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Influence of production on the presence of patulin and ochratoxin A in fruit juices and wines of Argentina

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ABSTRACT

In this study, the relative frequency and concentration of patulin (PAT) and ochratoxin A (OTA) in fruit juices and wines collected in Argentina between 2005 and 2013 were determined by high performance liquid chromatography. PAT was detected in 1997 of 5958 samples (ranging from 3.0 to 19,622 μg/L), and 510 samples presented PAT levels above 50 μg/L. The highest incidence of PAT was observed in 2005 (243 of 419 samples) while the lowest was quantified in 2009 (104 of 482 samples). OTA was detected in only 22 of 1401 samples at concentrations ranging from 0.15 to 3.6 μg/L, and the highest incidence was observed in 2007 (8 of 153 samples). The concentration of PAT and OTA in the beverages analyzed was found to be affected by the type of fruit product, fruit commodity and production year. A great amount of data on the incidence of these mycotoxins in these matrixes can be further used in the development and reinforcement of measures to reduce the burden of their presence in juices and wines. This is important since PAT levels above the limit set by regulations were high and fruit juices are quite consumed by children. Although OTA contamination was low, effective ways to safeguard consumer exposure to PAT and OTA and consequently to protect public health are essential and indispensable.

1. Introduction

Patulin (PAT), a mycotoxin produced by some species of *Penicillium* and *Byssochlamys*, features different harmful functions such as toxic, antibiotic, carcinogenic and mutagenic properties (Luque, Córdoba, Rodríguez, Núñez, & Andrade, 2013). PAT is highly soluble in water and highly stable in aqueous acid media, so it penetrates mainly into apple derivative products, such as juices (Gökmen & Acar, 2000). In pasteurized juices, some *Byssochlamys* species are potential producers of PAT, due to their capability to resist thermal processing usually applied to fruit juices (Sant'Ana, Rosenthal, & Massaguer, 2008). Cleaning of fruits by washing and removal of decayed parts are two main low-cost procedures to mitigate PAT in processed fruit products like juices and concentrates (Forouzan & Madadlou, 2014).

Ochratoxin A (OTA), produced by species of *Aspergillus* and *Penicillium*, is an important nephrotoxic mycotoxin with carcinogenic, teratogenic, immunotoxic, genotoxic and possibly neurotoxic effects (Al-Hazmi, 2010). The occurrence of OTA in fruit juices results from poor agriculture and harvesting practices, especially in the case of physical and physiological damage (Delage, d'Harlingue, Colonna Ceccaldi, & Bompeix, 2003). Also, OTA-producing fungi and final OTA amounts may be affected by climatic conditions. OTA normally occurs in subtropical regions and temperate climate and can be found in diverse foodstuffs of these regions, such as wines and grape products. Like other mycotoxins, OTA is relatively heat resistant within the range of applied thermal processing conditions. Nonetheless, OTA is partially destroyed during fermentation procedures, so it can also be found in various industrial food products (Soufleros, Tricard, & Bouloumpasi, 2003). Data suggest

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that OTA occurrence in subtropical regions of Argentina, Australia, and Brazil, is caused by black aspergilli.

There are several factors that affect PAT and OTA contamination levels in fruits and fruit products such as type and cultivar of fruit, climate conditions, geographical location, year of production, pre- and post-harvest treatments, use of pesticides, surface damage on the fruit, and storage conditions (Jackson & Al-Taher, 2008; Varga & Kozakiewicz, 2006). As result of their potential occurrence in several foodstuffs and their threats to human health, maximum levels of PAT and OTA have been established. The maximum tolerable level of PAT in nectars and fruit juices, particularly apple juice ingredients and apple juices in other drinks sold in Europe is 50 μg/kg and the provisional maximum tolerable daily intake for PAT set by the Scientific Committee on Food is 0.4 μg/kg of body weight (bw) (European Commission, 2006). The maximum level for OTA in wine, wine-based drinks, and grape juice is 2.0 μg/kg, also the European Food Safety Authority (EFSA) established that a tolerable weekly intake (TWI) for OTA is 120 ng/kg bw (European Commission, 2006). Because of these standards, several studies have been conducted in different countries to assess the levels of these mycotoxins in foods (Amézqueta, González-Peñas, Murillo-Arbizu, & López de Cerain, 2009; Delage et al., 2003; Iha, Barbosa, Heck, & Trucksess, 2014; Makun et al., 2013; Nguyen & Ryu, 2014; Wu, Tan, Wang, & Xu, 2011).

Surveys on the occurrence and levels of mycotoxins are of chief importance because they are reliable approaches to unveil real incidence of these contaminants in foodstuffs as affected by several factors. These comprise data of major relevance for food safety as the findings of surveys may allow comparisons with previous results and make it possible to assess contributor factors for the occurrence of mycotoxins. In addition, the findings of surveys will also allow decisions to be taken based on objective data. Given the above, the present study was performed aiming to report on the occurrence of PAT and OTA in many samples collected throughout nine years in Argentina. The objective of this study was to define the incidence and concentration of PAT and OTA in different fruit juices and wine as affected by production year, type of fruit and fruit commodity.

2. Material and methods

2.1. Samples collection

A total of 5958 samples of different types of fruit commodities (apple, apricot, grape, orange, peach, pear and pineapple) and type of fruit products (cloudy concentrated juice, cloudy single strength juice, concentrated juice, concentrated pulp, single strength pulp and sulphited juice) were obtained from 2005 to 2013 for the determination of PAT concentrations. Samples, collected for process verification or quality control, were obtained directly from 19 juice and pulp producers, located in different Argentinean provinces ($n = 10$) (Table 1). Industries provided samples in 1 L sterile containers, which were transported to the laboratory under adequate conditions (cooled or frozen).

During the same period (2005–2013), for determination of OTA level, a total of 1401 samples of different types of fruit juices (grape, apricot, lemon, orange and tangerine) and fruit products (concentrated juice cloudy, concentrated juice sulphited, concentrated juice, concentrated pulp, single strength juice, and red wine) were analyzed. Samples, also collected for process verification or quality control, were acquired directly from 13 juice and 36 wine producers located in eight Argentinean provinces (Table 2).

Prior to analysis, samples were diluted with distilled water to the soluble solids (°Brix) recommended in the Code of Practice for Fruit and Vegetable Juices of the Association of the Industries of Juices and Nectars from Fruits and Vegetables of the European Union, European Fruit Juice Association (AIJN, 2015). Reference values of soluble solids of juices and/or pulps were as follows: apple, 11.2, pear, 11.9, peach, 10.0, apricot, 11.2, pineapple, 12.8, grape, 15.9 and orange, 11.2) (AIJN, 2015). Sample pH and Brix values were measured using pH-meter (Model pH-2005, Selecta, Barcelona, Spain) and a refractometer (Model RFM 330+, Bellingham-Stanley Ltd, Tunbridge Wells, UK), respectively (data not shown).

2.2. Sample preparation

Juices, pulps, and wines were collected under aseptic conditions, placed in pouches or plastic sterile flasks (Low-density polyethylene) and transported to the lab under refrigeration (4 ± 0.2 °C). In the case of non-clarified juices and pulps, 750 μL of 20 g/L pectinase solution (Pectinex®, Novozymes, Bagsvaerd, Denmark) was added to 100 mL of diluted juices, homogenized and kept at 40 °C for 2 h in a water bath. After this, treated samples were centrifuged at $120 \times g$ for 10 min in an ultracentrifuge (Model Suprafuge 22, Heraeus Sepatech, Osterode, Germany) and the supernatant was collected.

PAT was extracted from juice samples (5 mL) with ethyl acetate (10 mL) (Merck, Darmstadt, Germany) in a shaker (Model SK-300, Jeio Tech, Seoul, South Korea) for 5 min. The extraction procedure was done according to MacDonald, Long, and Gilbert (2000). The supernatant layer was recovered and evaporated at 40–45 °C before the addition of 2 mL of 15 g/L sodium carbonate. The dried contents were re-suspended in 1 mL of acetate buffer pH 4.9 (acetic acid 0.2 mol/L + sodium acetate 0.2 mol/L; 49 mL/9 mL). The recovery of PAT varied between 87 and 100% for juices and pulps.

OTA extraction from wines and must samples was done using the official method proposed by Visconti, Pascale, and Centonze (2001). The pH of samples was adjusted to 7.2 with 40 g/L NaOH solution and a 10 mL portion was taken and added to an immunoaffinity column (OchraTestTM; Vicam, Digen Ltd, Oxford, UK). The column was washed with 10 mL of phosphate buffer solution containing 10 mL/L Tween 20 and then with 10 mL of double distilled water. OTA was eluted from the column with 1.5 mL of methanol/acetic acid (98 mL:2 mL), at a flow rate of 1 drop per second. Samples were analyzed by High Performance Liquid Chromatography (HLPC) in duplicate and two injections were made for each sample extract. The average was then reported. The recovery of OTA varied between 70 and 114% for wine and juices.

2.3. HPLC quantification

PAT quantification was performed as recommended by MacDonald et al. (2000). PAT standard (pure crystalline) was obtained from Sigma (St. Louis, MO, USA) and a stock standard solution (200 μg/mL) of this mycotoxin was prepared by dissolving the pure crystalline toxin in double distilled water (pH 4.0) acidified with acetic acid. Working standard solutions (0.05; 0.1; 0.2; 0.5 and 1.0 μg/mL) were made by appropriate dilution of this solution with acetate buffer (pH 4.0). PAT was detected using a HPLC (Shimadzu, Kyoto, Japan) equipment comprised of a PolyLCReliasil C-18 column (254 × 4.6 mm, 5 μm, Phenomenex, Torrance, USA), system controller (CBM-20A), solvent delivery unit (LC-20A), auto-sampler (SIL-20A), column oven (CTO 20 AC), liquid chromatographic pump (LC 20 AD), UV-VIS detector (SPD-20A) and photo-diode array detector (SPD-M20A) at 276 nm. The mobile phase was methanol 30 mL/L at 1 mL/min at 40 °C. The injection volume for PAT samples was 40 μL and its retention time was 8.52 min.

OTA quantification was done based on the recommendations of International Federation of Fruit Juice Producers (IFU, 2005) and the