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Effect of Spanish smoked paprika "Pimentón de La Vera" on control of ochratoxin A and aflatoxins production on a dry-cured meat model system

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ABSTRACT

Environmental conditions during ripening of dry-cured meat products favour growth of fungal population on their surface. Some of these moulds can produce mycotoxins. Paprika is one of the ingredients usually used in the formulation of raw-cured sausages, and its addition could influence the growth and production of mycotoxins of the moulds present in these products. In this work the effect of Spanish smoked paprika "Pimentón de la Vera" on growth of *Aspergillus parasiticus* and *Penicillium nordicum* and production of aflatoxins B_1 (AFB_1), G_1 (AFG_1) and ochratoxin A (OTA) respectively, was evaluated. Moulds were grown in a culture medium made from lyophilized fresh pork meat added with 4% salt and different concentrations of Spanish smoked paprika (1, 2 and 3%) at several water activity values (0.98, 0.94 and 0.87) and temperature (20–25 °C), to simulate conditions usually found during ripening of dry-cured meat products. Mould growth was evaluated by measuring the diameter of the colony every 24 h, and the production of mycotoxins by UHPLC-MS/MS every 2 days, during 10 days of incubation. Addition of paprika favours growth of the two mould species tested. However, the synthesis of mycotoxins was reduced at 0.94 and 0.98 a_w when at least a 2% of paprika was added. Therefore, the addition of Spanish smoked paprika at 2–3% in the formulations may help to minimize AFs and OTA production in dry-cured meat products such as loins or "chorizo" sausages.

1. Introduction

Environmental conditions during ripening of dry-cured meat products favour a massive moulds development on their surface (López-Díaz et al., 2001; Rojas et al., 1991). This fungal population plays a key role in sensorial characteristics of these products by proteolytic activity, oxidation prevention and generation of desirable volatile compounds (Martín et al., 2006, 2004). However, some moulds are objectionable by their toxigenic potential (Núñez et al., 1996; Tabuc et al., 2004), principally those producer of ochratoxin A (OTA) and aflatoxins (AFs) (Rodríguez et al., 2012). The nephrotoxic OTA is the mycotoxin most frequently found in dry-cured meat products, being produced by *Aspergillus* and *Penicillium* species (Battilani et al., 2007; Bogs et al., 2006; Vipotnik et al., 2017). Among OTA-producer moulds, *Penicillium nordicum* is the most commonly found in NaCl and protein rich foods, such as dry-cured meat products (Battilani et al., 2007; Rodríguez et al., 2012, 2014; Schmidt-Heydt et al., 2011; Sonjak et al., 2011). In addition, *Aspergillus flavus* and *Aspergillus parasiticus*, the main producing moulds of hepatotoxic AFs can be isolated from dry-cured meat products (Aziz et al., 1991; Cvetnić and Pepeljnjak, 1995; Rojas et al., 1991), and concerning amounts of AFs in these foods have been reported (Markov et al., 2013; Pleadin et al., 2015; Rodríguez et al., 2012).

Mycotoxins synthesis in foods is influenced by abiotic factors such as temperature, a_w, pH, and substrate composition. The effect of environmental conditions, mainly temperature and a_w on OTA and AFs production during dry-cured meat products manufacturing has been addressed in several studies (Comi and Iacumin, 2013; Peromingo et al., 2016; Rodríguez et al., 2015; Sánchez-Montero et al., 2019). However, the information about the role of different ingredients such as paprika, usually added to these dry-cured meat products, on mould development and the synthesis of mycotoxins is scarce.

Paprika is one of the major ingredients used in redness of food like dry-fermented sausage "chorizo" and dry-cured loin. This spice, obtained from red pepper *Capsicum annuum* L., is added in the mixing stage of these sausages and it is thoroughly mixed with the chopped meat and fat mass and the remaining ingredients before stuffing (Vignolo et al., 2010). Its addition has an important effect on sensory

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properties on the above products. Its content of carotenoids pigments gives it a high colourant power, being responsible to obtain the characteristic redness of "chorizo" and dry-cured loin (Minguez-Mosquera et al., 1992). The volatile compounds such as acetic acid, phenols, ethyl acetate, aldehydes and carbonyls have a deep impact on flavour and taste (Gómez et al., 2008). Moreover, the lower redox potential of this seasoning likely due to a high level of antioxidants (Daood et al., 1996), contributes to prevent oxidation reactions of meat products (Aguirrezábal et al., 2000).

The manufacturing of traditional Spanish smoked paprika from the region of La Vera in central-west of Spain involves a smoking stage for drying of peppers. This processing step involves the incorporation of some phenolics compounds and essential fatty acids with antimicrobial activity into paprika (Vega-Gálvez et al., 2009). Paprika components have showed antagonistic activity against bacteria such as *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Bokaeian et al., 2014; Shayan and Saeidi, 2013) as well as against different yeasts (Iorizzi and Marino, 2002). Therefore, it could be of interest to study the potential effect of paprika to prevent the development of toxigenic moulds and mycotoxin production on dry-cured and dry-fermented meat products.

There are several approaches to reduce fungal growth in foods, however most of them are not suitable for application on dry-cured meat processing (Asensio et al., 2014). The addition of *Capsicum* powder with a combination of environmental treatments according to the stages of meat products, could be a useful strategy to control the development of toxigenic moulds.

The objectives of this study were to evaluate the effect of Spanish smoked paprika "Pimentón de La Vera" on *Penicillium nordicum* and *Aspergillus parasiticus* development, and to analyse its influence on OTA and AFs production.

2. Materials and methods

2.1. Fungal strains and inoculum preparation

In the study, aflatoxigenic *A. parasiticus* CECT2682 from the Spanish Type Culture Collection (CECT, Spain) and ochratoxigenic *P. nordicum* FHSCC4 isolated from dry-cured meat products belonging to the Culture Collection of Food Hygiene and Safety (FHSCC) of the University of Extremadura (Spain) were used.

To obtain the inocula, the moulds were incubated at 25 °C during 7 days on Meat Extract Agar (MEA, Scharlab, Spain). The conidia were collected with phosphate-buffered saline (PBS) by rubbing the colonies surface with a glass rod. The spore suspensions were quantified using a Thoma counting chamber Blaubrand® (Brand, Wertheim, Germany) and adjusted to 10^6 spores/mL and used as an inoculum.

2.2. Culture conditions and inoculation assay

The study was carried out in a meat-based agar (MBA) designed according to Peromingo et al. (2016), made-up by mixing 30 g/L of lyophilized fresh pork meat, 40 g/L NaCl to simulate salt contents of dry-cured meat products during ripening, and 20 g/L of Bacto agar (Scharlab S.L., Spain). Different amount (0, 1, 2 and 3%) of commercial sweet paprika powder "Pimentón de la Vera" usually found on dry-cured meat products, was included in culture medium.

Meat based agar medium with initial water activity (a_w) of 0.98 was used as a_w of dry-cured meat products at the beginning of processing. To reach usual a_w values of the middle (0.94 a_w) and the end of ripening (0.87 a_w), 151.4 and 401.4 g/L respectively of glycerol (Fisher Scientific, UK), were added. These amounts were calculated according to a standard curve done with different amounts of glycerol in water (from 0 to 70%). Water activity was checked using the a_w meter "Lab Master" (Novasina AG, Switzerland).

Plates were one-point centrally inoculated separately by each of the strains tested by applying 3 µL of the inoculum (10^6 spores/mL) and incubated for 10 days at 20 °C for *P. nordicum* and at 25 °C for *A. parasiticus*. Each species was incubated at its optimum temperature for mycotoxin production according to preliminary assays (Peromingo et al., 2019; Sánchez-Montero et al., 2019).

To evaluate fungal growth the colony diameter was measured each 24 h in plates kept unchanged during incubation time. Sampling to analyse OTA and aflatoxins production was done each two days. All experiments were realized in triplicate for each treatment.

2.3. Growth assessment

Plates were examined each 24 h for 10 days, and fungal growth was determined by measuring of the diameter of the colonies in two directions at right angles to each other (Akbar and Magan, 2014). Data obtained were used to determine the relative growth rate (μ_m) of moulds. A linear regression plotting colony radius against time was applied to obtain the growth rate (mm/day) as the slope of the line. The lag phase (λ) in days was calculated by equalling the regression line formula to the size of the original inoculum (Peromingo et al., 2016).

2.4. Extraction and quantification of mycotoxins

2.4.1. Sample preparation

Four to ten agar plugs (4 mm diameter) collected with a cork borer were removed from each fungal cultures each two days, placed in 2 mL microcentrifuge tubes (Eppendorf, Germany), weighted and kept at −20 °C till mycotoxins extraction.

2.4.2. Mycotoxins extraction method

Thawed agar plugs were transferred to 10 mL glass tubes. Then, 5 mL of chloroform was added, and samples were shaken and macerated for 24 h. Then, chloroform layer was transferred to an amber flask and evaporated to dryness according to Peromingo et al. (2016). Extracts were resuspended in 200 µL of HPLC-grade acetonitrile (Scharlab S.L.) and filtered through a 0.45 mm pore size nylon membrane (MSI, Westboro, USA). This method was initially validated by analysis of different amounts of OTA, AFB_1 and AFG_1, with a recovery rate above 90%.

Moreover, the absence of mycotoxins on the commercial paprika "Pimentón de la Vera" used to make the culture media was confirmed carrying out this method.

2.4.3. Detection and confirmation of OTA and AFs

Analysis for mycotoxins were carried out by ultrahigh performance liquid chromatography (uHPLC-MS/MS) analysis in a Thermo Scientific Dionex UltiMate 3000 Rapid Separation LC (RSLC) system with an autosampler UltiMate® 3000 Rapid Separation Autosampler (Thermo Scientific, USA) coupled to an ion Trap Mass Spectrometer System amaZon SL (Bruker Daltonics Inc., Bremen, Germany). A reversed-phase column C_{18} (100 mm × 2.1 mm, 2 µm; Agilent Technologies, USA) was used. The mobile phase was 1% formic acid and 10 mM ammonium formate in water (Solvent A) and acetonitrile (solvent B) at a flow rate of 0.25 mL/min. To achieve the separation of metabolites, the flow rate was set at a 0.25 mL/min and the following gradient was performed from 2 to 98% B: ([0 min] 2% B, [0–0.1 min] 40% B, [0.1–4 min] 60% B, [4–7 min] 80% B, [7–8.5 min] 80% B, [8.5–8.51 min] 98% B, [8.51–12.5 min] 98% B, [12.5–13.51 min] 2% B and [13.51–15 min] 2% B).

The runtime for detecting and quantifying was 15 min, being detected OTA at 6.5 ± 0.5 min whereas AFB_1 and AFG_1 were detected at 6.4 ± 0.5 and 6.3 ± 0.5 respectively. Signals were processed by Hystar v. 3.2 software (Bruker Daltonics Inc.).

The calibration curves for OTA (0.5–100 ppb) and AFB_1 and AFG_1 (1–500 ppb) by uHPLC-MS revealed a linear relationship ($r^2 \geq 0.99$) between detector response and amounts of mycotoxins standards.

The limit of detection (LOD) was estimated from the calibration curve, according to Long and Winefordner (1983) equation: LOD = 3 $(s_B^2 + s_i^2 + (i/m)^2\ s_m)^{1/2/m}$, being "m" the slope of the calibration curve, "i" the intercept term and "sB", "si" and "sm" the standard errors of the blank, the intercept term and the slope of the calibration curve, respectively. Assuming a normal distribution of the estimated quantities, α (error of the first type) = β (error of the second type) = 0.05, the quantification limit (LOQ) was 3.04 LOD (Currie, 1999). The LODs obtained were 1.3 ppb (OTA), 4 ppb (AFB_1) and 1.5 ppb (AFG_1), and LOQs 3.9 ppb (OTA), 12 ppb (AFB_1) and 4.5 ppb (AFG_1).

2.5. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows v.22.0 (IBM Corporation, USA). Data of lag time, relative growth rates and mycotoxin production were tested for normality using the Shapiro–Wilk test. Normality test was unsuccessful for all data sets, thus non-parametric data analysis was performed using the Kruskal–Wallis rank sum test. After that, the Mann–Whitney test was applied to compare the mean values obtained. The statistical significance was set at $p \leq 0.05$.

3. Results

3.1. Influence of paprika on Lag time and growth of A. parasiticus and P. nordicum

Both mould strains grew fairly at any condition of a_w and concentration of paprika tested. The values of lag time prior to growth for *P. nordicum* and *A. parasiticus* on dry-cured meat-based media in relation to a_w (0.98–0.87) and paprika (0–3%) are shown in Table 1. In general, lag phases of each mould increased as a_w decreased, being the shortest lag time at 0.98 a_w. Otherwise, the addition of paprika provokes a reduction of lag phases, reaching the lower values with 3% of this ingredient.

Regarding to growth of *A. parasiticus* and *P. nordicum*, the higher values were found at the greater a_w and amount of paprika tested (Fig. 1). Then, the maximum growth rate was recorded at 0.98 a_w and 2–3% of paprika concentration, for both moulds, although the growth of *A. parasitus* was generally faster in every condition tested (Table 2). The analysis of different culture conditions revealed that lag phase and growth rate of *A. parasiticus* and *P. nordicum* were affected by a_w level, paprika concentration, and the interactions of both parameters (Table 3).

3.2. Mycotoxins production

A. parasiticus CECT2682 produce AFs in all tested conditions (Fig. 2), although at 0.87 a_w these mycotoxins were only detected after 10 days of incubation. Similar profiles of AFB_1 and AFG_1 production was observed, showing the highest values at 0.94 a_w. The addition of paprika resulted in the decrease of AFB_1 and AFG_1 on culture media ($p \leq 0.05$) at every sampling time at 0.98 a_w and at days 6 and 8 at 0.94 a_w. At 0.87 a_w no changes on AFs concentration were observes, since the production was very low in all conditions tested. The minimum production of AFs was recorded after 10 days at 0.98 a_w and 3% of paprika (AFB_1: non-detected; AFG_1: 0.13 μg/kg).

P. nordicum FHSCC4 produced OTA in all conditions evaluated (Fig. 2). In general, the amount of OTA was higher at lower a_w values. At 0.98 a_w, the addition of paprika at 1–3% caused a decrease of OTA accumulation in comparison with control without paprika, showing lower amount at 3% concentration of paprika. At 0.94 a_w the reduction of OTA in comparison with control was observed when the concentration of paprika was at least 2%. The analysis about the influence of the studied conditions on mycotoxins production (Table 4) showed that the synthesis of AFB_1, AFG_1 and OTA was mainly affected by a_w and the interaction of a_w × paprika ($p \leq 0.005$).

4. Discussion

Paprika, especially the Spanish traditional smoked "Pimentón de la Vera" is a frequently used seasoning on the formulation of several dry-cured meat products. The antimicrobial effect of some paprika components has been demonstrated (Bokaeian et al., 2014; Shayan and Saeidi, 2013; Vega-Gálvez et al., 2009). However, the influence of paprika in fungal growth and mycotoxins production in meat substrate is not known. This is the first study designed to explore the impact of paprika on growth of *A. parasiticus* and *P. nordicum* and mycotoxins production in a dry-cured meat-based substrate at a_w conditions usually found during the ripening of dry-cured meat products (Andrade et al., 2010; Benito et al., 2007).

A. parasiticus needed less time prior to growth than *P. nordicum*, but both moulds had similar patterns of growth adaptation, and the lag phase was influenced by Spanish paprika "Pimentón de la Vera" and a_w interaction. The colonization of *A. parasiticus* and *P. nordicum* was faster on dry-cured meat-based medium with 1–3% of paprika and higher a_w values.

The joint interaction of the two factors studied influenced significantly the growth of the moulds, obtaining optimum values of development at 0.98 a_w and 3% paprika. Therefore, higher amounts of paprika and 0.98 and 0.94 a_w favour the adaptation of the genera, while under water stress conditions (0.87 a_w) and without adding paprika, moulds studied need more time to adaptation in the environment before starting their development.

The rate of growth at the different a_w studied was similar to previously described for both *A. parasiticus* (Peromingo et al., 2016) and *P. nordicum* (Leggieri et al., 2011; Rodríguez et al., 2015; Sánchez-Montero et al., 2019). Furthermore, the present work adds evidences about paprika effect on the development of moulds on dry-cured meat-based medium. In both tested genera a clear stimulus of its development has been observed, mainly from 2% concentration.

There are discrepancies in the literature regarding the influence of

Table 1
Effect of paprika on lag phases (days) of *Aspergillus parasiticus* CECT2682 and *Penicillium nordicum* FHSCC4 at different values of a_w.

Species	a_w	Paprika			
		0%	1%	2%	3%
A. parasiticus CECT2682	0.87	11.01 ± 0.17^{1a}	9.81 ± 0.08^{1b}	9.02 ± 0.10^{1c}	8.84 ± 0.07^{1c}
	0.94	3.82 ± 0.04^{2}	3.20 ± 0.20^{2}	3.46 ± 0.09^{2}	3.36 ± 0.12^{2}
	0.98	0.81 ± 0.21^{3a}	0.30 ± 0.15^{3b}	0.29 ± 0.24^{3b}	0.29 ± 0.19^{3b}
P. nordicum FSHCC4	0.87	10.30 ± 0.34^{1a}	8.44 ± 0.19^{1b}	6.70 ± 0.31^{1c}	6.17 ± 0.46^{1c}
	0.94	5.99 ± 0.18^{2}	4.73 ± 0.15^{2}	4.48 ± 0.68^{2}	5.04 ± 0.41^{1}
	0.98	4.13 ± 0.11^{3a}	3.55 ± 0.11^{3b}	3.10 ± 0.2^{3b}	2.83 ± 0.11^{2c}

[1,2,3]Batches with statistically significant differences ($p \leq 0.05$) among a_w values at the same paprika concentration.
[a,b,c]Batches with statistically significant differences ($p \leq 0.05$) among paprika concentration at the same a_w value.

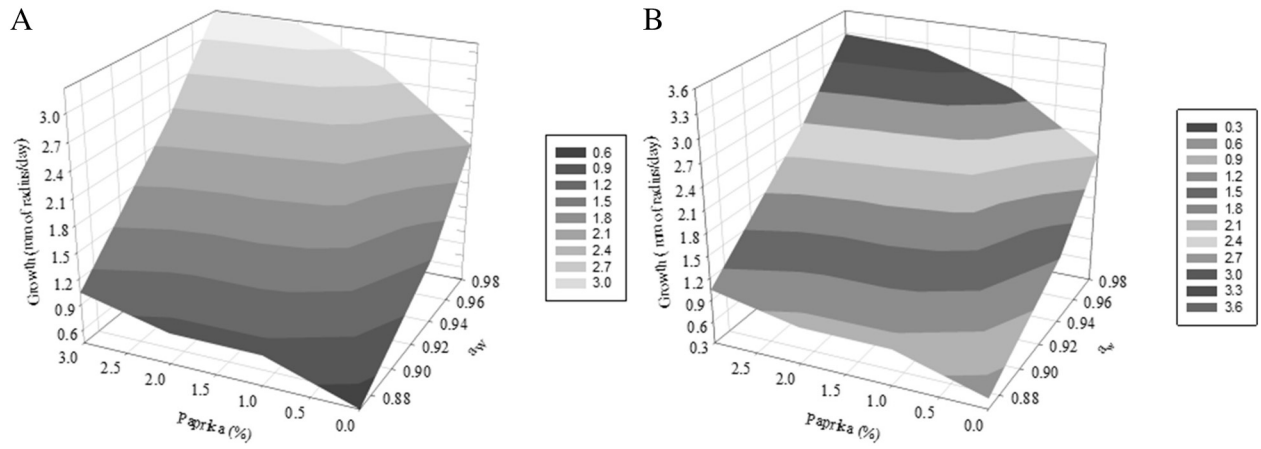

Fig. 1. Effect of paprika on growth (radium mm/day) of *Aspergillus parasiticus* CECT2682 (A) and *Penicillium nordicum* FHSCC4 (B) at different values of a_w.

paprika on the mould growth. Both an antifungal effect of paprika and a lack of antagonistic effect have been reported. On one side, an inhibitory effect on mould development has been attributed to some components usually found in paprika, such as capsanthin, capsorubin, saponins and phenolic compounds. Capsanthin completely inhibited *A. parasiticus* on synthetic medium during four days, and reduced 39% of its growth after 10 days (Masood et al., 1994). A carotenoid mixture containing capsanthin and capsorubin, obtained from red pepper powder, increased lag phases for *A. ochraceus*, *A. westerdijkiae*, *A. tubingensis* and *A. flavus* at 15 °C and reduced their growth rates at 25 °C (Santos et al., 2010a, 2010b). Furthermore, other compounds found in different plants belonging to the genus *Capsicum*, such as saponins, showed antifungal activity against *A. parasiticus* and *P. expansum* (Bautista-Baños et al., 2004; De Lucca et al., 2006). Likewise, phenolic compounds extracted from different varieties of peppers can inhibit the growth of moulds such as *Fusarium oxysporum* and *Alternaria alternata* by disrupting the cell wall or interfering some enzymatic routes (Rodriguez-Maturino et al., 2015).

However, the antifungal activity of some of these compounds is related to other environmental factors, such as temperature. Thus, the mixture of capsanthin and capsorubin did not affect the growth rates of some aflatoxigenic *A. flavus* and ochratoxigenic *Aspergillus* spp. isolates at 15 °C. In this sense, some researches did not found a worthy effect of red pepper powder (Karapinar, 1985) or paprika (Azzouz and Bullerman, 1982). Azzouz and Bullerman (1982) studied the inhibitory effect on *A. parasiticus* growth of different spices, and paprika was among them, but they only said that paprika is one of the less effectiveness against fungal growth. Karapinar (1985) did not use paprika really, but red pepper. The increase in mould growth with paprika addition, reported in the present study, could be related to the high amount of carbohydrates in the culture medium as consequence of paprika chemical composition (54 g carbohydrates/100 g) against that reported for red pepper (6 g carbohydrates/100 g) (USDA, 2018), since the mycelial growth are influenced by available C sources (Zong et al., 2015). Finally, Norton (1997) reported that growth of some strains of *A. flavus* was not affected by carotenoids.

Table 3
Summary of statistical analyses by Kruskal-Wallis test on the effect of factors on lag phase and growth for *Aspergillus parasiticus* CECT2682 and *Penicillium nordicum* FHSCC4.

Species	Factor studied	*p*-Value	
		Lag phase	Growth
A. parasiticus CECT2682	a_w	0.000	0.000
	Paprika	0.000	0.005
	a_w × paprika	0.000	0.001
P. nordicum FSHCC4	a_w	0.000	0.004
	Paprika	0.000	0.001
	a_w × paprika	0.001	0.000

Mycotoxins production was affected by a_w and the interaction of a_w and paprika. Addition of paprika reduced the amounts of AFs and OTA at 0.98 and 0.94 a_w. The AFs and OTA levels significantly decreased when paprika was added at a concentration of at least 2% of paprika. Furthermore, the relationship between growth and mycotoxin production is similar to that obtained with other seasoning such as thyme, ginger or bay leaf which stimulated the mould development while AFs production was being reduced (Mabrouk and El-Shayeb, 1981). It has been suggested that production of mycotoxins could be not associated with the rapid growth of moulds, and higher growth rates may restrict mycotoxin production (Haggblom, 1982). Moreover, in meat substrate, the production of some mycotoxins increases under suboptimal growing conditions (Núñez et al., 2000; Rodríguez et al., 2015; Sánchez-Montero et al., 2019; Sosa et al., 2002).

According to reported results in the literature, the paprika effects on mycotoxins production are not clear. The existence of an inhibitory

Table 2
Effect of paprika on growth rates (mm of radium/day) of *Aspergillus parasiticus* CECT82 and *Penicillium nordicum* FHSCC4 at different values of a_w.

Species	a_w	Paprika			
		0%	1%	2%	3%
A. parasiticus CECT2682	0.87	1.50 ± 0.13^{3c}	1.75 ± 0.25^{3c}	2.83 ± 0.38^{2b}	4.59 ± 0.25^{3a}
	0.94	2.68 ± 0.19^{2b}	2.82 ± 0.18^{2b}	3.14 ± 0.03^{2ab}	3.25 ± 0.16^{2a}
	0.98	3.30 ± 0.15^{1b}	4.23 ± 0.30^{1a}	4.12 ± 0.29^{1a}	4.59 ± 0.31^{1a}
P. nordicum FSHCC4	0.87	0.44 ± 0.12^{3}	0.79 ± 0.14^{3}	0.80 ± 0.25^{3}	1.04 ± 0.41^{3}
	0.94	1.21 ± 0.19^{2c}	2.09 ± 0.16^{2b}	2.22 ± 0.29^{2b}	2.34 ± 0.15^{2a}
	0.98	2.10 ± 0.32^{1c}	2.84 ± 0.12^{1b}	3.22 ± 0.09^{1a}	3.28 ± 0.14^{1a}

[1,2,3]Batches with statistically significant differences ($p \leq 0.05$) among a_w values at the same paprika concentration.
[a,b,c]Batches with statistically significant differences ($p \leq 0.05$) among paprika concentration at the same a_w value.

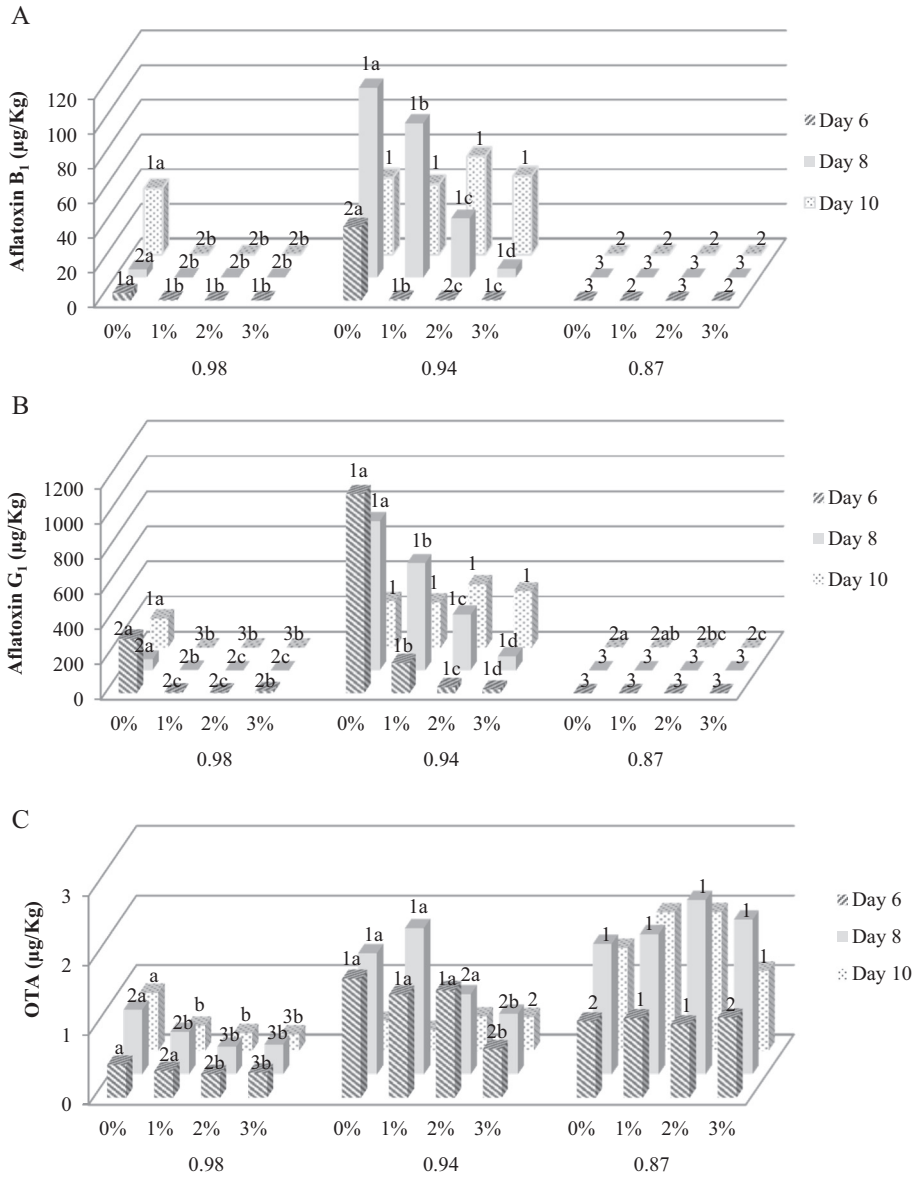

Fig. 2. Effect of paprika on AFB_1 (A) and AFG_1 (B) production by *Aspergillus parasiticus* CECT2682 and OTA production (C) by *Penicillium nordicum* FHSCC4 at different values of a_w at days 6, 8 and 10 on meat-based agar.

Table 4
Summary of statistical analyses by Kruskal-Wallis test on the effect of factors on aflatoxins B_1 (AFB_1), G_1 (AFG_1) and ochratoxin A (OTA) production.

Mycotoxin	Factor studied	*p*-Value		
		Day 6	Day 8	Day 10
AFB_1	a_w	0.000	0.000	0.052
	Paprika	0.203	0.576	0.690
	a_w × paprika	0.001	0.000	0.002
AFG_1	a_w	0.000	0.000	0.017
	Paprika	0.257	0.614	0.247
	a_w × paprika	0.000	0.000	0.001
OTA	a_w	0.004	0.167	0.000
	Paprika	0.105	0.820	0.510
	a_w × paprika	0.005	0.002	0.002

principle of the synthesis of AFs in red pepper (*Capsicum annuum*) was suggested (Madhyastha and Bhat, 1985). Among compounds present in paprika, that effect for reducing AFs biosynthesis has been attributed to carotenoids pigments such as capsanthin (Masood et al., 1994; Norton, 1997), although its mechanism of action is not fully defined. The antioxidant capacity of carotenoids (Baenas et al., 2019) would be involved in the inhibition of AFs synthesis, since the oxidative stress stimulates the biosynthesis of aflatoxins (Kim et al., 2008; Reverberi et al., 2005). Then, two characteristics of the carotenoids from paprika would be involved in the inhibition of AFB_1: the conjugated tail and the double-bond arrangement of the ring (Norton, 1997). On the other hand, carotenoids could modify cell membranes to indirectly affect to polyketide synthase complex (Dutton, 1988), associate specifically with hydrophobic domains of the synthase or in the enzymatic pathway of aflatoxin, and, consequently, affect their synthesis (Norton, 1997). However, the effect of some carotenoids obtained from red pepper over OTA and AFs production are inconclusived depending on ecological conditions and incubation time (Santos et al., 2010a, 2010b).

The discrepancy of these results with those obtained in the present work can be attribute to the differences in the mould strains and the ecophysiological conditions tested. It has been demonstrated that strains belonging to the same species can have a different behaviour with respect to their growth and mycotoxin production (Rodríguez et al., 2014). With regard to growing conditions, OTA (Pardo et al.,

2006) and AFs production (Klich, 2007; Madhyastha and Bhat, 1985; Pardo et al., 2006), are influenced by substrate and factors like temperature and a_w are determining of the synthesis of mycotoxins (Medina et al., 2014; Núñez et al., 2000). In the present work the culture medium and environmental conditions were designed to simulate usual condition during processing of dry cured meat products. However, studies such us Karapinar (1985) used a liquid medium with glucose to pH 6.6–6.7; Azzouz and Bullerman (1982) inoculated the moulds on PDA for 21 days.

In addition, it should be highlighted the special characteristic of the Spanish traditional smoked "Pimentón de la Vera" tested in this work as smoked product, due to the presence of phenolics compounds and essential fatty acids with antimicrobial activity (Vega-Gálvez et al., 2009), that could influence the reduction of AFs and OTA production.

According to the obtained results, the effect of Spanish traditional smoked paprika "Pimentón de la Vera" was not enough for fully suppress AFs and OTA production by *A. parasiticus* and *P. nordicum* respectively but it contributed to reduce accumulation of these mycotoxins. The protective effect diminishes with time and is not observed after 10 days at 0.98 and 0.94 a_w for both toxins, and at any time against OTA at 0.87. However, 0.87 a_w is achieved in dry-cured meat products after several weeks and even months of ripening. During this time of processing mould growth has usually been happened on the surface of the products. Then, the effect of paprika "Pimentón de la Vera" could contribute to control mycotoxins accumulation together with other preventive measures, such as the use of protective cultures. In this sense, some yeasts strains isolated from dry-cured meat product have proved their ability to reduce mycotoxin contamination in dry-cured sausages (Delgado et al., 2018; Peromingo et al., 2019, 2018). Therefore, according to our results a delay in mycotoxin production can be expected by using paprika in dry-cured meat products for long enough to allow the development of protective cultures that can help to effectively control this hazard.

5. Conclusions

In conclusion, although more studies are needed to establish the mechanism of action to which inhibition of mycotoxin synthesis is due, our results suggest that, the addition of Spanish traditional smoked "Pimentón de la Vera" at 2–3% in the formulations may is a promising strategy to minimize AFs and OTA production in dry-cured meat products such as loins or "chorizo" sausages in combination with protective cultures.

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Declaration of competing interest

The authors declare that have no conflicts of interest.

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