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ORIGINAL ARTICLE

Mycobiota and mycotoxins in Portuguese pork, goat and sheep dry-cured hams

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Abstract

The objectives of the present work were to survey, for the first time, the contamination of Portuguese fresh and dry-cured meat products with ochratoxin A (OTA) and aflatoxin B_1 (AFB_1), and to determine the fungi potentially responsible for this contamination. A total of 128 samples including pork fresh legs, dry-cured legs and shoulders, as well as goat and sheep dry-cured legs were analysed. Mycological analysis of these samples yielded a total of 630 fungal isolates. *Penicillium* sp. was the dominant fungal genus in all products (66% of all isolates). *Penicillium nordicum* and *Aspergillus westerdijkiae* were only rarely isolated from pork ham samples. In fresh pork meat, 40% of the samples were contaminated with OTA at levels below 1 μg/kg. In pork dry-cured legs with 20 to 25 months of ripening, 43% of the samples showed detectable contamination, while 18% of the shoulder hams were contaminated. OTA was not detected in any of the goat and sheep samples. OTA contamination does not seem to be a risk in small-piece and short-ripe products like goat and sheep legs, but affects longer ripe products like pork legs and shoulders. Although aflatoxigenic fungi were identified, AFB_1 was not detected in any sample, and it should not be considered a risk in dry-cured hams.

Keywords *Penicillium* · *Aspergillus* · Ochratoxin A · Aflatoxins · Food safety · Meat products

Introduction

Production of pigs, goats and sheep plays an important role in the economy of Portugal, with an average annual contribution of 23,000 t (Paulos et al. 2015). *Bísaro* is a highly appreciated breed of pig from the Northeast of Portugal. *Serrana* and *Churra Galega Bragançana* are the most important Portuguese goat and sheep breeds, and the production and commercialization of derived products have been promoted as viable strategies for the development of rural economy (Paulos et al. 2015). As such, meat from older animals with low commercial value is also used, usually by being diverted to process and cure with salts (Teixeira et al. 2017), to obtain the highly appreciated dry-cured hams.

Dry-cured hams constitute one of the most representative traditional foods that are produced and consumed all over the world. The reduced water activity, salt content and pH achieved during processing and storage of these products usually render them safe, since they together inhibit the development of pathogenic bacteria. But these characteristics are also inductive of superficial colonization by fungi. The hygienic quality of raw materials, the manufacturing practices and the environmental factors (such as temperature and relative humidity) during ripening determine the type and growth rate of fungi growing on the surface of dry-cured meats. A complex mycobiota is generally observed on the surface of these products with *Aspergillus*, *Eurotium* and *Penicillium* as the most frequently isolated genera (Comi et al. 2004; Samson et al. 2004a; Battilani et al. 2007; Sørensen et al. 2008; Sonjak et al. 2011; Alapont et al. 2014). These fungi are generally assumed to improve taste and quality, due to their lipolytic and proteolytic activities (Ludemann et al. 2004; Martín et al. 2004, 2006; Sonjak et al. 2011). But, besides these features, the development of fungi may also lead to the accumulation of toxigenic secondary metabolites, namely mycotoxins such as ochratoxin A (OTA), aflatoxins (AFs) and others, which result in health hazards to the consumers.

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OTA has been frequently reported as contaminant of dry-cured meat products, either due to carry-over effects of animals exposed to contaminated feed (Dall'Asta et al. 2010; Pleadin et al. 2013) or to the growth of toxigenic fungi during ripening, which generally occurs under environmental conditions that are inductive of mycotoxin production (Iacumin et al. 2009, 2011; Rodríguez et al. 2012b). Aflatoxin B_1 (AFB_1) contamination has not been clearly associated with dry-cured hams, but aflatoxigenic species have been detected in these type of products (Comi et al. 2004), and their ability to produce this toxin has been reported by Rodríguez et al. (2012a).

OTA can be nephrotoxic, hepatotoxic, neurotoxic, teratogenic and immunotoxic (Petzinger and Ziegler 2000), and is produced by several species of *Aspergillus* and *Penicillium* (Vipotnik et al. 2017). AFs are a group of mutagenic, teratogenic and immunosuppressive mycotoxins that include the most widely studied AFB_1. AFB_1 is considered the most carcinogenic compound naturally produced, and is produced by some species belonging to *Aspergillus* section *Flavi* (Rodrigues et al. 2011). The International Agency for Research on Cancer (IARC 1993) has classified OTA as possible carcinogenic to humans (group 2B), while AFB_1 is classified as carcinogenic (group 1). Mycotoxins have not been regulated by European legislation in meat and meat products. But OTA, and to a lesser extent AFs, have been detected in numerous meat products, like sausages, salami and dry-cured ham, in varying concentrations, as recently reviewed by Montanha et al. (2018).

The meat industry aims at developing innovative products to react to consumer's demand, and product's safety, including mould toxicity, is an underlying concern. The objectives of the present work were to reply to this concern, by surveying the contamination with OTA and AFB1 of several innovative meat products: long-ripe pork dry-cured hams (legs), pork dry-cured shoulders and goat and sheep dry-cured hams (legs). The fungi potentially responsible for this contamination were also studied.

Materials and methods

Conditions of dry-cured ham production

The fabrication process of sheep and goat legs (with approximate raw weight of 3 kg) was made in three batches, one at LTQCC (Carcass and Meat Quality and Technology Laboratory of Agriculture School of Bragança, Polytechnic Institute of Bragança, Portugal) and two at Bísaro Salsicharia Tradicional (traditional production), and consisted of (1) salting, between 2 and 5 °C and relative humidity of 80–90%, for a period of 1 day of salting for each 1 kg of meat; (2) cleaning and washing; (3) curing and drying, 1 to 2 months at 8–10 °C and relative humidity of 60–70%; and (4) ripening, 7 to 8 months at 17–20 °C and relative humidity of 60–70%.

The fabrication process of pork dry-cured hams (legs with average weight of 19.2 kg) and shoulders (with average weight of 10.4 kg) took place at the industry Bísaro Salsicharia Tradicional and was made as follows: (1) salting, 2 and 4 °C and relative humidity of 90–95%, for a period of 1 day of salting for each 1 kg of meat; (2) cleaning and washing; (3) drying-maturation, 6 months at 14–16 °C and relative humidity of 55–75%; and (4) ripening, 15–20 °C and relative humidity of 65–75%. Legs were cured for up to 25 months, and shoulders up to 15 months.

Sampling

Samples of pork legs were taken at two different stages: fresh (in the abattoir; $n = 15$), 14 months of ripening ($n = 9$) and 20–25 months of ripening ($n = 47$). Samples of pork shoulders, sheep hams and goat hams were taken at the end of ripening: 13 to 15 months for pork shoulders ($n = 40$), and 8 months for sheep and goat legs ($n = 11$ and $n = 21$, respectively).

Superficial pieces of meat (fresh meat or dry-cured ham) with 3 to 4 cm depth were cut from the meat pieces and put in sterile bags. Samples were immediately transported to the laboratory and preserved at 4 °C for up to 3 days until mycological analysis, and at − 18 °C until mycotoxin analysis. The measurement of pH was performed according to the Portuguese standard NP-ISO 3441 (2008) using a potentiometer with an electrode equipped with a penetrator cell calibrated. Water activity was assessed with an aw probe (HygroPalm Aw1 rotronic 8303, Basserdorf, Switzerland) according to AOAC (1990).

Mycological analysis

Fungal isolation

Fungal contamination of dry-cured hams was determined by swabbing a surface of approximately 100 cm^2. Samples were plated in Dichloran Rose Bengal Chloramphenicol (DRBC, Oxoid) and Dichloran Glycerol 18% (DG18, Merck), and incubated at 25 °C in the dark for 7 days. After incubation, representative isolates of genera *Aspergillus* and *Penicillium* were isolated as described by Rodrigues et al. (2013) and grouped in morphotypes.

Fungal identification

Morphological identification followed the taxonomic keys and guides available for genera *Aspergillus* and *Penicillium* (Klich 2002; Frisvad and Samson 2004; Samson et al. 2004a; Samson and Varga 2007; Pitt and Hocking 2009; Samson and Houbraken 2011; Samson et al. 2011). All isolates were

screened for AFs and OTA production by thin-layer chromatography (TLC) as described by Samson et al. (2004b). Those showing fluorescence (LOD = 2 ng/spot) were considered positive and were confirmed by HPLC (Rodrigues et al. 2011; Vipotnik et al. 2017).

For the molecular identification of fungi, genomic DNA of the selected isolates ($n = 186$) was obtained as described by Rodrigues et al. (2018). Isolates were grouped based on genomic fingerprinting by enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) following Alves et al. (2007), and at least two isolates representative of each group were further identified by DNA sequencing following Rodrigues et al. (2011), in a total of 54 sequenced isolates.

Aflatoxin B_1 and ochratoxin A analyses of meat samples by HPLC-FLD

Chemicals and materials

The standard solution of AFB_1 was purchased from Sigma-Aldrich (St. Louis, USA), and OTA standard solution was from AppliChem (Darmstadt, Germany). HPLC grade solvents (methanol and acetonitrile) were used in the preparation of mycotoxin standards, in sample extraction and in the preparation of mobile phase. For extract purification of AFB_1 and OTA, AflaTest WB and OchraTest WB immunoaffinity columns (IACs) were obtained from VICAM (Watertown, MA, USA). Phosphate buffer saline 0.1 M with Tween 80 (PBS-T) pH 7.0 was prepared by adding 500 mL of 0.1 M NaH_2PO_4, 500 mL of 0.1 M $Na_2HPO_4.12H_2O$ and 0.1% Tween 80. All necessary safety considerations were taken when handling mycotoxins (Pereira et al. 2017).

Aflatoxin B_1 and OTA co-extraction

AFB_1 and OTA were co-extracted following the methodology for OTA extraction proposed by Chiavaro et al. (2002), with some modifications. Comminuted samples (10 g) were added to 40 mL of methanol:1% sodium bicarbonate (70:30, v/v) and stirred for 30 min. The filtered extract (10 mL) was diluted with 40 mL of 0.1 M PBS-T pH 7 and further filtered through a glass microfibre filter. The extract was divided into two portions used to purify each of the mycotoxins AFB_1 and OTA with IAC. Twenty millilitres of the extract were passed through each IAC by gravity, at a rate of about 1–2 drops/s and further processed as recommended by the manufacturer. AFs and OTA were eluted from the IAC with 2 mL of HPLC grade methanol.

Mycotoxin detection and quantification by HPLC-FLD

Chromatographic separations of AFB_1 and OTA were performed independently, by high performance liquid chromatography coupled to a fluorescence detector (HPLC-FLD) using the conditions described by Pereira et al. (2017).

In-house HPLC methods validation

Different sets of standard solutions and of spiked samples were used for the validation of the AFB_1 and OTA extraction methods. Precision and recovery were performed by spiking blank samples with 10 μg/kg of both AFB_1 and OTA. Each sample set was composed of six replicates, tested in two different days.

Linearity, limit of detection (LOD) and limit of quantification (LOQ) were determined by three series of analyses. For OTA, seven standard solutions were used, at concentrations from 0.5 to 15.0 ng/mL. For AFB_1, 5 standard solutions were used, at concentrations from 0.5 to 15.6 ng/mL. The calibration curves for each mycotoxin revealed linear relationships ($r^2 = 0.996$ and 0.998 for OTA and AFB_1 respectively). LOD and LOQ were calculated as 0.3 and 0.9 μg/kg for OTA, and 0.5 and 1.5 μg/kg for AFB_1, respectively. Recovery rates of OTA and AFB_1 were calculated by the ratio of recovered mycotoxin concentration relative to the spiked concentration. Precision was calculated in terms of intra-day repeatability ($n = 3$) and intermediate precision (inter-day within-laboratory reproducibility; $n = 6$). Recovery rate, performance and precision of the extraction method are presented in Table 1. In-house method validation demonstrated the conformity of the method for OTA and AFB_1 analysis with provisions of Regulation (EC) no. 401/2006 (EC 2006a).

Results and discussion

Physico-chemical characterization of samples

pH and aw of the various types of cured products (pork, goat and sheep legs and pork shoulders) at the end of the curing period are presented in Table 2.

Table 1 Recovery, performance and precision of aflatoxin B_1 and ochratoxin A extraction method for spiking with 10 μg/kg of each mycotoxin

	Fresh meat	Ham	
	OTA	OTA	AFB_1
Recovery at 10 μg/kg (%)	115.5	100.9	97.9
Repeatability (RSD_r; $n = 3$)	1.3	1.8	15.9
Intermediate precision (RMD_{INT}; $n = 6$)	1.1	1.7	16.1

Table 2 pH and aw (average ± standard deviation) of pork, goat and sheep cured legs, and pork-cured shoulder

Category of product	Pork leg		Pork shoulder	Goat leg	Sheep leg
Curing period (# samples)	14 months ($n = 9$)	20–25 months (n = 47)	13–15 months ($n = 40$)	8 months ($n = 21$)	8 months ($n = 11$)
pH	6.00 ± 0.07	5.93 ± 0.18	5.91 ± 0.16	6.07 ± 0.09	5.89 ± 0.12
aw	0.91 ± 0.01	0.89 ± 0.01	0.86 ± 0.01	0.84 ± 0.06	0.90 ± 0.03

Mycobiota of meat samples

A total of 630 fungi (334 from pork leg, 126 from pork shoulder, 118 from goat and 52 from sheep) were isolated and grouped by morphotypes, and 186 of these fungi were molecularly identified. The identified fungal species, as well as their incidence in each product category (determined as number of samples of each category contaminated with at least one isolate of the species) and the ability to produce AFs and OTA are shown in Table 3. *Penicillium* isolates dominated the fungal population in all products (66% of the total isolates), followed by *Aspergillus* (34%).

The high incidence of *Penicillium* is supported by other studies on dry-cured hams and similar products (Nuñez et al. 1996; Comi et al. 2004; Battilani et al. 2007; Asefa et al. 2009; Sonjak et al. 2011; Alapont et al. 2014) given the ripening conditions of these products. The predominantly isolated species was *P. commune*, which corresponded to 53% of all isolates, and was detected in all samples. *P. commune* has generally been reported in very high frequencies in other studies (Nuñez et al. 1996; Comi et al. 2004; Battilani et al. 2007; Asefa et al. 2009; Sonjak et al. 2011; Alapont et al. 2014), and it is a cyclopiazonic acid (CPA) producing species (Frisvad and Samson 2004).

Other species with overall high incidence in our samples are also associated with mycotoxin production: *P. brevicompactum* (mycophenolic acid), *P. carneum* (mycophenolic acid, patulin, roquefortin C and penitrem A), *P. chrysogenum* (PR-toxin and roquefortin C), *P. coprobium* (patulin and roquefortin) C (Frisvad and Samson 2004).

P. nordicum, which was detected in low amounts and in pork samples only (four isolates in 20–25 months pork legs and one isolate in pork shoulders), is a producer of OTA, and is a common contaminant of protein-rich foods, which can grow well at low temperatures (15 °C) and increased salt content (> 5% NaCl) (Dall'Asta et al. 2010; Sonjak et al. 2011; Ferrara et al. 2015; Vipotnik et al. 2017). For this reason, foods like dry-cured ham and related meat products are typical habitats of this species (Larsen et al. 2001; Bogs et al. 2006) and it is generally considered the major risk in terms of mycotoxin contamination.

Considering the *Aspergillus* genus, it was less frequently isolated than *Penicillium*, as also reported by others (Comi et al. 2004; Sonjak et al. 2011; Alapont et al. 2014). Only six *Aspergillus* species were isolated from the samples, representing 31% of all isolates. The succession of *Aspergillus* species throughout ripening followed an expected trend: longer ripening processes led to the reduction of less xerophilic aflatoxigenic species (*A. flavus*, *A. parasiticus* and *A. nomius*) and to an increase in the more xerophilic ones (*A. proliferans* and *A. pseudoglaucus*). Among the aflatoxigenic species, *A. flavus* was detected in all animal sources, with higher incidence in the less ripened products (100%). On the other hand, *A. parasiticus* was detected only in goat and sheep samples with low representation, and *A. nomius* was detected in sheep samples only. To our knowledge, this is the first report of these two species in dry-cured hams. All isolates belonging to these three species were tested for their aflatoxin production ability. All *A. parasiticus* and *A. nomius* isolates were strong AFB and AFG producers, but only 11% of the *A. flavus* isolates were able to produce AFB, and at lower amounts than the other two species, as also described for isolates from other sources (Rodrigues et al. 2011).

In the present study, *A. westerdijkiae* was detected in one 20-month ripened pork leg sample. Other types of cured meat products seem to be more affected by this fungus, as Iacumin et al. (2011) and Canel et al. (2013) reported that up to 34% and 93%, respectively, of Italian sausage casings were contaminated with *A. ochraceus*/*A. westerdijkiae*. More recently, Merla et al. (2018) also reported the identification of three *A. westerdijkiae* isolates in Italian traditional salami samples. Given its relatively low detection, *A. westerdijkiae* has rarely been studied as a potential risk of OTA contamination in ripened meat products. Nonetheless, *A. westerdijkiae* has been recently reported to be well adapted to dry-cured ham and other ripened meat products (Vipotnik et al. 2017; Meftah et al. 2018; Merla et al. 2018), and should be considered a high sanitary risk in products of animal origin with high salt content, such as dry-cured ham (Vipotnik et al. 2017).

Mycotoxin contamination of meat samples

The results of OTA contamination of pork fresh meat and dry-cured ham samples are shown in Table 4. OTA was not detected in any of the goat and sheep samples. No AFB_1 was detected in any of the pork, goat and sheep samples.

Table 3 Representative mycobiota isolated from cured ham samples: total number of isolates, incidence (samples contaminated with the species, in percentage) and mycotoxin production ability (in bold, the detected aflatoxigenic and ochratoxigenic species, confirmed by HPLC)

Category of product	Pork leg	Pork leg	Pork shoulder	Goat	Sheep	Mycotoxin production
Curing period (# samples)	14 months ($n = 9$)	20–25 months ($n = 47$)	13–15 months ($n = 40$)	3 months ($n = 21$)	3 months ($n = 11$)	
A. flavus	30 (100%)	14 (30%)	8 (20%)	30 (100%)	13 (100%)	Aflatoxins B (11% positive)
A. nomius	–	–	–	–	3 (27%)	Aflatoxins B + G (100% positive)
A. parasiticus	–	–	–	5 (24%)	3 (27%)	Aflatoxins B + G (100% positive)
A. proliferans	11 (100%)	58 (100%)	4 (10%)	6 (29%)	4 (36%)	n.d.
A. pseudoglaucus	–	5 (11%)	–	–	–	n.d.
A. westerdijkiae	–	1 (2%)	–	–	–	Ochratoxin A (positive)
Total *Aspergillus*	41	78	12	41	23	
P. aethopicum	1 (11%)	–	2 (5%)	–	–	n.d.
P. brevicompactum	–	2 (4%)	4 (10%)	3 (14%)	1 (9%)	n.d.
P. carneum	–	13 (28%)	–	5 (24)	2 (18%)	n.d.
P. chrysogenum	3 (33%)	–	–	7 (33%)	3 (27%)	n.d.
P. citrinum	–	2 (4)	–	–	–	n.d.
P. commune	20 (100%)	92 (100%)	75 (100%)	25 (100%)	12 (100%)	n.d.
P. coprobium	3 (33%)	21 (45%)	13 (33%)			n.d.
P. crustosum	5 (56%)	6 (13%)	4 (10%)	5 (24%)	9 (82%)	n.d.
P. cyclopium	–	–	–	11 (52%)	–	n.d.
P. echinulatum	–	4 (9%)	15 (38%)	21 (100%)	–	n.d.
P. nalgiovense	–	–	–	–	1 (9%)	n.d.
P. nordicum	–	4 (9%)	1 (3%)	–	–	Ochratoxin A (100% positive)
P. polonicum	–	13 (28%)	–	–	–	n.d.
P. solitum	1 (11%)	12 (26%)	–	–	1 (9%)	n.d.
P. thymicola	–	9 (19%)	–	–	–	n.d.
P. viridicatum	–	4 (9%)	–	–	–	n.d.
Total *Penicillium*	33	182	114	77	29	
Total fungal isolates	74	260	126	118	52	

n.d. AFs and OTA not detected

Dall'Asta et al. (2010) reported mycotoxin accumulation in body fluids (blood and urine) and tissues (muscle, kidney, liver) at relatively low concentrations in animals fed with contaminated feedstuffs. Our results showed that as much as 40% of the fresh pork leg samples were contaminated with detectable levels of OTA (but below the limit of

Table 4 Quantification of ochratoxin A (in μg/kg) of pork dry-cured ham samples

Category of product	Fresh meat	Leg		Shoulder
Curing period (months)	0	14	20–25	13–15
# samples	15	9	47	40
# positive samples (> LOD)	6	1	20	7
% positive samples	40	11	43	18
Mean OTA (μg/kg ± SD) (>LOQ)	< LOQ	< LOQ	14.9 ± 27.9	6.6 ± 6.2
Median OTA (μg/kg) (>LOQ)	–	–	3.3	5.2
Min-max OTA (μg/kg)	< LOQ	< LOQ	< LOQ–99.1	< LOQ–14.7

LOD limit of detection

LOQ limit of quantification

SD standard deviation

quantification, 0.9 μg/kg), denoting a limited level of OTA carry-over into pork muscle. In fact, the real risk due to a carry-over from OTA-contaminated feed to pork meat has been considered negligible by the European Food Safety Authority (EFSA-Q-2003-039 2004), due to the generally low level of contamination of animal feed in Europe.

On the other side, the long ripening periods to which cured meat products are subjected, as well as their intrinsic features (low aw and pH, high salt content) are conducive to the growth of numerous fungi on the food surface, and to the potential production of mycotoxins. In the present study, 29% of all pork dry-cured ham samples were found to be contaminated with OTA. Of these, 21% were contaminated with more than 1 μg/kg of OTA.

Under the Regulation 1881/2006/EC (EC 2006b), the Commission of the European Communities defined OTA maximum permissible levels (MPLs) in different foodstuffs and underlines the importance of defining OTA MPLs in other products, meat products included. However, no European regulation on OTA contamination of meat products has emerged, and Italy is currently the only EU country where guidelines for the maximal recommended OTA level of 1 μg/kg in pork and pork-derived products have been provided by the Ministero della Sanità (1999).

In our study, the five isolates of *P. nordicum* originated from different cured meat samples showing levels of OTA ranging from < LOQ to 5.9 μg/kg. The sample showing the highest level of OTA contamination (99 μg/kg) was the one from where *A. westerdijkiae* was isolated. Iacumin et al. (2011) found that 34% of casings of Italian sausages showing high levels of OTA were contaminated with *A. ochraceus* (possibly *A. westerdijkiae*). More recently, Merla et al. (2018) reported 10% (13) of OTA-contaminated samples from Italian salami and, with the exception of one sample, samples contaminated with *A. westerdijkiae* showed the highest levels of OTA contamination. In two previous reports (Vipotnik et al. 2017; Meftah et al. 2018), it was possible to determine the strong ability of this species to produce OTA in dry-cured meat products under a wide range of environmental conditions. More than that, these studies showed that this fungus produces OTA in much higher levels and in a wider range of temperature and water activity than the widely studied *P. nordicum*, and its ochratoxigenic ability can be stimulated by the presence of other existing or added microbiota (Meftah et al. 2018). In the study by Vipotnik et al. (2017), *P. nordicum* produced OTA over a narrow range of selected conditions (0.97 aw, 3% salt, between 15 and 20 °C), while *A. westerdijkiae* was able to produce OTA over a wider range of aw and temperature conditions, with optimum conditions at 0.97–0.93 aw (3–6% salt) at all temperatures, with maximum at 20–24 °C. Considering the ripening conditions of the various types of cured products (17–20 °C for sheep and goat legs; 15–20 °C for pork legs and shoulders) as well as their intrinsic conditions (shown in Table 2) of low aw (0.90 for sheep, 0.84 for goat and between 0.89 and 0.91 for pork legs and shoulders) and slightly acidic pH (around 6.0 for all species), the two fungi find adequate conditions for growth and OTA production in all of them. The fact that no contamination was detected in goat and sheep samples can to be due to the apparent absence of ochratoxigenic fungi. Also, these legs are smaller than pork pieces, with shorter ripening periods. Their aw reduces rapidly (in average from 0.98 to 0.91 in the first 24 h after salting; Teixeira et al. 2017), which is a limiting factor for OTA production. On the other side, pork pieces, being larger, take longer to reduce aw to safe values, and that might be the reason for higher levels of OTA contamination.

In what concerns aflatoxin contamination, Pleadin et al. (2015) results showed the risk of AFB_1 presence in fresh meat products to be minimal, and it is assumed that a low rate of carry-over to edible tissues occurs, given that AFB_1 primarily targets the liver (Markov et al. 2013). For that reason, AFB_1 contamination of fresh pork meat was not determined in our study. On the other side, the hazard of AFB_1 production on ripened meat products as dry-cured ham has been demonstrated previously in model systems (Rodríguez et al. 2012a), where the inoculated aflatoxigenic fungi were able to produce high amounts of this toxin at both high and low aw (0.92 and 0.84). In our study, however, the high incidence of aflatoxigenic fungi in all types of products was not translated into AFB_1 sample contamination. Goat and sheep samples were contaminated with a low number of the strong aflatoxin producers *A. parasiticus* and *A. nomius*, but the presence of non-aflatoxigenic strains of *A. flavus* was much more significant. In pork samples, only *A. flavus* strains were detected, and only a low proportion of these were aflatoxigenic.

The ability of fungi to produce toxins is highly dependent on intrinsic as well as environmental conditions, as has been proven widely (Nuñez et al. 2007; Vipotnik et al. 2017; Meftah et al. 2018), and for such reason not all toxigenic fungi can be considered a real risk in all food matrices. This seems to happen with AFs production in dry-cured hams. AFs production has been determined to occur at temperatures between 20 and 37 °C, and for aw > 0.85, but they were found to be optimally produced at 28–30 °C and > 0.95 (Schmidt-Heydt et al. 2009). These conditions are not concordant with the observed conditions during ripening.

The fact that OTA was found in pork products only provides a hint with regard to the source of contamination. In the present study, only six OTA producing fungal isolates (1 × *A. westerdijkiae*, 5 × *P. nordicum*) were obtained, all from pork samples. This means that there were 22 OTA-contaminated pork samples from which no ochratoxigenic fungal isolates could have been obtained. The most plausible explanation for this finding would be primary contamination, meaning an in vivo carry-over of OTA from swine feed into swine blood and tissues. Limitations of culture-dependent

methods of fungal detection and consequently qualitative and quantitative underestimation could be another explanation for undetected secondary contamination.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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