



## Toxicogenic fungi and the occurrence of mycotoxins in traditional meat products

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### ABSTRACT

During ripening, the surface of dry traditional meat products (TMPs) becomes overgrown by fungi of the *Penicillium* spp., *Aspergillus* spp. and *Eurotium* spp. whose spores mostly come from the environment in which the ripening chambers are placed. Certain fungi species is often responsible for the occurrence of toxic compounds termed the mycotoxins, among which of the outermost importance in connection with meat products are aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) and ochratoxin A (OTA). Besides, some other mycotoxins such as citrinin (CIT), cyclopiazonic acid (CPA) and sterigmatocystin (STC) can also be present, but their impact on the quality and safety of meat products, and therefore also on human health, has still not been fully clarified. As control and prevention of toxicogenic fungi growth are key factors to the prevention of mycotoxin presence in dry-cured TMPs, levels of mycotoxin contamination, mycotoxin-producing mould species and factors of relevance for mycotoxin production, such as climate, should be determined.

### Introduction

Standardization of quality and safety of traditional meat products (TMPs) calls for research into sensory and physicochemical features and potential contamination sources, especially given an ever more increasing TMP consumption, since they represent in general nutritionally rich products. The most prominent TMPs represent various types of fermented sausages, dry rack and blade, bacon, pancetta, prosciuttos, hams and some other. During the production process, not only differences in recipes observed by different households, but also major differences in hygienic and environmental production settings conditioning the growth of specific microflora and resulting in differences in TMP quality and safety are encountered (Asefa et al., 2010; Asefa et al., 2011).

During ripening, the surface of dry TMPs becomes overgrown by fungi whose spores mostly come from

the environment in which the ripening chambers are placed. The intensity of the overgrowth is thereby being enhanced by ripening longevity and traditional production environment in which no microbiological filters and no pneumatic barriers are used, and in which temperature and relative air humidity are virtually uncontrollable. Research in general has shown a favourable impact of superficial fungi (e.g. *P. chrysogenum*, *P. nalgiovense* and *P. aurantiogriseum*) on product quality, coming as a result of active participation of fungi enzymes in fermentation and ripening either by own virtue or in synergy with endogenous enzymes present in stuffing. This favourable impact is also to be attributed to fungi's ability to retain moisture, which, in turn, prevents drying of the product's surface and incrustation that might arise due to protein coagulation (Toldrá, 1998; Ockerman et al., 2000; Bruna et al., 2003). On top of the above, superficial fungi contribute to the development of product-specific

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flavour and taste (Asefa et al., 2009; Comi and Iacumin, 2013; Pleadin et al., 2017).

However, literature data have shown that certain fungi species are often responsible for unfavourable taste and smell of a product and may be the reason behind contamination of meat products with toxic compounds termed by the mycotoxins (Pitt and Hocking, 2009). The mycotoxins considered to be of the outermost importance from the public health standpoint in connection with meat products are aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), ochratoxin A (OTA), sterigmatocystin (STC), cyclopiazonic acid (CPA) and citrinin (CIT). AFB<sub>1</sub> is the most potent mammalian liver carcinogen, therefore classified by the International Agency for Research on Cancer as a Group 1 definite human carcinogen (IARC, 2002), OTA and STC fall within the 2B Group of possible human carcinogens (IARC, 1993; 2012), while CPA and CIT cause necrotic changes of parenchyma organs (Varga et al., 2015; Ostry et al., 2018). OTA poses as the major contaminant of meat products, while other mycotoxins are seen less often and in lower concentrations (Iacumin et al., 2009; Duarte et al., 2010). On top of AFB<sub>1</sub> and OTA, other listed mycotoxins can also be present, but their impact on the quality and safety of meat products, has still not been fully clarified (Bailly and Guerre, 2009; Markov et al., 2013). Moreover, mycotoxins tend to persist in raw materials and final products and accumulate in the human body causing severe health impairments coming as a result of contaminated food consumption (Hussein and Brasel, 2001; Richard, 2007; Duarte et al., 2010).

This paper provides an overview of toxicogenic fungi, which can be present on the surface of dry-cured TMPs and cause their contamination with different mycotoxins that compromise the safety of the final product as well as the consumers health.

### Fungi on TMP surfaces

Quality of raw materials, physical and biochemical factors, production practices and hygiene of the production environment determine fungi species and type of mycotoxin that will occur on the surface of dry-cured meat products. Recent studies have shown that surface fungi spontaneously overgrow TMPs, including dry-cured meat products and fermented sausages, during ripening. Toxicogenic fungi most commonly found on TMP surfaces are those of the *Penicillium* and *Aspergillus* genera (Comi et al., 2004; Sørensen et al., 2008; Asefa et al., 2009; Iacumin et al., 2009; Frece et al., 2010; Sonjak et al., 2011; Markov et al., 2013; Pleadin et al., 2017).

These fungi species are xerophytic, mesophilic and aerobic organisms, which means that their growth

requires water activity ( $a_w$ ) of 0.6 at the minimum, the temperature range of 10-45 °C, and media that have a positive redox potential, whereas the addition of spices inhibits their growth (Table 1). Due to their vast presence in production environments, fungi most commonly encountered on TMPs are those of the *Penicillium* genus (Andersen, 1995; Battilani et al., 2007; Sørensen et al., 2008; Sonjak et al., 2011). Their vast presence in production environments is attributed to their higher ability to grow at low  $a_w$  (0.78 - 0.83), and low-to-moderate temperatures, which indeed are the conditions typical of sausage ripening chambers (Frisvad and Samson, 2004; Sørensen et al., 2008; Berni, 2015).

**Table 1.** Conditions favouring mould growth on meat product surfaces (Hamad, 2012)

Parameters	Moulds growth
Temperature	10 - 45 °C
pH	1.5 - 10
$a_w$	Min 0.6
Redox potential	Aerobic
Salt content	Up to 20%
Spices	Inhibition

### Important toxicogenic fungi and mycotoxins

#### *The Penicillium genus*

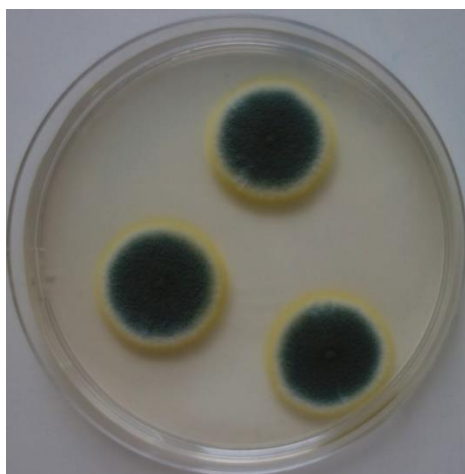
*Penicillium* is a well-known and very large genus currently embracing 354 accepted species (Visagie et al., 2014). Many *Penicillium* species are common contaminants of various food products and are known as potential mycotoxin producers. Therefore, whenever investigating possible *Penicillium*-induced food contamination, correct fungi identification is of the utmost importance.

The most important mycotoxins that can be produced by fungi of the *Penicillium* genus are OTA, patulin (PAT), CIT and CPA. The only well-studied OTA producers are *Penicillium verrucosum* (Figure 1) and *Penicillium nordicum* (Cabañes et al., 2010; Ostry et al., 2013; Wang et al., 2016). Their colonies grown on nutrition agars are very similar, but their ecology is quite different (Cabañes et al., 2010). *P. nordicum* can grow at low temperatures (10-25 °C) and on substrates with high salt (5% NaCl) and protein content, such as chilled and salted foods (meat and cheese) (Frisvad and Samson, 2004; Sonjak et al., 2011). Contrary to the above, *P. verrucosum* mainly contaminates cereals, but is also found on hams and salted olives (Pitt and Hocking, 2009; Cabañes et al., 2010; Schmidt-Heydt et al., 2012; Ostry et al., 2013). This fungus has a scent described as earthy and tart, and sometimes aromatic or fruity (Samson et al.,

2004; Mycobank Database, 2017). *P. verrucosum* is an important species that can produce OTA and CIT (Sweeney and Dobson, 1998) in moderate and colder climates (Pitt and Hocking, 2009; El Khoury and Atoui, 2010; Ostry et al., 2013), whereas *Penicillium citrinum* is the major CIT producer. Because of its mesophilic nature, the presence of *P. citrinum* is global, so that it can be isolated from cereals, nuts, fermented & cured meats, and cheese. *Penicillium commune* is an indoor fungus belonging to the *Penicillium* genus, isolated from the surface of various TMPs including dry-fermented sausages and prosciutto originating from many European countries (Andersen, 1995; Núñez et al., 1996; Peintner et al., 2000). It is also known as one of the most common cheese-spoiling fungi. Like many other *Penicillium* species, *P. commune* is able to grow at temperatures resembling that of a refrigerator. *P. commune* produces the CPA mycotoxin (Sosa et al., 2002) and has been proven to be implicated into the „phenol defect“ seen in hams during ripening (Spotti et al., 1988).

### The *Aspergillus* genus

The *Aspergillus* genus is a genus consisting of a few hundred species spread worldwide, mostly in tropical and subtropical rather than moderate climates, primarily present in the soil and various stored products, such as cereals, nuts, spices, but also dry-cured meat. The members of the *Aspergillus* genus are of huge importance in the synthesis of chemicals, biosynthetic transformation and enzyme production, but their involvement into the decomposition of a large number of foodstuffs and their production of mycotoxins, above all aflatoxins (AFs), OTA, STC and CPA, have an immense economic and social impact (Pitt and Hocking, 2009). Furthermore, the genus is frequently reported to be both human and animal pathogen (Samson et al., 2014). The most important species belonging to this genus are *Aspergillus flavus* and *Aspergillus parasiticus*, which are AFs-producers, *Aspergillus ochraceus* and *Aspergillus niger*, which are OTA-producers, and *Aspergillus versicolor*, which is a STC- and CPA- producer.



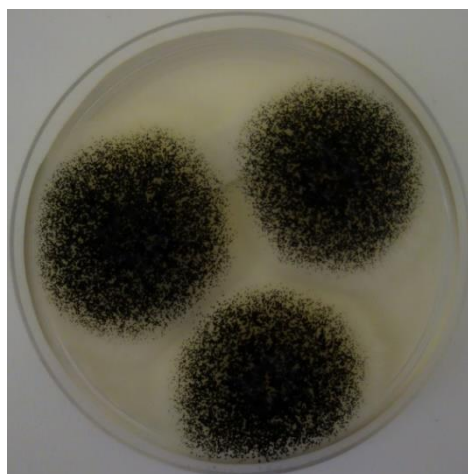
**Fig. 1.** *P. verrucosum* on dicloran glycerine (DG) 18 agar



**Fig. 2.** *A. flavus* on dicloran glycerine (DG) 18 agar



**Fig. 3.** *A. ochraceus* on dicloran glycerine (DG) 18 agar



**Fig. 4.** *A. niger* on dicloran glycerine (DG) 18 agar

*A. flavus* (Figure 2) and *A. parasiticus* are the most significant producers of AFs. These species are physiologically and macroscopically very similar, hence the studies reporting on *A. flavus* are probably equally applicable to *A. parasiticus*; definitive differences between the two can be made based on their microscopic characteristics, and surely using molecular methods (Pitt and Hocking, 2009). Both species are widespread due to the production of a large number of wind-spread spores. Their natural habitat is soil, but they also infect crops and contaminate stored grains, and can often be found in groundnuts, spices, oil seeds, cereals and dry-cured meat (Klich, 2007). High temperatures and relatively low air humidity favour the growth of these fungi species (Pitt and Hocking, 2009). *A. flavus* is also known as a CPA-producer (Chang et al., 2009), while *Aspergillus versicolor* can also produce the STC (EFSA, 2013). *A. versicolor* can be found on stored grains, spices, nuts and fermented & cured meat (Pitt and Hocking, 2009). *A. ochraceus* (Figure 3) is one of the most common OTA-producers, which contaminates different foodstuffs including grains, peanuts, nuts, fruits and meat products (Comi et al., 2004; Iacumin et al., 2009; Iacumin et al., 2011). The possibility of *A. ochraceus* to synthesize mycotoxins was detected in laboratory settings; on the occasion, three important toxins were found – OTA (the most toxic among them) and ochratoxins B & C (less toxic as compared to OTA and produced in smaller amounts). *Aspergillus niger* (Figure 4) has usually been regarded as a benign fungus and has been widely used in enzyme production and food processing ingredients (Pitt and Hocking, 2009), but Abarca et al. (1994) reported that 2 out of 19 *A. niger* isolates are able to produce OTA. This species is easy recognizable by black conidial heads and white mycelium. The black spores provide protection from sunlight and UV light, providing a competitive advantage in warmer climates (Valero et al., 2007).

*A. niger* is frequently isolated from sun-dried products, dried, smoked and cured fish and meat products, and spices (Abarca et al., 2003; Mandeel, 2005; Pitt and Hocking, 2009).

### Conditions favouring mycotoxin occurrence

Contamination with mycotoxins is possible in all phases of production and storage of food and feed. So far, more than 220 fungi species have been recognised as mycotoxigenic (Duraković and Duraković, 2003; Pleadin et al., 2018). A number of mould species can produce the same mycotoxin, but, likewise, a single fungi species can produce more than one type of mycotoxin (Bennett and Klich, 2003). The absence of visible fungi on a product does not necessarily mean that mycotoxins are not there, because these toxins are very stable and resistant, and manage to survive during food processing. Equally, the growth of mycotoxigenic fungi on food does not necessarily mean that mycotoxins are present in the food.

Table 2 displays the conditions under which most commonly encountered mycotoxigenic fungi species may produce certain mycotoxins. Numerous studies have demonstrated that, under certain circumstances, such as temperature, pH-value, water activity, casing cracking, presence or absence of crust (in case of prosciuttos) or cracks, as well as with insufficient washing and brushing of the TMP surface (i.e. uncontrolled fungi growth), superficial fungi of the *Penicillium* and the *Aspergillus* genera produce exactly the mycotoxins discussed above (Iacumin et al., 2009; Asefa et al., 2011; Rodríguez et al., 2012; Rodríguez et al., 2015). At the same time, technological operations pursued along the line of TMP production, such as thermal processing, salting, drying and ripening, as well as storage practices, have no significant impact on the quantity of these potent toxins in final meat products (Bullerman and Bianchini, 2007; Amézqueta et al., 2009; Pleadin et al., 2014a).

**Table 2.** Range of water activity and temperature within which certain moulds have been established to produce meat products-contaminating mycotoxins (Sørensen et al., 2009)

Mould species	Mycotoxin	a <sub>w</sub> range	T/°C range	Reference
<i>Aspergillus flavus</i>	AFB <sub>1</sub>	≥ 0,84; ≥ 0,80	12 – 35	Northolt et al., 1982; Ribeiro et al., 2006
<i>Aspergillus parasiticus</i>	AFB <sub>1</sub>	≥ 0,84	12 - 35	Northolt et al., 1982
<i>Aspergillus ochraceus</i>	OTA	≥ 0,87	12 - 35	Northolt et al., 1982; Ribeiro et al., 2006
<i>Penicillium verrucosum</i>	OTA	≥ 0,85	2 - 34	Northolt et al., 1982
<i>Penicillium nordicum</i>	OTA	-	15 - 30	Geisen, 2004
<i>Penicillium commune</i>	Cyclopiazonic acid	≥ 0,90	12 - 30	Sosa et al., 2002

AFB<sub>1</sub> – aflatoxin B<sub>1</sub>; OTA - ochratoxin A

## Occurrence of mycotoxins in TMPs

Mycotoxins present in TMPs may also originate from farm animal feed, in case a farm animal was fed on contaminated feed or feed mixture (the *carry-over effect*), or from spices used during TMP production (Gareis and Scheuer, 2000; Bertuzzi et al., 2013; Pleadin et al., 2013; Perši et al., 2014a). Following an OTA treatment of fattening pigs, a significant transfer of this mycotoxin from OTA-spiked feed into raw biological materials sampled from the treated animals was observed, the mycotoxin thereby being primarily transferred into offal, but also into meat of different categories and final meat products (Pleadin et al., 2013; Perši et al., 2014). Nevertheless, the presence of mycotoxins should primarily be attributed to the presence of mycotoxin-producing fungi overgrowing TMP surfaces (Comi et al., 2004; Iacumin et al., 2009; Iacumin et al., 2011; Asefa et al., 2010). It is not uncommon for mycotoxins to be present in TMPs in substantial concentrations, the outer casing cracking thereby facilitating their diffusion into the product interior (Dall'Asta et al., 2010; Pleadin et al., 2015a; Pleadin et al., 2015b).

AFs and OTA are found in varying concentrations in beef luncheon, beef burger, sausages, prosciutto and hams in different countries. This warrants the need for the implementation of certain control norms, so as to minimize the risk of mycotoxin exposure resulting from the consumption of these products. The maximum concentrations found in commercial salami samples were 7.83 µg/kg for OTA and 3.0 µg/kg for AFB<sub>1</sub> (Iqbal et al., 2014). Out of a total of 110 samples of different types of Italian cured hams, OTA was found on the surface of 84 samples in concentrations of 0.53 µg/kg, while in 32 samples the toxin was found in the innermost layers of the product in concentration below 0.1 µg/kg (Dall'Asta et al., 2010). AFB<sub>1</sub> was found in beef luncheon and burger (Aziz and Youssef, 1991; Abd-Elghany and Sallam, 2015) in Egypt, and in salami both in Egypt and Spain (Aziz and Youssef, 1991; Bernáldez et al., 2013). OTA was also found in salamis in Italy (Iacumin et al., 2009) and Spain, but in the latter case in concentrations below the LOD and LOQ (Bernáldez et al., 2013). When it comes to Spain, OTA was found in dry-cured Iberian ham, too (Rodríguez et al., 2012; Rodríguez et al., 2015).

Research on mycotoxin presence in meat products available on the Croatian market proved the presence of AFB<sub>1</sub> and OTA in the following meat products: Slavonian sausage (OTA 2.03-6.68 µg/kg (Markov et al., 2013; Vulić et al., 2014); AFB<sub>1</sub> < 1-1.2 µg/kg (Markov et al., 2013) and Slavonian Kkulen (OTA = 0.9-5.17 µg/kg (Frece et al., 2010; Vulić et al., 2014; Pleadin et al., 2016) and AFB<sub>1</sub> = 0.1-4.49 µg/kg (Frece

et al., 2010; Pleadin et al., 2015b). The research on mycotoxin prevalence conducted on a larger number of various dry and semi-dry TMPs sampled within the 2011-2014 timeframe, showed OTA span of 1.23 µg/kg in Slavonian sausage to 9.95 µg/kg in Istrian prosciutto, and AFB<sub>1</sub> span from 1.06 µg/kg in Istrian prosciutto to 1.69 µg/kg in a cooked sausage (Pleadin et al., 2015c). The above research mostly dealt with TMP samples of an unknown origin, while circumstances favouring mycotoxin production failed to be analysed. Although the research we have conducted insofar demonstrated TMP contamination with AFB<sub>1</sub> to be negligible, it should be borne in mind that the subsequent Croatian research showed an extremely high contamination of corn and feed mixtures with this mycotoxin (Pleadin et al., 2014b; Pleadin et al., 2014c; Pleadin et al., 2015d), which might have contributed to high meat products' contamination.

However, further research on AFB<sub>1</sub> transfer along the production chain (from farm animal feed to final meat products), attributable to the *carry over* effect, have not been carried out yet. The studies referring to the above mentioned came up with the conclusion that meat products' contamination with AFB<sub>1</sub> and OTA arises on the grounds of inadequate production control and inadequate storage practices, imposing the need for prevention, systematic control and further monitoring so as to be able to pinpoint the circumstances facilitating the production of these potent mycotoxins. On top of that, as of now no Maximum Levels (ML) of mycotoxins in meat and meat products have been stipulated by regulatory bodies (Commission Regulation (EC) No 1881/2006), making the data on mycotoxin prevalence virtually unavailable. OTA-contaminated samples were also a subject to investigation into the possibility of reducing the levels of this mycotoxin using physical methods applicable in households (cooking, baking) (Pleadin et al., 2014a) and/or gamma irradiation (Domijan et al., 2015). The research demonstrated the applied methods to be of a limited value for OTA reduction (20 to 30%-reduction achieved), proving their low efficiency and the need to resort to prevention of contamination rather than reduction.

Markov et al. (2013) in research of CIT presence in meat products, showed that this mycotoxin was not proven to be a significant meat products' contaminant (only 4.44% of the samples were positive), the established concentrations thereby mostly approximating to the Limit-of-Detection of the analytical technique used (1.0-1.3 µg/kg). However, given that the CIT nascence is also climate-conditioned, so that its representation definitely varies across the TMP production years, the production of

this mycotoxin deserves further research on a larger number of various meat products. On top of that, the concurrent CIT/OTA presence reported in the literature should be investigated into, as well. Furthermore, it has been established that outer casing damages also facilitate mycotoxin diffusion into the dry fermented sausage interior (Pleadin et al., 2015a; Pleadin et al., 2015b).

### Measures preventing mycotoxin contamination

Control and prevention of toxicogenic fungi growth are key factors to the prevention of mycotoxin presence in dry-cured TMPs (Núñez et al., 2015). In general, fungi growth can be efficiently controlled in meat products into which preservatives are added during production, or are packaged in a modified atmosphere. However, these procedures are not suitable for dry-cured TMPs, since fungi activity is of key importance for their sensory profile (Toldrá, 1998; Ockerman et al., 2000; Núñez et al., 2015). Given that  $a_w$  of a substrate affects fungi ability to produce mycotoxins, the latter production can be prevented by virtue of control of this parameter during the production process, in terms of drying and ripening temperature adjustments (Asefa et al., 2011).

Fungi overgrowing the product surface should be brushed off and washed off during the entire ripening stage, so as to prevent an excessive mouldiness of the product surface (Sørensen et al., 2008). Producers tend to rinse semi-dry meat products at some point between the drying and the ripening stage, so as to remove visible fungi colonies growing on the product surface (Asefa et al., 2011), since consumers are more prone to buy products free of surface fungi. Iacumin and co-workers (2009) have proposed an initial brushing and subsequent washing of dry-cured sausages' surface to decrease OTA concentration and eliminate a potential consumer health hazard. It is also common to spray rice flour over ripen sausages' surface following fungi layer brush-off, wash-off or pneumatic cleansing.

On top of that, in order to prevent an excessive mouldiness of products during ripening, a distance between the products should be ensured in order to prevent their contact and provide for an unhindered air flow. Products should ripen within ripening chambers equipped with biological micro-filters enabling the fresh air to flow in. Prior to products' intake and ripening commencement, ripening chamber surfaces should be coated with fungicide coats, while the ripening chamber entrance point should be provided with a pneumatic barrier capable of preventing the outer air inflow (Kovačević et al., 2014). Croatian rural households mostly produce TMPs in uncontrolled production environment, which enables a

significant impact of outer factors on the prevalence of surface fungi in terms of facilitation of toxicogenic fungi colonisation and mycotoxin contamination of TMPs.

### Analyses of fungi and mycotoxins

Upon the implementation of preventive measures during production and storage, final products should be analysed for the presence of toxicogenic fungi. Fungus identification can be carried out based on macro- and micromorphology (traditional method), extrolitic profile, sequencing of a specific gene region (molecular method) and determination of fungal antigens and metabolites, most commonly used in medical mycology (Hajsig and Delaš, 2016). With traditional method, the growth of colonies on MEA and CYA is to be expected and checked for after a seven-day incubation at  $25\pm 1^\circ\text{C}$ . After the incubation period, the colour of the agar, as also the colour, reverse, and diameter size of the fungi colony, should be checked. The texture of the fungi colony and exudates, as well as the smell, should be determined. On the microscopic level, all microscopic structures (spores, conidiophores, vesicles) should be analysed and, if possible, measured. In the subsequent course, the established characteristics of the grown colonies have to be compared to those given in most commonly used atlases, such as that of Samson et al. (2004) and that of Pitt and Hocking (2009). Traditional methods require a lot of experience and consume time.

In addition to traditional fungi identification methods, according to our experience, molecular methods such as PCR are needed to confirm the fungi species. PCR is a method widely used in molecular method in order to make numerous copies of a specific DNA sequence, which, upon sequencing, can be compared to the reference sequences deposited in, for example, the GenBank and the CBS databases. Genes encoding nuclear and mitochondrial ribosomal RNA and associated spacer regions were identified as ideal for fungus identification (Geiser, 2004). Based on the universal and conserved nature of these genes, primers applicable to a broad range of fungi can be designed (White et al., 1990). Primers widely used for fungi identification purposes are directed to the amplification of genes encoding the ITS and  $\beta$ -tubulin gene regions (Asefa et al., 2009).

In order to determine a possible mycotoxin contamination of TMPs, preventive measures have to include the detection of mycotoxins at all critical points of TMPs production and storage. With this aim, validated screening and confirmatory analytical methods can be implemented. When it comes to mycotoxin determination, the most commonly used

orientation technique is the enzyme-linked immunosorbent assay (the ELISA) (Pleadin et al., 2014c). Literature data have shown numerous strong suits of the ELISA, such as swift performance, the possibility of analysing a large number of samples in a short time-window, simplicity, cost-effectiveness and safe reagents usage (Zheng et al., 2005; Goryacheva et al., 2007). However, the quality of an ELISA kit may vary across producers, and even across batches released by the same producer (Pleadin et al., 2014d).

Other techniques used to determine mycotoxin concentrations include high-performance liquid chromatography (HPLC), gas chromatography (GC), liquid chromatography/mass spectrometry (LC/MS), and gas chromatography/mass spectrometry (GC/MS). Unlike orientation techniques, these techniques call for sophisticated laboratory equipment (Krska et al., 2008; Shephard et al., 2011). The HPLC technique is characterised by high performance features, high sensitivity and high distinction capability, the most commonly used HPLC detectors thereby being the fluorescent ones (FLDs) (Herzallah, 2009; Pleadin et al. 2014c,d). For the purpose of mycotoxin analysis, the HPLC has recently been more and more frequently combined with mass spectrometry (MS) (Li et al., 2009), because of its high sensitivity and selectivity (Sun et al., 2015). Nevertheless, the limitations faced within this context are high prices of the necessary equipment and the complexity of extraction, separation, detection and quantification procedures (Turner et al., 2009). On rare occasions, mycotoxin determination employs capillary electrophoresis and biosensor-based techniques. In the last decade, fluorimetric method with immunoaffinity columns clean up procedure has been recognised as an accurate, safe and rapid method for the determination of mycotoxins in meat products (Abd-Elghany and Sallam, 2015).

## Conclusion

Research on the presence of mycotoxins in TMPs conducted in European countries insofar has mostly been focused on OTA and AFB<sub>1</sub>, while notions on the prevalence of, and consumer exposure to, CIT, STC and CPA are undoubtedly scarce and inadequate, although the toxicity of these mycotoxins has been proven beyond doubt. In addition, analytical techniques sensitive and specific enough to allow for STC and CPA testing in meat products have not been developed yet, despite the fact that some authors emphasize the possibility of their substantial TMP representation. Beyond doubt, when it comes to TMP analysis, not only the levels of mycotoxin

contamination, but also the mycotoxin-producing fungi species and factors of relevance for mycotoxin production, such as climate, should be determined.

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