



## Ochratoxin A production by *Aspergillus westerdijkiae* in Italian-type salami

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### ABSTRACT

The occurrence of ochratoxin A (OTA) in matured meat products can be attributed to mycotoxin coming from raw materials and/or molds that develop on the product surface during ripening. This work aimed to evaluate OTA production by *Aspergillus westerdijkiae* inoculated on the surface of sausages and its diffusion into the product throughout ripening, study the effect of relative humidity (RH) on the production of this mycotoxin, and investigate the presence of OTA in dry fermented sausages that naturally present intense contamination by *A. westerdijkiae*. Italian-type sausages were surface inoculated with *A. westerdijkiae* and two distinct experiments were performed. In the first, the sausages were matured in a chamber with decreasing RH (from 95 to 75%) for 35 days. In the other, the sausages were incubated under different RH (79, 85 or 95%) for 21 days. Samples were taken at the beginning of the experiments and every 7 days, subdividing into casing, outer border, and core for analyses. Sausage samples naturally spoiled by ochratoxigenic fungi were collected during sanitary inspection. Even in the presence of *A. westerdijkiae* mycelia, no OTA was detected for up to 7 days of sausage maturation. On the other hand, this study demonstrated that the growth of *A. westerdijkiae* on salami surface produces high amounts of OTA on the casing and allows its diffusion through the casing with contamination to the outer border of sausages. In the same way, it shows that under similar water activity values of substrate, RH influences the amount of OTA produced. Conversely, OTA was restricted to the casing in the naturally contaminated sausages.

### 1. Introduction

Ochratoxin A (OTA) is a high toxicity mycotoxin that increases the incidence of hepatocellular tumor in mice. This metabolite produces renal-cell adenomas and carcinomas in male mice and rats and is considered a possible carcinogenic agent in humans (IARC, 1993). The World Health Organization (FAO/WHO, 2007) states that the OTA Provisional Tolerable Weekly Intake (PTWI) is 112 ng/kg bw/week. The ingestion of food with high levels of OTA may cause proximal renal tubule degeneration and interstitial nephropathy (FAO/WHO, 2007).

The name of this mycotoxin is attributed to the fungal isolate from which this compound was first detected by Van der Merwe et al. (1965), which was *Aspergillus ochraceus* CBS 263.67. However, Frisvad et al. (2004) found that this strain was a distinct species renamed *Aspergillus westerdijkiae* CBS 263.67. It is now believed that many fungi previously identified as *A. ochraceus* can be *A. westerdijkiae* (Samson et al., 2006), which are species that usually produce OTA in higher amounts and more consistently than *A. ochraceus* (Samson et al., 2006).

Ochratoxin A is the mycotoxin that most commonly occurs in cured meat products. Its presence in these products is attributed to contamination by raw materials [e.g. spices (Gareis and Scheuer, 2000; Pleadin et al., 2015) and meat from animals exposed to OTA through their diet (Dall'Asta et al., 2010; Perši et al., 2014)] and, mainly, due to the growth of molds on the surface of meat products during ripening (Iacumin et al., 2009; Dall'Asta et al., 2010; Sørensen et al., 2008; Rodríguez et al., 2012; Berni et al., 2017; Parussolo et al., 2019), since environmental conditions in manufacturing rooms are suitable for mold development.

Most studies available in the literature on OTA production in meat products or meat-based media are directed to the species *Penicillium nordicum* and *Penicillium verrucosum*, as they are traditionally more prevalent in European countries, which have a temperate climate (Spotti et al., 1999; Rodríguez et al., 2012; Battilani et al., 2010; Rodríguez et al., 2015a, b; Ferrara et al., 2016; Sánchez-Montero et al., 2018; Andrade et al., 2019; Peromingo et al., 2019). However, since the occurrence of *A. westerdijkiae*/*A. ochraceus* has already been described

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in salami production from Argentina (Vila et al., 2016; Castellari et al., 2010; Canel et al., 2013), Brazil (Parussolo et al., 2019), Italy (Iacumin et al., 2009; 2011), as well as on the surfaces of other meat products matured in several countries (Strzelecki and Badura, 1972; Sutic et al., 1972; Huerta et al., 1987; Rojas et al., 1991; Nunez et al., 1996; Comi et al., 2004; Wang et al., 2006; Costa et al., 2014), more attention should be paid to this species. Additionally, studies have highlighted important differences in the behavior of OTA-producing species (*P. verrucosum*, *P. nordicum*, and *A. westerdijkiae*) when growing on substrates based on meat products and incubated under different water activity (aw) of substrates and temperatures (Battilani et al., 2007; Rodríguez et al., 2015a, b; Vipotnik et al., 2017). *A. westerdijkiae* showed to be able to produce OTA in larger quantities and under broader conditions when compared to *P. nordicum* in culture medium based on cured ham (Vipotnik et al., 2017), which is a cause for concern.

Intense contamination by yellow aspergilli, which were later identified as *A. westerdijkiae*, was observed on the surface of dry fermented sausages produced in a small-scale industry in Southern Brazil during sanitary inspection. Despite knowledge on the huge ochratoxigenic potential of yellow aspergilli, no previous reports have been found on OTA occurrence in sausages produced in Brazil, South America or around the world evaluating OTA production by this species in salami ripening after surface inoculation. Therefore, the objective of this work is to evaluate the presence of OTA in dry fermented sausages naturally contaminated or artificially inoculated with *A. westerdijkiae* on the surface, their diffusion into the product during ripening, and the production of this mycotoxin in different relative humidity (RH) conditions.

## 2. Materials and methods

### 2.1. Natural occurrence of ochratoxin A in sausages

Four dried fermented sausages showing intense development of fungi on their surface, predominantly in shades of yellow to orange, were collected during sanitary inspection in a manufacturing industry from Santa Maria-RS, Brazil (L – 29.6914, L – 53.8008, 29° 41' 29" S, 53° 48' 3" W). Our group has published problems with the development of unwanted fungi in sausages from this manufacturing industry (Supp. Fig. 1), where the air of the facility was found to be the source of contamination (Parussolo et al., 2019). Previous mycological analyses of this lot revealed fungal counts in the order of  $10^6$  UFC/cm<sup>2</sup>, with predominance of *A. westerdijkiae* (Visagiel et al., 2014). The fermented sausages were sent to the Federal University of Santa Maria (UFSM) for OTA analyses. These sausages were produced according to Brazilian legislation for Italian-type salami (Brasil, 2000) and no mold starter was added during their production.

#### 2.1.1. Analyses of ochratoxin A (OTA) in sausages

The sausages were fractionated in the casing, outer border, and core and sent to the Laboratory of Mycotoxicological Analyses (LAMIC, UFSM) for OTA detection and quantification.

Twelve milliliters of extraction solvent (acetonitrile/water/acetic acid 84: 16: 1, v/v/v) were added to 3 g of the minced and homogenized sausage samples arranged in a 50 mL polypropylene tube. The extraction was done for 10 min in a vortex shaker (MA-541, Brazil). After sedimentation by gravity, each sample was diluted 1:9 with dilution solvent (acetonitrile/water/formic acid 49: 50: 1, v/v/v) and 5 µL of the diluted extract was injected into an LC-MS/MS.

For the recovery experiments, 3 g of 3 salami samples and 3 casing samples were weighed and spiked at a concentration level of 5 µg/kg. After homogenization of the material, the spiked samples were kept overnight at room temperature.

Analyses were performed by LC-MS/MS based on Sulyok et al. (2006) with minor adaptations. In particular, an API 5000 LC-MS/MS

System (Applied Biosystems, Foster City, CA, USA) equipped with a Turbo Ion Spray electrospray ionization (ESI) and 1200 Series HPLC System (Agilent, Waldbronn, Germany) were used. Chromatographic separation was performed at 35 °C with an Agilent Zorbax C18 150 × 4.6 mm i.d. and 5-µm particle size column equipped with a 4 × 3 mm C18 guard column i.d. (Agilent, USA).

Acetonitrile and methanol (LC grade) and phosphoric acid (P.A.) were purchased from J.T. Baker (Brazil). The analytical standard of OTA was obtained from Romer Labs (Tulln, Austria). The water was successively purified by reverse osmosis in a Milli-Q Millipore system (USA).

The OTA quantification limit (1 µg/kg) and detection limit (0.25 µg/kg) were calculated by the signal-to-noise ratio (LOQ = 10/1, LOD = 3/1), respectively. For calculation of the recovery estimate of the method (95%), triplicate analyses of fortified samples with 5 µg/kg of OTA standard were performed. Linearity was evaluated through the coefficient of determination (R<sup>2</sup>), which was calculated after triplicate injection of analytical curves with seven different concentration levels. For quantification, analytical curves with R<sup>2</sup> > 0.99 were used. Because there was no certified reference material (MRC) for the analyzed matrix, barley MRC (FAPAS 17173) was analyzed along with the sample sequence as well as artificially contaminated salami samples.

### 2.2. Experiments on OTA production in sausages

#### 2.2.1. Fungal strains

Three *A. westerdijkiae* ochratoxin-producing strains previously isolated from spoiled salami (Parussolo et al., 2019) were used for sausage inoculation: *A. westerdijkiae* 01/17 (AW 01), *A. westerdijkiae* 02/17 (AW 02), and *A. westerdijkiae* 03/17 (AW 03). Their capacity to produce OTA was confirmed according to the method reported by Alcano et al. (2016).

Isolates were lyophilized and kept under refrigeration (7 °C ± 1 °C) at the Laboratory of Food Mycology of UFSM in order to preserve the original characteristics and stability of viable cells.

#### 2.2.2. Inocula preparation

Each single strain was separately cultivated in Petri dishes containing Malt Extract Agar (MEA) [glucose, 20 g (Neon, São Paulo, Brazil); peptone, 1 g (Himedia, Mumbai, India); malt extract, 30 g (Bacto™, MD, USA); metal traces, 1 ml; distilled water, 1l] for 7 day at 25 °C.

An initial inoculum was prepared for each strain by scraping the conidia from the media surface and transferring them to flasks containing peptone water solution (0.01%). Then, the content was filtered with sterile gaze and standardized at a spore concentration of 10<sup>4</sup> conidia/mL with the aid of Neubauer's chamber (Sigma-Aldrich), followed by the mixture of the 3 strain suspensions. The final concentration of conidia was confirmed by plating the suspension in MEA (25 °C/ 5 days).

#### 2.2.3. Inoculation of salami

A total of 32 freshly produced Italian-type salami, which was made with at least 60% of pork meat ground in a mean particle size between 6 and 9 mm (Brasil, 2000) embedded in collagen wrap (with an average length of 16 cm and diameter of 50 mm), were acquired from a local producer and kept overnight under refrigeration.

The 30 sausages were individually surface inoculated with 1 mL of the spore suspension (10<sup>4</sup> conidia/mL), dropped with the aid of an automatic pipette, and spread with a sterile loop in a sterile flow cabinet.

Two non-inoculated and two freshly inoculated sausages were segregated for analyses to verify the possible influence of the initial inoculum in the OTA contamination of salami before ripening (T0). The remaining 28 salami were separated into two groups for different experiments.

### 2.2.4. Experiment I: OTA production during salami ripening in chamber

Ten inoculated sausages were arranged in a maturation chamber (Menoncin Ind. Com. Ltda, Erechim, Brazil) at 20 °C for 35 days. The ripening protocol consisted of an eight-phased ripening process according to Cichoski et al. (2009), with adaptations: on the first 5 days RH = 95%, on the 6th day RH = 93%, on the 7th day RH = 90%, on the 8th day RH = 87%, on the 9th day RH = 84%, on the 10th day RH = 81%, on the 11th day RH = 78%, and from the 12th to 35th maturation day RH = 75%.

Duplicate salami were randomly removed after 7, 14, 21, 28, and 35 days of maturation for subsequent partitioning and analyses.

### 2.2.5. Experiment II: OTA production in sausages exposed to different RH

Solutions with different concentrations of sodium chloride (23.5, 19, and 8%) were prepared in order to reach aw of 0.79, 85, and 0.95. The solutions were placed in three 30L transparent plastic boxes (42 cm × 20 cm × 27 cm) adapted for sausage suspension and kept hermetically sealed for 48 h before beginning the experiment.

After aw verification, six sausages were suspended in each box and placed in a chamber at 20 °C.

Duplicate salami were randomly removed from each box after 7, 14, and 21 days for subsequent partitioning and analyses.

### 2.2.6. Partitioning of salami for analyses

After each sampling, the salami of about 40 mm diameter were frozen and fractionated using a tool built to facilitate partitioning in casing, outer border, and core (20 mm diameter) (Fig. 1).

Sausage portions of the casing, border, and core were homogenized and subsamples were collected for aw, pH, and OTA analyses.

### 2.3. Measurement of water activity (aw)

The aw of different portions of salami and NaCl solutions along the experiment were directly measured in AquaLab 4 TE (Decagon Devices, USA), previously calibrated according to the manufacturer instructions, at 25 °C ± 1, in triplicate.

### 2.4. pH

The pH was determined by homogenizing 10 g of the sample with

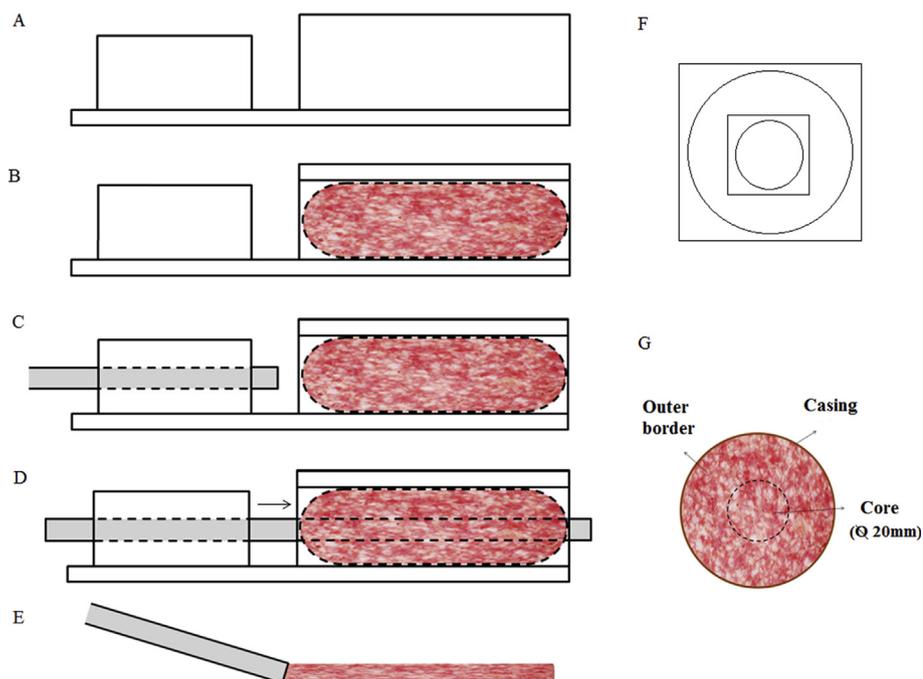


Fig. 1. The cutting and extraction of the center (core) of the salami: (A) Extraction tool; (B) Extraction tool with sample inside; (C) Extraction tool with sample and cylinder (20 mm) for cutting the salami; (D) Cylinder cutting sample from side to side; (E) Sample cylinder being withdrawn; (F) Transversal view of extraction tool; (G) Transversal scheme of salami partition for analyses.

distilled water (1:10 sample/water). The homogenate was submitted to pH-meter electrodes Tecnopon (mPA 210, Piracicaba, São Paulo, Brazil) for 5 min (Terra and Brum, 1988).

### 2.5. Analyses of ochratoxin A (OTA)

Detection and quantification of OTA were done at the Laboratory of Mycotoxicological Analyses (LAMIC, UFSM).

### 2.6. Data analyses

Variance analysis (ANOVA) was performed. Means of OTA production by *A. westerdijkiae* in salami in different RH were analyzed using the Tukey test ( $p < 0.05$ ). Statistical analyses were performed using version 5.6 of SISVAR® Software (Ferreira, 2011).

## 3. Results and discussion

### 3.1. Natural occurrence of ochratoxin A in sausages

Regarding the 4 naturally infected sausages analyzed for OTA presence, this mycotoxin was only detected in the casings, as it did not reach the stuffed meat. The contamination level of the casings ranged from 2.79 to 32.9 µg/kg. As far as we know, this is the first report of the natural occurrence of OTA in dry fermented sausages produced in Brazil.

Iacumin et al. (2009) showed that OTA contamination (up to 18 µg/kg) was restricted to the casings in about 50% of 160 artisanal, industrial, and dry fermented sausages produced in Italy, with the absence of OTA in 0.5 cm depth from the border of those sausages (Limit of Detection, LOD- 0.1 µg/kg). The authors verified that the washing and brushing of salami surface reduced the contamination of OTA to values below LOD. However, other studies (Armorini et al., 2016; Merla et al., 2018; Altafini et al., 2019) reported the presence of this toxin in dry fermented sausages, even the spices used in the formulation were also suspected to be the source of these product contamination with OTA (Markov et al., 2013; Altafini et al., 2019).



Fig. 2. Sausages surface inoculated with *Aspergillus westerdijkiae* and matured in chamber at 20 °C for 35 days.

### 3.2. Experiment I: OTA production during salami ripening in chamber

The development of *A. westerdijkiae* in salami was light at day seven, although it dominated the casing surface on the following days (Fig. 2). Ripening evolution is shown in Table 1.

Values of OTA produced in the different portions of salami superficially inoculated with *A. westerdijkiae* and incubated during 35 days in maturation chamber (experiment I) are shown in Fig. 3. No differences were detected between the freshly produced sausages with and without *A. westerdijkiae* inoculum on T0, and no OTA was detected until 7 days of maturation, which was when the growth of *A. westerdijkiae* was perceived. The mycotoxin was mainly present on the casing, with peak contamination ( $676.5 \pm 179 \mu\text{g}/\text{kg}$ ) occurring on the 21st day of ripening and decreasing by time in the casings. A small migration of the toxin from the casing to the salami meat border ( $1.45 \pm 0.07 \mu\text{g}/\text{kg}$ ) was detected at the end of maturation, although no OTA was detected in the central part of the salami.

Berni et al. (2017) also reported the absence of OTA for up to 7 days of maturation in a study conducted with salami casings inoculated with different proportions of toxigenic and atoxigenic *P. nordicum* and matured in RH 75–85% between 14 and 20 °C. The peak of OTA contamination occurred at 18 days (mean =  $177.79 \mu\text{g}/\text{kg}$ ), decreasing and remaining close to  $50 \mu\text{g}/\text{kg}$  at 29 and 40 days of maturation.

Spotti et al. (1999) also checked differences in OTA production during ripening of salami elaborated in natural and artificial casings and inoculated with *P. verrucosum*. The highest OTA level was at 28 days of maturation ( $1879 \mu\text{g}/\text{kg}$ ) in salami with artificial casing, which reduced along the time of ripening. These authors also observed the migration of small amounts to the outer layer of salami. The maximum amount of OTA detected in the meat was  $2.9 \mu\text{g}/\text{kg}$  at 35 days of maturation.

This reduction in OTA contamination may be related to microbial metabolism, especially by acid-lactic bacteria (Spotti et al., 1999).

Another possibility for such reduction in the casing during maturation may be because of its diffusion from the thin layer of the casing to the meat sausage border (about 10 mm), even though this mycotoxin may only be detected after 35 days of ripening (LOD  $0.25 \mu\text{g}/\text{kg}$ ). Ferrara et al. (2016) reported that OTA appeared at high levels in the mycelia of *P. nordicum* grown over salami casings, although it did not accumulate significantly, gradually spreading into the stuffed meat. The lower detected levels may also be influenced by the stronger OTA binding to proteins at neutral or alkaline pH (Valenta, 1998), since pH increases as a result of the utilization of lactate and acetate together with the production of ammonia from amino acid breakdown related to the fungal metabolism (Grazia et al., 1986; Bruna et al., 2001).

### 3.3. Experiment II: OTA production in sausages exposed to different RH

The mean values of aw and pH at different maturation times of sausages according to the RH of the incubation environment is shown in Table 1. A slight reduction in the aw of the product was observed along the time, with the exception of the sausages maintained at 95% RH, which remained constant.

The aqueous content of a substrate is influenced by the humidity of the environment in which it is inserted in (Pitt and Hocking, 2009), although the sausage casings and fruit peels can act as barriers to retard water exchange. Authors (Grazia et al., 1986; Leistner and Ayres, 1968) have also attributed the superficial fungal layer as a barrier effect against dehydration. The absence of airflow inside the boxes (differently from experiment I, matured in the chamber) may have also affected evaporative water loss in products. Reduction of pH on day 7 reflects the microbial metabolism, which usually reaches the lowest value on the 3rd day of fermentation, the increase in the following measurements are likely related to the growth and metabolic activity of fungi on the sausage surface (Grazia et al., 1986; Bruna et al., 2001), as previously mentioned.

Table 1  
Average water activity (aw) and pH of the salami on different days.

	Relative Humidity (RH)							
	95 to 75% (chamber)		79%		85%		95%	
	aw	pH	aw	pH	aw	pH	aw	pH
Day 0	0.98 ± 0	6.35 ± 0.01	0.98 ± 0	6.35 ± 0.01	0.98 ± 0	6.35 ± 0.01	0.98 ± 0	6.35 ± 0.01
Day 7	0.97 ± 0	5.83 ± 0.05	0.98 ± 0	5.67 ± 0.04	0.98 ± 0	6.05 ± 0.02	0.98 ± 0	5.85 ± 0.04
Day 14	0.95 ± 0	6.27 ± 0.00	0.97 ± 0	6.55 ± 0.01	0.97 ± 0	6.78 ± 0.00	0.98 ± 0	6.92 ± 0.08
Day 21	0.92 ± 0	6.52 ± 0.05	0.97 ± 0	7.04 ± 0.10	0.97 ± 0	7.10 ± 0.04	0.98 ± 0	7.01 ± 0.01
Day 28	0.91 ± 0	6.68 ± 0.01	-	-	-	-	-	-
Day 35	0.90 ± 0	6.89 ± 0.04	-	-	-	-	-	-

not checked.

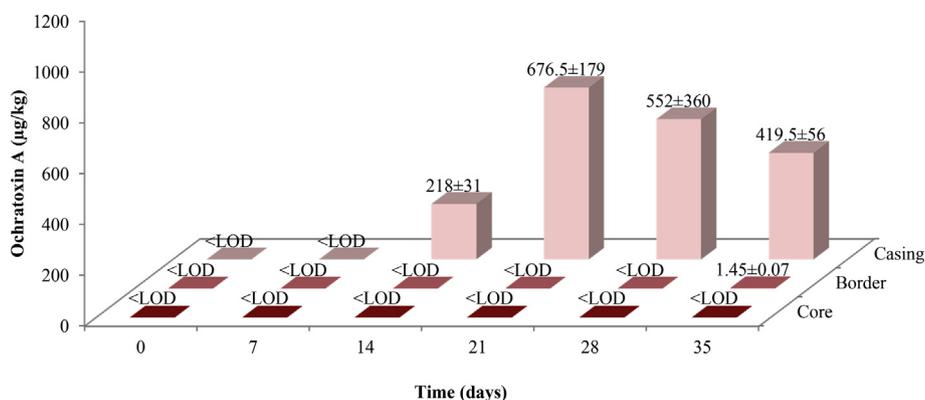


Fig. 3. Ochratoxin A (OTA) contamination in different portions of Italian-type salami (casing, outer border, and core) superficially inoculated with *Aspergillus westerdijkiae* and matured for 35 days in chamber.

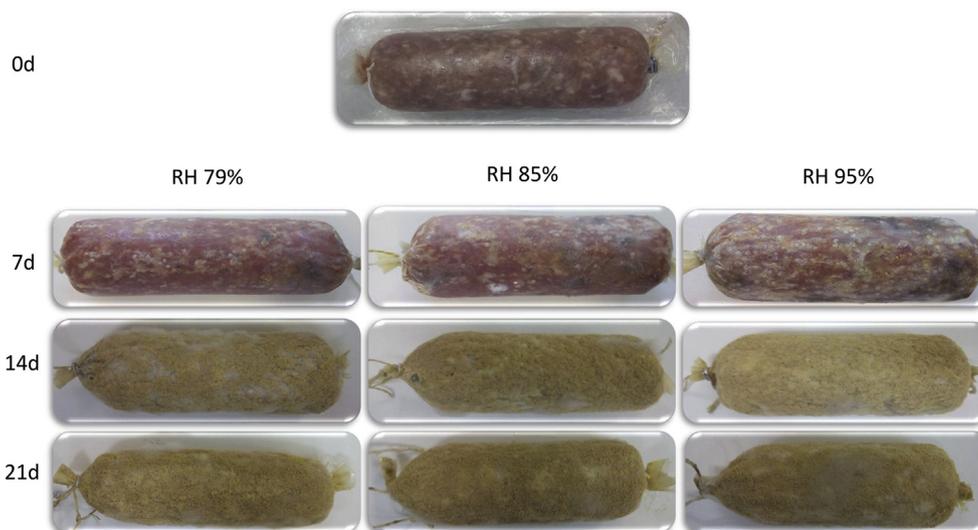


Fig. 4. Sausage surfaces inoculated with *Aspergillus westerdijkiae* and incubated under different relative humidity after 21 days at 20 °C.

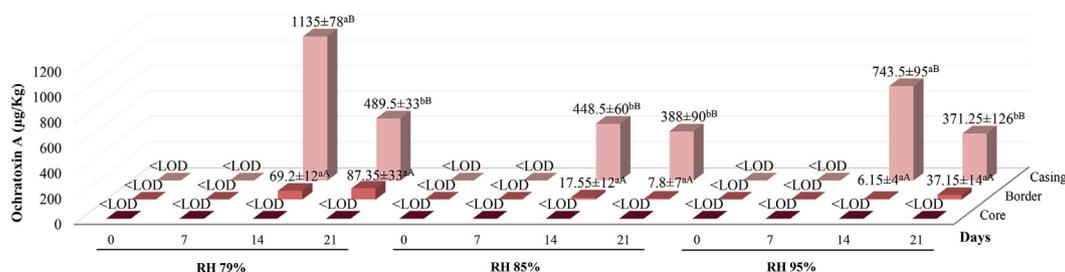


Fig. 5. Ochratoxin A (OTA) produced in different portions of Italian-type salami (casing, outer border, and core) superficially inoculated with *Aspergillus westerdijkiae* and maintained under different RH (79, 85, and 95%) at 20 °C for up to 14 days. Supplementary material 1: Picture of the maturation chamber where the samples of sausages naturally contaminated with ochratoxin A were produced with ripening sausages showing intense contamination by *Aspergillus westerdijkiae*.

An intense layer of *A. westerdijkiae*, which was covering the entire surface of the sausages, was observed on the 10th day after inoculation. Sausages incubated in boxes under different RH showed similar fungi development (Fig. 4), however, differences were observed regarding OTA production (Fig. 5).

Vipotnik et al. (2017) reported that environmental conditions had different effects on OTA production and growth of *A. westerdijkiae* in a sterile dry-cured ham-based medium, showing that mycotoxin production does not correlate with growth. The optimal ecophysiological conditions for OTA production in this model system were at 0.94–0.97 aw, which was achieved with a combination of ionic (NaCl) and non-

ionic (glycerol) solutes and 20–25 °C. Fungal growth was affected by temperature, substrate aw, and aw × temperature.

As observed in the dry fermented sausages matured in chamber (experiment I), no OTA was detected until 7 days of ripening. Both the casing and outer border of sausages showed OTA contamination after 14 days. Under all RH conditions evaluated, no OTA was detected in the core of the salami. In all RH tested (79, 85, and 95%) the peak OTA contamination was detected in the casings at 14 days of maturation. For this portion, the highest amount of OTA ( $p < 0.05$ ) was produced at RH 79%, followed by 95%, and the lowest production occurred at 85%, showing that under similar substrate aw, the RH of the environment

appears to play an important role in OTA production by perhaps acting as a stress factor. Reduction in casing contamination by OTA at 21 days ( $\rho < 0.05$ ) was observed for all sausages except for the ones maintained at RH 85%, which remained unaltered ( $\rho < 0.05$ ). On the other hand, the contamination of the sausage meat on the outer border, which touches the casing, increased at 14–21 days of incubation ( $\rho < 0.05$ ), excepted for the sausages maintained at RH 85%, which remained unaltered ( $\rho < 0.05$ ), suggesting diffusion of the toxin from the casing to the meat as the main responsible for this decrease.

For comparison purposes, no studies were found regarding the influence of RH in OTA production on meat products or meat based media. Pardo et al. (2005) studied the influence of RH (80, 90, and 100%) on visible growth of *A. ochraceus* in sterile grapes and OTA accumulation after 14 days of incubation at different temperatures. A significant influence of RH and temperature in visible fungal growth of ochratoxigenic *A. ochraceus* isolates in grapes was observed. Although differences were found in OTA content at different conditions, OTA accumulation was not significantly modified by the assayed levels of these abiotic factors.

Furthermore, RH may have had influenced the aw of the collagen casing, affecting the microbial community developing on the sausage surface and species interactions, also exerting effects in OTA production. Meftah et al. (2018) showed that *A. westerdijkiae* growing under axenic conditions was unable to produce detectable amounts of OTA at 20 °C in culture media based on meat. However, it was significantly stimulated to produce OTA when co-inoculated with four yeasts (*Candida krusei*, *Candida zeylanoides*, *Rodothorula glutinis*, and *Rodothorula mucilaginosa*) isolated from dry-cured sausages and a commercial starter culture (composed of *Pediococcus pentosaceus*, *Lactobacillus sakei*, *Staphylococcus carnosus*, *Staphylococcus xylosus*, and *Debaryomyces hansenii*). On the other hand, some microorganisms, especially acid-lactic bacteria, may metabolise OTA (Spotti et al., 1999). Fuchs et al. (2008) showed OTA *in vitro* detoxification by adding lactic acid bacteria (BAL) with a global reduction of about 95% of this mycotoxin content.

The fermentation of meat sausages is a complex biological process. Ecological studies considering the microbial interactions in casings under different physico-chemical parameters of RH, pH, and aw may help elucidate factors affecting fungal growth as well influencing OTA production in sausages and, consequently, improve the quality of these meat products.

#### 4. Conclusion

Besides reporting for the first time the presence of ochratoxin A (OTA) in the casing of dry fermented sausages produced in Brazil, this work evaluated OTA production by *Aspergillus westerdijkiae* inoculated on the surface of sausages, its diffusion into the product throughout ripening, and the effect of relative humidity (RH) on the production of this mycotoxin. This work demonstrated that the growth of *A. westerdijkiae* on salami surfaces produces high amounts of OTA in casings and allows the diffusion of OTA with contamination to the outer borders of sausages. In the same way, RH influenced the amount of OTA produced by *A. westerdijkiae* in sausages.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fm.2019.05.007>.

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#### References

- Alcano, M.J., Jahn, R.C., Scherer, C.D., Wigmann, E.F., Moraes, V.M., Garcia, M.V., Mallmann, C.A., Copetti, M.V., 2016. Susceptibility of *Aspergillus* spp. to acetic and sorbic acids based on pH and effect of sub-inhibitory doses of sorbic acid on ochratoxin A production. *Food Res. Int.* 81, 25–30.
- Altafini, A., Fedrizzi, G., Roncada, P., 2019. Occurrence of ochratoxin A in typical salami produced in different regions of Italy. *Mycotoxin Res.* 35, 141–148.
- Andrade, M.J., Peromingo, B., Rodríguez, M., 2019. Effect of cured meat product ingredients on the *Penicillium verrucosum* growth and ochratoxin A production. *Food Control* 96, 310–317.
- Armorini, S., Altafini, A., Zaghini, A., Roncada, P., 2016. Ochratoxin A in artisan salami produced in Veneto (Italy). *Food Addit. Contam. B* 9, 9–14.
- Battilani, P., Pietri, V.A., Giorni, P., Formenti, S., Bertuzzi, T., Toscani, T., et al., 2007. *Penicillium* populations in dry-cured ham manufacturing plants. *J. Food Prot.* 70, 975–980.
- Battilani, P., Formenti, S., Toscani, T., Virgili, R., 2010. Influence of abiotic parameters on ochratoxin A production by a *Penicillium nordicum* strain in dry-cured meat model systems. *Food Control* 21, 1739–1744.
- Berni, E., Montagna, I., Restivo, F.M., Degola, F., 2017. Ochratoxin A control in meat derivatives: intraspecific biocompetition between *Penicillium nordicum* strains. *J. Food Qual.* 8.
- Brasil, 2000. Aprovar os regulamentos técnicos de identidade e qualidade de copa, de jerked beef, de presunto tipo parma, de presunto cru, de salame, de salaminho, de salame tipo alemão, de salame tipo calabrés, de salame tipo friolano, de salame tipo napolitano, de salame tipo hamburguês, de salame tipo italiano, de salame tipo milano, de língua colonial e pepperoni. P. 15, Instrução Normativa n° 22. Diário Oficial, Brasília.
- Bruna, J.M., Ordóñez, J.A., Fernández, M., Herranz, B., de la Hoz, L., 2001. Microbial and physico-chemical changes during the ripening of dry fermented sausages superficially inoculated with or having added an intracellular cell-free extract of *Penicillium aurantiogriseum*. *Meat Sci.* 59, 87–96.
- Canel, R.S., Wagner, J.R., Stenglein, S.A., Ludemann, V., 2013. Indigenous filamentous fungi on the surface of Argentinean dry fermented sausages produced in Colonia Caroya (Córdoba). *Int. J. Food Microbiol.* 164, 81–86.
- Castellari, C., Quadrelli, A.M., Laich, F., 2010. Surface mycobiota on Argentinean dry fermented sausages. *Int. J. Food Microbiol.* 142, 149–155.
- Cichoski, A.J., Zis, L.C., Franceschetto, C., 2009. Physicochemical and microbiological characteristics of the surface of Italian salami containing potassium lactate. *J. Food Sci. Technol.* 29, 546–552.
- Comi, G., Orlic, S., Redzepovic, S., Urso, R., Iacumin, L., 2004. Moulds isolated from Istrian dried ham at the pre-ripening and ripening level. *Int. J. Food Microbiol.* 96, 29–34.
- Costa, P.M.C., 2014. Identificação de fungos filamentosos e quantificação de ocratoxina A em produtos cárneos ao longo do período de cura. Master Dissertation (Master's Degree in Quality and Food Safety) Instituto Politécnico de Bragança.
- Dall'Asta, O., Galaverna, G., Bertuzzi, A., Moseriti, A., Pietri, A., Dossena, A., et al., 2010. Occurrence of ochratoxin A in raw ham muscle, salami and dry-cured ham from pigs fed with contaminated diet. *Food Chem.* 120, 978–983.
- Ferrara, M., Magistà, D., Lippolis, V., Cervellieri, S., Susca, A., Perrone, G., 2016. Effect of *Penicillium nordicum* contamination rates on ochratoxin A accumulation in dry-cured salami. *Food Control* 67, 235–239.
- Fuchs, S., Sontag, G., Stidl, R., Ehrlich, V., Kundi, M., Knasmüller, S., 2008. Detoxification of patulin and ochratoxin A, two abundant mycotoxins, by lactic acid bacteria. *Food Chem. Toxicol.* 46, 1398–1407.
- Ferreira, D.F., 2011. Sisvar: a computer statistical analysis system. *Ciênc. agrotec.* 35, 1039–1042.
- Frivstad, J.C., Frank, M.J., Houbraeken, J.A.M.P., Kuijpers, A.F.A., Samson, R.A., 2004. New ochratoxin producing species in *Aspergillus* section *Circumdati*. *Stud. Mycol.* 50, 2343.
- Gareis, M., Scheuer, R., 2000. Ochratoxin A in meat and meat products. *Arch. Leb.* 51, 102–104.
- Grazia, L., Romano, P., Bagni, A., Roggiani, D., Guglielmi, G., 1986. The role of moulds in the ripening process of salami. *Food Microbiol.* 3, 19–25.
- Huerta, T., Sanchis, V., Hernandez, J., Hernandez, E., 1987. Mycoflora of dry-salted Spanish ham. *MAN* 5, 247–252.
- 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 Microbiol.* 26, 65–70.
- Iacumin, L., Milesi, S., Pirani, S., Comi, G., Chiesa, L.M., 2011. Ochratoxigenic mold and ochratoxin A in fermented sausages from different areas in Northern Italy: occurrence, reduction or prevention with ozonated air. *J. Food Saf.* 31, 538–545.
- International Agency of Research on Cancer (IARC), 1993. Monographs on the Evaluation of the Carcinogenic Risks to Humans, vol. 56.
- Leistner, L., Ayres, J.C., 1968. Molds and meats. *Fleischwirtschaft* 1, 62–65.
- Markov, K., Pleadin, J., Bevardi, M., Vahčić, N., Sokolić-Mihalak, D., Frece, J., 2013. Natural occurrence of aflatoxin B1, ochratoxin A and citrinin in Croatian fermented meat products. *Food Control* 34, 312–317.
- Meftah, S., Abid, S., Dias, T., Rodrigues, P., 2018. Effect of dry-sausages culture and endogenous yeasts on *Aspergillus westerdijkiae* and *Penicillium nordicum* growth and OTA production. *LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.)* 87, 250–258.
- Merla, C., Andreoli, G., Garino, C., Vicari, N., Tosi, G., Guglielminetti, M.L., Moretti, A.,

- Biancardi, A., Arlorio, M., Fabbri, M., 2018. Monitoring of ochratoxin A and ochratoxin-producing fungi in traditional salami manufactured in Northern Italy. *Mycotoxin Res.* 34, 107–116.
- Nunez, F., Rodriguez, M.M., Bermudez, M.E., Cordoba, J.J., Asensio, M.A., 1996. Composition and toxigenic potential of the mould population on dry-cured Iberian ham. *Int. J. Food Microbiol.* 32, 185–197.
- Pardo, E., Marín, S., Sanchis, V., Ramos, A.J., 2005. Impact of relative humidity and temperature on visible fungal growth and OTA production of ochratoxigenic *Aspergillus ochraceus* isolates on grapes. *Food Microbiol.* 22, 383–389.
- Parussolo, G., Garcia, M.V., Stefanello, A., Silva, T.S., Bernardi, A.O., 2019. Fungal microbiota in environmental air, raw materials and surface of dry fermented sausage produced in Southern Brazil. *LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.)* 108, 190–198.
- Peromingo, B., Sulyok, M., Lemmens, M., Rodríguez, A., Rodríguez, M., 2019. Diffusion of mycotoxins and secondary metabolites in dry-cured meat products. *Food Control* 101, 144–150.
- Perši, N., Pleadin, J., Kovačević, D., Scortichini, G., Milone, S., 2014. Ochratoxin A in raw materials and cooked meat products made from OTA-treated pigs. *Meat Sci.* 96, 203–210.
- Pitt, J.I., Hocking, A.D., 2009. *Fungi and Food Spoilage*. Blackie Academic and Professional, London, pp. 593.
- Pleadin, J., Kovačević, D., Perši, N., 2015. Ochratoxin A contamination of the autochthonous dry-cured meat product “Slavonski Kulen” during a six-month production process. *Food Control* 57, 377–384.
- Rodríguez, A., Rodríguez, M., Martín, A., Delgado, J., Córdoba, J.J., 2012. Presence of ochratoxin A on the surface of dry-cured Iberian ham after initial fungal growth in the drying stage. *Meat Sci.* 92, 728–734.
- Rodríguez, A., Bernáldez, V., Rodrigues, M., Andrade, M.J., Núñez, F., Córdoba, J.J., 2015a. Effect of selected protective cultures on ochratoxin A accumulation in dry-cured Iberian ham during its ripening process. *LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.)* 60, 923–928.
- Rodríguez, A., Capela, D., Medina, Á., Córdoba, J.J., Magan, N., 2015b. Relationship between ecophysiological factors, growth and ochratoxin A contamination of dry-cured sausage based matrices. *Int. J. Food Microbiol.* 194, 71–77.
- Rojas, F.J., Jodral, M., Gosálvez, F., Pozo, R., 1991. Mycoflora and toxinogenic *Aspergillus flavus* in Spanish dry-cured ham. *Int. J. Food Microbiol.* 13, 249–256.
- Samson, R.A., Hong, S.B., Frisvad, J.C., 2006. Old and new concepts of species differentiation in *Aspergillus*. *Med. Mycol.* 44, 133–148.
- Sánchez-Montero, L., Córdoba, J.J., Peromingo, B., Álvarez, M., Núñez, F., 2018. Effects of environmental conditions and substrate on growth and ochratoxin A production by *Penicillium verrucosum* and *Penicillium nordicum*: relative risk assessment of OTA in dry-cured meat products. *Food Res. Int. (in press)* manuscript.
- Sørensen, L.M., Jacobsen, T., Nielsen, P.V., Frisvad, J.C., Kock, A.G., 2008. Mycobiota in the processing areas of two different meat products. *Food Microbiol.* 124, 58–64.
- Spotti, E., Busolli, C., Palmia, F., 1999. Sviluppo di colture fungine di importanza rilevante nell'industria dei prodotti carnei. *Ind. Conserve* 74, 23–34.
- Strzelecki, E.L., Badura, L., 1972. Occurrence of aflatoxinogenic molds on dry Cracower sausage. *Acta Microbiol. Pol. B* 4, 233–239.
- Sulyok, M., Berthiller, F., Krska, R., Schuhmacher, R., 2006. Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of 39 mycotoxins in wheat and maize. *Rapid Commun. Mass Spectrom.* 20, 2649–2659.
- Sutic, M., Ayres, J.C., Koehler, P.E., 1972. Identification and aflatoxin production of moulds isolated from country cured hams. *Appl. Microbiol.* 23, 656–658.
- Terra, N.N., Brum, M.A.R., 1988. *Carne e Seus Derivados*. Nobel, São Paulo, pp. 119p.
- Valenta, H., 1998. Chromatographic methods for the determination of ochratoxin A in animal and human tissues and fluids. *J. Chromatogr.* 815 (1), 875–892.
- Van der Merwe, K.J., Steyn, P.S., Fourie, L., 1965. Mycotoxins Part II the constitution of ochratoxin A, B, and C, metabolites of *Aspergillus ochraceus*. *Wilh. J. Chem. Soc.* 7083–7088.
- Vila, G.S., Pose, G.N., Segura, J.A., Ludemann, V., 2016. Diversidad de hongos filamentosos en el emplume de embutidos secos producidos en la región pampeana. *SNS* 10, 40–49.
- Vipotnik, Z., Rodríguez, A., Rodrigues, P., 2017. *Aspergillus westerdijkiae* as a major ochratoxin A risk in dry-cured ham based-media. *Food Microbiol.* 241, 244–251.
- Visagie, C.M., Varga, J., Houbraken, J., Meijer, M., Kocsis, S., Yilmaz, N., Fotedar, R., Seifert, K.A., Frisvad, J.C., Samson, R.A., 2014. Ochratoxin production and taxonomy of the yellow aspergilli (*Aspergillus* section *Circumdati*). *Stud. Mycol.* 78, 1–61.
- Wang, X., Ma, P., Jiang, D., Peng, Q., Yang, H., 2006. The natural microflora of Xuanwei ham and the no-mouldy ham production. *J. Food Eng.* 77, 103–111.
- World Health Organization (FAO/WHO), 2007. Evaluation of Certain Food Additives and Contaminants, vol. 68 FAO/WHO Expert Committee on Food Additives.