 1.0 L. They were stored in their original packages at 4–5 °C until analysis. The samples were thoroughly homogenized and opened the same day of the analysis.

2.2. Reagents

PAT standard was provided by Sigma Chemicals (St. Louis, MO, USA) and dissolved in methanol. All reagents (ethyl acetate, acetic acid and sodium carbonate) were obtained from Prolabo, Merck and Sigma–Aldrich (France). All solvents (acetonitrile, methanol and water) were purchased from Fisher Scientifics (Fisher chemicals HPLC, France) with HPLC grade.

2.3. Preparation of standard solutions

The standard solutions of PAT were prepared by dissolving 5 mg of pure crystalline PAT in 1 ml of methanol. The concentration of the PAT stock solution was determined by measuring the UV absorbance at 276 nm and calculated by using the molar extinction coefficient ε of 14,600 L/mol/cm. The standard curve solutions were freshly prepared from appropriate dilutions of the stock solution with methanol (150, 100, 50, 25 and 10 μg/ml). All the solutions were stored at +4 °C.

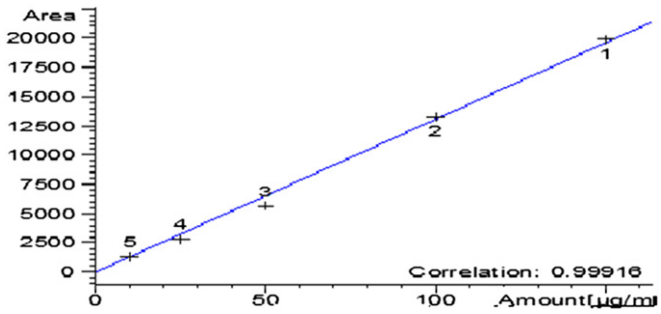

Fig. 1. Calibration curve of patulin standard concentrations (150, 100, 50, 25 and 10 μg/ml).

2.4. Extraction and clean-up steps

PAT analyses were performed by HPLC with UV detector according to the procedure described by Yuan, Zhuang, Zhang, and Liu (2010) with some modifications. Ten milliliters of apple juice were extracted with 20 ml of ethyl acetate by mixing vigorously for 10 min using a vortex mixer. The mixture was centrifuged at 4500 rpm at 25 °C for 5 min. The organic upper layer was transferred to a centrifuge tube and the aqueous phase was twice re-extracted with 20 ml of ethyl acetate. The organic layers were combined and 2 ml of 2% sodium carbonate solution was added and the tube was three times shacked vigorously. The pH value of the solution was adjusted to 4 with 36% acetic acid. The solution was evaporated to dryness at 60 °C and 5 ml of acetonitrile solution (5%) was dissolved to the residue. Then, the reconstituted extract was purified with MycoSep® 228 AflaPat Column and finally the purified extract was evaporated to dryness at 60 °C. For analysis in the HPLC system, 500 μl of methanol were added.

2.5. HPLC quantification

HPLC analyses of PAT were performed with an 1100 series HPLC system from Agilent Technologies equipped with a UV detector (λ = 276 nm) controlled by the Chemstation 3D software (Agilent Technologies Chemstation family software products) and equipped with an auto-injector (Injection Valve Assembly G1313A). The separation was carried out on a C18 reversed-phase (Spherisorb ODII, Leonberg) (250 × 4 mm, 5 μm). The system was run isocratically with a mobile phase consisting of a mixture of water: acetonitrile (90:10, v/v) at a flow rate of 1 ml/min. The injection volume of the standard and sample extract was 50 μl. The quantification of PAT was performed by the measurement of the peak area at PAT retention time and the comparison with the relevant calibration curve (150, 100, 50, 25 and 10 μg/ml).

Table 1
Average recoveries for PAT in all apple products.

Concentrations of PAT (ng/ml)	150	100	50
Recoveries ± RSD[a] (%) of apple juice	71 ± 8.1	77 ± 4.5	72 ± 7.2
Recoveries ± RSD[a] (%) of mixed juice	75 ± 6.2	82 ± 3.5	76 ± 6.5
Recoveries ± RSD[a] (%) of baby food	70 ± 3.3	81 ± 5.5	77 ± 5.3
Average recovery ± RSD[a] (%)	75 ± 4		

[a] RSD: Relative Standard Deviation.

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