Food Chemistry 120 (2010) 978–983

Contents lists available at ScienceDirect

Food Chemistry

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

# Occurrence of ochratoxin A in raw ham muscle, salami and dry-cured ham from pigs fed with contaminated diet

Chiara Dall'Asta [a], Gianni Galaverna [a], Terenzio Bertuzzi [b], Alessandra Moseriti [c], Amedeo Pietri [b], Arnaldo Dossena [a,*], Rosangela Marchelli [a]

[a] Dipartimento di Chimica Organica e Industriale, Università Degli Studi di Parma, Viale Usberti 17/A, I-43100 Parma, Italy
[b] Istituto di Scienze Degli Alimenti e Della Nutrizione, Via Emilia Parmense, 84, I-29100 Piacenza, Italy
[c] Joint Research Centre, Institute for Reference Materials and Measurements, Retieseweg, 111, B-2440 Geel, Belgium

ARTICLE INFO

*Article history:*
Received 11 June 2009
Received in revised form 30 September 2009
Accepted 18 November 2009

*Keywords:*
Ochratoxin
Dry-cured ham
Pigs
Carry-over
Salami

ABSTRACT

Pork meat-derived products can contribute to the overall ochratoxin A intake, either by carry-over effect, or by environmental mould population cross-contamination. In order to assess the role of these different contamination routes, a study was carried out with pigs challenged orally with OTA contaminated feed at subchronical level. After slaughtering, thighs and minced meat from control and treated groups were transformed into dry-cured hams and salami, respectively, which were analysed for OTA determination after ripening. From collected data, the carry-over in muscle was generally low, whereas a significant contribution to the OTA contamination in dry-cured hams was due to toxinogenic mould population growing on their surface during ripening. Finally, a survey of different types of dry-cured ham ($n$ = 110), from the Italian market, was performed, showing the occurrence of OTA on the surface portion in 84 out of 110 samples with a median value of 0.53 μg/kg and in the inner core in 32 out of 110 samples with a median value lower than 0.1 μg/kg.



## 1. Introduction

Ochratoxin A (OTA) is a well-known mycotoxin, produced mainly by *Aspergillus ochraceus*, *Penicillium verrucosum* and *Penicillium nordicum*, occurring in many foods, such as cereals, coffee, cocoa, dried fruits, wine and grape juice, beer, spices, meat and meat products. In particular, *P. nordicum* can mainly be isolated from proteinaceous foods, such as cheeses and fermented meats (Castella et al., 2002; Lund & Frisvad, 2003). Several studies have shown that the toxin has nephrotoxic properties, causing both acute and chronic lesions of kidneys, and has been suspected to be involved in the aetiology of Balkan endemic nephropathy in humans, a disease characterised by progressive renal fibrosis. On account of this toxic activity, OTA has been classified by IARC as a possible carcinogen to human (Group 2B) (1993). Concerning the EU legislation for human consumption, the Commission of the European Communities fixed, in Regulation (EC) 1881/2006, maximum admissible levels for OTA in several foodstuffs and expressed the appropriateness of setting a maximum level in others, among which are meat products. Previously, the Scientific Opinion on OTA contamination of the European Food Safety Authority (EFSA) stated that the risk associated with the consumption of food derived from animals fed with OTA contaminated feeds is negligible (EFSA-Q-2003-039, 2004). In Italy, a guideline value of 1 μg/kg in pork meat and derived products has been recommended by the Italian Ministry of Health since 1999 (Ministero della Sanità, 1999).

Animal food products can contribute to the OTA intake via indirect transmission from animals exposed to naturally contaminated feed (carry-over effect) (Gareis, 1996), but the risk is limited to monogastric species because the activity of symbiotic microorganisms in ruminants can hydrolyse the amidic bond of OTA into phenylalanine and ochratoxin α, which is generally considered to be non-toxic (Karlovsky, 1999). Among farmed animals, pigs are known to be particularly sensitive to OTA accumulation, with a tissue distribution following the pattern: kidney > liver > muscle > fat (Gareis & Scheuer, 2000; Lusky, Tesch, & Gobel, 1993). Data concerning OTA occurrence in meat products have underlined that carry-over should be considered the contamination route of major concern (Gareis & Scheuer, 2000; Gareis & Wolff, 2000; Lusky et al., 1993; Yiannikouris & Jouany, 2002). However, OTA can also be produced by moulds growing on pork products during ripening: *P. nordicum* has been proven to be able to grow on meat (Battilani et al., 2007; Sorensen, Jacobsen, Nielsen, Frisvad, & Koch, 2008), and OTA was found in hams sampled during the curing time (Chiavaro et al., 2002; Curtui, Gareis, Usleber, & Martlbauer, 2001; Iacumin et al., 2009). In particular, few fungal species have been

* Corresponding author. Tel.: +39 0521 905413; fax: +39 0521 905472.
*E-mail address:* arnaldo.dossena@unipr.it (A. Dossena).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.11.036

isolated from dry-cured hams during the cold salting phase, while abundant yeast and mould growth is often observed on exposed muscle surfaces during ripening. The ham surface mould population includes several species, mainly belonging to *Aspergillus* and *Penicillium* genera. The growth of moulds on the surface of salami and dry-cured meat products during the ripening time is generally appreciated because their enzymatic activities contribute to the development of the characteristic flavour of these products (Ockerman, Sanchez, & Crespo, 2000), although it is proven that an extensive and uncontrolled mycobiota growth may cause off-flavour production and mycotoxin contamination. In addition, the use of contaminated spice mixtures may contribute to the total occurrence of mycotoxins in meat products.

Several studies have been carried out, in different countries, on the occurrence of OTA in edible tissues of pigs and in pork products. Jorgensen (2005) evaluated a mean contamination of 0.052 μg/kg for products of animal origin (mainly pork products) in the European Union. The contribution of this food category and the total OTA intake in Europe were calculated to be about 1.5 and 45 μg/kg b.w. per week, respectively. An interesting survey reported by Gareis and Scheuer (see Gareis & Scheuer, 2000) evaluated the occurrence of OTA in meat and meat products from the German market: concerning pork meat, 10 out of 58 muscle samples were found to be positive, with a median OTA concentration of 0.01 μg/kg and a maximum level of 0.14 μg/kg. Regarding pork-derived products, liver-type ($n$ = 53, 68% positive), Bologna-type ($n$ = 45, 46.7% positive) and blood sausages ($n$ = 57, 77.2% positive) were considered for OTA evaluation: for liver-type sausages, the OTA median concentration was 0.02 μg/kg with a maximum level of 4.56 μg/kg; blood sausages showed a median contamination of 0.04 μg/kg (maximum level 3.16 μg/kg) and Bologna-type sausages had a median OTA concentration of 0.01 μg/kg (maximum level 0.38 μg/kg). According to the authors, the occurrence of OTA was mainly due to the use of blood, liver or kidney in the sausages recipe, as well as to the addition of OTA contaminated spices, such as paprika, nutmeg, peppers and coriander.

Among the pork-derived sausages produced in Italy, the use of offal or blood is singular, since the most consumed products are dry-cured ham and salami: the former are prepared without addition of herbs or spices, whereas the latter may have added seasoning, according to the traditional recipe.

Recently, Matrella, Monaci, Milillo, Palmisano, and Tantillo (2006) studied the occurrence of OTA in muscles ($n$ = 54) from pigs slaughtered in southern Italy, reporting very low contamination levels (mean concentration: 0.024 μg/kg, maximum concentration: 0.9 μg/kg). From these data, the authors concluded that the OTA incidence was far from representing a real concern for consumers.

In the same period, Pietri, Bertuzzi, Gualla, and Piva (2006) reported a small survey about the occurrence of OTA in muscles and pork-derived products from northern Italy: in this case, according to Matrella et al. (2006) raw muscles ($n$ = 22, positive 9%) were only mildly contaminated (mean value: 0.05 μg/kg, maximum concentration: 0.06 μg/kg). On the other hand, the OTA occurrence in derived products, such as dry-cured ham ($n$ = 30, positive 40%) seemed to be of serious concern (mean value: 4.06 μg/kg, maximum concentration: 28.4 μg/kg). Starting from these results, the authors suggested that only direct contamination with OTA-producing strains during the ripening of dry-cured hams can explain such high levels found in several samples.

In order to investigate the origin of OTA occurrence in pork-derived products, a multidisciplinary study was carried out with pigs challenged orally with OTA contaminated feed at subchronical level. After slaughtering, thighs and minced meat from control and treated groups were transformed into dry-cured hams and salami, respectively, which were used for OTA determination after ripening. At the same time, to gain a realistic picture of OTA occurrence in dry-cured hams produced in Italy, a survey of hams ($n$ = 110) from the market was performed.

## 2. Materials and methods

### 2.1. Chemicals and reagents

OTA (solid standard 1 mg) and bovine pancreas carboxypeptidase A (50 units/mg protein) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Immunoaffinity columns (Ochraprep™) were purchased from R-Biopharm Rhône LTD (Glasgow, UK). All solvents used (LC grade) were obtained from Carlo Erba (Milan, Italy); bi-distilled water was produced in our laboratory using an Alpha-Q system from Millipore (Marlborough, MA, USA). The 33% ammonia solution, $NaHCO_3$ and *o*-phosphoric acid were from Riedel-de-Haen (Seelze, Germany). PBS buffer was prepared by dissolving 8 g of NaCl, 1.2 g of $Na_2HPO_4$, 0.2 g of $K_2HPO_4$ and 0.2 g of KCl in 1 l of bi-distilled water and adjusting pH to 7.6 with 2 N HCl. Tris–HCl buffer (0.2 M, pH 8.5) was prepared by dissolving the proper amount of Trizma base (Sigma Chemical Co., St. Louis, MO, USA) in bi-distilled water and then adjusting the pH with 1 N HCl.

### 2.2. Experimental design

The experimental design involved 20 adult pigs, divided into control (T1) and treated (T2) groups, each of 10 animals (five males and five females). The pigs were fed 3.4 kg of commercial feed, according to the Consortium of Parma Ham Regulation. In the T2 group, the commercial feed was supplemented with crystalline OTA to have a contamination of 200 μg/kg and, consequently, an ingestion of 0.68 mg/day of OTA. The pigs were fed the two diets for 40 days; after slaughtering, carcasses and target organs were inspected; then liver, kidney and blood were analysed for OTA occurrence (Rossi, Sardi, Zaghini, & Rizzi, 2006). Felino-type salami and dry-cured hams were produced from control and treated group carcasses.

### 2.3. Preparation of hams and salami

Dry-cured hams were prepared and stored in plants of Parma Province for ripening according to the Regulation of the Consortium. Basic technological treatments applied to thighs are as follows (Parma Ham Consortium website): (a) trimming: removal of some skin and fat to give the ham its typical "chicken drumstick" shape; (b) ham salting (manual meat rubbing and removal of the residual salt) and resting (2–4 months) at low temperatures (0–4 °C) and high relative humidity (75–90%); (c) drying and maturing (3–4 months) at mild temperatures (12–18 °C) and relative humidity (50–70%); (d) application of a spreadable mince onto the exposed muscle surface to prevent crusting of the outer muscle layer; (e) mid- to long-term aging (8–10 months) at temperatures in the range of 15–20 °C and relative humidity in the range 40–70%. Drying, maturing and aging phases were carried out in separate rooms. For each group, dry-cured hams were divided and ripened in two different plants (10 hams in plant A and 10 in plant B). Each plant was checked for mycobiota environmental contamination during the storage time (Battilani et al., 2007). Dry-cured hams were ripened for 16 months.

Felino-type salami are fermented sausages produced from minced pork muscle (75%) and fat (25%) with added salt, lactose, black pepper in grains and selected starter culture (lactic acid bacteria and Staphylococci). The mixture is then placed in a pork gut,

used as a casing, and then salami are stored in selected rooms for ripening (3 months).

All the collected data were corrected for the weight loss during ripening.

### 2.4. Sampling strategy

The analyses were performed on fresh muscles (*Semimembranosus* and *Biceps Femoris*; see Fig. 1) ($n = 8$), on dry-cured hams ($n = 32$) and on ripened salami ($n = 16$). In particular, for ripened meat products, both the inner (I) and the outer (O) portions were considered: (a) a portion was cut from the core; (b) a portion was taken from the outer face (1.5 cm depth) of each product. Afterwards, meat samples (100 g) were minced ($Ø_{particles} \sim 1$ mm) and stored under vacuum at −20 °C. The considered variables, thus, were the treatment (T1 or T2), the sampling position (I or O) and, for hams, the ripening plant (A or B).

### 2.5. Sample preparation

The extraction procedure was performed according to Toscani et al. (2007). Briefly, a minced meat aliquot of 10 g was extracted with a solution of chloroform (100 ml) acidified with 85% *o*-phosphoric acid solution (0.75 ml) by homogenising the sample with an Ultraturrax (T50 basic IKA®-WERKE, Staufen, Germany) for 2 min. After filtration, an aliquot of the extract (60 ml) was transferred into a separating funnel and extracted twice with 15 ml of 0.2 M Tris–HCl, buffer pH 8.5. The upper aqueous phases were carefully collected and mixed. To avoid the growth of OTA-degrading microorganisms in the aqueous phase, a volume of $CH_3CN$ was added to achieve a 0.2 M Tris–HCl: $CH_3CN$ (90:10, v/v) ratio.

### 2.6. HPLC-FLD analysis

The validated method proposed by Toscani et al. (2007) was followed. Briefly, HPLC analysis was performed using a C18 column (Waters XTerra®, 250 mm × 2.1 mm, 3 μm) in a Waters Alliance 2695 chromatographic system under isocratic conditions at room temperature, with an aqueous $NH_3/NH_4Cl$ (20 mM, pH 9.8):$CH_3CN$ (85:15, v/v) mobile phase; the flow rate was 0.2 ml/min and the injected volume was 20 μl. The FLD detection was obtained by means of a Waters 474 Scanning Fluorescence Detector ($\lambda_{ex}$ = 380 nm, $\lambda_{em}$ = 440 nm; gain = ×100; attenuation = 32; band width = 40 nm). The retention time of the analyte was 20 times the retention time corresponding to the void volume of the column.

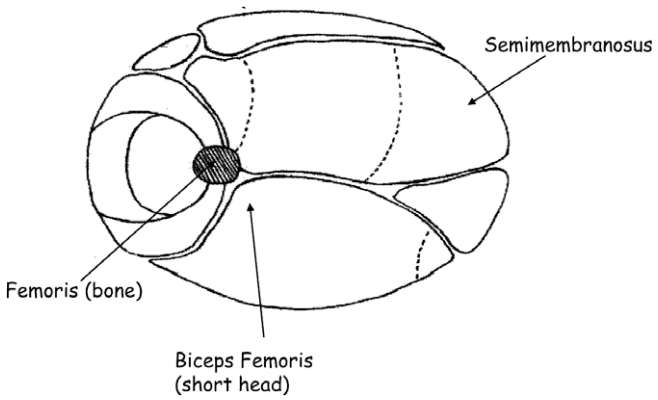


**Fig. 1.** Sampling scheme of the ham muscles.

### 2.7. OTA identity confirmation by the carboxypeptidase A method

The confirmation test for OTA identification was performed using carboxypeptidase A (Frohlich, Marquardt, & Clarke, 1997; Stander, Steyn, van der Westhuizen, & Payne, 2001). Four blank samples of dry-cured pork meat were spiked at 3 μg/kg and extracted in 0.2 M Tris–HCl, pH 8.5; then, the extracts were adjusted to pH 7.5 and divided into two aliquots (1.4 and 1.5 ml, respectively). 100 μl of the carboxypeptidase A were added to the 1.4 ml fraction. Both aliquots were incubated at 37 °C for 3 h and analysed by HPLC-FLD. Percent degradation of the OTA peak was calculated and the onset of the OTα signal was monitored.

### 2.8. Dry-cured ham survey from the market

A survey of OTA occurrence in dry-cured hams from the market, was carried out by purchasing different typologies of dry-cured hams ($n = 110$) produced in Italy. Each ham underwent the sampling and extraction procedure, as previously described. Each analysis was performed in triplicate.

### 2.9. Statistical evaluation of the data

Statistical analysis was carried out using SPSS v.16.0.

## 3. Results and discussion

### 3.1. Method of OTA analysis in dry-cured ham

Analysis of pork meat was performed according to the method recently proposed by Toscani et al. (2007), fully validated according to the requirements reported by the EU Commission (Commission, 2002). The method performances (LOD, LOQ, recovery at 1 μg/kg) were also evaluated for raw muscle and salami, in order to check the reliability of this procedure also for these products. Briefly, the extraction procedure was developed, taking into account the protonation equilibrium of OTA, which is negatively charged at alkaline pH whereas it is neutral at acidic pH. In particular, a crude extract was obtained from meat by a solid–liquid extraction with chloroform after acidification of the sample (pH 3), in order to keep OTA in its neutral form and, thus, soluble in organic solvent. Then, OTA was extracted from the organic phase by liquid–liquid partition, using a slightly alkaline buffer solution (0.2 M Tris–HCl) (pH 8.5). The slightly alkaline pH allowed for the back-extraction of the dianionic form of OTA, and purification of the analyte from most of the interferents. The validation parameters of the method are summarised in Table 1. The data obtained were corrected for recovery.

The HPLC profile of a naturally contaminated dry-cured ham (outer layer) is shown in Fig. 2.

**Table 1**
Validation parameters for ochratoxin A detection in pork meat.

| | Dry-cured ham | Raw muscle | Salami |
|---|---|---|---|
| Limit of detection (LOD) | 0.1 μg/kg | 0.1 μg/kg | 0.1 μg/kg |
| Limit of quantification (LOQ) | 0.3 μg/kg | 0.4 μg/kg | 0.3 μg/kg |
| Recovery (%) at 0.5 μg/kg | 90.5% | – | – |
| Recovery (%) at 1 μg/kg | 71.1% | 77.8% | 78.4% |
| Recovery (%) at 2 μg/kg | 67.2% | – | – |
| Repeatability at 0.5 μg/kg | 18.6% | – | – |
| Within-laboratory reproducibility at 0.5 μg/kg | 23.7% | – | – |

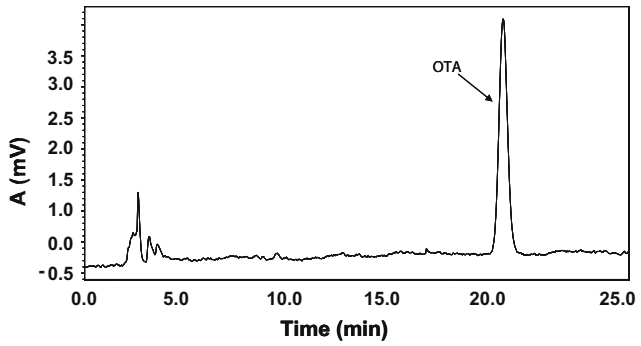


**Fig. 2.** Chromatographic analysis of an OTA contaminated ham (2.3 μg/kg).

### 3.2. Determination of OTA in raw ham muscle

Raw ham muscle was analysed for the occurrence of OTA. *Semimembranosus* and *Biceps Femoris* muscles were considered for sampling, since these portions are the most representative of dry-cured hams. Moreover, their sampling is easily performed, giving reliable results. A scheme of ham muscles is shown in Fig. 1. The analyses were performed on samples from the control group (T1) and from the treated group (T2). OTA was found in all the samples obtained from animals fed OTA contaminated feed (T2) with an average OTA concentration of 2.21 ± 0.78 μg/kg, whereas no contamination was observed for the control group.

These data are in agreement with data published by Rossi et al. (2006), concerning the occurrence of OTA in body fluids and tissues from the same challenged animals. Very briefly, the authors found the following medium levels of OTA in the analysed tissues (T2 group): blood 48.5 ± 13.2 μg/kg, kidney 9.6 ± 2.7 μg/kg, liver 6.3 ± 1.7 μg/kg, urine 4.0 ± 4.1 μg/kg, muscle 1.9 ± 0.6 μg/kg, fat 1.1 ± 0.6 μg/kg. No OTA was found in tissues or fluids from the control group.

The OTA levels found in muscle are quite low when compared with the OTA concentration in feed ingested by the animals (0.68 mg/day OTA for 40 days). Since the average weight of the treated pigs was about 140 kg during the trial (162.1 kg at slaughtering), the average daily ingestion in the experimental period was about 4.8 μg/kg body weight. Comparing these results with those obtained by Lusky et al. (1993) the levels were lower, probably due to the shorter ingestion time of contaminated feed in our trial.

### 3.3. Determination of ochratoxin A in Felino-type salami

OTA occurrence was evaluated in Felino-type salami produced from control (T1) and treated groups (T2). Since the minced meat and fat mixture was added with black pepper before ripening, the occurrence of OTA in pepper was evaluated and found to be negligible (<LOD). No contamination was found in samples ($n = 8$) of T1; only one sample, obtained from the outer layer, was found to be contaminated by OTA (0.62 μg/kg), probably as a consequence of an environmental contamination. OTA was always detected in salami ($n = 8$) obtained from T2; the OTA median value was found to be 2.64 μg/kg (maximum level 3.14 μg/kg) regarding the inner part, and 2.87 μg/kg (maximum level 3.32 μg/kg) for the outer part as shown in Table 2 and Fig. 3.

T2 data for the inner part (I) and the outer part (O) were statistically evaluated using a t-paired test ($\alpha = 0.05$): no significant differences were observed. These results suggest that the OTA level is not affected by environmental contamination in Felino-type salami, probably on account of the presence of the casing which acts as a protective system against external contamination during ripening. These data are in agreement with those recently reported by Iacumin et al. (2009): the authors investigated the occurrence of OTA in dry sausages from northern Italy. Although very high

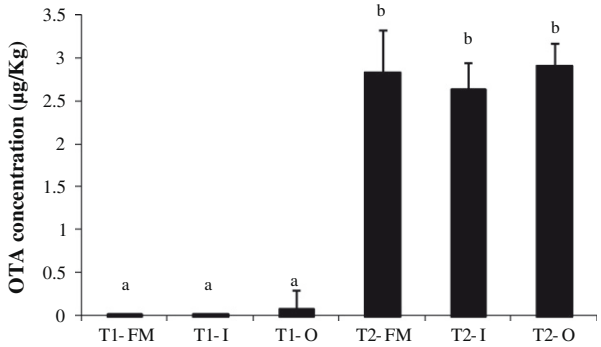


**Fig. 3.** Comparison of OTA levels found in fresh minced meat (FM) and in ripened salami from control (T1) and mycotoxin-treated groups (T2), by sampling both the inner core (I) and the outer layer (O) of each sample ($n = 16$). Significant differences ($\alpha = 0.05$) are reported as a letter.

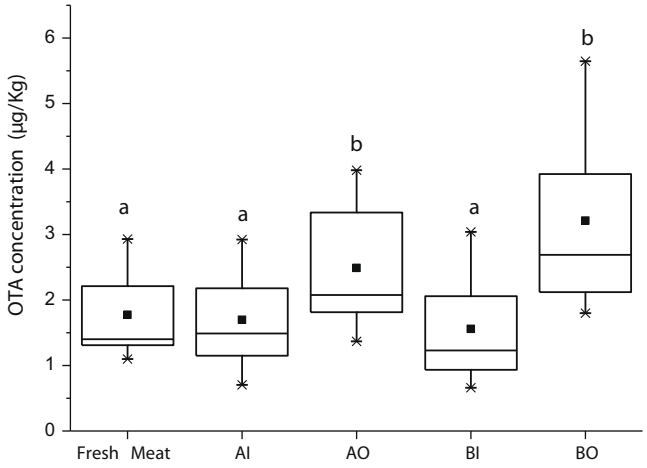


**Fig. 4.** Box plot obtained by comparison of the OTA levels found in fresh muscles and final products from group T2. Hams were ripened into two different plants (A and B) and samples were taken from the inner core (I) and from the outer layer (O). Each sample ($n = 20$) was prepared in duplicate and injected in triplicate. Significant differences ($\alpha = 0.05$) are reported as a letter.

**Table 2**
OTA (μg/kg) occurrence in ripened salami. The data are shown for control (T1) and OTA-treated groups (T2). For each group, sampling was performed from the inner part (I) and at the outer part (O) of the sausage.

| | Positive/total samples | Range of contamination (μg/kg) | Median (μg/kg) | Mean (μg/kg) | SD (μg/kg) |
|---|---|---|---|---|---|
| T1–I | 0/8 | n.d. | – | – | – |
| T1–O | 1/8 | LOD–0.62 | n.d. | 0.08 | 0.22 |
| T2–I | 8/8 | 2.40–3.14 | 2.64 | 2.65 | 0.31 |
| T2–O | 8/8 | 2.58–3.32 | 2.87 | 2.915 | 0.27 |

**Table 3**
OTA (μg/kg) occurrence in ripened dry-cured hams. The data are shown for the control (T1) and for OTA-treated groups (T2). For each group, sampling was performed from the inner (I) and the outer part (O). Moreover, for each group, two ripening plants were considered (A and B).

| | Sample | Positive/total samples | Range of contamination (μg/kg) | Median | Mean | SD |
|---|---|---|---|---|---|---|
| T1 | A–I | 0/8 | – | – | – | – |
| | A–O | 3/8 | LOD–0.70 | n.d.[a] | n.d.[a] | – |
| | B–I | 0/8 | – | – | – | – |
| | B–O | 4/8 | LOD–1.67 | n.d.[a] | 0.255 | 0.515 |
| T2 | A–I | 8/8 | 1.255–2.925 | 2.25 | 2.22 | 0.61 |
| | A–O | 8/8 | 1.715–3.98 | 3.42 | 3.11 | 0.72 |
| | B–I | 8/8 | 1.325–3.04 | 2.14 | 2.13 | 0.47 |
| | B–O | 8/8 | 1.99–5.645 | 3.935 | 4.12 | 0.985 |

[a] Calculated values lower than 0.1 μg/kg (limit of detection).

**Table 4**
OTA (μg/kg) occurrence in dry-cured hams from the market. Determinations were performed in triplicate.

| | Positive/total | Media | Median | SE | 1st Quartile | 3rd Quartile | Maximum |
|---|---|---|---|---|---|---|---|
| Inner | 32/110 | 0.24 | n.d.[a] | 0.07 | 0 | 0.15 | 4.66 |
| Outer | 84/110 | 0.98 | 0.53 | 0.16 | 0.06 | 1.13 | 12.51 |

[a] Calculated values lower than 0.1 μg/kg (limit of detection).

OTA levels were found on the surface of the products (ranging from 3 to 18 μg/kg), the mycotoxin has been never detected inside the product, indicating that casings are a proper protection against environmental contamination.

### 3.4. Determination of OTA in dry-cured hams

The results obtained in the ham study are shown in Table 3.

Surprisingly, OTA occurred in the outer parts of three and four samples belonging to the control group from plants A and B, respectively: in particular, the maximum OTA concentration in the outer part of T1 hams was 1.67 μg/kg, although the median value was negligible (0.01 μg/kg). No contamination was found in the inner part. In the dry-cured hams obtained from T2, OTA was found in both the inner and the outer samples, although at different levels. The median OTA concentration detected for the inner part samples was 2.19 μg/kg with a 75% percentile of 2.85 μg/kg and a maximum value of 3.04 μg/kg; for the outer part samples, the median concentration was 3.62 μg/kg with a 75% percentile of 3.96 μg/kg and a maximum value of 5.64 μg/kg. These data indicated that an OTA contamination occurred on the ham surface in both plants. Contrary to the case of salami, dry-cured ham is not protected by a casing; thus OTA, produced from superficial mycobiota, can contaminate the outer part of dry-cured ham (see Fig. 4).

Statistical analyses of the T2 data, using a one way-Anova test ($\alpha = 0.05$) and a Tukey test ($\alpha = 0.05$) for means comparison showed no significant differences between OTA content in raw ham muscle and the inner samples after ripening (both plants), whereas the OTA concentration was significantly higher ($p < 0.0001$) in the outer parts in comparison with the inner ones in both ripening plants. Thus, OTA was stable during ripening, and the contribution of external contamination is both feasible and in agreement with that observed for the untreated samples.

A two way-Anova test ($\alpha = 0.05$) was performed, considering the plant (A or B) as factor A and the sampling positions (I or O) as factor B: the test results showed a significant difference for the sampling position ($p = 7.05 \times 10^{-7}$) whereas the ripening plant did not exhibit any significant difference ($p = 0.064$). The significant differences occurring between the inner and the outer portion underline the role played by the environmental mould population in OTA contamination of dry-cured hams. Indeed, studies on the environmental contaminating mould population of the two ripening plants (Battilani et al., 2007) have shown that the number of penicillia (colonies/cm$^2$) potentially able to produce OTA, detected in maturing and aging rooms, were not statistically different, although slight differences in temperature and relative humidity were monitored for plants A and B.

To further substantiate the environmental origin of OTA contamination, we analysed a large number of dry-cured hams of different typologies ($n = 110$) purchased from the market. All these samples were checked for OTA contamination according to the experimental protocols, separately analysing both the inner and the outer parts of the dry-cured ham. Data obtained from this survey confirmed a diffuse contamination in the majority of the hams, although generally at low levels: OTA occurred in 84 out of 110 outer samples and in 32 out of 110 inner samples (Table 4). Once more, the one way-Anova test ($\alpha = 0.05$) and the Tukey test ($\alpha = 0.05$), performed on the data obtained from those hams, showing both inner and outer contamination, gave a significant difference ($p = 0.00111$) between the two values, the outer part showing the highest OTA level.

The overall descriptive statistics obtained for the ham survey are given in Table 4.

In any case, the average contamination found in commercial samples was below the Italian legal limit for pork meat (1 μg/kg): nevertheless, the presence of samples showing a very high contamination level (inner maximum level found: 4.66 μg/kg; outer maximum level found: 12.5 μg/kg) underlines the importance of environmental hygienic control in the ripening plants.

## 4. Conclusions

Dry-cured ham and salami produced from pigs fed with OTA contaminated feed for 40 days (OTA intake: 0.68 mg/day, 4.8 μg/kg of body weight) were found to contain OTA due to a carry-over effect. OTA concentrations in muscles after slaughtering and in final products were low (range 2.40–3.32 μg/kg) although higher than the Italian guidelines (1 μg/kg). In any case, feeds commercialized in the EU normally have an OTA contamination lower than that used in this study; thus, the real risk, due to a carry-over from OTA contaminated feed to pork meat, is negligible, as already outlined by the European Food Safety Authority (EFSA-Q-2003-039, 2004). Nevertheless, this study showed that an important contribution to the overall OTA contamination in dry-cured hams seems

to be due to environmental toxinogenic mould population growing on the surface of the products during ripening. In the environmental conditions used in ripening plants, *Penicillium* mould population could contaminate hams and produce OTA, underlying the importance of monitoring the general hygienic conditions of the manufacturing rooms.

## Acknowledgement

The present work has been performed within the project "Safety and quality in the typical pork products food chain (2002–2004)" granted by the Emilia-Romagna Region (Italy).

## References

Battilani, P., Pietri, A., Giorni, P., Formenti, S., Bertuzzi, T., Toscani, T., et al. (2007). Penicillium populations in dry-cured ham manufacturing plants. *Journal of Food Protection, 70*(4), 975–980.

Castella, G., Larsen, T. O., Cabanes, J., Schmidt, H., Alboresi, A., Niessen, L., et al. (2002). Molecular characterization of ochratoxin A producing strains of the genus Penicillium. *Systematic and Applied Microbiology, 25*(1), 74–83.

Chiavaro, E., Lepiani, A., Colla, F., Bettoni, P., Pari, E., & Spotti, E. (2002). Ochratoxin A determination in ham by immunoaffinity clean-up and a quick fluorometric method. *Food Additives and Contaminants, 19*(6), 575–581.

Commission, E. (2002). Commission Decision 2002/657/EC. *Official Journal of European Community, L221*.

Curtui, V. G., Gareis, M., Usleber, E., & Martlbauer, E. (2001). Survey of Romanian slaughtered pigs for the occurrence of mycotoxins ochratoxins A and B, and zearalenone. *Food Additives and Contaminants, 18*(8), 730–738.

EFSA-Q-2003-039, R. N. (2004). Opinion of the scientific panel on contaminants in food chain on a request from the commission related to ochratoxin A (OTA) as undesirable substance in animal feed. In *EFSA, The EFSA Journal* (vol. 101, pp. 1–36). European Food Safety Authority.

Frohlich, A. A., Marquardt, R. R., & Clarke, J. R. (1997). Enzymatic and immunological approaches for the quantitation and confirmation of ochratoxin A in swine kidneys. *Journal of Food Protection, 60*(2), 172–176.

Gareis, M. (1996). *Fate of ochratoxin A on processing of meat products* (pp. 35–37).

Gareis, M., & Scheuer, R. (2000). Ochratoxin A in meat and meat products. *Archiv Fur Lebensmittelhygiene, 51*(4–5), 102–104.

Gareis, M., & Wolff, J. (2000). Relevance of mycotoxin contaminated feed for farming animals and carry over of mycotoxins in food of animal origin. *Mycoses, 43*, 79–83.

Iacumin, L., Chiesa, L., Boscolo, D., Manzano, M., Cantoni, C., Orlic, S., et al. (2009). Moulds and ochratoxin A on surfaces of artisanal and industrial dry sausages. *Food Microbiology, 26*(1), 65–70.

IARC/WHO (1993). *IARC monographs on the evaluation of the carcinogenic risks to humans* (Vol. 56, p. 449). Lyon, France: IARC Press.

Jorgensen, K. (2005). *Occurrence of ochratoxin A in commodities and processed food – A review of EU occurrence data* (pp. 26–30).

Karlovsky, P. (1999). Biological detoxification of fungal toxins and its use in plant breeding, feed and food production. *Natural Toxins, 7*(1), 1–23.

Lund, F., & Frisvad, J. C. (2003). Penicillium verrucosum in wheat and barley indicates presence of ochratoxin A. *Journal of Applied Microbiology, 95*(5), 1117–1123.

Lusky, K., Tesch, D., & Gobel, R. (1993). Influence of the mycotoxin ochratoxin-A on animal health and formation of residues in pigs and different types of sausages derived from these animals. *Archiv Fur Lebensmittelhygiene, 44*(6), 131–134.

Matrella, R., Monaci, L., Milillo, M. A., Palmisano, F., & Tantillo, M. G. (2006). Ochratoxin A determination in paired kidneys and muscle samples from swines slaughtered in southern Italy. *Food Control, 17*(2), 114–117.

Ministero della Sanità (1999). Circolare 09.06.1999. In M. D. Sanità (Ed.), *Circolare 09.06.1999* (vol. 135) 11.06.1999. Gazzetta Ufficiale Repubblica Italiana.

Ockerman, H. W., Sanchez, F. J. C., & Crespo, F. L. (2000). Influence of molds on flavor quality of Spanish ham. *Journal of Muscle Foods, 11*(4), 247–259.

Parma Ham Consortium website: <http://www.prosciuttodiparma.com/eng/info/making/>.

Pietri, A., Bertuzzi, T., Gualla, A., & Piva, G. (2006). Occurrence of ochratoxin a in raw ham muscles and in pork products from Northern Italy. *Italian Journal of Food Science, 18*(1), 99–106.

Rossi, A., Sardi, L., Zaghini, A., & Rizzi, L. (2006). Diete contaminate da micotossine nel suino: Effetti in vivo e al macello. *Suinicoltura, 10*, 131–134.

Sorensen, L. M., Jacobsen, T., Nielsen, P. V., Frisvad, J. C., & Koch, A. G. (2008). Mycobiota in the processing areas of two different meat products. *International Journal of Food Microbiology, 124*(1), 58–64.

Stander, M. A., Steyn, P. S., van der Westhuizen, F. H., & Payne, B. E. (2001). A kinetic study into the hydrolysis of the ochratoxins and analogues by carboxypeptidase A. *Chemical Research in Toxicology, 14*(3), 302–304.

Toscani, T., Moseriti, A., Dossena, A., Dall'Asta, C., Simoncini, N., & Virgili, R. (2007). Determination of ochratoxin A in dry-cured meat products by a HPLC-FLD quantitative method. *Journal of Chromatography B – Analytical Technologies in the Biomedical and Life Sciences, 855*(2), 242–248.

Yiannikouris, A., & Jouany, J. P. (2002). Mycotoxins in feeds for ruminants; fate and effects on animals. *Productions Animales, 15*(1), 3–16.