Food Control 26 (2012) 259–268

Contents lists available at SciVerse ScienceDirect

Food Control

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

Review

OTA-producing fungi in foodstuffs: A review

S. Amézqueta [a,1], S. Schorr-Galindo [b], M. Murillo-Arbizu [a,2], E. González-Peñas [a,*], A. López de Cerain [c], J.P. Guiraud [b]

[a] Organic and Pharmaceutical Chemistry Department, Ed. CIFA, Faculty of Pharmacy, University of Navarra, C/Irunlarrea s/n 31008 Pamplona, Spain
[b] Qualisud Food Quality Joint Research Unit, Food Safety Team cc023, University of Montpellier 2, 34095 Montpellier Cedex 5, France
[c] Department of Nutrition and Food Sciences, Physiology and Toxicology, Faculty of Pharmacy, University of Navarra, 31008 Pamplona, Spain

ARTICLE INFO

Article history:
Received 7 October 2011
Received in revised form
16 January 2012
Accepted 21 January 2012

This document summarizes the OTA-producing ability of the different toxigenic fungi in several crops.Keywords:
Mycotoxins
Ochratoxin A
Fungi
Analysis
Review

ABSTRACT

Ochratoxin A (OTA) is a secondary metabolite produced by filamentous fungi of the genera *Aspergillus* and *Penicillium* present in a wide variety of foodstuffs. The most relevant OTA-producing species are *Penicillium verrucosum* (*P. verrucosum*), *Aspergillus ochraceus* (*A. ochraceus*), *Aspergillus niger* and *Aspergillus carbonarius* due to their prevalence in foodstuffs (cereals, grapes, coffee, etc.) and the number of strains able to produce OTA. To target pre- and post-harvest control programs, studies concerning the toxigenic fungi in each foodstuff are essential. This paper summarizes the state-of-the-art and the requirements in OTA control.

© 2012 Elsevier Ltd. All rights reserved.

Contents

1. Introduction 260
2. Fungi producing OTA 260
3. OTA analysis in fungal cultures 260
   3.1. Extraction and purification 260
   3.2. Determination 260
   3.3. Method validation 261
4. Influence of culture conditions in OTA production 261
   4.1. Cereals 262
   4.2. Coffee 263
   4.3. Grapes 263
   4.4. Other matrices 263
5. Fungal OTA-producing ability 263
   5.1. Fungi isolated from cereals 264
   5.2. Fungi isolated from coffee 265
   5.3. Fungi isolated from grapes 265
   5.4. Fungi isolated from other matrices 265
6. Conclusion 266
   Acknowledgements 266
   References 266

* Corresponding author. Tel.: +34 948 425653; fax: +34 948 425652.
E-mail addresses: samezqu@alumni.unav.es (S. Amézqueta), galindo@univ-montp2.fr (S. Schorr-Galindo), mmurarb@alumni.unav.es (M. Murillo-Arbizu), mgpenas@unav.es (E. González-Peñas), acerain@unav.es (A. López de Cerain), guiraud@univ-montp2.fr (J.P. Guiraud).
[1] Current address: Bioglane SLNE, c/Jordi Girona 18-26, 08034 Barcelona, Spain.
[2] Current address: 3P Biopharmaceuticals, S.L. Polígono Mocholí, C/Mocholí 2, 31110 Noáin Navarra, Spain.

0956-7135/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodcont.2012.01.042

1. Introduction

Ochratoxin A (OTA) is a secondary metabolite produced by filamentous fungi of the genera *Aspergillus* (*A.*) and *Penicillium* (*P.*) present in a wide variety of foodstuffs (Hesseltine, Vandegraft, Fennell, Smith, & Shotwell, 1972) such as cereals, coffee, cocoa, grapes or spices and, due to OTA stability, in processed products such as bread, coffee beverages, chocolate or wine (Miraglia & Brera, 2002).

This mycotoxin has been described as nephrotoxic, carcinogenic, teratogenic, immunotoxic and hepatotoxic in laboratory and domestic animals, as well as the probable causal agent in the development of nephropathies and urothelial tumours in humans (O'Brien & Dietrich, 2005). In consequence, the European Union has established regulatory levels in order to control the presence of OTA in cereal, grape and coffee-derived products (Commission Regulation (EC) 1881/2006).

It is known that certain enzymes, yeasts and *Aspergillus* and *Rhizopus* species are able to degrade OTA (Abrunhosa, Serra, & Venancio, 2002; Bejaoui, Mathieu, Taillandier, & Lebrihi, 2005; Masoud & Kaltoft, 2006; Varga, Rigó, & Téren, 2000; Varga, Péteri, Tábori, Téren, & Vágwölgyi, 2005). However, in order to reduce human exposure to this mycotoxin, fungal control and preventive measures in raw materials need to be established. For this purpose, the identification of the OTA-producing species and the ecophysiological factors that make possible the production of the toxin is needed. A summary of the state-of-the-art is presented in this review.

2. Fungi producing OTA

OTA was first isolated from *Aspergillus ochraceus* (Van der Merwe, Steyn, & Fourie, 1965). Other fungi able to produce OTA are: *Petromyces alliaceus* (Lai, Semeniuk, & Hesseltine, 1970), *Aspergillus sulphureus* (Hesseltine et al., 1972); *Penicillium verrucosum* (Pitt, 1987); *Aspergillus niger* (Abarca, Bragulat, Castellá, & Cabañes, 1994), *Aspergillus carbonarius* (Téren, Varga, Hamari, Rinyu, & Kevei, 1996), *Penicillium nordicum* (Larsen, Svendsen, & Smedsgaard, 2001), *Aspergillus lacticoffeatus* and *Aspergillus sclerotioniger* (Samson, Houbraken, Kuijpers, Frank, & Frisvad, 2004), *Aspergillus cretensis*, *Aspergillus flocculosus*, *Aspergillus pseudoelegans*, *Aspergillus roseoglobulosus*, *Aspergillus westerdijkiae*, *Aspergillus sulphurous*, *Neopetromyces muricatus* and *Aspergillus steynii* (Frisvad, Frank, Houbraken, Kuijpers, & Samson, 2004).

The most relevant OTA-producing species among the aforementioned are *P. verrucosum*, *A. ochraceus* (it belongs to *A.* section *Circumdati*), *A. niger* and *A. carbonarius* (both belong to *A.* section *Nigri*) due to their incidence in foodstuffs and the number of strains able to produce OTA.

Two different approaches have been used to examine the OTA production ability of fungi. Some studies are specifically directed to determine how different parameters (temperature, water activity, etc.) influence mold growth and OTA production. These studies are reviewed in Section 4. The aim of other works is, first, identify the genera and species of fungi isolated from raw materials and, then, quantify the OTA they are able to generate under controlled culture conditions. These studies are reviewed in Section 5.

In all of these works the OTA synthesized by fungi isolated from different raw materials is studied in culture medium by applying several analytical techniques.

3. OTA analysis in fungal cultures

OTA analysis in foodstuffs has been carried out by the application of different extraction, purification and determination techniques.

3.1. Extraction and purification

Téren et al. (1996) suggested an extraction method based in culture extraction with chloroform and liquid–liquid extraction with sodium hydrogen carbonate. Magnoli et al. (2007) and coworkers have applied this method in their studies avoiding liquid–liquid extraction step.

Park, Choi, Hwang, and Kim (2005) and Trung, Bailly, Querin, Le Bars, and Guerre (2001) extracted the mycotoxin with acetonitrile/KCl and chloroform/fosforic acid, respectively, and then carried out a liquid–liquid extraction with toluene/ethyl acetate/formic acid and sodium hydrogen carbonate, respectively, to obtain clearer extracts. Afterwards, Park et al. (2005) cleaned up the partitioned extract with a C_{18} cartridge.

Medina, Mateo, López-Ocaña, Valle-Algarra, and Jiménez (2005) suggested an extraction of the solid media with methanol/sodium hydrogen carbonate and a high speed blender followed by filtration and cleaning up with a C_{18} cartridge. In the case of liquid media, they extracted the toxin with aqueous phosphoric acid followed by liquid–liquid extraction with chloroform.

Extractions from the matrix followed by liquid–liquid extraction or SPE (solid phase extraction) purification offer clean extracts but are time consuming and recoveries are not always high. In addition, some of these extractions are made with chloroform, a solvent that should be substituted by safer organic solvents due to its toxicity.

In most studies, extraction is carried out according to the method proposed by Bragulat, Abarca, and Cabañes (2001). After incubation of the fungi in the selected medium, some agar plugs are removed from the medium, weighed and introduced into a small vial. Then, they are extracted with methanol or methanol/formic acid during a maximum of 60 min and then passed through a filter. This method is very simple, selective, shows good recovery results and allows fast screenings. Other solvents employed for OTA extraction are methanol/water (Tjamos, Antoniou, & Tjamos, 2006) and acetate/dichloromethane/methanol/formic acid (Noonim, Mahakarnchanakul, Nielsen, Frisvad, & Samson, 2008). Extraction time can be reduced to several minutes by vortexing (Tjamos et al., 2006) or applying an ultrasound treatment (Suárez-Quiroz et al., 2004a). To obtain representative data, agar plugs have to be appropriately selected in the culture taking into account the aim of the study and the concrete fungus growth pattern.

3.2. Determination

The technique most spread in OTA determination is a FLD (fluorescence detector) coupled to an HPLC (high pressure liquid chromatography). Selected excitation wavelength is 254 or 330 nm and selected emission wavelength is 460 nm. HPLC columns are C_{18}-type and mobile phases are gradients of different percentages of acetonitrile and acidified water. HPLC allows the separation of OTA from interferences due to their different interactions with the stationary/mobile phases. FLD contributes to the method selectivity and sensitivity because not all the molecules can be excited at 254/330 nm and emit at 460 nm. Moreover, HPLC–FLD is a quantitative technique: results are precise and accurate in a wide range of concentrations for OTA samples.

Díaz, Torres, Vega, and Latorre (2009), Kapetanakou, Panagou, Gialitaki, Drosinos, and Skandamis (2009) and Tjamos et al. (2006) alternatively analyzed the OTA by ELISA (enzyme-linked immunosorbent assay). This technique is fast, cheap, it does not require high-skilled technician and is usually employed for screening studies. Compared to HPLC–FLD, it provides lower LODs (Díaz et al., 2009) and often demands the false-positive confirmation.

OTA can also be analyzed by TLC (thin layer chromatography) placing the plug with the mycelium side towards the gel and using an organic solvent mixture to perform the chromatography (Masoud & Kaltoft, 2006). The toxin is detected under UV light at 336 nm. Recently, a new TLC method has been developed using photosensor detection (De Rossi et al., 2011). TLC is fast and appropriate in preliminary screenings as it provides semi-quantitative data.

Finally, Leggieri, Planas, Battilani, and Magan (2011) have prepared an electronic nose able to detect and distinguish between OTA producer and non-producer strains of *P. nordicum* on a ham-based medium. This technique could be used in preliminary screenings in the ham industry.

3.3. Method validation

The validation of analytical procedures, which is the proof of their suitability for the intended purpose, is essential to obtain reliable data, to compare results, to avoid additional work and to help in the correct decision-making process (Ermer, 2001; Valenta, 1998). In consequence, validation parameters have to be studied for every analytical method used. Published *in vitro* OTA analyses do not always include a validation study. In order to compare results obtained in different laboratories, at least the recovery, and the limit of detection (LOD) or the limit of quantification (LOQ) should be provided.

Recovery parameter allows the correction of the data taking into account the losses during the sample preparation. Although it is not imperative to correct the analytical data, recovery value is necessary to compare results. Not all the studies about *in vitro* OTA production indicate this parameter, but values in the range 52–106% have been reported (see Tables 4–6).

The LOD and LOQ are different depending on the procedure employed. Some studies do not include either of these parameters but values in the range 0.02–20 μg/kg culture medium have been stated in the literature (see Tables 4–6). Lower limits would permit the detection of smaller OTA quantities and, in consequence, establish as OTA-producers fungal strains not considered before. In any case, OTA *in vitro* levels in the most relevant fungal strains are higher than the limits provided by the available current techniques.

In long-term studies, between-day precision and accuracy should also be considered to assure the validity of the methodology throughout the entire working period. In this sense, matrix spiking with different OTA levels should be repeated in different days to prove adequate RSD (relative standard deviation) and RE (relative error) values.

With respect to the selectivity of the analytical method employed, it is the most important aspect to avoid false-positive results. The selectivity can be proven by changing the chromatographic conditions, analyzing “blank” matrix samples or, alternatively, confirming positive results with high selective devices such as HPLC or GC coupled to MS (mass spectrometry) detectors. Mass spectrometry is the novelty in mycotoxin determination. It provides very reliable results because, depending on the detector, it can inform about the exact mass or mother/daughter/neutral loss masses of the molecule eluting during the chromatography. However, it is expensive, technically complex and requires highly skilled technician.

Table 1
Summary of the conditions under which OTA is produced by different fungi.

	P. verrucosum	*A. ochraceus*	*A. carbonarius*	*A. niger*
Minimum temperature	4–10 °C	5–10 °C	5–15 °C	10–15 °C
Maximum temperature	21–31 °C	30–40 °C	30–45 °C	35–41 °C
Optimal temperature	24–25 °C	20–35 °C	15–30 °C	15–35 °C
Minimum a_w	0.80–0.83	0.87–0.90	0.85–0.94	0.90–0.95
Optimal a_w	0.95–0.99	0.95–0.99	0.95–0.99	0.95–0.99
Minimum time	7 days	3 days	2–5 days	3–7 days
Optimal time	>14 days	9–21 days	10–15 days	5–30 days

4. Influence of culture conditions in OTA production

Aspergillus and *Penicillium* are able to develop under a wide variety of environmental conditions (Sweeney & Dobson, 1998). However, the synthesis of secondary metabolites is conditioned by several factors such as temperature, water activity (a_w) (Palacios-Cabrera, Taniwaki, Menezes, & Iamanaka, 2004; Romero, Patriarca, Fernández-Pinto, & Vaamonde, 2007) and medium composition, in particular its micronutrients, its pH and the presence of competitive agents (Esteban, Abarca, Bragulat, & Cabañes, 2004, 2006a; Kokkonen, Jestoi, & Rizzo, 2005; Suárez-Quiroz et al., 2004a; Valero, Oliván, Marín, Sanchis, & Ramos, 2007).

The influence of these factors has been studied by culturing fungi under different environmental conditions in suitable growing substrates: synthetic media (MEA (malt extract agar), YES (yeast extracts), CYA (Czapek yeast extract agar), DYSG (dichloran yeast extract sucrose glycerol agar), PDA (potato-dextrose agar), DG18 (dichloran glicerlo), MY40G (malt yeast extract, 40% glucose agar), SNM (synthetic nutrient medium)), matrix-based synthetic media (cereal, rice, grape, grape juice, coffee based media) or sterilized foodstuffs (cereals, coffee). All of these substrates have shown good results in the OTA-producing fungi growth and the mycotoxin production stimulation.

With regard to temperature and water activity, a summary of the conditions that yield the highest OTA production for each strain is presented in Table 1. *P. verrucosum* and *A. ochraceus* are able to synthesize OTA at lower temperatures than *A. niger* and *A. carbonarius*. Nonetheless, *A. ochraceus* requires higher temperatures than *P. verrucosum* to attain an optimum OTA-producing rate. In this sense, the origin of OTA in cool and temperate climates could be attributed to *P. verrucosum* and *A. ochraceus* whereas in warm temperate and tropical zones it could be associated with *A. ochraceus* and black aspergilli, as previously suggested by other authors (Zimmerli & Dick, 1996).

OTA production occurs at high a_w levels: a minimum a_w of 0.80–0.95 is required, being synthesis optimal at 0.95–0.99. In consequence, to avoid this toxin, environmental conditions must be under strict control during product handling in humid regions (harvest, drying, fermentation and storage stages) and boat transport (Magan & Aldred, 2005; Taniwaki, Pitt, Teixeira, & Iamanaka, 2003).

Moreover, due to a short lag phase germination step in all OTA-producing fungi, significant amounts of OTA can be produced in a few days if certain environmental conditions are met. The FAO/WHO/UNEP (1999) recommends carrying out a fast drying step to avoid fungal invasion in most products. In tropical crops, it is imperative not to store fresh foodstuffs (Goryacheva et al., 2006; Teixeira, Taniwaki, Pitt, Iamanaka, & Martins, 2001) but subject them to a drying treatment. Humidity control devices in dried foodstuff storage areas are also necessary (FAO/WHO/UNEP, 1999).

The presence of competing flora could also influence in the production of OTA. In grapes, for example, competing flora such as non-toxigenic *A. niger* can inhibit the OTA synthesized by *A. carbonarius* (Valero et al., 2007).

Specific conditions for every foodstuff are summarized in Tables 2 and 3. Although more studies are needed to reach concluding results, published data can provide some general trends for each matrix.

Table 2
OTA-producing conditions by fungi isolated from cereals, coffee and grapes.

		Temperature (°C) [a]	Minimum a_w [a]	Medium [a]	Time (days) [a]	Authors
Cereals	*P. verrucosum*	4–31 [24]	0.83 [0.95–0.99]	Grains	NA [b]	Northolt, 1979
		10–25 [25]	0.80 [0.95–0.98]	Wheat, wheat medium	7–56 [>14]	Cairns-Fuller, Aldred, & Magan, 2005
		10–21	NA	DYSG	14	Czaban, Wróblewska, Stochmal, & Janda, 2006
	A. ochraceus	20–40 [30]	15% RH [25–30%]	Maize medium	15	Adebajo, Idowu, & Adesanya, 1994
		15–30 [25–30]	NA [0.98]	Barley medium	7–21 [14–21]	Ramos, Labernia, Marín, Sanchís, & Magan, 1998
		10–30 [30]	0.90 [0.99]	Barley	28	Pardo, Marín, Sanchís, & Ramos, 2004
	A. carbonarius	15–35 [15–20]	0.92 [0.98]	MEA	5–60 [15]	Alborch, Bragulat, Abarca, & Cabañes, 2011
	A. niger	15–30 [25–30]	0.91 [0.97–0.99]	MEA	7–21 [7]	Astoreca et al., 2007
		15–30 [25]	0.91 [0.97]	MEA	7–21 [7]	Astoreca, Barberis, Magnoli, Combina, & Dalcero, 2009a
		15–30 [25]	0.91 [0.97]	MEA	7–21 [7–14]	Astoreca, Barberis, Magnoli, Combina, & Dalcero, 2009b
		15–40 [15–30]	0.92 [0.96–0.98]	MEA	5–60 [10–30]	Alborch et al., 2011
Coffee	*A. ochraceus*	5–40 [35]	0.90 [0.95]	Coffee medium	NA	Suárez-Quiroz et al., 2004b
		28–40	0.90 [0.95]	Coffee	25	Suárez-Quiroz et al., 2004b
		25–35 [25–30]	NA	DG18, MY40G	3–18 [9–12]	Palacios-Cabrera et al., 2005
		>10 [20]	0.80 [0.99]	Coffee	28	Pardo, Marín, Ramos, & Sanchis, 2005b
	A. carbonarius	25–41 [30]	NA	CYA, DG18, MY40G	3–12 [6–8]	Palacios-Cabrera et al., 2005
		15 & 30 [15]	0.86 [0.98]	CYA/YES [CYA]	5–30 [5–10]	Esteban et al., 2006b
	A. niger	25–41 [35]	NA	CYA, DG18, MY40G	3–9 [6–9]	Palacios-Cabrera et al., 2005
Grapes	*A. ochraceus*	20 & 30 [30]	NA [80–100% RH]	MEA	14	Pardo, Marín, Sanchís, & Ramos, 2005a
		15–30 [20]	0.87 [0.99]	MEA	30	Kapetanakou et al., 2009
	A. carbonarius	5–45 [15–20]	0.86 [0.95–0.98]	Grape medium	10	Mitchell, Parra, Aldred, & Magan, 2004
		25	0.90 [0.95–0.98]	Grape medium	5–20 [5]	Bellí, Ramos, Sanchís, & Marín, 2004
		15–37 [20]	0.90 [0.95–0.99]	Grape medium	7	Bellí, Ramos, Coronas, Sanchís, & Marín, 2005
		15–35 [20]	NA	Grape medium	2–10 [8–10]	Marín et al., 2006
		15–35 [15]	0.92 [0.95–0.98]	Grape Juice Medium	7–40 [10–15]	Leong, Hocking, & Scott, 2006
		15 & 30 [15]	0.94 [0.98]	CYA/YES [CYA]	5–30 [10]	Esteban et al., 2006b
		20–30 [20]	[0.97]	CYA	4–18 [4–7]	Valero, Farré, Sanchís, Ramos, & Marín, 2006
		15–35 [25–30]	0.85 [0.95]	CYA	1–34 [3–5]	Romero et al., 2007
		15–30 [25–30]	0.91 [0.99]	MEA	7–21 [7]	Astoreca et al., 2007
		15–30 [25]	0.93 [0.93–0.99]	MEA	30	Kapetanakou et al., 2009
		15–35 [20–25]	0.85 [0.94–0.98]	SNM	5–25 [5–15]	Tassou, Natskoulis, Magan, & Panagou, 2009
		15–37 [15–25]	0.9 [0.99]	SNM	5–10 [5–10]	Lasram et al., 2010
		15–35 [15–20]	0.87 [0.95]	CYA	7–28 [21–28]	Romero, Fernández-Pinto, Patriarca, & Vaamonde, 2010
	A. niger	25	0.90–0.95 [0.98–0.995]	Grape medium	5–20 [5–10]	Bellí, Ramos, et al., 2004
		25	0.90–0.94 [0.96–0.98]	CYA/YES [YES]	5–30 [5]	Esteban, Abarca, Bragulat, & Cabañes, 2006c
		15–35 [15]	0.92 [0.95]	Grape Juice Medium	7–40 [15–20]	Leong et al., 2006

[a] In brackets, optimum production levels.
[b] NA: Data not available.

4.1. Cereals

P. verrucosum isolated from cereals produce OTA at an optimal temperature of 21–25 °C. Higher temperatures, 25–30 °C, favour OTA production in *A. ochraceus* and *A. niger* cultures. The minimum water activity necessary to produce the toxin is 0.80 in the case of *P. verrucosum*, 0.90 in the case of *A. ochraceus* and 0.91 in the case of *A. niger*. Optimal humidity is 0.95–0.99 in the three cases. Maximum production rate is attained after 14 days of *P. verrucosum* and *A. ochraceus* cultures and after shorter times (5 days) in the case of *A. niger*.

To avoid fungal invasion, overripe or damaged cereals should not be collected as the inner part is exposed to risky environmental temperature or humidity. Cereals have to be quickly dried or

Table 3
OTA-producing conditions by fungi isolated from other foodstuffs.

		Temperature (°C) [a]	Minimum a_w [a]	Medium [a]	Time (days) [a]	Authors
A. carbonarius	Apples	15 & 30 [15]	0.94 [0.98]	CYA/YES [CYA]	5–30 [10]	Esteban et al., 2006b
	Several matrices	15–35 [15–20]	NA [b]	CYA/YES [CYA]	5–30 [10]	Esteban et al., 2004
	Cocoa beans	28	0.85 [0.99]	PDA	7–24 [7–14]	Amézqueta et al., 2008
		25	NA	Rice/cocoa medium [rice medium]	7–20 [20]	Mounjouenpou et al., 2008
A. niger	Peanut and maize	15–30 [25–30]	0.91 [0.97–0.99]	MEA	7–21 [7]	Astoreca et al., 2007
	Several matrices	10–35 [20–25]	NA	CYA/YES [YES]	5–30 [5–10]	Esteban et al., 2004
	Cocoa beans	25	NA	Rice/cocoa medium [rice medium]	7–20 [20]	Mounjouenpou et al., 2008

[a] In brackets, optimum production levels.
[b] NA: Data not available.

Table 4
OTA-producing fungi present in cereals and coffee.

	Culture conditions	LOD Recovery	Mold isolated	OTA-producers/total of isolates	OTA amount (μg/L or kg culture medium)	Authors
Cereals	YES, 30 °C	1 μg/L culture medium	*A. ochraceus*	1/4	8	Magnoli et al., 2006
		87%	*A. niger* var. *niger*	10/171	>LOD–32	
			A. niger var. *awamori*	20/220	2–22	
	CYA, 25 °C	0.02 μg/kg culture	*A. ochraceus*	12/12	0.23–11.5	Riba et al., 2008
		medium	*A. carbonarius*	8/10	>LOD–9.35	
		NA[a]	*A. niger* var. *niger*	7/25	>LOD–0.03	
	YES, 35 °C	NA	*A. ochraceus*	3/3	11–178	Trung et al., 2001
		NA				
	PDA, 25 °C	NA	*P. verrucosum*	4/10	700–2000	Park et al., 2005
		NA				
	YES, 25 °C	NA	*A. ochraceus*	3/20	2.2–254	Medina et al., 2006
		NA	*A.* section *Nigri*	30/40	0.036–0.567	
Coffee	CMEA, 25 °C	NA	*A. ochraceus*	3/5	0.3–680	Suárez-Quiroz et al., 2004a
		NA	*A. niger* var. *niger*	1/39	2.2–9.8	
	YES, 28 °C	1 μg/kg culture	*A. ochraceus*	2/2	510–740	Moslem, Mashraqi, Abd-Elsalam,
		medium	*A. carbonarius*	3/3	820–1900	Bahkali, & Elnagaer, 2010
		NA	*A. niger* var. *niger*	4/5	200–2100	
	CYA, 28 °C	NA	*A. westerdijkiae*	1/1	77	Gil-Serna et al., 2011
		NA	*A. steynii*	5/5	720–44,000	

[a] NA: Data not available.

processed after collection. Storage and transport must be done in suitable containers and in environmentally controlled and well-drained installations. Taking into account the previously described data, temperatures over 5 °C and water activities over 0.80 must be avoided. In packed products, temperature should not exceed 15 °C.

4.2. Coffee

A. ochraceus produces optimally the toxin in the range 20–35 °C and a water activity of 0.95–0.99. This fungus requires a minimum of 0.80 of water activity. The period to reach a maximum producing rate is variable and depends on the medium used. In the case of *A. carbonarius*, two studies are available in the literature (Palacios-Cabrera, Taniwaki, Hashimoto, & Menezes, 2005 and Esteban, Abarca, Bragulat, & Cabañes, 2006b) and report optimal temperatures of 15 and 30 °C, respectively, and a minimum value of water activity of 0.86 to synthesize the mycotoxin. Regarding *A. niger*, Palacios-Cabrera et al. (2005) found that optimal production rate is reached at 35 °C employing different culture media. *A.* section *Nigri* fungi are able to produce OTA at shorter period times than *A. ochraceus*.

Coffee is cultivated in tropical regions and is highly hygroscopic when damaged. Due to the high humidity in these areas, overripe, fermented, damaged or fallen onto the soil grains must be discarded and eliminated even off the field to avoid toxigenic fungi and their metabolites. Coffee cannot be stored as a fresh product and has to be quickly and in-place dried to achieve good quality standards. During drying, piles have to be turned over in order to promote their aeration and to prevent the ochratoxigenic mold development. Drying has to be carried out in appropriate surfaces and with efficient equipment. Once the coffee dried, it is fermented and dehulled. These two steps must be carried out in environmentally controlled areas to avoid rewetting or cross contamination. Storage and transport must be done in suitable containers and in environmentally controlled and well-drained installations (Amézqueta, Gonzalez-Penas, Murillo-Arbizu, & López de Cerain, 2009). In concrete, and taking into account the previously described data, temperatures over 10 °C and water activities over 0.75 must be avoided during coffee grain handling. In packed products, temperature should not exceed 20 °C.

4.3. Grapes

A. carbonarius and *A. niger* are able to produce the toxin in the range 15–35 °C. *A. ochraceus* does not generate the toxin over 30 °C. The minimum water activity necessary to produce the toxin is 0.87 in the case of *A. ochraceus*, 0.85 in the case of *A. carbonarius* and 0.90 in the case of *A. niger*. Optimal humidity is over 0.95 in all cases. In general, *A. ochraceus* requires longer incubation periods to produce significant OTA levels.

When grapes are damaged, high sugar and moisture contents are available for fungal development and promote OTA production. In consequence, overripe, fermented, damaged or fallen onto the soil fruits must be discarded and eliminated even off the field. Grapes' skin is very sensitive to damage so collecting machinery has to be carefully selected and reviewed before fruit collection. Once collected, grapes must be stored in suitable containers and in environmentally controlled areas. As this fruit is not generally dried, temperature and moisture control are imperative. Taking into account the data above described, grapes must be maintained under 5 °C of temperature and 0.80 of water activity. When grapes are dried, rewetting must be avoided and, once packed, surrounding temperature must not exceed 15 °C.

4.4. Other matrices

In apples, 15 °C, 0.98 of humidity and 10 days are optimum conditions for OTA production in CYA medium. In the case of cocoa beans, different culture mediums have been used but, in general, mild temperatures and high humidity environments favour OTA production. In peanuts, mild temperatures, high humidity levels and 7 days of incubation are enough to appreciate OTA production.

Due to physical and growing/manufacturing similarities, apples should be controlled in the same manner as grapes and cocoa and peanuts in the same manner as coffee.

5. Fungal OTA-producing ability

A great number of studies have isolated and identified mold strains from cereals, grapes or coffee seeds. Their potential to produce OTA has also been evaluated. Cereals are the main foodstuff contributing to the human OTA consumption (Miraglia &

Table 5
OTA-producing fungi present in grapes.

Culture conditions	LOD Recovery	Mold isolated	OTA-producers/total of isolates	OTA amount (μg/L or kg culture medium)	Authors
YES, 30 °C	1 μg/L culture medium	*A. ochraceus*	2/5	140–2900	Da Rocha Rosa et al., 2002
	87%	*A. carbonarius*	8/32	180–2340	
YES, 30 °C	1 μg/L culture medium	*A. niger* var. *niger*	20/44	>LOD–24	Magnoli, Violante, Combina, Palacio,
	87%	*A. niger* var. *awamori*	4/15	>LOD–20	& Dalcero, 2003
YES, 30 °C	1 μg/L culture medium	*A. carbonarius*	20/23	>LOD–5202	Magnoli et al., 2004
	87%	*A. niger* var. *niger*	23/90	>LOD–61	
		A. niger var. *awamori*	18/98	>LOD–56	
CYA, 25 °C	10 μg/kg culture medium	*A.* section *Circumdati*	3/10	LOD–22,000	Bellí, Pardo et al., 2004
	52–106%	*A.* section *Nigri*	18/386	LOD–2900	
CYA, 25 °C	10 μg/kg culture medium	*A. carbonarius*	175/353	<LOD–477,000	Bellí et al., 2006
	52–106%	*A. niger*	27/671	<LOD–1940	
CYA, 30 °C	10 μg/kg culture medium	*A. ochraceus*	1/2	7300	Bau, Bragulat, Abarca, Minguez,
	52–106%				& Cabañes, 2005
CYA, 30 °C	20 μg/kg culture medium	*A. ochraceus*	1/1	45,000	Gómez, Bragulat, Abarca, Mínguez,
	52–106%	*A. carbonarius*	133/136	>LOD–36,000	& Cabañes, 2006
		A. niger	3/291	3800	
		A. niger	24/101	26,000–96,000	
CYA, 25 °C	20 μg/kg culture medium	*A. carbonarius*	41/47	2500–9600	Lasram et al., 2007
	52–106%	*A. niger*	3/84	40–530	
YES, 30 °C	1 μg/L culture medium	*A. carbonarius*	0/7	<LOD	Ponsone, Combina, Dalcero,
	87%	*A. niger* var. *niger*	27/84	>LOD–51	& Chulze, 2007
		A. niger var. *awamori*	7/38	>LOD–4.8	
		A. carbonarius	101/101	1920–19,5000	
		A. niger	0/474	0	
YES/CYA, 24 °C	1.5 μg/kg culture medium	*A. carbonarius*	14/15	>LOD–87,500	Sage, Krivobok, Delbos, Seigle-Murandi,
	NA[a]	*A.* section *Nigri*	0/8	<LOD	& Creppy, 2002
YES/CYA, 24 °C	1.5 μg/kg culture medium	*A. carbonarius*	10/10	>LOD–1900	Sage, Garon, & Seigle-Murandi, 2004
	NA	*A. niger*	0/29	<LOD	
GJ50, 25 °C	0.1 μg/kg culture medium	*A. carbonarius*	68/68	>LOD–1129	Serra, Braga, & Venancio, 2005
	NA	*A. niger*	23/571	>LOD–137	
CYA, 25 °C	LOQ: 0.2 μg/kg culture medium	*A. carbonarius*	21/99	>LOQ–>25	Tjamos et al., 2006
	NA	*A. niger*	27/60	0.25–15.9	
CYA, 25 °C	1 mg/kg culture medium	*A. carbonarius*	32/36	>LOD–37,500	Bejaoui, Mathieu, Taillandier,
	NA	*A. niger*	1/23	>LOD–100	& Lebrihi, 2006
PDA, 25 °C	0.023 μg/L culture medium	*A. carbonarius*	11/11	4.1–28	Dachoupakan et al., 2009
	NA	*A. niger*	8/13	>LOD–91	
PDA, 25 °C	1.9 μg/L culture medium	*A. wentii*	5/11	>LOD–<20	Díaz et al., 2009
	NA	*A. westerdijkiae*	7/7	10–20	
		A. carbonarius	14/24	>LOD–<20	
		A. niger	14/99	>LOD–<20	
CYA, 25 °C	0.05 μg/kg culture medium	*A. carbonarius*	28/54	1.2–1285	Chiotta, Ponsone, Combina, Torres,
	NA	*A. niger*	63/230	0.5–17	& Chulze, 2010
CYA, 25 °C	NA	*A. carbonarius*	49/86	>100	Battilani et al., 2003
	NA				
CYA, 25 °C	NA	*A. carbonarius*	46/48	>1000	Romero et al., 2005
	NA	*A. niger*	3/293	<100	
YES, 25 °C	NA	*A. carbonarius*	89/120	1.2–3500	Medina et al., 2005
	NA	*A. tubingensis*	3/21	46–111	
CYA, 25 °C	NA	*A. carbonarius*	22/23	6–7500	Perrone et al., 2006
	NA	*A. niger* var. *niger*	3/15	250–350	
		A. tubingensis	3/15	4–130	
CYA, 25 °C	NA	*A. carbonarius*	9/9	>LOD–8380	El Khoury et al., 2008
	NA				
CYA, 28 °C	NA	*A. westerdijkiae*	2/2	18–179	Gil-Serna et al., 2011
	NA	*A. steynii*	1/1	23,000	
YES, 28 °C	1 μg/L culture medium	*A. carbonarius*	12/12	50–6700	Palumbo, O'Keeffe, Vasquez,
	NA				& Mahoney, 2011

[a] NA: Data not available.

Brera, 2002). However, the majority of the studies concern grapes. There are very few studies in other foodstuffs.

5.1. Fungi isolated from cereals

Table 4 shows the number of strains isolated from cereals producing OTA and the amounts synthesized. Culture conditions and validation parameters are also indicated, if available. The percentages of strains isolated, positive for OTA production, were different for each species and also different between studies. For instance, between 15% and 100% of the *A. ochraceus* strains were found to produce OTA, depending on the study, and between 6% and 75% of the *A.* section *Nigri*. This difference may be due, at least in part, to the different culture conditions used, in particular the incubation temperature (25, 30 or 35 °C). *P. verrucosum* showed the highest OTA-producing ability: it was found in a concentration between 0.7 and 2 mg/kg (Park et al., 2005) whereas in other strains, OTA was in the range of μg/L or μg/kg (Magnoli et al., 2006; Medina et al., 2006; Riba, Mokrane, Mathieu, Lebrihi, & Sabaou, 2008; Trung et al., 2001).

Table 6
OTA-producing fungi present in other foodstuffs.

	Culture conditions	LOD Recovery	Mold isolated	OTA-producers/total of isolates	OTA amount (μg/L or kg culture medium)	Authors
Feeds	YES, 30 °C	1 μg/L culture medium	*A. niger* var. *niger*	21/37	13.3–24.7	Dalcero et al., 2002
		87%	*A. niger* var. *awamori*	20/42	13–23.6	
	YES/CYA, 25 °C	10 μg/kg culture medium	*A. ochraceus*	4/20	>LOD–50,500	Accensi, Abarca, & Cabañes, 2004
		52–106%	*A. niger* var. *niger*	30/52	11,600–20,500	
	YES, 30 °C	1 μg/L culture medium	*P. verrucosum*	8/61	18–97	Rosa et al., 2006
		87%	*A. ochraceus*	19/74	53–116	
			A. carbonarius	5/7	9–32	
			A. niger	44/175	10–26	
	CYA/YES, 25 °C	10 μg/kg culture medium 52–106%	*P. verrucosum*	11/155	<LOD–113,000	Bragulat, Martínez, Castellá, & Cabañes, 2008
Peanuts	YES, 30 °C	1 μg/L culture medium	*A. carbonarius*	2/4	9–20	Magnoli et al., 2007
		87%	*A. niger*	99/310	>LOD–24	
Cocoa	PDA, 28 °C	6 μg/kg culture medium 64%	*A. carbonarius*	3/3	>LOD–3520	Amézqueta et al., 2008
	PDA, 25 °C	NA[a]	*A. carbonarius*	3/3	573–2772	Mounjouenpou et al., 2008
		NA	*A. niger*	2/2	3.5–3.6	
	CYA, 25 °C	NA	*A. carbonarius*	6/6	200–8000	Sánchez-Hervás, Gil, Bisbal,
		NA	*A. niger*	59/132	>LOD-90,000	Ramón, & Martínez-Culebras, 2008
	CYA, 25 °C	NA	*A. tubingensis*	46/89	>LOD-120,000	Bisbal, Gil, Ramón, &
		NA				Martínez-Culebras, 2009
	YES, 25 °C	NA	*A. ochraceus*	2/NA	NA	Copetti, Pereira, Iamanaka,
		NA	*A. melleus*	6/NA	NA	Pitt, & Taniwaki, 2010
			A. westerdijkiae	2/NA	NA	
			A. niger	10/191	NA	
			A. carbonarius	92/92	NA	
Pistachios	CYA, 25 °C	0.2 μg/kg culture medium	*P. verrucosum*	3/7	1.2–77	Fernane, Sanchís, Marín, &
		52–106%	*A. niger*	20/62	0.3–1.2	Ramos, 2010
			A. carbonarius	1/1	1.2	
Herbal drugs	PDA, 28 °C	NA	*A. ochraceus*	1/20	80–240	Efuntoye, 1999
		NA				
Bee pollen	PDA, 25 °C	NA	*P. verrucosum*	14/57	1100–3900	González, Hinojo, Mateo, Medina,
		NA	*A. ochraceus*	5/15	4200–4500	& Jiménez, 2005
			A. carbonarius	7/7	2.6–3.1	
			A. niger var. *niger*	9/16	1.4–1.8	
Smoked red pepper	CYA, 28 °C	NA	*A. ochraceus*	1/3	12	Gil-Serna et al., 2011
		NA	*A. westerdijkiae*	1/1	20	

[a] NA: Data not available.

5.2. Fungi isolated from coffee

The studies concerning green coffee are described in Table 4. Suárez-Quiroz et al. (2004b), identified 39 strains of the *A. niger* var. *niger* and 5 of *A. ochraceus*; they found that 2% of the former and 60% of the later were able to synthesize the mycotoxin. *A. ochraceus* presented the highest producing rate (up to 0.68 mg/kg culture medium). Noonim et al. (2008) carried out a semiquantitative work in YES medium (LOD = 0.025 μg/kg culture medium) where *A. steynii*, *A. carbonarius* and *A. westerdijkiae* were able to produce high OTA levels and *A. niger* intermediate OTA levels compared to previous OTA-producing studies. More recently, Gil-Serna, Vázquez, Sardiñas, González-Jaén, and Patiño (2011) have found the highest OTA-producing rate (up to 44 mg/kg culture medium) in *A. steynii* strains.

5.3. Fungi isolated from grapes

In the past few years great attention has been paid to fungi producing OTA in grapes. Table 5 shows the percentage of *A.* section *Circumdati* and *A.* section *Nigri* strains isolated from grapes producing OTA and the amounts synthesized. Culture conditions are very similar between studies and incubation temperature has been 25 °C or 30 °C. The LOD of the analytical technique and the recovery are also indicated, if available.

Several *Aspergillus* section *Nigri* and *A. ochraceus* strains produced the mycotoxin. No strains of *Penicillium* able to produce OTA were isolated in any study. The percentage of positive strains for OTA production varied in a range between 0 and 100% in *A.* section *Nigri* (21–100% *A. carbonarius*, 0–62% *A. niger* and 14–20% *Aspergillus tubingensis*) and between 30% and 100% in *A.* section *Circumdati* (30–50% *A. ochraceus*, 45% *Aspergillus wentii* and 100% *A. westerdijkiae*). In general, the highest percentages were obtained with *A. carbonarius* strains in the majority of the studies. With respect to the amount of OTA detected, concentrations in the range of mg/L or mg/kg have been identified in several studies working on *A. carbonarius*, *A. niger* and *A.* section *Circumdati* strains. *A. carbonarius* was the most toxigenic fungi identified in grapes, up to 477 mg/kg culture medium were quantified in one study (Bellí et al., 2006).

5.4. Fungi isolated from other matrices

Table 6 shows the percentage of *A.* section *Circumdati* and *A.* section *Nigri* strains isolated from several matrices producing OTA and the amounts synthesized. OTA can be synthesised by *A.* section *Nigri*, *A.* section *Circumdati* and *P. verrucosum* isolated from feeds and bee pollen; *A.* section *Circumdati* isolated from herbal drugs and smoked red pepper; *A. carbonarius*, *A. niger*, *A. tubingensis* and *A.* section *Circumdati* isolated from cocoa; and *A. carbonarius* and

A. niger isolated from peanuts. Highest OTA levels have been found in strains isolated from feed and cocoa.

6. Conclusion

OTA quantification is generally carried out by HPLC coupled to a fluorimeter detector, after an extraction of the toxin from culture medium. To prove the reliability of the data and to permit the comparison between different studies, studies should include the validation data of the methodologies applied. Unfortunately, this piece of data is not always included.

In cereals, grapes, cocoa and feed, OTA-producing species are well-known. However, in other human OTA-intake contributing products such as coffee, spices or dried fruits more scientific work is necessary to have a deeper knowledge in OTA contamination. In cereals and feed, ochratoxigenic molds isolated from raw material belong principally to *A. ochraceus* and *P. verrucosum* species. In grapes and cocoa, *Aspergillus* section *Nigri* species strains are frequently isolated, being *A. carbonarius* ones the most efficient in producing the mycotoxin.

Many efforts have to be made in order to avoid OTA contamination in the most susceptible products, by an efficient pre- and post-harvest management that prevents mold growth and OTA production. In cereals, post-harvest measures are crucial, whereas in grapes, coffee and coffee emphasis has to be put in both pre- and post-harvest strategies. In general, unpacked products must be maintained at 5–10 °C and packed products at 15–20 °C and water content must be under 0.75–0.80.

Acknowledgements

Thanks are given to Ms. Laura Stokes for reviewing the English version of this manuscript. Susana Amézqueta and María Murillo-Arbizu thank the Navarra's Government for the grants awarded.

References

Abarca, M. L., Bragulat, M. R., Castellá, G., & Cabañes, F. J. (1994). Ochratoxin A production by strains of *Aspergillus niger* var. *niger*. *Applied and Environmental Microbiology, 60*, 2650–2652.

Abrunhosa, L., Serra, R., & Venancio, A. (2002). Biodegradation of ochratoxin A by fungi isolated from grapes. *Journal of Agricultural Food Chemistry, 50*, 7493–7496.

Accensi, F., Abarca, M. L., & Cabañes, F. J. (2004). Occurrence of *Aspergillus* species in mixed feeds and component raw materials and their ability to produce ochratoxin A. *Food Microbiology, 21*, 623–627.

Adebajo, L. O., Idowu, A. A., & Adesanya, O. O. (1994). Mycoflora, and mycotoxins production in Nigerian corn and corn-based snacks. *Mycopathologia, 126*, 183–192.

Alborch, L., Bragulat, M. R., Abarca, M. L., & Cabañes, F. J. (2011). Effect of water activity, temperature and incubation time on growth and ochratoxin A production by *Aspergillus niger* and *Aspergillus carbonarius* on maize kernels. *International Journal of Food Microbiology, 147*, 53–57.

Amézqueta, S., González-Peñas, E., Dachoupakan, C., Murillo-Arbizu, M., López de Cerain, A., & Guiraud, J. P. (2008). OTA-producing fungi isolated from stored cocoa beans. *Letters in Applied Microbiology, 47*, 197–201.

Amézqueta, S., Gonzalez-Penas, E., Murillo-Arbizu, M., & López de Cerain, A. (2009). Ochratoxin A decontamination: a review. *Food Control, 20*, 326–333.

Astoreca, A., Magnoli, C., Barberis, C., Chiacchiera, S. M., Combina, M., & Dalcero, A. (2007). Ochratoxin A production in relation to ecophysiological factors by *Aspergillus* section *Nigri* strains isolated from different substrates in Argentina. *Science of the Total Environment, 388*, 16–23.

Astoreca, A., Barberis, C., Magnoli, C., Combina, M., & Dalcero, A. (2009a). Influence of ecophysiological factors on growth, lag phase and ochratoxin A production by Aspergillus niger aggregate strains in irradiated corn grains. *International Journal of Food Microbiology, 129*, 174–179.

Astoreca, A., Barberis, C., Magnoli, C., Combina, M., & Dalcero, A. (2009b). Ecophysiological factor effect on growth rate, lag phase and ochratoxin A production by *Aspergillus niger* aggregate strains in irradiated peanut seeds. *International Journal of Food Microbiology, 129*, 131–135.

Battilani, P., Pietri, A., Bertuzzi, T., Languasco, L., Giorni, P., & Kozakiewicz, Z. (2003). Occurrence of ochratoxin A – producing fungi in grapes grown in Italy. *Journal of Food Protection, 66*, 633–636.

Bau, M., Bragulat, M. R., Abarca, M. L., Minguez, S., & Cabañes, F. J. (2005). Ochratoxigenic species from Spanish wine grapes. *International Journal of Food Microbiology, 98*, 125–130.

Bejaoui, H., Mathieu, F., Taillandier, P., & Lebrihi, A. (2005). Conidia of black aspergilli as new biological adsorbents for ochratoxin A in grape juices and musts. *Journal of Agricultural and Food Chemistry, 53*, 8224–8229.

Bejaoui, H., Mathieu, F., Taillandier, P., & Lebrihi, A. (2006). Black aspergilli and ochratoxin A production in French vineyards. *FEMS Microbiology Letters, 255*, 203–208.

Bellí, N., Ramos, A. J., Sanchís, V., & Marín, S. (2004). Incubation time and water activity effects on ochratoxin A production by *Aspergillus* section *Nigri* strains isolated from grapes. *Letters in Applied Microbiology, 38*, 72–77.

Bellí, N., Pardo, E., Marín, S., Farré, G., Ramos, A. J., & Sanchís, V. (2004). Occurrence of ochratoxin A and toxigenic potential of fungal isolates from Spanish grapes. *Journal of the Science of Food and Agriculture, 84*, 541–546.

Bellí, N., Ramos, A. J., Coronas, I., Sanchís, V., & Marín, S. (2005). *Aspergillus carbonarius* growth and ochratoxin A production on a synthetic grape medium in relation to environmental factors. *Journal of Applied Microbiology, 98*, 839–844.

Bellí, N., Bau, M., Marín, S., Abarca, M. L., Ramos, A. J., & Bragulat, M. R. (2006). Mycobiota and ochratoxin A producing fungi from Spanish wine grapes. *International Journal of Food Microbiology, 111*, 840–845.

Bisbal, F., Gil, J. V., Ramón, D., & Martínez-Culebras, P. V. (2009). ITS-RFLP characterization of black *Aspergillus* strains responsible for ochratoxin A contamination in cocoa beans. *European Food Research Technology, 229*, 751–755.

Bragulat, M. R., Martínez, E., Castellá, G., & Cabañes, F. J. (2008). Ochratoxin A and citrinin producing species of the genus *Penicillium* from feedstuffs. *International Journal of Food Microbiology, 126*, 43–48.

Bragulat, M. R., Abarca, M. L., & Cabañes, F. J. (2001). An easy screening method for fungi producing ochratoxin A in pure culture. *International Journal of Food Microbiology, 30*, 139–144.

Cairns-Fuller, V., Aldred, D., & Magan, N. (2005). Water, temperature and gas composition interactions affect growth and ochratoxin A production by isolates of *Penicillium verrucosum* on wheat grain. *Journal of Applied Microbiology, 99*, 1215–1221.

Chiotta, M. L., Ponsone, M. L., Combina, M., Torres, A. M., & Chulze, S. N. (2010). *Aspergillus* section *Nigri* species isolated from different wine-grape growing regions in Argentina. *International Journal of Food Microbiology, 136*, 137–141.

Commission Regulation (EC) 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union, L364*, 5–24.

Copetti, M. V., Pereira, J. L., Iamanaka, B. T., Pitt, J. I., & Taniwaki, M. H. (2010). Ochratoxigenic fungi and ochratoxin A in cocoa during farm processing. *International Journal of Food Microbiology, 143*, 67–70.

Czaban, J., Wróblewska, B., Stochmal, A., & Janda, B. (2006). Growth of *Penicillium verrucosum* and production of Ochratoxin A on nonsterilized wheat grain incubated at different temperatures and water content. *Polish Journal of Microbiology, 55*, 321–331.

Dachoupakan, C., Ratomahenina, R., Martinez, V., Guiraud, J. P., Baccou, J. P., & Schorr-Galindo, S. (2009). Study of the phenotypic and genotypic biodiversity of potentially ochratoxinogenic black aspergilli isolated from grapes. *International Journal of Food Microbiology, 132*, 14–23.

Da Rocha Rosa, C. A., Palacios, V., Combina, M., Fraga, M. E., De Oliveira Rekson, A., Magnoli, C. E., et al. (2002). Potential ochratoxin A producers from wine grapes in Argentina and Brazil. *Food Additives and Contaminants, 19*, 408–414.

Dalcero, A., Magnoli, C., Hallak, C., Chiacchiera, S. M., Palacio, G., & Rosa, C. A. R. (2002). Detection of ochratoxin A in animal feeds and capacity to produce this mycotoxin by *Aspergillus* section *Nigri* in Argentina. *Food Additives and Contaminants, 19*, 1065–1072.

De Rossi, P., Reverberi, M., Ricelli, A., Fabbri, A. A., Caputo, D., De Cesare, G., et al. (2011). Early detection of ochratoxigenic fungi in wine grapes and of ochratoxin A in wine. *Analytical Microbiology, 61*, 11–15.

Díaz, G. A., Torres, R., Vega, M., & Latorre, B. A. (2009). Ochratoxigenic *Aspergillus* species on grapes from Chilean vineyards and *Aspergillus* threshold levels on grapes. *International Journal of Food Microbiology, 133*, 195–199.

Efuntoye, M. O. (1999). Mycotoxins of fungal strains from stored herbal plants and mycotoxin contents of Nigerian crude herbal drugs. *Mycopathologia, 147*, 43–48.

El Khoury, A., Rizk, T., Lteif, R., Azouri, H., Delia, M. L., & Lebrihi, A. (2008). Fungal contamination and Aflatoxin B1 and Ochratoxin A in Lebanese wine-grapes. *Food and Chemical Toxicology, 46*, 2244–2250.

Ermer, J. (2001). Validation in pharmaceutical analysis. Part 1: an integrated approach. *Journal of Pharmaceutical and Biomedical Analysis, 24*, 755–767.

Esteban, A., Abarca, M. L., Bragulat, M. R., & Cabañes, F. J. (2004). Effects of temperature and incubation time on production of ochratoxin A by black aspergilli. *Research in Microbiology, 155*, 861–866.

Esteban, A., Abarca, M. L., Bragulat, M. R., & Cabañes, F. J. (2006a). Effect of pH on ochratoxin A production by *Aspergillus niger* aggregate species. *Food Additives and Contaminants, 23*, 616–622.

Esteban, A., Abarca, M. L., Bragulat, M. R., & Cabañes, F. J. (2006b). Study of the effect of water activity and temperature on ochratoxin A production by *Aspergillus carbonarius*. *Food Microbiology, 23*, 634–640.

Esteban, A., Abarca, M. L., Bragulat, M. R., & Cabañes, F. J. (2006c). Effect of water activity on ochratoxin A production by *Aspergillus niger* aggregate species. *International Journal of Food Microbiology, 108*, 188–195.

FAO/WHO/UNEP. (1999). Mycotoxin prevention and decontamination, HACCP and its mycotoxin control potential: an evaluation of ochratoxin A in coffee

production. In *Third Joint FAO/WHO/UNEP international conference on mycotoxins 6 c, 1–13*, Available at ftp://ftp.fao.org/es/esn/food/myco6c.pdf Accession date: 10.03.09.

Fernane, F., Sanchís, V., Marín, S., & Ramos, A. J. (2010). First report on mould and mycotoxin contamination of pistachios sampled in Algeria. *Mycopathologia, 170*, 423–429.

Frisvad, J. C., Frank, J. M., Houbraken, J. A. M. P., Kuijpers, A. F. A., & Samson, R. A. (2004). New ochratoxin A producing species of *Aspergillus* section *Circumdati*. *Studies in Mycology, 50*, 23–43.

Gil-Serna, J., Vázquez, C., Sardiñas, N., González-Jaén, M. T., & Patiño, B. (2011). Revision of ochratoxin A production capacity by the main species of *Aspergillus* section *Circumdati*. *Aspergillus steynii* revealed as the main risk of OTA contamination. *Food Control, 22*, 343–345.

Gómez, C., Bragulat, M. R., Abarca, M. L., Mínguez, S., & Cabañes, F. J. (2006). Ochratoxin A-producing fungi from grapes intended for liqueur wine production. *Food Microbiology, 23*, 541–545.

González, G., Hinojo, M. J., Mateo, R., Medina, A., & Jiménez, M. (2005). Occurrence of mycotoxin producing fungi in bee pollen. *International Journal of Food Microbiology, 105*, 1–9.

Goryacheva, I. Y., De Saeger, S., Lobeau, M., Eremin, S. A., Barna-Vetró, I., & Van Peteghem, C. (2006). Approach for ochratoxin A fast screening in spices using clean-up tandem immunoassay columns with confirmation by high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS). *Analytica Chimica Acta, 577*, 38–45.

Hesseltine, C. W., Vandegraft, E. E., Fennell, D. I., Smith, M. L., & Shotwell, O. L. (1972). *Aspergilli* as ochratoxin producers. *Mycologia, 64*, 539–550.

Kapetanakou, A. E., Panagou, E. Z., Gialitaki, M., Drosinos, E. H., & Skandamis, P. N. (2009). Evaluating the combined effect of water activity, pH and temperature on ochratoxin A production by *Aspergillus ochraceus* and *Aspergillus carbonarius* on culture medium and Corinth raisins. *Food Control, 20*, 725–732.

Kokkonen, M., Jestoi, M., & Rizzo, A. (2005). The effect of substrate on mycotoxin production of selected *Penicillium* strains. *International Journal of Food Microbiology, 99*, 207–214.

Lai, M., Semeniuk, G., & Hesseltine, C. W. (1970). Conditions for production of ochratoxin A by *Aspergillus* species in a synthetic medium. *Applied Microbiology, 19*, 542–544.

Larsen, T. O., Svendsen, A., & Smedsgaard, J. (2001). Biochemical characterization of ochratoxin A-producing strains of the genus *Penicillium*. *Applied Environmental Microbiology, 67*, 3630–3635.

Lasram, S., Bellí, N., Chebil, S., Nahla, Z., Ahmed, M., Sanchís, V., et al. (2007). Occurrence of ochratoxigenic fungi and ochratoxin A in grapes from a Tunisian vineyard. *International Journal of Food Microbiology, 114*, 376–379.

Lasram, S., Oueslati, S., Valero, A., Marín, S., Ghorbel, A., & Sanchís, V. (2010). Water activity and temperature effects on fungal growth and ochratoxin A production by ochratoxigenic *Aspergillus carbonarius* isolated from Tunisian grapes. *Journal of Food Science, 75*, 89–97.

Leggieri, M. C., Planas, N., Battilani, P., & Magan, N. (2011). Detection and discrimination between ochratoxin producer and non-producer strains of *Penicillium nordicum* on a ham-based medium using an electronic nose. *Mycotoxin Research, 27*, 29–35.

Leong, S. L., Hocking, A. D., & Scott, E. S. (2006). Effect of temperature and water activity on growth and ochratoxin A production by Australian *Aspergillus carbonarius* and A. niger isolates on a simulated grape juice medium. *International Journal of Food Microbiology, 110*, 209–216.

Magan, N., & Aldred, D. (2005). Conditions of formation of ochratoxin A in drying, transport and in different commodities. *Food Additives and Contaminants, 1*, 10–16.

Magnoli, C., Violante, M., Combina, M., Palacio, G., & Dalcero, A. (2003). Mycoflora and ochratoxin-producing strains of *Aspergillus* section *Nigri* in wine grapes in Argentina. *Letters in Applied Microbiology, 37*, 179–184.

Magnoli, C., Astoreca, A., Ponsone, L., Combina, M., Palacio, G., Rosa, C. A. R., et al. (2004). Survey of mycoflora and ochratoxin A in dried vine fruits from Argentina markets. *Letters in Applied Microbiology, 39*, 326–331.

Magnoli, C., Hallak, C., Astoreca, A., Ponsone, L., Chiacchiera, S., & Dalcero, A. M. (2006). Occurrence of ochratoxin A – producing fungi in commercial corn kernels in Argentina. *Mycopathologia, 161*, 53–58.

Magnoli, C., Astoreca, A., Ponsone, M. L., Fernández-Juri, M. G., Barberis, C., & Dalcero, A. M. (2007). Ochratoxin A and *Aspergillus* section *Nigri* in peanut seeds at different months of storage in Córdoba, Argentina. *International Journal of Food Microbiology, 119*, 213–218.

Marín, S., Bellí, N., Lasram, S., Chebil, S., Ramos, A. J., Ghorbel, A., et al. (2006). Kinetics of ochratoxin a production and accumulation by *Aspergillus carbonarius* on synthetic grape medium at different temperature levels. *Journal of Food Science, 71*, 196–200.

Masoud, W., & Kaltoft, C. H. (2006). The effects of yeasts involved in the fermentation of *Coffea arabica* in East Africa on growth and ochratoxin A (OTA) production by *Aspergillus ochraceus*. *International Journal of Food Microbiology, 106*, 229–234.

Medina, A., Mateo, R., López-Ocaña, L., Valle-Algarra, F. M., & Jiménez, M. (2005). Study of Spanish grape mycobiota and ochratoxin A production by isolates of *Aspergillus tubingensis* and other members of *Aspergillus* section *Nigri*. *Applied and Environmental Microbiology, 71*, 4696–4702.

Medina, A., Valle-Algarra, F. M., Mateo, R., Gimeno-Adelantado, J. V., Mateo, F., & Jiménez, M. (2006). Survey of the mycobiota of Spanish malting barley and evaluation of the mycotoxin producing potential of species of *Alternaria*, *Aspergillus* and *Fusarium*. *International Journal of Food Microbiology, 108*, 196–203.

Miraglia, M., & Brera, C. (2002). Assessment of dietary intake of ochratoxin A by the population of EU member states. In *Reports on tasks for scientific cooperation, task 3.2.7* (pp. 69–86). Directorate-General Health and Consumer Protection. http://europa.eu.int/comm/food/fs/scoop/3.2.7_en.pdf.

Mitchell, D., Parra, R., Aldred, D., & Magan, N. (2004). Water and temperature relations of growth and ochratoxin A production by *Aspergillus carbonarius* strains from grapes in Europe and Israel. *Journal of Applied Microbiology, 97*, 439–445.

Moslem, M. A., Mashraqi, A., Abd-Elsalam, K. A., Bahkali, A. H., & Elnagaer, M. A. (2010). Molecular detection of ochratoxigenic *Aspergillus* species isolated from coffee beans in Saudi Arabia. *Genetics and Molecular Research, 9*, 2292–2299.

Mounjouenpou, P., Gueule, D., Fontana-Tachon, A., Guyot, B., Tondje, P. R., & Guiraud, J. P. (2008). Filamentous fungi producing ochratoxin A during cocoa processing in Cameroon. *International Journal of Food Microbiology, 121*, 234–241.

Northolt, M. D. (1979). The influence of water activity on microorganisms in foods. *International Journal of General and Molecular Microbiology, 45*, 159.

Noonim, P., Mahakarnchanakul, W., Nielsen, K., Frisvad, J. C., & Samson, R. A. (2008). Isolation, identification and toxigenic potential of ochratoxin A – producing *Aspergillus* species from coffee beans grown in two regions of Thailand. *International Journal of Food Microbiology, 128*, 197–202.

O'Brien, E., & Dietrich, D. R. (2005). Ochratoxin A: the continuing enigma. *Critical Reviews in Toxicology, 35*, 33–60.

Palacios-Cabrera, H., Taniwaki, M. H., Menezes, H. C., & Iamanaka, B. (2004). The production of ochratoxin A by *Aspergillus ochraceus* in raw coffee at different equilibrium relative humidity and under alternating temperatures. *Food Control, 15*, 531–535.

Palacios-Cabrera, H., Taniwaki, M. H., Hashimoto, J. M., & Menezes, H. C. (2005). Growth of *Aspergillus ochraceus*, *A. carbonarius* and *A. niger* on culture media at different water activities and temperatures. *Brazilian Journal of Microbiology, 36*, 24–28.

Palumbo, J. D., O'Keeffe, T. L., Vasquez, S. J., & Mahoney, N. E. (2011). Isolation and identification of ochratoxin A-producing *Aspergillus* section *Nigri* strains from California raisins. *Letters in Applied Microbiology, 52*, 330–336.

Pardo, E., Marín, S., Sanchís, V., & Ramos, A. J. (2004). Prediction of fungal growth and ochratoxin A production by *Aspergillus ochraceus* on irradiated barley grain as influenced by temperature and water activity. *International Journal of Food Microbiology, 95*, 79–88.

Pardo, E., Marín, S., Sanchís, V., & Ramos, A. J. (2005a). Impact of relative humidity and temperature on visible fungal growth and OTA production of ochratoxigenic *Aspergillus ochraceus* isolates on grapes. *Food Microbiology, 22*, 383–389.

Pardo, E., Marín, S., Ramos, A. J., & Sanchis, V. (2005b). Effect of water activity and temperature on mycelial growth and ochratoxin A production by isolates of *Aspergillus ochraceus* on irradiated green coffee beans. *Journal of Food Protection, 68*, 133–138.

Park, J. W., Choi, S., Hwang, H., & Kim, Y. (2005). Fungal mycoflora and mycotoxins in Korean polished rice destined for humans. *International Journal of Food Microbiology, 103*, 305–314.

Perrone, G., Mulè, G., Susca, A., Battilani, P., Pietri, A., & Logrieco, A. (2006). Ochratoxin A production and amplified fragment length polymorphism analysis of *Aspergillus carbonarius*, *Aspergillus tubingensis*, and *Aspergillus niger* strains isolated from grapes in Italy. *Applied and Environmental Microbiology, 72*, 680–685.

Pitt, J. I. (1987). *Penicillium viridicatum*, *Penicillium verrucosum*, and production of ochratoxin A. *Applied and Environmental Toxicology, 53*, 266–269.

Ponsone, M. L., Combina, M., Dalcero, A., & Chulze, S. (2007). Ochratoxin A and ochratoxigenic *Aspergillus* species in Argentinean wine grapes cultivated under organic and non-organic systems. *International Journal of Food Microbiology, 114*, 131–135.

Ramos, A. J., Labernia, N., Marín, S., Sanchís, V., & Magan, N. (1998). Effect of water activity and temperature on growth and ochratoxin production by three strains of *Aspergillus ochraceus* on a barley extract medium and on barley grains. *International Journal of Food Microbiology, 44*, 133–140.

Riba, A., Mokrane, S., Mathieu, F., Lebrihi, A., & Sabaou, N. (2008). Mycoflora and ochratoxin A producing strains of *Aspergillus* in Algerian wheat. *International Journal of Food Microbiology, 122*, 85–92.

Romero, S. M., Comerio, R. M., Larumbe, G., Ritieni, A., Vaamonde, G., & Fernández Pinto, V. (2005). Toxigenic fungi isolated from dried vine fruits in Argentina. *International Journal of Food Microbiology, 104*, 43–49.

Romero, S. M., Patriarca, A., Fernández-Pinto, V., & Vaamonde, G. (2007). Effect of water activity and temperature on growth of ochratoxigenic strains of *Aspergillus carbonarius* isolated from Argentinean dried vine fruits. *International Journal of Food Microbiology, 115*, 140–143.

Romero, S. M., Fernández-Pinto, V., Patriarca, A., & Vaamonde, G. (2010). Ochratoxin A production by a mixed inoculum of *Aspergillus carbonarius* at different conditions of water activity and temperature. *International Journal of Food Microbiology, 140*, 277–281.

Rosa, D. A. R., Ribeiro, J. M. M., Fraga, M. J., Gatti, M., Cavaglieri, L. R., Magnoli, C. E., et al. (2006). Mycoflora of poultry feeds and ochratoxin-producing ability of isolated *Aspergillus* and *Penicillium* species. *Veterinary Microbiology, 113*, 89–96.

Sage, L., Krivobok, S., Delbos, E., Seigle-Murandi, F., & Creppy, E. E. (2002). Fungal flora and ochratoxin A production in grapes and musts from France. *Journal of Agricultural and Food Chemistry, 50*, 1306–1311.

Sage, L., Garon, D., & Seigle-Murandi, F. (2004). Fungal microflora and ochratoxin A risk in French vineyards. *Journal of Agricultural and Food Chemistry, 52*, 5764–5768.

Samson, R. A., Houbraken, J. A. M. P., Kuijpers, A. F. A., Frank, J. M., & Frisvad, J. C. (2004). New ochratoxin A or sclerotium producing species in *Aspergillus* section *Nigri*. *Studies in Mycology, 50*, 45–61.

Sánchez-Hervás, M., Gil, J. V., Bisbal, F., Ramón, D., & Martínez-Culebras, P. V. (2008). Mycobiota and mycotoxin producing fungi from cocoa beans. *International Journal of Food Microbiology, 125*, 336–340.

Serra, R., Braga, A., & Venancio, A. (2005). Mycotoxin-producing and other fungi isolated from grapes for wine production, with particular emphasis on ochratoxin A. *Research in Microbiology, 156*, 515–521.

Suárez-Quiroz, M. L., González-Ríos, O., Barel, M., Guyot, B., Schorr-Galindo, S., & Guiraud, J. P. (2004a). Effect of chemical and environmental factors on *Aspergillus ochraceus* growth and toxigenesis in green coffee. *Food Microbiology, 21*, 629–634.

Suárez-Quiroz, M. L., González-Ríos, O., Barel, M., Guyot, B., Schorr-Galindo, S., & Guiraud, J. P. (2004b). Study on ochratoxin A – producing strains in coffee processing. *International Journal of Food Science and Technology, 39*, 501–507.

Sweeney, M. J., & Dobson, A. D. W. (1998). Mycotoxin production by *Aspergillus*, *Fusarium* and *Penicillium* species. *International Journal of Food Microbiology, 43*, 141–158.

Taniwaki, M. H., Pitt, J. I., Teixeira, A. A., & Iamanaka, B. T. (2003). The source of ochratoxin A in Brazilian coffee and its formation in relation to processing methods. *International Journal of Food Microbiology, 82*, 173–179.

Tassou, C. C., Natskoulis, P. I., Magan, N., & Panagou, E. Z. (2009). Effect of temperature and water activity on growth and ochratoxin A production boundaries of two *Aspergillus carbonarius* isolates on a simulated grape juice medium. *Journal of Applied Microbiology, 107*, 257–268.

Teixeira, A. A., Taniwaki, M. H., Pitt, J. I., Iamanaka, B. T., & Martins, C. P. (2001). The presence of ochratoxin A in coffee due to local conditions and processing in four regions of Brazil. In *ASIC congress*, Trieste (Italy). May 14th–18th.

Téren, J., Varga, J., Hamari, Z., Rinyu, E., & Kevei, F. (1996). Immunochemical detection of ochratoxin A in black *Aspergillus* strains. *Mycopathologia, 134*, 171–176.

Tjamos, S. E., Antoniou, P. P., & Tjamos, E. C. (2006). *Aspergillus* spp., distribution, population composition and ochratoxin A production in wine producing vineyards in Greece. *International Journal of Food Microbiology, 111*, S61–S66.

Trung, T. S., Bailly, J. D., Querin, A., Le Bars, P., & Guerre, P. (2001). Fungal contamination of rice from South Vietnam, mycotoxinogenesis of selected strains and residues in rice. *Revue de Medecine Veterinaire, 152*, 555–560.

Valenta, H. (1998). Chromatographic methods for determination of ochratoxin A in animal and human tissues and fluids. *Journal of Chromatography A, 815*, 75–92.

Valero, A., Farré, J. R., Sanchís, V., Ramos, A. J., & Marín, S. (2006). Kinetics and spatial distribution of OTA in *Aspergillus carbonarius* cultures. *Food Microbiology, 23*, 753–756.

Valero, A., Oliván, S., Marín, S., Sanchis, V., & Ramos, A. J. (2007). Effect of intra and interspecific interaction on OTA production by *A.* section *Nigri* in grapes during dehydration. *Food Microbiology, 24*, 254–259.

Van der Merwe, K. J., Steyn, P. S., & Fourie, L. (1965). Ochratoxin A, a toxic metabolite produced by *Aspergillus ochraceus*. *Nature, 205*, 1112–1113.

Varga, J., Rigó, K., & Téren, J. (2000). Degradation of ochratoxin A by *Aspergillus* species. *International Journal of Food Microbiology, 59*, 1–7.

Varga, J., Péteri, Z., Tábori, K., Téren, J., & Vágwölgyi, C. (2005). Degradation of ochratoxin A and other mycotoxins by *Rhizopus* isolates. *International Journal of Food Microbiology, 99*, 321–328.

Zimmerli, B., & Dick, R. (1996). Ochratoxin A in table wine and grape-juice: occurrence and risk assessment. *Food Additives and Contaminants, 13*, 665–668.