LWT - Food Science and Technology 80 (2017) 200–207

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

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

Juan M. Oteiza [a, **], Amin M. Khaneghah [b], Fernanda B. Campagnollo [b], Daniel Granato [c], Mohammad R. Mahmoudi [d], Anderson S. Sant'Ana [b, *], Leda Gianuzzi [e]

[a] Centro de Investigación y Asistencia Técnica a la Industria (CIATI AC), Neuquén, Argentina
[b] Department of Food Science, Faculty of Food Engineering, University of Campinas, Campinas, SP, Brazil
[c] Department of Food Engineering, State University of Ponta Grossa, Ponta Grossa, PR, Brazil
[d] Department of Statistics, College of Science, Fasa University, Fasa, Iran
[e] Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), CCT-La Plata, Facultad Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina

ARTICLE INFO

Article history:
Received 16 October 2016
Received in revised form
12 February 2017
Accepted 13 February 2017
Available online 17 February 2017

Keywords:
Occurrence
Survey
Beverage
Mycotoxin
Exposure
Food processing

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

* Corresponding author. Rua Monteiro Lobato, 80, Cidade Universitária Zeferino Vaz, CEP: 13083-862, Campinas, SP, Brazil.
** Corresponding author.
E-mail addresses: juano@ciati.com.ar (J.M. Oteiza), and@unicamp.br (A.S. Sant'Ana).

http://dx.doi.org/10.1016/j.lwt.2017.02.025
0023-6438/

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

Table 1
Type of product, used fruit and number of collected samples per each year for determination of patulin level.

Type of product	Fruit	2005	2006	2007	2008	2009	2010	2011	2012	2013
Cloudy concentrated juice	Apple	0	2	7	9	16	0	1	3	3
	Orange	0	0	0	0	0	0	0	0	16
	Pear	0	0	0	2	2	0	0	2	0
Cloudy single strength juice	Apple	0	4	4	6	58	13	4	3	6
Concentrated juice	Apple	387	327	330	315	267	253	604	691	630
	Apricot	4	2	0	0	0	0	0	0	0
	Grape	0	1	0	0	3	8	7	16	11
	Peach	4	5	25	17	12	15	3	1	1
	Pear	12	32	29	25	72	53	161	273	316
	Pineapple	0	0	2	4	0	0	0	0	0
Concentrated pulp	Apple	4	8	41	21	42	47	39	81	42
	Apricot	0	1	0	2	2	7	3	1	0
	Peach	0	1	0	4	2	2	0	0	0
	Pear	5	9	14	10	4	5	4	11	8
Single strength pulp	Apple	3	7	256	7	1	3	11	46	32
	Pear	0	0	46	28	1	1	4	5	4
Sulphited juice	Grape	0	0	0	0	0	0	0	0	4

Table 2
Type of product, used fruit and number of collected samples per each year for determination of ochratoxin A level.

Type of product	Fruit	2005	2006	2007	2008	2009	2010	2011	2012	2013
Concentrated juice Cloudy	Grape	0	1	0	0	0	0	0	0	0
	Lemon	0	0	0	0	0	0	0	16	0
	Orange	0	0	0	0	0	0	0	0	2
	Tangerine	0	0	0	0	0	0	0	0	2
Concentrated juice sulphited	Grape	1	39	35	64	23	12	28	85	85
Concentrated juice	Grape	11	12	25	16	13	9	19	21	21
	Lemon	0	0	0	0	0	0	0	0	16
Concentrated pulp	Apricot	0	0	0	0	0	9	0	0	0
Single strength juice	Grape	3	0	13	0	0	0	13	3	3
Wine	Grape	53	43	80	111	88	100	145	91	90

International Organization of Vine and Wine (Method OIV-MA-AS315-10) (OIV, 2016). The HPLC system used for OTA determination was a Shimadzu (Kyoto, Japan) equipped with a C18 column (150 × 4.6 mm, 5 μm particle size; Supelcosil LC-ABZ, Supelco, Bellefonte, PA, USA), connected to a pre-column (20 × 4.6 mm, 5 μm particle size; Supelguard LC-ABZ, Supelco), system controller (CBM-20A), solvent delivery unit (LC-20A), auto-sampler (SIL-20AHT), column oven (CTO 20AC), liquid chromatographic pump (LC 20AD), spectrofluorescence detector (RF-10AxL, excitation at 333 nm; emission at 460 nm) and photo-diode array detector (SPD-M20A). The mobile phase was pumped at 1 mL/min and consisted of an isocratic system of acetonitrile/water/acetic acid (99 mL:99 mL:2 mL). The injection volume for OTA samples was 100 μL and its retention time was 10 min. OTA standard solution was prepared using OTA standard (purity > 99%; Sigma-Aldrich Co., St Louis, MO, USA) at a concentration of 40 μg/mL.

2.4. Statistical analysis

All statistical analyses and graphs were performed by means of SPSS software (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp, USA). The data were processed using descriptive statistical parameters and relative frequencies (proportions) were calculated to express the prevalence of PAT and OTA in the diverse samples marketed in Argentina (Granato, Calado, & Jarvis, 2014). In order to compare the relative frequencies, the goodness of fit test (z-score test) was used. Samples with an amount of PAT or OTA higher than the LOQ were deemed positive, while samples with quantities between the LOD and the LOQ were deemed negative. Mean PAT and OTA contents were calculated by using LOD/2 for negative samples according to Succop, Clark, Chen, and Galke (2004).

Experimental results were reported as percentage of positive samples, mean, standard deviation, median, minimum and maximum concentrations for each mycotoxin based on years, type of fruit and type of product, separately. To compare the OTA and PAT levels between the years, type of fruit and type of product, one-way analysis of variance (ANOVA) followed by Z-score test with Bonferroni's method (Agresti, 2007, p. 744) or Duncan's multiple comparison test were applied for multiple comparisons of treatments (Nunes, Alvarenga, Sant'Ana, Santos, & Granato, 2015).

3. Resuts and discussion

3.1. Quality assurance of the method

HPLC methodology was evaluated for quality assurance using parameters such as Limit of Detection (LOD), Limit of Quantification (LOQ), recovery, precision (SD), repeatability relative standard deviations (RSDr) and measurement. For PAT, values found for all kind of products were the same and included a LOD of 3 μg/L, LOQ of 10 μg/L, 94% of recovery, SD of 4,5 μg/L, RSDr of 4.7% and a measurement of 22%. For OTA, values were the same for all products (LOD of 0.87 μg/L, LOQ of 1.7 μg/L, 92% of recovery, SD of 12.1 μg/L, RSDr of 13.2% and measurement of 36%) excluding single strength juice and wine, for which values found were LOD of 0.15 μg/L, LOQ of 0.3 μg/L, 92% of recovery, SD of 7,6 μg/L, RSDr of 8.3% and measurement of 26%.

3.2. Incidence and concentration of PAT in fruit juices and pulps

The results of the incidence and concentration of PAT in analyzed samples obtained in 2005–2013 are shown in Table 3. PAT could be detected in 1997 out of 5958 samples at concentrations

Table 3
Occurrence, positive samples, mean, standard deviation, median and maximum concentration of patulin in fruit juices and pulps during 2005 up to 2013.

Year (total/positive samples)	Positive (%)	10 < × < 50 µg/L (% positive)	50–60 µg/L (% positive)	60–70 µg/L (% positive)	70–80 µg/L (% positive)	80–90 µg/L (% positive)	90–100 µg/L (% positive)	100–200 µg/L (% positive)	>200 µg/L (% positive)	Mean (µg/L)	Standard deviation	Median (µg/L)	Max (µg/L)
2005 (419/243)	58.0[a]	175 (41.8)	23 (5.5)	15 (3.6)	11 (2.6)	4 (1.0)	2 (0.5)	13 (3.1)	0	22[a]	29	13.12	170
2006 (399/161)	40.4[bc]	107 (26.8)	14 (3.5)	8 (2.0)	4 (1.0)	6 (1.5)	2 (0.5)	8 (2.0)	12 (3.0)	31[a]	160	0	2982
2007 (754/346)	45.9[b]	203 (26.9)	55 (7.3)	24 (3.2)	23 (3.1)	9 (1.2)	8 (1.1)	16 (2.1)	8 (1.1)	23[a]	42	0	87
2008 (450/165)	36.7[bc]	131 (29.1)	16 (3.6)	4 (0.9)	3 (0.7)	0	0	6 (1.3)	5 (1.1)	48[a]	449	0	7339
2009 (482/104)	21.6[d]	62 (12.9)	9 (1.9)	6 (1.2)	2 (0.4)	4 (0.8)	8 (1.7)	9 (1.9)	4 (0.8)	13 [a]	44	0	469
2010 (407/150)	36.9[bc]	108 (26.5)	15 (3.7)	6 (1.5)	2 (0.5)	4 (1.0)	3 (0.7)	4 (1.0)	8 (2.0)	33[a]	211	0	3338
2011 (841/274)	32.6[c]	246 (29.3)	13 (1.5)	4 (0.5)	1 (0.1)	1 (0.1)	2 (0.2)	7 (0.8)	0	9[a]	18	0	158
2012 (1133/284)	25.1[d]	241 (21.3)	15 (1.3)	8 (0.7)	8 (0.7)	1 (0.1)	2 (0.2)	7 (0.6)	2 (0.2)	20[a]	410	0	13,808
2013 (1073/270)	25.2[d]	214 (19.9)	12 (1.1)	8 (0.7)	7 (0.7)	2 (0.2)	3 (0.3)	17 (1.6)	7 (0.7)	29[a]	600	0	19,622

[a–d] Different letters in the Positive (%) and Mean (µg/L) columns indicate significant differences ($p < 0.05$) according to Z-score test (with Bonferroni's method) and Duncan's test, respectively.

ranging from 3.0 to 19,622 µg/L. In addition, an overall incidence of 33.5% was observed in different fruit juices. Regarding each analyzed year, the highest incidence of PAT was observed in 2005 (243 of 419 samples), differing significantly from other values, and in this year 16.2% (68 of 419 samples) of samples were determined above the EU limit for PAT (50 µg/L). The years of 2009 presented the lowest incidence of PAT (104 of 482 samples), although it did not differ significantly from 2012 and 2013.

The highest PAT level was detected in 2013 (19,622 µg/L) and the highest mean concentration was found in 2008 (48.5 µg/L), however there was no significant difference between the mean concentrations of all years. Factors such as different cultivation procedures, climate conditions and different periods of storage at chilling temperature could explain the differences observed in the positive samples for the years analyzed (Table 3). It could be also observed that the median values were almost all zero, except for the year of 2005 (13.1 µg/L). The explanation for this data is that 66.4% of samples analyzed for PAT have concentration values equal to zero, which pulls the median values also to zero.

Analyzing Table 4, regarding the effect of fruit commodity, the significantly highest percentage of positive samples was detected for apple (1866 of 4634 samples), whereas samples of pineapple and orange did not show positive samples. Apple also had the maximum PAT concentration between fruit commodities (19,622 µg/L). However, grape samples presented the highest mean concentration for PAT (283 µg/L), differing significantly from others. Again, median values were all equal to zero.

Table 4 also shows the results regarding type of products. Values of positive samples were significantly higher for cloudy single strength juice and single strength pulp (67.4% and 54.1%, respectively). In contrast, none of the sulphited juices were reported as positive in PAT contamination. The highest mean concentration for PAT in the different products was found in cloudy concentrated juice and cloudy single strength juice ($p < 0.05$). Also, cloudy concentrated juice presented the highest maximum PAT concentration (19,622 µg/L) and the median values were different from zero just for cloudy single strength juice and single strength pulp (36.9 µg/L and 21 µg/L, respectively).

Generally, there is an extensive variability in the fraction of positive samples among several reported investigations, because of different techniques and LOQ used, becoming complex to compare these values. According to the literature, about 50% of apple juice samples analyzed have been contaminated up to PAT detectable levels (Prieta, Moreno, Diaz, Suarez, & Dominguez, 1994). Sometimes, contamination of apple juice products might reach 8000 µg/L, especially when the partly rotten apples are used for production of juices (Brackett & Marth, 1979), and even higher concentration of PAT has already been detected when decayed apples were not separated through the processing at industrial premises (Wilson & Nuovo, 1973). Gökmen and Acar (2000) observed that all 215 samples of apple juice concentrate produced in Turkey in 1994 were contaminated with PAT at levels ranging from 7 to 376 µg/L, and 98 of these samples contained PAT levels above 50 µg/L. A survey conducted in South Africa demonstrated that the maximum PAT level was 45 µg/L in 60 commercial apple products (Leggott & Shephard, 2001). PAT determination in solid and semisolid apple and pear products commercialized in Argentina revealed that only 11 out of 51 analyzed samples were positive with a mean contamination level of 61.7 µg/kg (Funes & Resnik, 2009).

In the present study, PAT contamination in 1487 (25%) samples out of 5958 analyzed samples was below 50 µg/L, in addition 464 (7.8%) of the fruit juice and pulps samples had PAT levels between 50 and 200 µg/L. Only 46 (0.8%) of investigated samples were contaminated with levels higher than 200 µg/L. In a similar study in the Northeast China, 95 samples of apple products were examined

Table 4
Percentage of positive samples, mean, standard deviation, median and maximum concentration for patulin according to type of product and fruit.

Category		Total/positive samples	Positive samples (%)	Mean (μg/L)	Standard deviation	Median (μg/L)	Max (μg/L)
Type of product	Cloudy concentrated juice	63/16	25.4[b]	322[a]	2470	0	19,622
	Cloudy single strength juice	98/68	67.4[a]	327[a]	1045	36.93	7339
	Concentrated juice	4918/1551	31.5[b]	14[b]	199	0	13,808
	Concentrated pulp	420/122	29.0[b]	12[b]	45	0	708
	Single strength pulp	455/246	54.1[a]	35[b]	93	21.05	1750
	Sulphited juice	4/0	0[c]	0[b]	0	0	0
Fruit	Apple	4634/1866	40.3[a]	26[b]	330	0	19,622
	Apricot	22/1	4.5[b]	0.7[b]	3.5	0	16
	Grape	50/5	10.0[b]	283[a]	1951	0	13,808
	Orange	17/0	0[b]	0[b]	0	0	0
	Peach	93/9	9.7[b]	5[b]	5	0	24
	Pear	1138/122	10.7[b]	54[b]	53	0	1749
	Pineapple	6/0	0[b]	0[b]	0	0	0

[a-c] Different letters in the Positive (%) and Mean (μg/L) columns indicate significant differences ($p < 0.05$) according to Z-score test (with Bonferroni's method) and Duncan's test, respectively.

for PAT content and the mycotoxin was detected in all samples at concentrations ranging from <1.2 to 94.7 μg/kg. The results showed that 16% of apple products had PAT level higher than the permitted EU regulation level and the mean PAT contamination was 20.4 μg/kg (Yuan, Zhuang, Zhang, & Liu, 2010). The occurrence of PAT in apple juice samples was also determined by Murillo-Arbizu, Amézqueta, González-Peñas, & López de Cerain (2009) and 66% of samples contained PAT with an average level of 19.4 μg/L. In addition, 11% of analyzed samples had PAT level exceeding the EU maximum permitted level.

According to Cano-Sancho, Marin, Ramos, and Sanchis (2009), who studied PAT in apple juice and apple products in Catalonia, Spain, the mean PAT concentration recorded in apple juices was 8.05 μg/kg, and none of the samples had levels greater than the permitted EU level. Similar result was found by Tangni et al. (2003), PAT contamination of domestic and imported apple-based drinks in Belgium was analyzed and PAT was detected in 79%, 86% and 43% of 29 samples of national apple juice, 14 samples of imported apple juice and 7 samples of ciders, respectively. However, none of the contaminated samples was over the EU limit and mean concentration of PAT was 9.0 μg/L for apple juices (national and imported) and 3.4 μg/L for ciders. Findings of Spadaro, Ciavorella, Frati, Garibaldi, and Gullino (2007) at Italy indicated that the mean level of PAT in pure apple juices was 9.3 μg/kg and 47.2% of samples were positive. The occurrence of PAT in 69 organic and 100 conventional fruit products in Italy was investigated by Piemontese, Solfrizzo, and Visconti (2005). According to the authors, the incidence of positive samples was higher in organic (45%) than in conventional products (26%), as well as the mean concentration of PAT in apple, pear and other juices and fruit purees.

PAT determination in apple juice concentrates in Shaanxi (China) showed that 1941 out of 1987 examined samples were positive, but only four samples contained the PAT level above 50 μg/kg (Guo, Zhou, Yuan, & Yue, 2013). Additionally, 85 samples of different apple juice products, largely consumed by Tunisian population, were investigated and the incidence of PAT contamination was 35% with a mean value of 20 μg/L. Only 18% of total juice samples exceeded the established EU limit (Zaied, Abid, Hlel, & Bacha, 2013). The differences between the levels of contamination among the different studies may be because of the influence of different apple raw materials, processing steps and storage conditions of apple juices (Sant'Ana et al., 2008).

Comparing the results in the present study of products obtained in Argentina with studies conducted in other countries showed that the fairly high incidence of PAT was observed in fruit juice samples and the maximum concentrations of PAT were higher than the maximum recommended level of Codex Alimentarius (50 μg/L). According to Beretta, Gaiaschi, Galli, and Restani (2000), Burda (1992) and Cheraghali et al. (2005), which analyzed samples of apple juice in Australia, Italy, and Iran, the highest levels of PAT found were 646, 1150 and 285 μg/kg, respectively. However, our findings showed that the highest PAT detected level was 19,622 μg/L. Due to the high level of contamination; improvements in the process of production are strongly recommended.

3.3. Incidence and concentration of OTA in fruit juices and wine

The results for OTA in the analyzed samples through the years of 2005–2013 are shown in Table 5. OTA was detected in 22 out of 1401 analyzed samples (ranging from 0.1 to 3.6 μg/L). Only one out of 68 samples (1.5%) in the year of 2005 presented OTA level higher than the permitted EU level (2 μg/L) (European Commission, 2006). The highest incidence was observed in 2007 with 8 positive samples in a total of 153 samples (5.2%), however no significant differences were found between the years analyzed. Samples from 2006, 2008 and 2009 did not show any contamination. Samples obtained in 2005 and 2007 presented the highest mean OTA concentration, 0.05 μg/L and 0.03 μg/L, respectively ($p < 0.05$), while the highest maximum OTA concentration (3.6 μg/L) was found in 2005. Median was equal to zero for all years, since 98.4% of all samples analyzed for OTA did not presented any concentration level, which, as mentioned before, pulls the median values to zero.

Results regarding the type of fruit for OTA contamination were in Table 6. Grape showed the highest number of contaminated samples (19 of 1356 samples), however orange and tangerine presented the significantly highest positive samples, both with 1 contaminated sample in 2 analyzed samples (50%). Apricot and lemon samples had no OTA contamination. Mean OTA concentration was also significantly higher for orange and tangerine. Maximum OTA concentration level was found for grape (3.6 μg/L) and median values were all zero.

Analyzing Table 6 for type of product, cloudy concentrated juice had the significantly higher number of positive samples (2 out of 21 samples). There was no OTA contamination for concentrated pulp products. Single strength juice presented the highest mean OTA concentration (0.1 μg/L) ($p < 0.05$) and the highest maximum OTA value (3.6 μg/L). Again, median values were zero for all samples.

Our results have good confirmation in comparison with previous investigations. The levels were not high when compared with the data reported by Czerwiecki, Wilczynska, and Kwiecien (2005), who demonstrated that the incidence of OTA in wine samples from Polish market was 92% and its concentration ranged from 2.2 to

Table 5
Occurrence, positive samples, mean, standard deviation, median and maximum concentration of ochratoxin A in fruit juices and wines during 2005 up to 2013.

Year (total/positive samples)	Positive (%)	0.1–1.0 μg/l (% positive)	1.1–1.5 μg/l (% positive)	1.6–2.0 μg/l (% positive)	>2 μg/l (% positive)	Mean (μg/l)	Standard deviation	Median (μg/l)	Max (μg/l)
2005 (68/1)	1.5[ab]	0	0	0	1 (1.5)	0.05[a]	0.4	0	4
2006 (95/0)	0[b]	0	0	0	0	0[b]	0	0	0
2007 (153/8)	5.2[a]	5 (3.3)	3 (2.0)	0	0	0.03[ab]	0.1	0	1
2008 (191/0)	0[b]	0	0	0	0	0[b]	0	0	0
2009 (124/0)	0[b]	0	0	0	0	0[b]	0	0	0
2010 (130/1)	0.8[ab]	1 (0.8)	0	0	0	0.004[b]	0.04	0	0.5
2011 (205/5)	2.4[ab]	5 (2.4)	0	0	0	0.01[b]	0.07	0	0.5
2012 (216/3)	1.4[ab]	3 (1.4)	0	0	0	0.007[b]	0.06	0	0.6
2013 (219/4)	1.8[ab]	4 (1.8)	0	0	0	0.006[b]	0.05	0	0.6

[a-b] Different letters in the Positive (%) and Mean (μg/L) columns indicate significant differences ($p < 0.05$) according to Z-score test (with Bonferroni's method) and Duncan's test, respectively.

Table 6
Percentage of positive samples, mean, standard deviation, median and maximum concentration for ochratoxin A according to type of product and fruit.

Category		Total/positive samples	Positive samples (%)	Mean (μg/l)	Standard deviation	Median (μg/l)	Max (μg/l)
Type of product	Cloudy concentrated juice	21/2	9.5[a]	0.02[b]	0.06	0	0.2
	Concentrated juice	163/6	3.7[a]	0.02[b]	0.09	0	0.5
	Concentrated pulp	9/0	0[b]	0[b]	0	0	0
	Single strength juice	35/2	5.7[a]	0.1[a]	0.6	0	4
	Sulphited concentrated juice	372/10	2.7[a]	0.02[b]	0.1	0	1
	Wine	801/2	0.2[b]	0.001[b]	0.02	0	0.5
Fruit	Apricot	9/0	0[b]	0[a]	0	0	0
	Grape	1356/19	1.4[b]	0.01[a]	0.1	0	4
	Lemon	32/0	0[b]	0[a]	0	0	0
	Orange	2/1	50[a]	0.1[a]	0.1	0	0.2
	Tangerine	2/1	50[a]	0.1[a]	0.1	0	0.2

[a-b] Different letters in the Positive (%) and Mean (μg/L) columns indicate significant differences ($p < 0.05$) according to Z-score test (with Bonferroni's method) and Duncan's test, respectively.

6710 ng/L with a mean of 39 ng/L. Nevertheless, in the case of grape juice and fruit drinks, OTA was identified in all samples with concentrations varying from 1.6 to 64.7 ng/L. Ng, Mankotia, Pantazopoulos, Neil, and Scott (2004) determined OTA in samples of wine and grape juice collected over three years in Canada. Authors observed that 47 of 180 samples of wine and 4 of 71 grape juice samples showed OTA levels higher than the LOQ. All positive samples were below the maximum levels of EU.

Rosa, Magnoli, Fraga, Dalcero, and Santana (2004) investigated the level of OTA contamination in grape and grape products in Brazil and found that 25% of 64 samples of grape juice and frozen pulps were positive for OTA with mean and maximum concentration of 37 ng/L and 100 ng/L, respectively. For wine samples, the mean concentration observed in positive samples (28.8%) was 34.4 ng/L. The present study had a higher mean concentration and lower incidence of OTA in comparison with results of Rosa et al. (2004). Also, our results showed that the level of OTA in grape juice and wine (Tables 5 and 6) in Argentina was not of main concern of food safety, but the improvement of the quality of raw materials and standardization could be used to keep the safe situation.

In Spain, a survey of Bellí, Marín, Duaigües, Ramos, & Sanchis (2004) on 240 samples of grape-based beverages was carried out and 43 (17.9%) of all samples presented measurable levels of OTA. The authors also concluded that the percentage of wine samples with detectable quantities of OTA was higher for red (18.3%) than for white (10%) wines. Chiodini, Scherpenisse, and Bergwerff (2006) reported the median OTA concentrations of 0.073 μg/L, 0.092 μg/L and 0.066 μg/L for red, rosé and white wines, respectively. The authors did not find significant differences between OTA concentrations in organic and conventional wines. Samples of wine ($n = 30$), beer ($n = 5$) and fruit juices ($n = 14$) from Morocco were evaluated for OTA level, and all wine samples were contaminated at a mean of 0.65 μg/L, just one sample of fruit juices were contaminated (1.16 μg/L) and none contamination was found for beer samples (Filali et al., 2002).

Pietri, Bertuzzi, Pallaroni, and Piva (2001) analyzed OTA contamination in 96 samples of red wine and 15 samples of white dessert wine produced in the years of 1995–1997 in 19 Italian regions. It was observed that OTA amount ranged from <1 to 3856 ng/L, and the mean OTA concentration for red wine was 90 ng/L and for white dessert wine was 8 ng/L. It was also concluded that the geographical region of origin has a strong influence on OTA contamination for both types of wines. In another study, also using Italian wines and South African wines, 2 Italian red wines from 8 samples analyzed were contaminated with OTA at a mean level of 0.58 μg/L, while all South African wines were contaminated with OTA at a mean level of 0.16 μg/L in the white wines and 0.24 μg/L in the red wines (Shephard, Fabiani, Stockenström, Mshicileli, & Sewram, 2003).

4. Conclusion

In this extensive study on the incidence of PAT and OTA in fruit juices and wines in Argentina, it has been showed that 33.5% of fruit juice samples contained PAT in the level above of LOD and just 1.6% of fruit juices and wines were contaminated with OTA. PAT and OTA concentrations values varied greatly among samples regarding year, type of fruit and type of product. It should be emphasized that a great amount of data on the incidence of these mycotoxins in these matrixes can be further used in the developments and reinforcement of measures to reduce the burden of their presence in juices and wines and also used to establishing new maximum tolerable levels. It is worrying that 8.6% of the samples analyzed for

PAT were above the limit imposed by the European legislation, mainly because fruit juices are quite consumed by children. Although OTA contamination was low, effective ways to safeguard consumers against PAT and OTA toxic effects and consequently to protect public health are important and necessary. The use of good agricultural practices in order to avoid fungal growth in the field and during storage of fruits as well as the improvement of processing conditions are ways to mitigate the occurrence and concentration of these mycotoxins in fruit juices and wines.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgments

The authors would like to acknowledge the support by the "Consejo Nacional de Investigaciones Científicas y Técnicas, Centro de Investigación y Asistencia Técnica a la Industria (CIATI AC); "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq) (under Grant #302763/2014-7); and CNPq-TWAS Postgraduate Fellowship (under Grant #3240274290); "Fundação de Amparo à Pesquisa do Estado de São Paulo (under Grant #2014/14891-7); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

References

Agresti, A. (2007). *An introduction to categorical data analysis* (2nd ed.). Hoboken, NJ: John Wiley & Sons.

AIJN – Association of the Industries of Juices and Nectars from Fruits and Vegetables of the European Union. (2015). Available from: www.aijn.org. (Accessed 15 September 2015.

Al-Hazmi, N. A. (2010). Determination of patulin and Ochratoxin A using HPLC in apple juice samples in Saudi Arabia. *Saudi Journal of Biological Sciences, 17*(4), 353–359. http://dx.doi.org/10.1016/j.sjbs.2010.06.006.

Amézqueta, S., González-Peñas, E., Murillo-Arbizu, M., & López de Cerain, A. (2009). Ochratoxin A decontamination: A review. *Food Control, 20*(4), 326–333. http://dx.doi.org/10.1016/j.foodcont.2008.05.017.

Bellí, N., Marín, S., Duaigües, A., Ramos, A. J., & Sanchis, V. (2004). Ochratoxin A in wines, musts and grape juices from Spain. *Journal of the Science of Food and Agriculture, 84*(6), 591–594. http://dx.doi.org/10.1002/jsfa.1702.

Beretta, B., Gaiaschi, A., Galli, C. L., & Restani, P. (2000). Patulin in apple-based foods: Occurrence and safety evaluation. *Food Additives & Contaminants, 17*, 399–406. http://dx.doi.org/10.1080/026520300404815.

Brackett, R. E., & Marth, E. H. (1979). Ascorbic acid and ascorbate cause disappearance of patulin from buffer solutions and apple juice. *Journal of Food Protection, 42*(11), 864–866. http://dx.doi.org/10.4315/0362-028x-42.11.864.

Burda, K. (1992). Incidence of patulin in apple, pear, and mixed fruit products marketed in New South Wales. *Journal of Food Protection, 55*, 796–798. http://dx.doi.org/10.4315/0362-028x-55.10.796.

Cano-Sancho, G., Marin, S., Ramos, A. J., & Sanchis, V. (2009). Survey of patulin occurrence in apple juice and apple products in Catalonia, Spain, and an estimate of dietary intake. *Food Additives & Contaminants. Part B, Surveillance, 2*(1), 59–65. http://dx.doi.org/10.1080/02652030902897747.

Cheraghali, A. M., Mohammadi, H. R., Amirahmadi, M., Yazdanpanah, H., Abouhossain, G., Zamanian, F., et al. (2005). Incidence of patulin contamination in apple juice produced in Iran. *Food Control, 16*, 165–167. http://dx.doi.org/10.1016/j.foodcont.2004.01.006.

Chiodini, A. M., Scherpenisse, P., & Bergwerff, A. A. (2006). Ochratoxin A contents in wine: Comparison of organically and conventionally produced products. *Journal of Agriculture and Food Chemistry, 54*, 7399–7404. http://dx.doi.org/10.1021/jf0613482.

Czerwiecki, L., Wilczynska, G., & Kwiecien, A. (2005). Ochratoxin A: An improvement clean-up and HPLC method used to investigate wine and grape juice on the polish market. *Food Additives and Contaminants, 22*(2), 158–162. http://dx.doi.org/10.1080/02652030500038066.

Delage, N., d'Harlingue, A., Colonna Ceccaldi, B., & Bompeix, G. (2003). Occurrence of mycotoxins in fruit juices and wine. *Food Control, 14*(4), 225–227. http://dx.doi.org/10.1016/S0956-7135(03)00010-0.

European Commission. (2006). Commission regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union, L364*, 5–24. Available from: eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF (Accessed 5 January 2017).

Filali, A., Betbeder, A. M., Baudrimont, I., Benayada, A., Soulaymani, R., & Creppy, E. E. (2002). Ochratoxin A in human plasma in Morocco: A preliminary survey. *Human & Experimental Toxicology, 21*(5), 241–245. http://dx.doi.org/10.1080/0265203011003513-8.

Forouzan, S., & Madadlou, A. (2014). Incidence of patulin in apple juices produced in west Azerbayjan province, Iran. *Journal of Agricultural Science and Technology, 16*, 1613–1622. http://dx.doi.org/10.1016/j.foodcont.2004.01.006.

Funes, G. J., & Resnik, S. L. (2009). Determination of patulin in solid and semisolid apple and pear products marketed in Argentina. *Food Control, 20*(3), 277–280. http://dx.doi.org/10.1016/j.foodcont.2008.05.010.

Gökmen, V., & Acar, J. (2000). Long-term survey of patulin in apple juice concentrates produced in Turkey. *Food Additives and Contaminants, 17*(11), 933–936. http://dx.doi.org/10.1080/026520300750038117.

Granato, D., Calado, V. M. A., & Jarvis, B. (2014). Observations on the use of statistical methods in food science and technology. *Food Research International, 55*, 137–149. http://dx.doi.org/10.1016/j.foodres.2013.10.024.

Guo, Y., Zhou, Z., Yuan, Y., & Yue, T. (2013). Survey of patulin in apple juice concentrates in Shaanxi (China) and its dietary intake. *Food Control, 34*(2), 570–573. http://dx.doi.org/10.1016/j.foodcont.2013.05.029.

IFU – International Federation of Fruit Juice Producers. (2005). *Recommendations for analysis of Ochratoxin in fruit juices* (Vol. 10, pp. 1–2). IFU Recommendation.

Iha, M. H., Barbosa, C. B., Heck, A. R., & Trucksess, M. W. (2014). Aflatoxin M_1 and ochratoxin A in human milk in Ribeirão Preto-SP, Brazil. *Food Control, 40*, 310–313. http://dx.doi.org/10.1016/j.foodcont.2013.12.014.

Jackson, L. S., & Al-Taher, F. (2008). Factors affecting mycotoxin production in fruits. In R. Barkai-Golan, & N. Paster (Eds.), *Mycotoxins in fruits and vegetables* (pp. 75–104). San Diego, CA: Academic Press.

Leggott, N. L., & Shephard, G. S. (2001). Patulin in South African commercial apple products. *Food Control, 12*, 73–76. http://dx.doi.org/10.1016/S0956-7135(00)00023-2.

Luque, M. I., Córdoba, J. J., Rodríguez, A., Núñez, F., & Andrade, M. J. (2013). Development of a PCR protocol to detect ochratoxin A producing moulds in food products. *Food Control, 29*(1), 270–278. http://dx.doi.org/10.1016/j.foodcont.2012.06.023.

MacDonald, S., Long, M., & Gilbert, J. (2000). Liquid chromatographic method for determination of patulin in clear and cloudy apple juices and apple puree: Collaborative study. *Journal of AOAC International, 83*, 1387–1394.

Makun, H. A., Adeniran, A. L., Mailafiya, S. C., Ayanda, I. S., Mudashiru, A. T., Ojukwu, U. J., et al. (2013). Natural occurrence of ochratoxin A in some marketed Nigerian foods. *Food Control, 31*(2), 566–571. http://dx.doi.org/10.1016/j.foodcont.2012.09.043.

Murillo-Arbizu, M., Amézqueta, S., González-Peñas, E., & López de Cerain, A. (2009). Occurrence of patulin and its dietary intake through apple juice consumption by the Spanish population. *Food Chemistry, 113*(2), 420–423. http://dx.doi.org/10.1016/j.foodchem.2008.07.054.

Ng, L., Mankotia, M., Pantazopoulos, P., Neil, R. J., & Scott, P. M. (2004). Ochratoxin A in wine and grape juice sold in Canada. *Food Additives & Contaminants, 21*(10), 971–981. http://dx.doi.org/10.1080/02652030400000653.

Nguyen, K. T. N., & Ryu, D. (2014). Concentration of ochratoxin A in breakfast cereals and snacks consumed in the United States. *Food Control, 40*, 140–144. http://dx.doi.org/10.1016/j.foodcont.2013.11.041.

Nunes, C. A., Alvarenga, V. O., Sant'Ana, A. S., Santos, J. S., & Granato, D. (2015). The use of statistical software in food science and technology: Advantages, limitations and misuses. *Food Research International, 75*, 270–280. http://dx.doi.org/10.1016/j.foodres.2015.06.011.

OIV – The International Organisation of Vine and Wine. (2016). Method OIV-MA-AS315–10. Measuring ochratoxine A in wine after going through an immunoaffinity column and HLPC with fluorescence detection (Oeno 16/2001 revised by Oeno 349-2011). Edition 2016. In *The compendium of international methods of analysis of wines and musts* (Vol. 2). Paris.

Piemontese, L., Solfrizzo, M., & Visconti, A. (2005). Occurrence of patulin in conventional and organic fruit products in Italy and subsequent exposure assessment. *Food Additives and Contaminants, 22*(5), 437–442. http://dx.doi.org/10.1080/02652030500073550.

Pietri, A., Bertuzzi, T., Pallaroni, L., & Piva, G. (2001). Occurrence of ochratoxin A in Italian wines. *Food Additives and Contaminants, 18*(7), 647–654. http://dx.doi.org/10.1080/02652030119480.

Prieta, J., Moreno, M. A., Diaz, S., Suarez, G., & Dominguez, L. (1994). Survey of patulin in apple juice and children's apple food by the diphasic dialysis membrane procedure. *Journal of Agricultural and Food Chemistry, 42*(8), 1701–1703. http://dx.doi.org/10.1021/jf00044a022.

Rosa, C. A. R., Magnoli, C. E., Fraga, M. E., Dalcero, A. M., & Santana, D. M. N. (2004). Occurrence of ochratoxin A in wine and grape juice marketed in Rio de Janeiro, Brazil. *Food Additives & Contaminants, 21*(4), 358–364. http://dx.doi.org/10.1080/02652030310001639549.

Sant'Ana, A. S., Rosenthal, A., & Massaguer, P. R. (2008). The fate of patulin in apple juice processing: A review. *Food Research International, 41*(5), 441–453. http://dx.doi.org/10.1016/j.foodres.2008.03.001.

Shephard, G. S., Fabiani, A., Stockenström, S., Mshicileli, N., & Sewram, V. (2003). Quantitation of ochratoxin A in South African wines. *Journal of Agricultural and Food Chemistry, 51*(4), 1102–1106. http://dx.doi.org/10.1021/jf0259866.

Soufleros, E. H., Tricard, C., & Bouloumpasi, E. C. (2003). Occurrence of ochratoxin A in Greek wines. *Journal of the Science of Food and Agriculture, 83*(3), 173–179. http://dx.doi.org/10.1002/jsfa.1300.

Spadaro, D., Ciavorella, A., Frati, S., Garibaldi, A., & Gullino, M. L. (2007). Incidence and level of patulin contamination in pure\rand mixed apple juices marketed in

Italy. *Food Control, 18*(9), 1098–1102. http://dx.doi.org/10.1016/j.foodcont.2006.07.007.

Succop, P. A., Clark, S., Chen, M., & Galke, W. (2004). Imputation of data values that are less than a detection limit. *Journal of Occupational and Environmental Hygiene, 1*(7), 436–441. http://dx.doi.org/10.1080/15459620490462797.

Tangni, E. K., Theys, R., Mignolet, E., Maudoux, M., Michelet, J. Y., & Larondelle, Y. (2003). Patulin in domestic and imported apple-based drinks in Belgium: Occurrence and exposure assessment. *Food Additives & Contaminants, 20*(5), 482–489. http://dx.doi.org/10.1080/0265203031000093204.

Varga, J., & Kozakiewicz, Z. (2006). Ochratoxin A in grapes and grape-derived products. *Trends in Food Science and Technology, 17*, 72–81. http://dx.doi.org/10.1016/j.tifs.2005.10.007.

Visconti, A., Pascale, M., & Centonze, G. (2001). Determination of ochratoxin A in wine and beer by immunoaffinity column cleanup and liquid chromatographic analysis with fluorometric detection: Collaborative study. *Journal of AOAC International, 84*(6), 1818–1827.

Wilson, D. M., & Nuovo, G. J. (1973). Patulin production in apples decayed by *Penicillium expansum*. *Applied Microbiology, 26*(1), 124–125.

Wu, J., Tan, Y., Wang, Y., & Xu, R. (2011). Occurrence of ochratoxin A in wine and beer samples from China. *Food Additives & Contaminants. Part B, Surveillance, 4*(1), 52–56. http://dx.doi.org/10.1080/19393210.2010.535909.

Yuan, Y., Zhuang, H., Zhang, T., & Liu, J. (2010). Patulin content in apple products marketed in Northeast China. *Food Control, 21*(11), 1488–1491. http://dx.doi.org/10.1016/j.foodcont.2010.04.019.

Zaied, C., Abid, S., Hlel, W., & Bacha, H. (2013). Occurrence of patulin in apple-based-foods largely consumed in Tunisia. *Food Control, 31*(2), 263–267. http://dx.doi.org/10.1016/j.foodcont.2012.10.005.