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Influence of abiotic parameters on ochratoxin A production by a *Penicillium nordicum* strain in dry-cured meat model systems

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ABSTRACT

OTA production from an ochratoxigenic *Penicillium nordicum* strain was studied in artificial inoculation trials managed both in a dry-cured pork-based medium (DCM) and in dry-cured pork cores. The experimental region defined by 7–23 °C temperature, 0.83–0.97 a_w and 7–21 days of incubation was considered for DCM in the applied Central Composite Design (CCD). $A_w > 0.92$, temperature > 18 °C and incubation time protracted to 21 days strongly enhanced OTA production. The conditions of CCD central point ($a_w = 0.90$, temperature = 15 °C and days of incubation = 14) and those predicted as the most suitable for OTA production in DCM, were tested in dry-cured pork samples incubated up to 190 days. A ten-fold increase in OTA (0.04–0.41 μg/g) was achieved when the temperature rose from 15 °C up to 20 °C, an over twenty-fold (0.02–0.43 μg/g) when switching from 0.90 to 0.93 a_w. The results can be useful as guidelines for critical control points (CCPs) detection in dry-curing processing to prevent OTA contamination of the products.

1. Introduction

Pork meat and pork derivatives are known for being a possible source for human exposure to ochratoxin A (OTA) (Govaris, Solomakos, & Pexara, 2007), reported as an immunosuppressive agent, a nephrotoxin and a renal and urinary tract carcinogen (Lock & Hard, 2004; Pfohl-Leszkowicz & Manderville, 2007). Ochratoxin A is controversially reported as involved in Balkan Endemic Nephropathy (BEN) a chronic tubulointerstitial disease of unknown aetiology; a high frequency of urothelial atypia, occasionally culminating in tumours of the renal pelvis and urethra, is associated with this disorder. Affected patients most commonly reside in south eastern Europe, including the areas traditionally considered to comprise the Balkans (Mally, Hard, & Dekant, 2007). OTA is the most toxic of the known ochratoxins and is regarded as a cumulative toxic molecule; a TDI of 5 ng OTA/kg BW/day is recommended by the World Health Organization.

Ochratoxin A contamination can be the result of either carry over from animals exposed to naturally contaminated feed or direct contamination with moulds (Gareis, 1996).

Pig exposure to OTA is reported by several authors with kidneys and liver as the most contaminated tissues (Dragacci, Grosso, Bire, Fremy, & Coulon, 1999; Matrella, Monaci, Milillo, Palmisano, & Tantillo, 2006) and blood sausages and liver-type sausages as the most contaminated meat products in a German market survey (Gareis & Scheuer, 2000).

Escher, Koehler, and Ayres (1973) ascribed OTA presence to direct contamination by mold toxigenic strains. More recently *Penicillium nordicum* strains were isolated from air and products stored in dry-curing plants; 50% of strains proved to be able to produce OTA in vitro (Battilani et al., 2007).

P. nordicum, morphologically similar to *Penicillium verrucosum*, was recently identified as a separate species, according to its secondary metabolite profile (Larsen, Svendsen, & Smedsgaard, 2001) and genomic sequences (Bogs, Battilani, & Geisen, 2006). Studied strains of *P. nordicum* all originate from proteinaceous, dry-cured salt rich foods like cheese and meat products, while isolates of *P. verrucosum* derive from plants, suggesting a different ecology for the aforementioned species (Larsen et al., 2001). Studies on *P. nordicum* were mainly focused on molecular detection methods and the genes involved in OTA biosynthesis (Bogs et al., 2006; Schmidt-Heydt, Schunk, & Geisen, 2009) with less information on ecological conditions favorable to OTA production in model systems and in food products.

The aim of this research was to study the role of temperature and substrate water activity (a_w) on OTA production from a *P. nordicum* strain previously tested as OTA producer, both in a dry-cured pork-based medium and in a dry-cured pork model system closely approaching real samples. Investigated temperature,

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a_w and incubation time ranges represented variations occurring during dry-cured meat processing in order to assess potential risk of OTA contamination in dry-cured meat products.

2. Materials and methods

2.1. Selection of ochratoxin A producing strain of Penicillium nordicum

In a previous study carried out in several pork dry-curing plants, *P. nordicum* strains were isolated and tested for OTA production (Battilani et al., 2007; Bogs et al., 2006). The strains were identified at molecular level (Bogs et al., 2006) and stored in the fungal collection of the Institute of Entomology and Plant Pathology, Università Cattolica del Sacro Cuore in Piacenza (code MPVP) (Battilani et al., 2007). The highest OTA producing strain of *P. nordicum* (data not shown), labelled as MPVP1669, was selected for the trials conducted in this study.

2.2. Preparation of dry-cured pork-based medium (DCM)

Thirty g of lyophilised dry-cured pork (4% moisture, 11% NaCl, 67% proteins, 18% fat) were added to 1 L of bidistilled water and maintained 1 h in a thermostatic bath, shaken each 15 min, filtered with double layer gauze and added with 15 g of agar. The original a_w (0.97) of the DCM was modified by the non-ionic solute glycerol; 50 g of glycerol were added to 1 L of media to reduce 0.01 a_w; the pH of the medium, measured with a Hamilton glass electrode probe attached to pH meter (pH300 Hanna Instruments, Padova, Italy) was 5.80. To preserve the composition of DCM, 3 g of lyophilised dry-cured pork were added to each 100 g of glycerol. The a_w of media was determined with AquaLab model CX-2 (Decagon Devices, Inc., Washington, USA). Then the media were poured into 9 cm diam sterile plastic Petri dishes (10 ml/plate) for inoculation with the selected ochratoxigenic strain of *P. nordicum*.

2.3. Preparation of dry-cured pork model system

Ten muscle cuts, 3–4 kg in weight, including portions of *Semimembranosus, Semitendinosus* and *Biceps femoris* muscles and partly covered with outer rind, were removed from pork thighs and salted with dry salt (4% w/w added salt). Then, the cuts were stored at 0–2 °C and 85–90% relative humidity for 10 days (salting period), 0–2 °C and 65–70% relative humidity for 2 weeks (resting period) and ripened at 15 °C and 65–70% relative humidity in conditioned rooms for three–four months, according to weight, to achieve 0.90 ± 0.005 a_w (outer *Semimembranosus*) and 0.93 ± 0.005 a_w (inner *Biceps femoris*). Samples (30 mm in diameter and 5 mm in height) were excised using a hollow metal sampler with a cylindrical cutting edge. The water activity of samples was measured according to the ISO/FDIS 21807 method (2004). The pH of *Semimembranosus* and *Biceps femoris* muscles, was measured by insertion in the muscles, close to the excised cores, of a Hamilton glass electrode probe attached to a portable pH meter (WTW pH330, Weilheim, Germany); pH values of the muscles were in the range 5.65–5.90.

The muscle cores were dipped in absolute ethanol for 1 min, drawn out and flamed, to sterilize them before inoculation (Rojas, Jodral, Gosalvez, & Pozo, 1991). Preliminary tests confirmed this treatment as suitable to avoid undesirable contamination and to preserve the original a_w.

2.4. Inoculum preparation, inoculation and incubation

P. nordicum MPVP1669 was inoculated in Petri dishes with Czapek Yeast Agar (CYA: sucrose 30 g; yeast extract 5 g; $NaNO_3$ 2 g; KCl 0.5 g; $MgSO_4 \cdot 7H_2O$ 0.5 g; $FeSO_4 \cdot 7\ H_2O$ 0.01 g; K_2HPO_4 1 g; $ZnSO_4 \cdot 7H_2O$ 0.01 g; $CuSO_4 \cdot 7H_2O$ 0.005 g; agar 15 g; H_2O to 1000 ml), incubated at 25 °C for 7 days and used as inoculum. Spores were captured using a needle and transferred in small vials (1.5 mL) with Water Agar (agar 10 g, water 1 L) according to Pitt (1979). Then, the DCM and the dry-cured pork cores placed in Petri plates were inoculated in the center, put in sealed boxes equipped with beakers containing NaCl solutions (Multon & Bizot, 1980) with the same a_w as the dry-cured pork model systems, and incubated in the dark according to the experimental design (see below).

2.5. Experimental design for DCM

The trials were organized according to a blocked Central Composite Design (CCD). Independent factors were fixed to 5 levels as follows: temperature (7, 10, 15, 20, 23 °C), a_w (0.83, 0.86, 0.90, 0.94, 0.97), and incubation time (2, 7, 14, 21, 26 days).

The experimental design relies on two blocks, 28 thesis, with 7 central points in each block, set at temperature = 15 °C, a_w = 0.90 and incubation time = 14 days (Table 1). Each experimental condition was quadruplicated and a total of 112 Petri dishes was used.

At the end of each incubation time, the diameters of the growing colonies were measured in two directions at right angles to each other, crossing the inoculation point. Plates were frozen (−20 °C) until the OTA extraction and analysis. The maintenance of the original a_w was checked with AquaLab at the end of incubation.

2.6. Experimental design for dry-cured pork model system

A full factorial design with 3 factors was organized with dry-cured pork inoculated muscle cores. Factors involved were: temperatures (15 and 20 °C), a_w (0.90 and 0.93) and incubation time (14, 21, 42, 63, 84 and 190 days). The trial was done in quintuplicate. Fungal growth was observed and scored, in the

Table 1
Grid of the Central Composite Design (CCD) applied to DCM. The central point corresponds to temperature = 15 °C, a_w = 0.90 and incubation time = 14 days.

trial n°	block	temperature(°C)	time (days)	a_w
1	1	15	14	0.90
2	1	20	21	0.94
3	1	10	7	0.94
4	1	20	7	0.86
5	1	10	21	0.86
6	1	15	14	0.90
7	1	15	14	0.90
8	1	10	7	0.86
9	1	20	7	0.94
10	1	15	14	0.90
11	1	15	14	0.90
12	1	15	14	0.90
13	1	10	21	0.94
14	1	15	14	0.90
15	1	20	21	0.86
16	2	15	14	0.90
17	2	15	14	0.90
18	2	23	14	0.90
19	2	15	14	0.83
20	2	15	2	0.90
21	2	15	14	0.90
22	2	15	14	0.90
23	2	7	14	0.90
24	2	15	14	0.97
25	2	15	14	0.90
26	2	15	14	0.90
27	2	15	14	0.90
28	2	15	26	0.90

Table 2
Standardized coefficients of polynomial multiple regression for the factors temperature, water activity (a_w) and incubation time on ochratoxin A production in dry-cured pork-based medium (DCM).

Factors	Regression coefficients of *P. nordicum* MPVP1669 OTA production model (μg/g) [a]	
	sdz. coeff.	signif. *P*
temperature	4.42	**
incubation time	2.02	*
a_w	4.78	**
(temperature)^2	1.15	#
(incubation time)^2	−0.72	#
$(a_w)^2$	2.07	*
temperature × incubation time	2.91	*
temperature × a_w	3.57	*
incubation time × a_w	2.09	#

*P < 0.05; **P < 0.001; # not significant.
[a] Estimated model $P < 0.001$, $R^2 = 0.821$ (R^2 = percentage of variation explained by the model).

inoculation and reverse side of pork samples, after 14, 21 and 28 days of incubation.

At each established incubation time, visible mycelium was removed and dry-cured pork samples were frozen (−20 °C) until extraction and analysis of OTA produced in muscle samples. The maintenance of the original a_w was checked in all samples at the end of incubation.

2.7. Ochratoxin A extraction and analysis

Three plugs (diam. = 0.46 cm, height = 0.4 cm, and 0.18 g), were removed from the inner, middle and outer area of the fungal colony on DCM for OTA analysis. They were introduced in a vial containing 1 ml of methanol, extracted for 1 h and shaken each fifteen minutes (Bragulat, Abarca, & Cabañes, 2001). The extraction of OTA in dry-cured pork samples was carried out according to the method of Toscani et al., (2007). Briefly, an aliquot of finely minced sample was extracted (1:10 w/v ratio) with chloroform acidified with 85% ortophosphoric acid, homogenized for 2 min, filtered and back-extracted twice in a separating funnel with buffer 0.2 M Tris–HCl, pH 8.5. The aqueous phases were collected, added with CH_3CN (Tris–HCl:CH_3CN 90:10 v/v ratio) to improve sample stability.

Detection and quantitative determination of OTA from DCM and dry-cured pork muscle cores were made without a clean-up step, analyzing the final solutions by an HPLC-FLD quantitative method. HPLC analysis was performed with a C18 column (Waters XTerra ®, 250 × 2.1 mm, 3 μm) on an Agilent 1100 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 was 1 ml/min and the injected volume was 20 μl. The FLD detection was obtained by means of an Agilent 1100 Fluorescence Detector (λ_{ex} = 380 nm, λ_{em} = 440 nm).

OTA peak is well separated from background interferences in less than 20 min; in DCM and dry-cured pork the mean recoveries were 75.8% and 80.1% respectively. Limit of detection (LOD) = 0.1 ng/ml; limit of quantification (LOQ) = 0.3 ng/ml.

2.8. Data analysis

The CCD was generated by TRIAL RUN (TRIAL RUN 1.0, SPSS Inc. Chicago, IL), and the resulting response surface model for OTA production in DCM was calculated according to a polynomial multiple regression (MLR): response = $b_0 + b_1$ *temperature* $+ b_2$ *incubation time* $+ b_3\ a_w + b_{12}$ *temperature* × *incubation time* $+ b_{13}$ *temperature* × $a_w + b_{23}$ *incubation time* × *temperature* $+ b_{11}$ *temperature*$^2 + b_{22}$ *incubation time*$^2 + b_{33}\ a_w^2$.

Ochratoxin A accumulation in dry-cured pork muscle cores was analyzed by the general linear model (GLM) procedure of SPSS (SPSS 11.5, SPSS Inc. Chicago, IL). Main effects (temperature, a_w and incubation time) and their interactions were included in the model. Least Squares Means (LSM) were computed for the main effects, and the Least Significant Difference (LSD) t-test was used to statistically separate LSM ($P < 0.05$).

3. Results and discussion

3.1. Modeling OTA accumulation in DCM

The CCD model requires fewer runs than the corresponding full factorial design, and it is used when the experimental region in which the wanted response could lie is roughly known or pre-established. The assayed a_w range (0.83–0.97) of DCM is inclusive of the values commonly found in the surface layer of dry-cured pork derivatives during maturation (Rodriguez et al., 1994; Simoncini, Virgili, Quintavalla, Formenti, & Battilani, 2009), and the tested temperature values between 7 and 23 °C are in agreement with those used in plants for pork dry-curing practices (Battilani et al., 2007; Martin, Cordoba, Aranda, Cordoba, & Asensio, 2006;). The number of replicates of the central point (15°C temperature, 0.90 a_w and 14 days incubation time) ensures the orthogonality of the design, minimizing the average variance of prediction of the response surface equation.

The investigated factors temperature, a_w and incubation time, the two-way interactions temperature × time and temperature × a_w, and the quadratic factor a_w proved to have a significant effect on

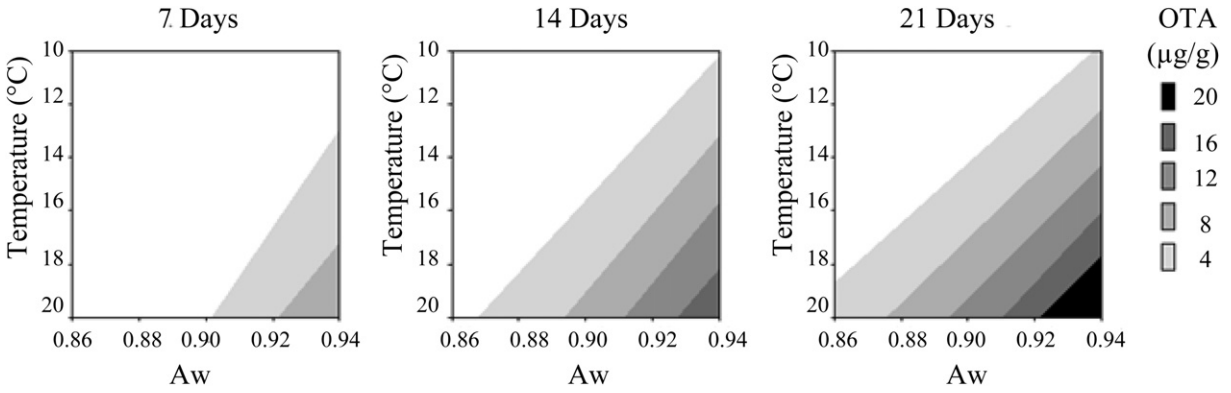

Fig. 1. Response surface contour plots showing the effect of increasing incubation time on OTA production by *P. nordicum* MPVP1669 in dry-cured pork-based medium (DCM).

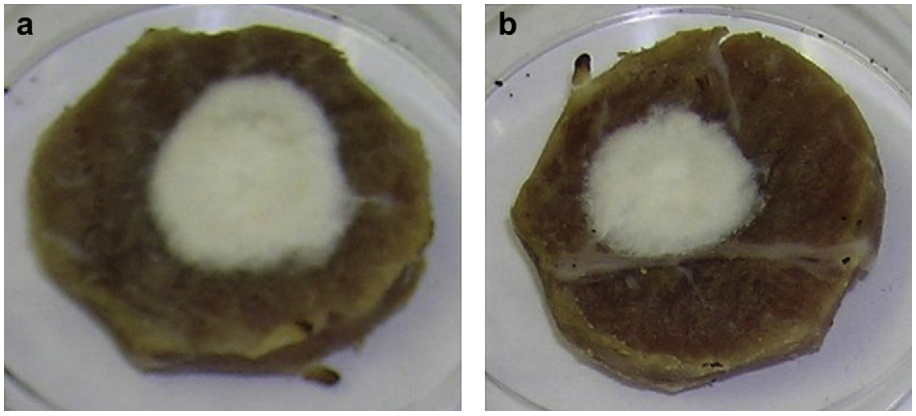

Fig. 2. a) Inoculation and b) reverse side of the model system (dry-cured pork muscle core at 0.93 a_w) inoculated in the centre with *P. nordicum* MPVP1669 incubated at 15 °C for 14 days.

OTA production (Table 2). Polynomial equation coefficients show a positive influence of all the assayed factors upon OTA accumulation, even if a_w seems to play the most important role. The contemporary presence of temperature > 18 °C and a_w > 0.92 resulted in a strong enhancement of OTA production in DCM. The response surface contour plots of Fig. 1 highlights, in addition to temperature and a_w, the influence of the increasing times of incubation on OTA accumulation. The OTA amount predicted by the model at 20 °C, 0.94 a_w, 21 days incubation time, increased sharply if compared to 7 days. The treatment carried out at 10 °C did not result in OTA production at reported a_w values, while no OTA was predicted at 15 °C up to 0.89 a_w and 21 days of incubation. An OTA amount in the range 0–4 μg/g was calculated in the CCD central point. Other authors reported the maximum OTA production by *Aspergillus* section *Nigri* grown in artificial media during the first fungal growth stages, 5 days for *A. carbonarius* and 7–13 days for *A. niger* aggregate, and a decrease during the late steps of incubation. It was probably due to early nutrient consumption in synthetic substrate (Belli, Ramos, Sanchis, & Marin, 2004), but more rapid growth of *Aspergilli*, compared to *Penicillia*, has to be considered.

The main information achieved with the DCM model is the sharp OTA accumulation occurring in the range 0.90–0.94 a_w (Fig. 1). These results are in agreement with recent findings about the influence of a_w on OTA biosynthesis gene expression in *P. verrucosum*, showing an activation of the biosynthetic genes and OTA production under a_w values regarded as mild stressful if compared to higher a_w (Schmidt-Heydt, Baxter, Geisen, & Magan, 2007).

3.2. OTA accumulation in dry-cured pork muscle cores

DCM composition (0.3% NaCl, 2.0% proteins, 0.5% fat, ≈ 90% water) differs markedly from rough composition of dry-cured pork muscle cores (3–6% NaCl, 20–30% proteins, 3–10% fat, 40–60% moisture), but the results obtained with DCM were taken into account for planning the design of the trial with dry-cured muscle cores. In dry-cured pork substrates, *P. nordicum* growth and OTA biosynthesis could be affected by chlorides presence, high availability of free amino acids, peptides, free fatty acids and minerals. High amounts of NaCl in the production substrate may influence the final biosynthetic step from OTB (without chlorine) to OTA (with incorporated chlorine) (Wei, Strong, & Smalley, 1971) and/or may be regarded as a stress factor leading to OTA genes activation and subsequent biosynthesis (stress adaptation). It was recently reported that stressful conditions corresponding to 0.93 a_w gave a peak of OTA production for *P. verrucosum*, indicating two possible ways of productions, one under optimal and one under stress conditions (Schmidt-Heydt et al., 2007). The dry-cured pork muscle cores have the contemporary advantage of closely approaching real samples in terms of nutrients and salt, minimizing the possible limitation due to the gradual impoverishment in nutrients occurring in synthetic media (Kekkonen, Jestoi, & Rizzo, 2005; Schmidt-Heydt et al., 2007), but preserving the established a_w and temperature values during the designed incubation times like a model system.

According to the results of the previous trial, dry-cured pork muscle cores were incubated at 0.93 a_w and 20 °C, conditions yielding the highest OTA production in DCM after 21 days of incubation (Fig. 1). The values of a_w and temperature set in the central point of the CCD (0.90 a_w and 15 °C temperature) were applied to dry-cured muscle cores as reference conditions; the combinations with the aforementioned values were also included.

Pork cores at 0.93 a_w were, on average, 5% salt, 62% moisture, 26% proteins, while samples at 0.90 a_w were 5% salt, 52% moisture, 35% proteins. The investigated a_w and temperature values are representative of variations occurring in dry-curing processing after the end of the early cold phases (Simoncini, Rotelli, Virgili, & Quintavalla, 2007). The incubation times lasted up to 190 days to simulate the long lasting manufacturing cycles of dry-cured meat products. Values < 0.90 a_w and 15 °C temperature were not taken into account in the present trial, due to the negligible OTA production predicted by the DCM model in that experimental region.

Visible moulding on the inoculated and reverse sides of the dry-cured pork cylinders was scored during incubation (Fig. 2). The scores given to fungal growth at the established deadlines of incubation are

Table 3
Results of visual assessment of dry-cured pork samples, with 0.90 and 0.93 a_w, inoculated with *P. nordicum* MPVP1669 and incubated at 15 and 20 °C for 14, 21 and 28 days.

	a_w	Temperature (°C)	Days of incubation		
			14	21	28
inoculation side	0.90	15	+ *	++	++++
	0.93	15	++	+++	++++
	0.90	20	+	++	++++
	0.93	20	+++	+++	++++
reverse side	0.90	15	−	−	++++
	0.93	15	+	++	++++
	0.90	20	−	+	++++
	0.93	20	+	++	++++

* Results of visual assessment.
+: ≈25% of sample surface is covered by fungal mycelium.
−: no visible mold. Scores take into account all replicates.

Table 4
Least Square Means (LSM) of the effect of temperature, a_w and incubation time (days) on ochratoxin A accumulation in dry-cured pork muscle cores inoculated with *P. nordicum* MPVP1669.

	Temperature (°C)		A_w		Days					
	15	20	0.90	0.93	14	21	42	63	84	190
OTA (μg/g)	0.04	0.41	0.02	0.43	0.08^{c}	$0.16^{b, c}$	0.13^{c}	$0.35^{a, b}$	0.44^{a}	$0.18^{b, c}$

Different letters along rows, for each effect, mean significant difference ($P < 0.05$).
The comparison was performed with LSD (Least Square Means) t-test.

displayed in Table 3. At 14 and 21 days, 0.90 a_w proved to be limiting both for fungal growth on inoculated surface and diffusion to the reverse side, while temperature variation did not generate a visible difference. Within the assayed values, the optimum condition for visible fungal colony growth was found at 0.93 a_w and 20 °C.

The ability of *P. nordicum* to grow in the assayed ecological conditions is consistent with its frequent detection on cured meat products (Battilani et al., 2007; Larsen et al., 2001), though few reports are available on the presence of OTA in cured meat products (Chiavaro et al., 2002; Pietri, Bertuzzi, Gualla, & Piva, 2006), if compared with the large number regarding food commodities like cereals, wine and coffee (Jorgensen, 2005). During the processing steps, the presence of moulds on the surface of dry-cured meat products is visible in a wide range of abiotic and biotic conditions, also in competition with other moulds, yeasts and bacteria (Battilani et al., 2007; Simoncini et al., 2007). Visible moulding was even required during the protracted times of maturation, to preserve the tradition in many typical products and, perhaps, to achieve mould contribution to the development of classic flavour.

A significant effect on OTA production has been shown for the factors a_w, temperature ($P < 0.001$), days of incubation and their two- and three-ways interactions ($P < 0.05$). In the assayed experimental conditions, a ten-fold increase in OTA amount was achieved when temperature rose from 15 °C up to 20 °C (Table 4), and the ratio between OTA generated at 0.93 a_w and 0.90 a_w was even higher (over twenty-fold increase). Up to the 84th incubation day OTA amount kept on rising, showing that dry-cured pork substrate composition, low in carbohydrates, but rich in amino acids and nitrogen sources, is suitable for OTA biosynthesis for protracted times (Núñez, Westphal, Bermúdez, & Asensio, 2007).

Fig. 3 summarizes the changes occurring in OTA content of dry-cured pork model system at the assayed conditions of temperature, a_w and incubation time: the outstanding finding is the strong enhancement in OTA production by *P. nordicum* when 20 °C and 0.93 a_w are kept for long times.

The significant interaction "incubation times × a_w" in OTA production, is graphically shown by the drop corresponding to the longest incubation time (190 days) at 0.93 a_w, and could most likely

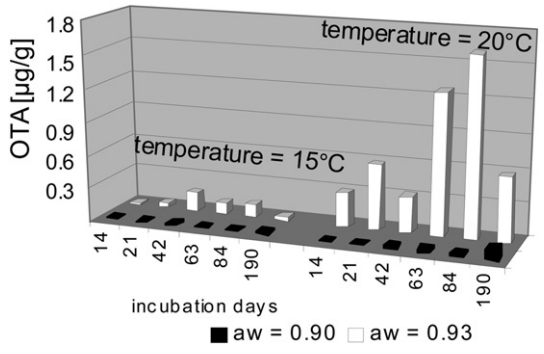

Fig. 3. Changes of OTA amounts produced by *P. nordicum* MPVP1669 in the model system (dry-cured pork muscle cores) during long lasting incubation time at two established temperature and a_w values. OTA analysis was performed in the muscle portion after mycelium removal.

be ascribed to nutrient decline, to OTA degradation by the inoculated mould itself or by other microorganisms, because the preservation of dry-cured pork cores from contamination for six months was not completely achieved in the described experimental conditions, or to other reasons deserving further investigations. In this respect, it was recently established that also light of a certain intensity can produce a partial degradation of OTA (Schmidt- Heydt, Bode, Raupp, & Geisen, 2010). Ochratoxin A production at 0.90 a_w, though much lower than at 0.93, kept on increasing up to the final deadline. This means that, in the presence of reduced fungal activity at 0.90 a_w, nutrients available in dry-cured muscle surface support OTA biosynthesis even for very prolonged incubation time.

During real and long lasting dry-curing process of meat products, OTA production *in situ* may be affected by interactions with environment and/or other contaminating populations. Microorganisms like micrococci, lactic acid bacteria, streptococci, staphylococci (Rodriguez et al., 1994), yeasts and fungi (Battilani et al., 2007; Simoncini et al., 2007;) can grow on dry-cured meat product surface. A strain of *Candida famata* isolated from dry-cured meat surface, was found capable of inhibiting *P. nordicum* growth and OTA production in YPD medium (Simoncini et al., 2009), while lactic acid bacteria exhibited antifungal activity against ochratoxin-producing *P. nordicum* in MRS medium (Schillinger & Varela Villareal, 2010). Environmental stress factors occurring in maturing rooms (fluctuating values of a_w, temperature, relative humidity and day/light oscillation) may affect *P. nordicum* growth and metabolites production if compared to controlled *in vitro* conditions. Furthermore, occasional events in manufacturing practices could raise temperature and/or surface a_w, potentially increasing dry-cured meat exposure to OTA contamination (Battilani et al., 2007).

In conclusion, even if the results obtained in dry-cured pork model systems cannot directly be extrapolated and applied to real conditions of dry-cured meat processing, data on OTA production by the assayed *P. nordicum* strain have to be taken into account when technological parameters of pork dry-curing steps are established, being indicative for the definition of control points during processing and storage.

The toxigenic potential of *P. nordicum* may be regarded as very weak in aged dry-cured meat products because the surface layers are generally < 0.90 a_w, but higher values are common during early manufacturing steps. The combination of temperature and a_w values favorable to OTA production may be a temporary condition during processing and storage, but the generated contamination could last until to the final outcome. It is confirmed by this study that OTA is stable for protracted times and the amount produced during growth accumulates in substrates (medium and muscle cores). Key points could be the control of events like temperature increase, scarce air circulation, presence of *P. nordicum* in air and on products during pork dry-curing and storage.

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