LWT - Food Science and Technology 213 (2024) 117030

Contents lists available at ScienceDirect

LWT

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

Debaryomyces hansenii reduces ochratoxin A production by *Penicillium nordicum* on dry-cured ham agar through volatile compounds

Elia Roncero [a], María J. Andrade [a], Micaela Álvarez [b,*], Eva Cebrián [a], Mar Rodríguez [a]

[a] Higiene y Seguridad Alimentaria, Instituto de Investigación de Carne y Productos Cárnicos, Facultad de Veterinaria, Universidad de Extremadura, Avda. de las Ciencias s/n, 10003, Cáceres, Spain

[b] Sección Departamental de Nutrición y Ciencia de los Alimentos, Facultad de Veterinaria, Universidad Complutense de Madrid, Avda. Puerta de Hierro s/n, 28040, Madrid, Spain

ARTICLE INFO

Keywords:
Mycotoxin
Protective yeast
Antifungal
Ochratoxigenic moulds

ABSTRACT

Ochratoxin A (OTA) produced by *Penicillium nordicum* during the processing of dry-cured ham represents a risk in the meat industry. To avoid it, yeasts as control agents are under study although their mechanisms of action are not elucidated yet. The aim of this work was to evaluate the antiochratoxigenic capacity of volatile compounds produced by *Debaryomyces hansenii* against three strains of *P. nordicum*. For this, three ochratoxigenic *P. nordicum* strains were inoculated in Petri dishes with a culture medium based on lyophilised ham. *D. hansenii* was inoculated on the surface of other plates with the same agar. The two plates were faced in such a way that microorganisms did not come into contact but shared a headspace between them. After 14 days at 20 °C, the growth and OTA production of *P. nordicum* were evaluated and the volatile compounds were analysed. The results revealed OTA reductions up to 99.9 % in the three tested strains exerted by the volatile compounds generated by *D. hansenii*. The 3-methyl butanol and the phenylethyl alcohol were negative correlated with OTA production, showing their implication in the antiochratoxigenic effect. These results can be used to establish new low-invasive strategies in the dry-cured ham industry.

1. Introduction

Dry-cured ham is a “ready to eat” (RTE) traditional product of high quality and social importance. Spain is known for its Serrano and Iberian hams, and Italy for its Parma ham, although ham production has now spread to other areas, such as China and United States.

Dry-cured ham is a product known for its specific sensory characteristics, which are developed throughout its processing because of molecular reactions from different origin. Many of these biochemical consequences are due to the growth of an abundant microbial population on the surface of the hams (Li et al., 2023). Among the wide variety of microorganisms of dry-cured ham bacteria, such as *Staphylococcus xylosus* and *Staphylococcus equorum*, yeasts, such as *Debaryomyces hansenii* and *Candida zeylanoides*, and moulds, such as *Penicillium*, *Aspergillus* and *Eurotium* spp. are found (Núñez et al., 1996a, 1996b; Rodríguez et al., 1994). Many of these microorganisms have the ability to trigger lipolytic, proteolytic and Maillard reactions and Strecker degradations, providing the final sensory characteristics desired by consumers (Munekata et al., 2021). Moulds are the microorganisms found in the greatest proportion on the surface of final pieces, strongly contributing to the generation of their organoleptic features (Alapont et al., 2014). Apart from having such beneficial effects, some of them can pose a risk due to the production of mycotoxins and consequently a great concern for the meat industry (Battilani et al., 2007; Chen et al., 2022). The ochratoxin A (OTA) is the most common mycotoxin found in dry-cured ham and is mainly synthesised by moulds belonging to the genera *Penicillium* and *Aspergillus* (Rodríguez et al., 2012a; Toman et al., 2024). Among all OTA-producing moulds, the major producer in dry-cured ham is *Penicillium nordicum* (Battilani et al., 2007; Rodríguez et al., 2012a; Rodríguez et al., 2012b). OTA was classified as a Group 2B human carcinogen by the International Agency for Research on Cancer (Ostry et al., 2016), although some studies suggest its reclassification in the group 2A as probably carcinogenic to humans (Ostry et al., 2016; Schrenk et al., 2020). OTA has been associated with immunotoxic, genotoxic, neurotoxic, carcinogenic, nephrotoxic and teratogenic effects, and is believed to be the most toxic compound of the ochratoxin family (Schrenk et al., 2020). Scientific evidence on the high prevalence of OTA in meat products (Vulić et al., 2016) led the European Food

---

* Corresponding author.
E-mail address: malvar54@ucm.es (M. Álvarez).

https://doi.org/10.1016/j.lwt.2024.117030
Received 29 July 2024; Received in revised form 2 November 2024; Accepted 7 November 2024

Safety Authority (EFSA) to consider them one of the main contributors to chronic exposure to this mycotoxin (Schrenk et al., 2020). However, meat derivatives are still not covered by European legislation (European Commission, 2023) and only Italy has established a guidance level for this toxin of 1 μg/kg in meat and meat products (Ministero della Sanità, 1999).

The prevention of the growth of toxigenic moulds on the surface of dry-cured ham must be controlled by antifungal methods compatible with the desired sensorial attributes (Chen et al., 2022). Thus, the preventive methods shall not drastically affect the growth of the beneficial moulds to the organoleptic characteristics of the product (Rodríguez et al., 2015) or cause an increase in the production of OTA in meat derivatives as a response to the mould stress caused (Ferrara et al., 2022). Currently, the most used techniques are those related to the control of environmental conditions inside the curing-maturing chambers, such as relative humidity, temperature, ventilation and product water activity (a_w) (Rodríguez et al., 2015). As an alternative to this, the strategy that is being most studied with the aim of being immediately applicable is the use of biopreservation techniques.

To reduce the OTA contamination in dry-cured meat products, the yeast *D. hansenii* FHSCC 253H has shown to be effective as biocontrol agent (BCA) in dry-cured fermented sausages through the alteration of the fungal cell wall integrity pathway and the mycotoxin biosynthetic pathway (Álvarez et al., 2022; Andrade et al., 2014; Cebrián et al., 2022; Núñez et al., 2015). Among the modes of action of yeasts studied as BCAs against toxigenic moulds, it is described the production of mycocins known as killer toxins, the competition for nutrients and space, the secretion of hydrolytic enzymes, the adsorption of mycotoxins to the cell wall, the production of both soluble and volatile antifungal metabolites and the effect on the relative gene expression of genes related to mycotoxin synthesis (Álvarez et al., 2020; 2022; Delgado et al., 2023; Medina-Córdova et al., 2018; Núñez et al., 2015; Peromingo et al., 2019; Ruiz-Moyano et al., 2020). Regarding volatile compounds (VOCs), many of them have shown antifungal capacity in a wide variety of food matrices because of decreasing the mycotoxin production (Núñez et al., 2015; Ruiz-Moyano et al., 2020). These VOCs can belong to different chemical groups, such as alcohols, esters, acids, aldehydes, ketones and phenols, among others (Delgado et al., 2021; Medina-Córdova et al., 2018; Ruiz-Moyano et al., 2020). Different yeast species, including *D. hansenii*, and some species of the genus *Hanseniaspora* are characterised by the production of antifungal compounds, such as acetic acid, 3-methylbutanoic, ethyl acetate, octanoic acid and the phenylethyl alcohol (Delgado et al., 2021; Huang et al., 2021; Ruiz-Moyano et al., 2020). The antimicrobial activity of some of these VOCs, such as alcohols, is due to their binding to the membrane of the undesirable mould. This mechanism of action causes an anomaly in the movement of essential ions across the membrane inhibiting its growth as well as spore germination (Hernández-Montiel et al., 2018). Furthermore, the addition of *D. hansenii* strains applied to meat derivatives has no negative sensory effects and reduces the lipid oxidation of dry-cured meat products (Iacumin et al., 2017; Álvarez et al., 2020, 2023a).

Therefore, the aim of this work was to evaluate the potential antifungal and antiochratoxigenic activity of the VOCs produced by *D. hansenii* FHSCC 253H in a dry-cured ham-based medium against different *P. nordicum* strains. For this purpose, their effects on growth, OTA production and the VOCs produced were evaluated.

2. Material and methods

2.1. Microorganisms

Three ochratoxigenic strains of *P. nordicum* were used in this study: *P. nordicum* FHSCC 15 (Pn15) and FHSCC 26 (Pn26) from the Food Hygiene and Safety Culture Collection (FHSCC) of the University of Extremadura (Spain) and *P. nordicum* BFE 856 (Pn856) from Max Rubner-Institut (Karlsruhe, Germany). The yeast *D. hansenii* FHSCC 253H also belonged to the FHSCC. All of them were isolated from dry-cured ham.

2.2. Culture media

Potato dextrose agar (PDA) was prepared following the manufacturer's instructions and Yeast Extract Sucrose broth (YES) was elaborated by adding 20 g/L of yeast extract and 125 g/L of sucrose to 1 L of distilled water. These media and components were supplied by Scharlab, S.L. (Madrid, Spain).

The dry-cured ham-based agar (DHA) was made using 150 g/L of lyophilised dry-cured ham and 20 g/L of bacteriological agar (Scharlab, S.L.) to emulate the nutrient composition of the product. All the media were autoclaved for 20 min at 121 °C and plated in Petri dishes.

2.3. Inocula preparation

P. nordicum strains were inoculated at 3-point per plate in PDA and incubated for 7 days at 25 °C. Spores were then collected by the addition of 2 mL of phosphate buffer saline (PBS; 0.32 g of NaH_2PO_4 (Scharlab, S.L.), 1.09 g of Na_2HPO_4 (Scharlab, S.L.), 9 g of NaCl (Scharlab, S.L.), 1 L of distilled water) scraping the plate surface with a glass spatula. The spores' concentrations were calculated using a Thoma counting chamber Blaubrand® (Brand, Germany), visualising them in a microscope (NIKON, Japan) and adjusted to 10^6 spores/mL.

D. hansenii was incubated in 10 mL of YES for 2 days at 25 ºC under stirring conditions. The culture was then centrifuged at 10640×*g* for 5 min and the pellet was resuspended in PBS. The yeast concentration was adjusted to 10^6 cells/mL using the same methodology described for moulds.

2.4. Antifungal volatile screening

The screening for antifungal VOCs was performed following the method described by Ruiz-Moyano et al. (2020) with some modifications. A volume of 3 μL of each strain of *P. nordicum* (Pn15, Pn26 and Pn856) were inoculated in the centre of DHA Petri plate (mould control batches: Pn15, Pn26 and Pn856). Moreover, 100 μL of *D. hansenii* were inoculated and spread with a Digralsky spatula on the surface of DHA (yeast control batch: Dh). Two DHA petri plates were faced sharing headspace between them per each confrontation to obtain co-inoculated batches Pn15+Dh, Pn26+Dh and Pn856+Dh. The two plates of DHA were confronted and joined with Parafilm™ (Bemis, USA) to create a close space to VOC interactions with the toxigenic moulds following the "mouth to mouth" method. The 7 batches were incubated at 20 °C for 14 days. Five replicates were done for each batch.

2.5. VOCs analysis

VOCs were extracted by solid-phase microextraction (SPME) using a fibre of carboxen/polydimethylsiloxane (Supelco, USA). This fibre was preconditioned at 220 °C for 50 min. For VCO collection, it was inserted into the space of the double-plates system for 30 min and analysed by gas chromatography-mass spectrometry (GC-MS) in a gas chromatograph 6890 GC (Agilent Technologies; USA) coupled to a mass spectrometer detector, 5975C (Agilent Technologies). Oven temperature started at 40 °C for 5 min and increased to 280 °C, with a rate of 7 °C/min. The desorption time was 30 min at 250 °C. The transfer line temperature was established at 280 °C. The carrier gas was helium with a flow rate of 1.2 mL/min. MS detection was performed in full scan (50–350 amu). The identification of the VOCs was achieved by comparing their mass spectra with the NIST/EPA/NIH library.

2.6. Growth assessment of ochratoxigenic moulds

Just after taking the samples for the VOCs analyses and before the sample collection for OTA analyses, the final diameters of mould colonies were measured in two perpendicular directions. These two directions had been marked on each of the plates prior to inoculation to ensure homogeneous measurements. The average of both perpendicular diameters was used to compare the co-inoculated batches (Pn15+Dh, Pn26+Dh and Pn856+Dh) with the respective control ones (Pn15, Pn26 and Pn856).

2.7. *Extraction and quantification of OTA*

After the VOCs analyses, half of the mycelium colony and the agar below were collected individually from each plate for OTA extraction. The samples were kept at −20 °C until their extraction using the QuEChERS procedure described by Delgado et al. (2018). The samples were diluted to 1:1 with ultrapure water and filtered with a 0.22 μm pore-size membrane (RephiLe Bioscience). Then, 10 μL were analysed using a Q-Exactive plus mass spectrometer Orbitrap (Thermo Fisher Scientific) following the method described by Cebrián et al. (2020). Briefly, a Thermo Fisher Accucore Aq C18 of 150 × 2.1 mm and 2.6 μm particle size column was used. The mobile phases were: A (H_2O 0.1 % formic acid) and B (acetonitrile 0.1 % formic acid) with a flow rate of 0.3 mL/min. OTA was eluted at 6.71 ± 0.1 min. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated by using the same acetonitrile matrix from untreated samples undergone to QuEChERS method and spiked with OTA standards, as the lowest evaluable concentration level at which the qualifier ion signal exceeds the noise level by a factor of 3 and 10, respectively. The LOD and LOQ were 0.1 ng/mL and 0.5 ng/mL, respectively.

2.8. *Statistical analysis*

SPSS software v. 22 (IBM Corporation, USA) was used for statistical analyses. After testing the normality using the Shapiro-Wilk test and homoscedasticity using Levene's test, data showed a non-normal distribution. Thus, the non-parametric tests Kruskal-Wallis and Mann-Whitney U were performed. Moreover, single correlation analysis between OTA level and VOCs was calculated using Spearman's rank correlation coefficients. A principal component analysis (PCA) was also performed to relate and compare the VOCs produced with each of the batches. The statistical significance was established at $p \le 0.05$ or $p \le 0.01$ depending on the results analysed.

3. Results and discussion

3.1. *Effects of VOCs on growth and OTA production*

The growth of the colonies of Pn26 and Pn856 was significantly diminished in the presence of the yeast with respect to the control samples, while Pn15 growth was not inhibited in the presence of *D. hansenii* (Table 1, Fig. 1). The significant inhibition observed indicates that the VOCs produced by *D. hansenii* directly affect the normal growth of these two *P. nordicum* strains. In this sense, different yeasts, such as *Aureobasidium pullulans* and *Hanseniaspora uvarum*, have shown to reduce the growth of the plant pathogen *Botrytis cinerea* when the same methodology described in the present work was used (Ruiz-Moyano et al., 2020). Moreover, different VOCs produced by other strains of *D. hansenii* affected the normal growth of *Cladosporium inversicolor* and *Penicillium roqueforti* due to decreases in growth rates up to 50 % and 70 %, respectively (Huang et al., 2021).

Table 1

Growth and ochratoxin A (OTA) production of different strains of *Penicillium nordicum* after 14 days of incubation at 20 °C in dry-cured ham medium in the absence and presence of *Debaryomyces hansenii* in "mouth to mouth" experiment.

Batches[a]	Growth (diameter in mm)	OTA (ng/g)	OTA reduction (%)
Pn856	47.30 ± 1.71	5369.31 ± 1167.35	–
Pn15	3.88 ± 0.14	1094.14 ± 539.31	–
Pn26	3.99 ± 0.40	43285.95 ± 3918.83	–
Pn856+Dh	37.12 ± 3.42[b]	9.75 ± 12.67[b]	99.93
Pn15+Dh	3.83 ± 0.59	0.75 ± 1.50[b]	99.93
Pn26+Dh	3.33 ± 0.33[b]	649.97 ± 30.05[b]	98.49

[a] Pn856, Pn15, Pn26 (control batches): 3 μL of 10^6 spores/mL of each strain of *P. nordicum* (BFE 856, FHSCC Pn15, FHSCC Pn26). Pn856+Dh, Pn15+Dh, Pn26+Dh: 3 μL of 10^6 spores/mL of each strain of *P. nordicum* plus 100 μL of 10^6 cells/mL of *D. hansenii* FHSCC 253H inoculated in the upper dry-cured ham medium plate. The results are presented as mean of quintuples ± standard deviation.

[b] Significant differences ($p \le 0.05$) between batches co-inoculated with *D. hansenii* (Pn856+Dh, Pn15+Dh and Pn26+Dh) and their corresponding control batches (Pn856, Pn15 and Pn26, respectively).

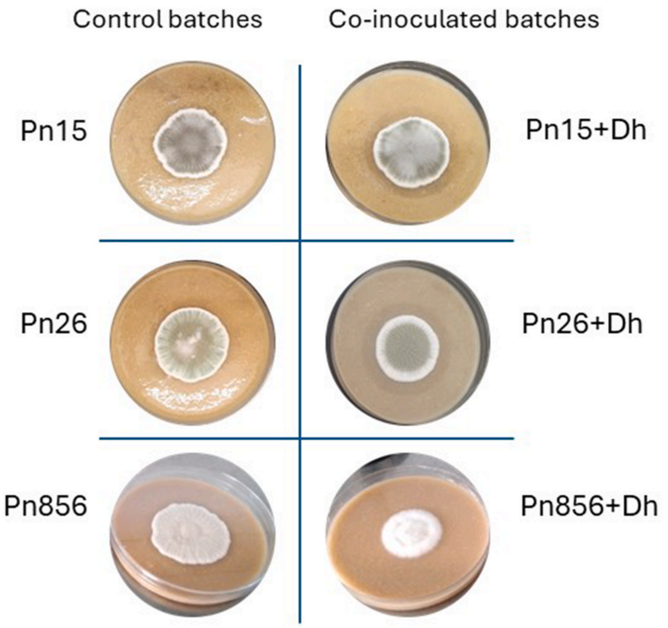

Fig. 1. Photographs showing the radial growth of *Penicillium nordicum* strains after 14 days of incubation at 20 °C in dry-cured ham-based medium in absence and presence of *Debaryomyces hansenii* in "mouth to mouth" experiment. Pn15, Pn26, Pn856 (control batches): 3 μL of 10^6 spores/mL of each strain of *P. nordicum* (FHSCC Pn15, FHSCC Pn26, BFE 856). Pn15+Dh, Pn26+Dh, Pn856+Dh (co-inoculated batches): 3 μL of 10^6 spores/mL of each strain of *P. nordicum* plus 100 μL of 10^6 cells/mL of *D. hansenii* FHSCC 253H inoculated in an upper dry-cured ham medium plate.

Regarding the OTA production, a significant decrease in its amount was observed when all the three *P. nordicum* strains were grown in the presence of *D. hansenii*, being achieved about a 99 % reduction when comparing to the batch containing only each of the ochratoxigenic strains (Table 1). The antiocratoxigenic effect of *D. hansenii* has been previously studied on several occasions with very good results in a variety of matrices, including meat matrices such as dry fermented sausages and dry-cured ham (Andrade et al., 2014; Cebrián et al., 2019; Gil-Serna et al., 2011; Iacumin et al., 2017, 2020; Peromingo et al., 2018). The ability of *D. hansenii* to decrease the synthesis of OTA in most of these studies is due to mechanisms of action like the blockage of genes related to OTA biosynthesis (Cebrián et al., 2019; Peromingo et al., 2018), adsorption to the yeast cell wall (Gil-Serna et al., 2011) and competition for nutrients and space (Iacumin et al., 2017, 2020). However, the antiochratoxigenic effect of the VOCs produced by *D. hansenii* has been poorly examined until now, and even less in meat matrices. The results in this study agree with previous ones establishing the additive or synergistic effect of several mechanisms of action of the antagonistic activity of *D. hansenii* against ochratoxigenic moulds (Andrade et al., 2014; Núñez et al., 2015). Similarly, the ability to reduce OTA synthesis by VOCs produced by different yeast species in commercial culture media and food-based medium has been previously demonstrated (Farbo et al., 2018; Galván et al., 2022). These results would be a great benefit to the meat industry due to the possibility of using a biocontrol agent that does not need to come into direct contact with the toxigenic mould to be effective.

Another remarkable fact is the difference in OTA production of each of the three *P. nordicum* strains, ranging from 1094.14 to 43285.95 ng/g (Table 1). This variety in the production of a secondary metabolite as OTA by strains from the same mould species has been previously reported by several studies performed in meat products (Andrade et al., 2019; Cebrián et al., 2020; Delgado et al., 2018). These differences are due to the different optimal conditions to produce OTA, such as temperature and a_w, as well as the different susceptibility of each of the strains to similar antagonistic conditions, which is also observed in the different growth behaviour against the same antifungal agent, as in Pn15 (Medina et al., 2007; Sánchez-Montero et al., 2019). It is worthy to note that the reduction in the mould growth is not proportional to the reduction in the mycotoxin production, which can be observed in the case of Pn15, with no reduction of its growth but a significant reduction in its OTA generation. This demonstrated that, whereas the three *P. nordicum* strains responded differently to the VOCs produced by the yeast, the OTA biosynthetic pathway was affected in the same way.

3.2. Volatile compounds profile

Twenty-four compounds were identified and quantified (Table 2). These compounds were classified depending on their origin in carbohydrate fermentation, lipid oxidation, amino acid catabolism and sulphur reduction. The volatile profile showed clear differences between *P. nordicum* strains, being more similar Pn15 and Pn26. This could be due to the fact that Pn15 and Pn26 are isolated from Spanish dry-cured ham while Pn856 was isolated from Italian dry-cured ham. The differences between Pn15 and Pn856 were also detected at proteome level (Álvarez et al., 2021).

With regard to compounds related to carbohydrate fermentation, acetone was the only detected. This VOC was produced by the strains Pn15 and Pn26 and the presence of Dh seems to decrease its concentration in Pn26 (Table 2).

Regarding the compounds derived from lipid oxidation, the 2-pentyl furan was the predominant compound in all batches, being produced by moulds and *D. hansenii* (Table 2). This compound has been found when a grey mould infection by *B. cinerea* occurred in winegrapes (Steel et al., 2020). Interestingly, this compound was increased in most of the co-inoculated batches with the *P. nordicum* strains and *D. hansenii*. In addition, the 2-pentyl furan was negatively correlated with the OTA production (Table 3), although we cannot know if its increase in 2-pentyl furan is because of the mould or the yeast metabolism. Other furans, such as 2-butyl furan, 2-ethyl furan and 2-hexyl furan, were only detected in the co-inoculated batches, showing negative correlations with OTA concentrations. On the other hand, the ketones were produced by *D. hansenii* and the 2-nonanone and 2-octanone were negatively

Table 2

Volatile compounds[a] of different strains of *Penicillium nordicum* and *Debaryomyces hansenii* from different treatments after 14 days of incubation at 20 °C in dry-cured ham-based medium in absence and presence of *Debaryomyces hansenii* in “mouth to mouth” experiment.

Compound			Batches[c]						
	Number	Id[b]	Pn856	Pn856+Dh	Pn15	Pn15+Dh	Pn26	Pn26+Dh	Dh
Carbohydrate fermentation									
Acetone	1	MS	n.d. [d]	n.d.	0.27 ± 0.25	1.31 ± 1.05	0.80 ± 0.23	0.14 ± 0.03[e]	n.d.
Lipid oxidation									
1-3- Octanediene	2	MS	n.d.	0.48 ± 0.47[e]	n.d.	0.55 ± 0.76	n.d.	n.d.	n.d.
Hexanol	3	MS	n.d.	0.77 ± 1.10[e]	n.d.	n.d.	n.d.	n.d.	n.d.
2-Ethyl-1-hexanol	4	MS	n.d.	n.d.	0.25 ± 0.19	n.d.	n.d.	n.d.	1.82 ± 1.46
1-Octen-3-ol	5	MS	2.38 ± 0.26	1.24 ± 0.84	4.58 ± 0.21	3.37 ± 3.24	3.08 ± 1.10	0.35 ± 0.07[e]	0.61 ± 0.14
2-Butanone	6	MS	n.d.	0.04 ± 0.00	n.d.	1.55 ± 1.16[e]	0.16 ± 0.24	0.13 ± 0.04	n.d.
2-Butyl furan	7	MS	n.d.	0.97 ± 0.52[e]	n.d.	1.94 ± 1.55[e]	n.d.	0.22 ± 0.20[e]	n.d.
2-Nonanone	8	MS	n.d.	n.d.	n.d.	n.d.	n.d.	0.12 ± 0.07	0.09 ± 0.05
2-Octanone	9	MS	n.d.	0.43 ± 0.76	n.d.	0.12 ± 0.44	n.d.	0.32 ± 0.24	0.37 ± 0.18
2-Pentanone	10	MS	n.d.	0.47 ± 0.38	n.d.	0.36 ± 0.73	n.d.	0.28 ± 0.34	n.d.
Decane	11	MS	n.d.	n.d.	n.d.	0.29 ± 0.22[e]	n.d.	n.d.	n.d.
Furan, 2-(1-pentenyl)-, (E)-	12	MS	0.18 ± 0.06	0.35 ± 0.24	n.d.	n.d.	n.d.	n.d.	0.27 ± 0.12
2-Ethyl furan	13	MS	n.d.	0.84 ± 0.23[e]	n.d.	0.58 ± 0.57[e]	n.d.	0.11 ± 0.04[e]	n.d.
2-Hexyl furan	14	MS	n.d.	0.20 ± 0.04[e]	n.d.	0.32 ± 0.34[e]	n.d.	n.d.	n.d.
2-Pentyl furan	15	MS	13.78 ± 1.75	42.92 ± 20.28[e]	2.93 ± 1.01	22.08 ± 19.10[e]	3.07 ± 0.57	4.46 ± 0.87[e]	14.6 ± 7.07
Heptane	16	MS	1.88 ± 0.41	3.95 ± 1.49	0.48 ± 0.23	4.6 ± 4.3[e]	0.58 ± 0.09	1.82 ± 0.58[e]	1.84 ± 0.42
Octane	17	MS	1.67 ± 0.33	6.46 ± 3.06[e]	0.59 ± 0.05	6.35 ± 5.58[e]	0.73±	2.34 ± 0.98[e]	1.46 ± 0.51
Pentane	18	MS	0.54 ± 0.15	1.35 ± 1.29	n.d.	0.37 ± 0.38	0.02±	0.31 ± 0.14[e]	0.27 ± 0.01
Amino acid catabolism									
3-Methyl butanol	19	MS/Rf	n.d.	0.10 ± 0.10	n.d.	1.43 ± 2.07[e]	n.d.	0.21 ± 0.20[e]	1.26 ± 0.8
Benzaldehyde	20	MS	n.d.	0.03 ± 0.06	0.19 ± 0.2	n.d.	n.d.	n.d.	n.d.
Heptane, 2,2,4,6,6-pentamethyl	21	MS	n.d.	n.d.	n.d.	0.98 ± 0.77[e]	0.35 ± 0.15	0.09 ± 0.06	n.d.
Methyl isocyanide	22	MS	n.d.	n.d.	n.d.	0.53 ± 0.68[e]	n.d.	0.09 ± 0.01[e]	n.d.
Phenylethyl alcohol	23	MS	n.d.	0.47 ± 0.93	n.d.	4.00 ± 3.43[e]	n.d.	0.42 ± 0.05[e]	3.59 ± 1.71
Sulphur reduction									
Dimethyl disulfide	24	MS	n.d.	0.02 ± 0.02	n.d.	1.42 ± 1.6[e]	n.d.	0.14 ± 0.13[e]	n.d.

[a] Results expressed in arbitrary area units ($x10^{-6}$) as means of 3 replicates.

[b] Id: reliability of identification; MS: chromatogram deconvolution and identification by comparing the mass spectrum of the compounds with the NIST/EPA/NIH database; Rf: mass spectrum and retention time identical to a reference compound.

[c] Pn856, Pn15, Pn26 (control batches): 3 μL of 10^6 spores/mL of each strain of *P. nordicum* (BFE 856, FHSCC Pn15, FHSCC Pn26). Pn856+Dh, Pn15+Dh, Pn26+Dh: 3 μL of 10^6 spores/mL of each strain of *P. nordicum* plus 100 μL of 10^6 cells/mL of *D. hansenii* FHSCC 253H inoculated in the upper dry-cured ham medium plate. The results are presented as mean of quintuples ± standard deviation.

[d] n.d.: not detected.

[e] Significant differences ($p \leq 0.05$) between batches Pn856+Dh, Pn15+Dh and Pn26+Dh and their control batches (Pn956, Pn15 and Pn26, respectively).

Table 3

Statistically significant Spearman's correlation coefficients (ρ) for ochratoxin A level and volatile compounds from *Penicillium nordicum* strains and *Debaryomyces hansenii* after 14 days of incubation at 20 °C in dry-cured ham-based medium in absence and presence of *D. hansenii* in "mouth to mouth" experiment.

	Correlation coefficients
3-Methyl butanol	-0.798^{b}
1-octen-3-ol	0.439^{a}
2-butyl furan	-0.435^{a}
2-nonanone	-0.454^{b}
2-octanone	-0.630^{b}
Furan, 2-(1-pentenyl)-,(E)	-0.350^{a}
2-ethyl furan	-0.409^{a}
2-hexyl furan	-0.388^{a}
2-pentyl furan	-0.467^{b}
Heptane	-0.479^{b}
Octane	-0.461^{b}
Phenylethyl alcohol	-0.758^{b}

[a] Indicates $p \leq 0.05$.

[b] indicates $p \leq 0.01$.

correlated with the OTA production (Table 3), suggesting that these compounds could be part of the antifungal mechanisms of the yeast. The 2-octanone had demonstrated in another study its antagonistic effect against the soil fungal pathogen *Setophoma terrestris* decreasing its growth as the concentration of the ketone increased (Albarracín et al., 2020). The alcohol 1-octen-3-ol was detected in all samples and is associated with the mould's growth (Sunesen et al., 2004). This compound, which contributes to the typical mushroom odour of dry-cured meat products, was the only positively correlated with the OTA production ($\rho = 0.439$; Table 3). However, this compound can be present in toxigenic and non-toxigenic strains so it cannot be used to differentiate OTA producers (Jeleń & Grabarkiewicz-Szczęsna, 2005). Furthermore, previous studies have even correlated the presence of this compound with the production of OTA by toxigenic moulds isolated from foods, such as grapes (Zhang et al., 2017). The aliphatic alkanes octane and heptane were increased in the batches with *D. hansenii* as it has been previously attributed to some yeast species (Zhu et al., 2017).

Concerning the derivatives from amino acid catabolism, the 3-methyl butanol and the phenylethyl alcohol were only detected in all the batches containing *D. hansenii*. These compounds showed a strong negative correlation with OTA amounts ($\rho = -0.798$ and $\rho = -0.758$, respectively; Table 3). Both VOCs has been previously related to the antifungal action of yeasts, such as *Saccharomyces cerevisiae* and *Candida stellimalicola* against *Penicillium digitatum* (Belinato et al., 2018) and *H. uvarum* against *B. cinerea* (Ruiz-Moyano et al., 2020). Therefore, these VOCs could be the main ones involved in the antiochratoxigenic activity of *D. hansenii* against *P. nordicum* strains. Previous studies confirmed the effectiveness of *D. hansenii* FHSCC 253H and its main targets of action on *P. nordicum*, affecting to the cell wall structures, but the possible compounds responsible for these effects were not known (Cebrián et al., 2019; Álvarez et al., 2022).

The dimethyl disulfide was the only compound detected from the sulphur reduction (Table 2). Surprisingly, this compound was identified in the presence of both the toxigenic mould and *D. hansenii* and was increased in the treatments Pn15+Dh and Pn26+Dh. Thus, it is needed the presence of both microorganisms to enhance the production of dimethyl disulfide. Due to *P. nordicum* strains and *D. hansenii* individually did not produce this compound, it is impossible to confirm the origin of this VOC. However, its producer could be *D. hansenii* owing to the fact that its production has been described as a mechanism of a biocontrol agent (*Burkholderia ambifaria* H8) to reduce the mycelial growth of toxigenic *Fusarium graminearum* (Chen et al., 2024).

Table 4

Results of principal component analysis (PCA) of volatile compounds from *Penicillium nordicum* strains and *Debaryomyces hansenii* after 14 days of incubation at 20 °C in dry-cured ham-based medium in absence and presence of *D. hansenii* in "mouth to mouth" experiment.

Principal component	Eigenvalue	Variance contribution rate (%)	Cumulative variance contribution rate (%)
1	15.83	51.07	51.07
2	7.59	24.51	75.58
3	4.49	14.51	90.09
4	1.68	5.44	95.53
5	0.91	2.96	98.49
6	0.47	1.51	100.00

The PCA component was performed using the VOCs identified to evaluate the similarities between batches. Six components were extracted (Table 4). The first principal component (PC1) comprised 51.07 % of the variance and the second principal component (PC2) 24.51 %, being the cumulative contribution of both 75.58 %. The findings showed that Pn15 and Pn26 had a similar volatile profile while Pn856 was more distant than the previous ones (Fig. 2). This can be explained due to the different origin of *P. nordicum* strains, which indicates that strains isolated from Spanish dry-cured ham have a different volatile profile than that from Italian ham despite growing in the same matrix in this assay. Interestingly, the volatilome changed in a greater extent in the batches Pn856+Dh and Pn15+Dh due to the addition of the yeast. Moreover, these two batches were those that presented the highest reduction in the OTA content (Table 1). On the other hand, the batch Pn26+Dh was highly correlated with the compounds from lipid oxidation 2-ethyl-1-hexanol (4) and 2-nonanone (8) and the benzaldehyde (20) derived from the amino acid catabolism. However, the batches with *D. hansenii* (Dh, Pn856+Dh, Pn15+Dh and Pn26+Dh) were not related to most of the lipid oxidation compounds that can be due to the antioxidant activity of the yeast in dry-cured meat products (Álvarez et al., 2023b). Interestingly, the 3-methylbutanol (19) and the phenylethyl alcohol (23), highly correlated with the antiochratoxigenic activity of *D. hansenii* (Table 3), were also correlated between them as it is shown in Fig. 2. This could show a synergistic effect of both compounds that would be involved in the effectiveness of *D. hansenii* against *P. nordicum.*

4. Conclusions

D. hansenii FHSCC 253H uses the production of antifungal VOCs as mode of action against OTA-producing *P. nordicum* strains, which have a high impact on the enormous reduction of the mycotoxin generation. The results indicate that the yeast is effective even at a distance, reducing toxin levels without direct contact with the mould. This characteristic suggests that *D. hansenii* FHSCC 253H could offer a broad protective effect throughout the product and potentially benefit nearby dry-cured hams within the curing chamber. These results would allow to enhance the antifungal effect of *D. hansenii* as a biocontrol agent in meat industries due to the presence of their antiochratoxigenic VOCs in all the space of ripening chambers.

Funding

This work is part of the grant PID2019-104260 GB-I00 funded by MCIN/AEI/10.13039/501100011033, and of the grant GR21130 funded by *Junta de Extremadura* and by "European Union ERDF A way of making Europe". Eva Cebrián is recipient of the grant PRE2020-093605 funded by MCIN/AEI/10.13039/501100011033 and by "ESF Investing in your future". Q-Exactive Plus mass spectrometer to OTA analysis was funded by the Spanish Ministerio de Economía y Competitividad (Ref. UNEX-AE-3394).

CRediT authorship contribution statement

Elia Roncero: Writing – original draft, Investigation, Formal

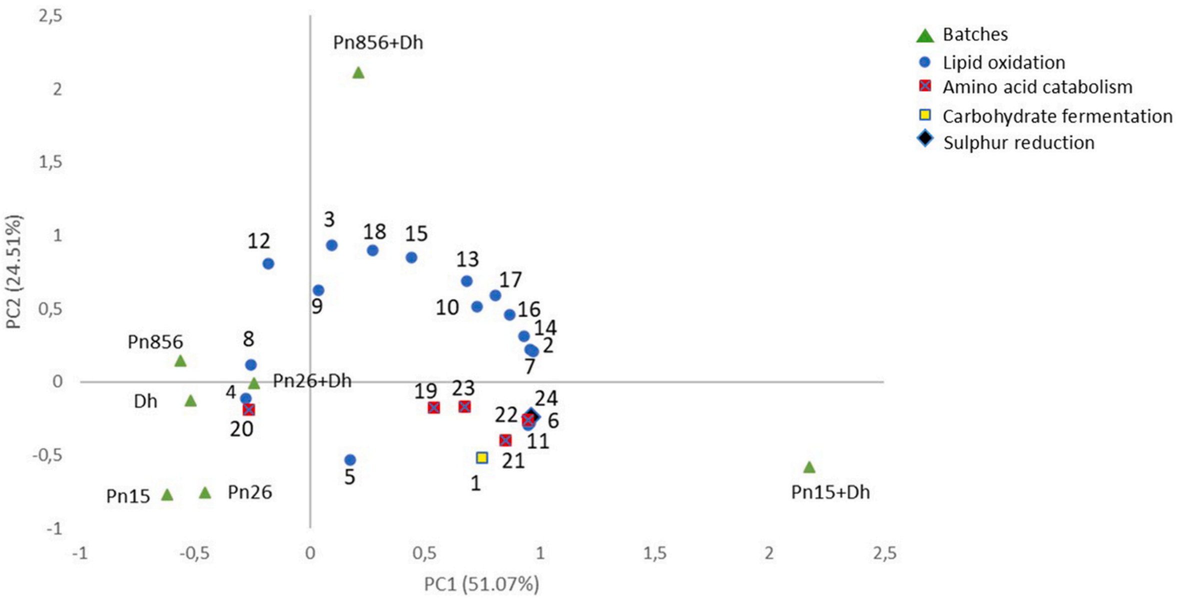

Fig. 2. Loading plot after principal component analysis (PCA) for discriminating volatile compounds from dry-cured ham medium inoculated with *Penicillium nordicum* and *Debaryomyces hansenii* after 14 days. Numbers correspond to those in the 'Number' column of Table 2. Triangles in green are the batches. The square in yellow is a volatile compound derived from the carbohydrate fermentation, plots in blue are volatile compounds from the lipid oxidation, crossed-out squares in red are volatile compounds from amino acid catabolism and diamond in black is a volatile compound from sulphur reduction. Batches: Pn15, Pn26, Pn856: 3 μL of 10^6 spores/mL of each strain of *P. nordicum* (FHSCC Pn15, FHSCC Pn26, BFE 856). Pn15+Dh, Pn856+Dh, Pn856+Dh: 3 μL of 10^6 spores/mL of each strain of *P. nordicum* plus 100 μL of 10^6 cells/mL of *D. hansenii* FHSCC 253H inoculated in an upper dry-cured ham medium plate. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

analysis. **María J. Andrade:** Writing – review & editing, Validation, Funding acquisition, Conceptualization. **Micaela Álvarez:** Writing – original draft, Investigation, Formal analysis. **Eva Cebrián:** Investigation. **Mar Rodríguez:** Writing – review & editing, Validation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Alapont, C., López-Mendoza, M. C., Gil, J. V., & Martínez-Culebras, P. V. (2014). Mycobiota and toxigenic *Penicillium* species on two Spanish dry-cured ham manufacturing plants. *Food Additives & Contaminants: Part A, 31*(1), 93–104. https://doi.org/10.1080/19440049.2013.849007

Albarracín, A. G., Petras, D., Tobares, R. A., Aksenov, A. A., Wang, M., Juncosa, F., Sayago, P., Moyano, A. J., Dorrestein, P. C., & Smania, A. M. (2020). Fungal–bacterial interaction selects for quorum sensing mutants with increased production of natural antifungal compounds. *Communications Biology, 3*(1), 670. https://doi.org/10.1038/s42003-020-01342-0

Álvarez, M., Andrade, M. J., Cebrián, E., Roncero, E., & Delgado, J. (2023b). Perspectives on the probiotic potential of indigenous moulds and yeasts in dry-fermented sausages. *Microorganisms, 11*(7), 1746. https://doi.org/10.3390/microorganisms11071746

Álvarez, M., Andrade, M. J., García, C., Rondán, J. J., & Núñez, F. (2020). Effects of preservative agents on quality attributes of dry-cured fermented sausages. *Foods, 9*(10), 1505. https://doi.org/10.3390/FOODS9101505

Álvarez, M., Delgado, J., Núñez, F., Cebrián, E., & Andrade, M. J. (2021). Proteomic analyses reveal mechanisms of action of biocontrol agents on ochratoxin A repression in *Penicillium nordicum*. *Food Control, 129*, Article 108232. https://doi.org/10.1016/j.foodcont.2021.108232

Álvarez, M., Delgado, J., Núñez, F., Roncero, E., & Andrade, M. J. (2022). Proteomic approach to unveil the ochratoxin A repression by *Debaryomyces hansenii* and rosemary on *Penicillium nordicum* during dry-cured fermented sausages ripening. *Food Control, 137*, Article 108695. https://doi.org/10.1016/J.FOODCONT.2021.108695

Álvarez, M., Núñez, F., Cebrián, E., Roncero, E., & Andrade, M. J. (2023a). Effect of selected agents for ochratoxin A biocontrol on the colour, texture and volatile profile of dry-cured fermented sausages. *Journal of the Science of Food and Agriculture, 103*(15), 7862–7868. https://doi.org/10.1002/jsfa.12868;
It is a key reference as it demonstrates that inoculation of a dry-cured meat derivative with D. hansenii would not have a negative effect on the colour, texture and volatile compounds of the product.

Andrade, M. J., Peromingo, B., Rodríguez, M., & Rodríguez, A. (2019). Effect of cured meat product ingredients on the *Penicillium verrucosum* growth and ochratoxin A production. *Food Control, 96*, 310–317. https://doi.org/10.1016/j.foodcont.2018.09.014

Andrade, M. J., Thorsen, L., Rodríguez, A., Córdoba, J. J., & Jespersen, L. (2014). Inhibition of ochratoxigenic moulds by *Debaryomyces hansenii* strains for biopreservation of dry-cured meat products. *International Journal of Food Microbiology, 170*, 70–77. https://doi.org/10.1016/J.IJFOODMICRO.2013.11.004

Battilani, P., Pietri, A., Giorni, P., Formenti, S., Bertuzzi, T., Toscani, T., Virgili, R., & Kozakiewicz, Z. (2007). *Penicillium* populations in dry-cured ham manufacturing plants. *Journal of Food Protection, 70*(4), 975–980. https://doi.org/10.4315/0362-028X-70.4.975

Belinato, J. R., Kupper, K. C., & Augusto, F. (2018). *In vivo* investigation of the volatile metabolome of antiphytopathogenic yeast strains active against *Penicillium digitatum* using comprehensive two-dimensional gas chromatography and multivariate data analysis. *Microchemical Journal, 141*, 362–368. https://doi.org/10.1016/j.microc.2018.05.047;
It is a key reference as it shows the beneficial effect of volatile compounds againstpathogenic moulds belonging to *Penicillium species*.

Cebrián, E., Núñez, F., Álvarez, M., Roncero, E., & Rodríguez, M. (2022). Biocontrol of ochratoxigenic *Penicillium nordicum* in dry-cured fermented sausages by *Debaryomyces hansenii* and *Staphylococcus xylosus*. *International Journal of Food Microbiology, 375*, Article 109744. https://doi.org/10.1016/J.IJFOODMICRO.2022.109744

Cebrián, E., Núñez, F., Gálvez, F. J., Delgado, J., Bermúdez, E., & Rodríguez, M. (2020). Selection and evaluation of *Staphylococcus xylosus* as a biocontrol agent against toxigenic moulds in a dry-cured ham model system. *Microorganisms, 8*(6), 793. https://doi.org/10.3390/MICROORGANISMS8060793

Cebrián, E., Rodríguez, M., Peromingo, B., Bermúdez, E., & Núñez, F. (2019). Efficacy of the combined protective cultures of *Penicillium chrysogenum* and *Debaryomyces hansenii* for the control of ochratoxin a hazard in dry-cured ham. *Toxins, 11*(12), 1–15. https://doi.org/10.3390/toxins11120710;
It is a key reference as it demonstrates the positive effect of *D. hansenii* in controlling ochratoxin A in dry-cured ham.

Chen, Y., Chen, J., Zhu, Q., & Wan, J. (2022). Ochratoxin A in dry-cured ham: OTA-producing fungi, prevalence, detection methods, and biocontrol strategies—a review. *Toxins, 14*(10), 693. https://doi.org/10.3390/TOXINS14100693

Chen, X., Liu, J., Chen, A. J., Wang, L., Jiang, X., Gong, A., Liu, W., & Wu, H. (2024). *Burkholderia ambifaria* H8 as an effective biocontrol strain against maize stalk rot via producing volatile dimethyl disulfide. *Pest Managment Science, 80*, 4125–4136. https://doi.org/10.1002/ps.8119

Delgado, J., Álvarez, M., Cebrián, E., Martín, I., Roncero, E., & Rodríguez, M. (2023). Biocontrol of pathogen microorganisms in ripened foods of animal origin. *Microorganisms, 11*(6), 1578. https://doi.org/10.3390/MICROORGANISMS11061578

Delgado, J., da Cruz Cabral, L., Rodríguez, M., & Rodríguez, A. (2018). Influence of ochratoxin A on adaptation of *Penicillium nordicum* on a NaCl-rich dry-cured ham-based medium. *International Journal of Food Microbiology, 272*, 22–28. https://doi.org/10.1016/j.ijfoodmicro.2018.02.020

Delgado, N., Olivera, M., Cádiz, F., Bravo, G., Montenegro, I., Madrid, A., Fuentealba, C., Pedreschi, R., Salgado, E., & Besoain, X. (2021). Volatile organic compounds (VOCs) produced by *Gluconobacter cerinus* and *Hanseniaspora osmophila* displaying control effect against table grape-rot pathogens. *Antibiotics, 10*(6), 663. https://doi.org/10.3390/antibiotics10060663

European Commission. (2023). Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum limits for certain contaminants in food and repealing Regulation (EC) No 1881/2006. *Official Journal of the European Union, L119*, 103–157.

Farbo, M. G., Urgeghe, P. P., Fiori, S., Marcello, A., Oggiano, S., Balmas, V., Hassan, Z. U., Jaoua, S., & Migheli, Q. (2018). Effect of yeast volatile organic compounds on ochratoxin A-producing *Aspergillus carbonarius* and *A. ochraceus*. *International Journal of Food Microbiology, 284*, 1–10. https://doi.org/10.1016/j.ijfoodmicro.2018.06.023;
It is a key reference as it demonstrates the antiochratoxigenic effect of certain volatile compounds produced by some yeast species.

Ferrara, M., Perrone, G., & Gallo, A. (2022). Recent advances in biosynthesis and regulatory mechanisms of principal mycotoxins. *Current Opinion in Food Science, 48*, Article 100923. https://doi.org/10.1016/J.COFS.2022.100923

Galván, A. I., Hernández, A., Córdoba, M. de G., Martín, A., Serradilla, M. J., López-Corrales, M., & Rodríguez, A. (2022). Control of toxigenic *Aspergillus* spp. in dried figs by volatile organic compounds (VOCs) from antagonistic yeasts. *International Journal of Food Microbiology, 376*, Article 109772. https://doi.org/10.1016/J.IJFOODMICRO.2022.109772

Gil-Serna, J., Patiño, B., Cortés, L., González-Jaén, M. T., & Vázquez, C. (2011). Mechanisms involved in reduction of ochratoxin A produced by *Aspergillus westerdijkiae* using *Debaryomyces hansenii* CYC 1244. *International Journal of Food Microbiology, 151*(1), 113–118. https://doi.org/10.1016/j.ijfoodmicro.2011.08.012

Hernández-Montiel, L. G., Gutiérrez-Perez, E. D., Murillo-Amador, B., Vero, S., Chiquito-Contreras, R. G., & Rincón-Enríquez, G. (2018). Mechanisms employed by *Debaryomyces hansenii* in biological control of anthracnose disease on papaya fruit. *Postharvest Biology and Technology, 139*, 31–37. https://doi.org/10.1016/J.POSTHARVBIO.2018.01.015

Huang, C., Zhang, L., Johansen, P. G., Petersen, M. A., Arneborg, N., & Jespersen, L. (2021). *Debaryomyces hansenii* strains isolated from Danish cheese brines act as biocontrol agents to inhibit germination and growth of contaminating molds. *Frontiers in Microbiology, 12*, Article 662785. https://doi.org/10.3389/FMICB.2021.662785/BIBTEX

Iacumin, L., Arnoldi, M., & Comi, G. (2020). Effect of a *Debaryomyces hansenii* and Lacto*bacillus buchneri* starter culture on *Aspergillus westerdijkiae* ochratoxin A production and growth during the manufacture of short seasoned dry-cured ham. *Microorganisms, 8*(10), 1623. https://doi.org/10.3390/MICROORGANISMS8101623

Iacumin, L., Manzano, M., Andyanto, D., & Comi, G. (2017). Biocontrol of ochratoxigenic moulds (*Aspergillus ochraceus* and *Penicillium nordicum*) by *Debaryomyces hansenii* and *Saccharomycopsis fibuligera* during speck production. *Food Microbiology, 62*, 188–195. https://doi.org/10.1016/J.FM.2016.10.017

Jeleń, H. H., & Grabarkiewicz-Szczęsna, J. (2005). Volatile compounds of *Aspergillus* strains with different abilities to produce ochratoxin A. *Journal of Agricultural and Food Chemistry, 53*(5), 1678–1683. https://doi.org/10.1021/jf0487396

Li, P., Bao, Z., Wang, Y., Su, X., Zhou, H., & Xu, B. (2023). Role of microbiota and its ecological succession on flavor formation in traditional dry-cured ham: A review. *Critical Reviews in Food Science and Nutrition*, 1–17. https://doi.org/10.1080/10408398.2023.2286634

Medina, Á., Jiménez, M., Mateo, R., & Magan, N. (2007). Efficacy of natamycin for control of growth and ochratoxin A production by *Aspergillus carbonarius* strains under different environmental conditions. *Journal of Applied Microbiology, 103*(6), 2234–2239. https://doi.org/10.1111/J.1365-2672.2007.03462.X

Medina-Córdova, N., Rosales-Mendoza, S., Hernández-Montiel, L. G., & Angulo, C. (2018). The potential use of *Debaryomyces hansenii* for the biological control of pathogenic fungi in food. *Biological Control, 121*, 216–222. https://doi.org/10.1016/J.BIOCONTROL.2018.03.002

Ministero della Sanità. (1999). Direttive in materia di controllo ufficiale sui prodotti alimentari: valori massimi ammissibili di micotossine nelle derrate alimentari di origine nazionale, comunitaria e Paesi terzi. *Gazzetta Ufficiale Della Repubblica Italiana, 135*, 52–57.

Munekata, P. E. S., Pateiro, M., Franco, D., & Lorenzo, J. M. (2021). *Pork: Meat quality and processed meat products*. CRC Press. https://doi.org/10.1201/9780429324031

Núñez, F., Lara, M. S., Peromingo, B., Delgado, J., Sánchez-Montero, L., & Andrade, M. J. (2015). Selection and evaluation of *Debaryomyces hansenii* isolates as potential bioprotective agents against toxigenic penicillia in dry-fermented sausages. *Food Microbiology, 46*, 114–120. https://doi.org/10.1016/j.fm.2014.07.019

Núñez, F., Rodríguez, M. M., Bermúdez, M. E., Córdoba, J. J., & Asensio, M. A. (1996a). Composition and toxigenic potential of the mould population on dry-cured Iberian ham. *International Journal of Food Microbiology, 32*(1–2), 185–197.

Núñez, F., Rodríguez, M. M., Córdoba, J. J., Bermúdez, M. E., & Asensio, M. A. (1996b). Yeast population during ripening of dry-cured Iberian ham. *International Journal of Food Microbiology, 29*(2–3), 271–280.

Ostry, V., Malir, F., Toman, J., & Grosse, Y. (2016). Mycotoxins as human carcinogens—the IARC Monographs classification. *Mycotoxin Research, 33*(1), 65–73. https://doi.org/10.1007/S12550-016-0265-7

Peromingo, B., Andrade, M. J., Delgado, J., Sánchez-Montero, L., & Núñez, F. (2019). Biocontrol of aflatoxigenic *Aspergillus parasiticus* by native *Debaryomyces hansenii* in dry-cured meat products. *Food Microbiology, 82*, 269–276. https://doi.org/10.1016/J.FM.2019.01.024

Peromingo, B., Núñez, F., Rodríguez, A., Alía, A., & Andrade, M. J. (2018). Potential of yeasts isolated from dry-cured ham to control ochratoxin A production in meat models. *International Journal of Food Microbiology, 268*, 73–80. https://doi.org/10.1016/J.IJFOODMICRO.2018.01.006

Rodríguez, A., Bernáldez, V., Rodríguez, M., Andrade, M. J., Núñez, F., & Córdoba, J. J. (2015). Effect of selected protective cultures on ochratoxin A accumulation in dry-cured Iberian ham during its ripening process. *LWT - Food Science and Technology, 60*(2), 923–928. https://doi.org/10.1016/J.LWT.2014.09.059

Rodríguez, M., Núñez, F., Córdoba, J. J., Sanabria, C., Bermúdez, E., & Asensio, M. A. (1994). Characterization of *Staphylococcus* spp. and *Micrococcus* spp. isolated from Iberian ham throughout the ripening process. *International Journal of Food Microbiology, 24*, 329–335.

Rodríguez, A., Rodríguez, M., Martín, A., Delgado, J., & Córdoba, J. J. (2012a). Presence of ochratoxin A on the surface of dry-cured Iberian ham after initial fungal growth in the drying stage. *Meat Science, 92*(4), 728–734. https://doi.org/10.1016/J.MEATSCI.2012.06.029

Rodríguez, A., Rodríguez, M., Martín, A., Núñez, F., & Córdoba, J. J. (2012b). Evaluation of hazard of aflatoxin B1, ochratoxin A and patulin production in dry-cured ham and early detection of producing moulds by qPCR. *Food Control, 27*(1), 118–126. https://doi.org/10.1016/J.FOODCONT.2012.03.009

Ruiz-Moyano, S., Hernández, A., Galvan, A. I., Córdoba, M. G., Casquete, R., Serradilla, M. J., & Martín, A. (2020). Selection and application of antifungal VOCs-producing yeasts as biocontrol agents of grey mould in fruits. *Food Microbiology, 92*, Article 103556. https://doi.org/10.1016/j.fm.2020.103556;
This is a key reference as it uses the same technique used in our study to assess the effect of volatile compounds.

Sánchez-Montero, L., Córdoba, J. J., Peromingo, B., Álvarez, M., & Núñez, F. (2019). Effects of environmental conditions and substrate on growth and ochratoxin A production by P*enicillium verrucosum* and *Penicillium nordicum*. Relative risk assessment of OTA in dry-cured meat products. *Food Research International, 121*, 604–611. https://doi.org/10.1016/j.foodres.2018.12.025

Schrenk, D., Bodin, L., Chipman, J. K., del Mazo, J., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L., Leblanc, J. C., Nebbia, C. S., Nielsen, E., Ntzani, E., Petersen, A., Sand, S., Schwerdtle, T., Vleminckx, C., Wallace, H., Alexander, J., Dall'Asta, C., Mally, A., … Bignami, M. (2020). Risk assessment of ochratoxin A in food. *EFSA Journal, 18*(5). https://doi.org/10.2903/j.efsa.2020.6113

Steel, C. C., Schwarz, L. J., Qiu, Y., Schueuermann, C., Blackman, J. W., Clark, A. C., & Schmidtke, L. M. (2020). Thresholds for *Botrytis* bunch rot contamination of Chardonnay grapes based on the measurement of the fungal sterol, ergosterol. *Australian Journal of Wine and Grapes Research, 26*, 79–89. https://doi.org/10.1111/ajgw.12417

Sunesen, L. O., Trihaas, J., & Stahnke, L. H. (2004). Volatiles in a sausage surface model-influence of *Penicillium nalgiovense, Pediococcus pentosaceus*, ascorbate, nitrate and temperature. *Meat Science, 66*(2), 447–456. https://doi.org/10.1016/S0309-1740(03)00133-5

Toman, J., Pickova, D., Rejman, L., Ostry, V., & Malir, F. (2024). Investigation of ochratoxin A in air-dry-cured hams. *Meat Science, 109605*. https://doi.org/10.1016/J.MEATSCI.2024.109605

Vulić, A., Vahčić, N., Hengl, B., Gross-Bošković, A., Jurković, M., Kudumija, N., & Pleadin, J. (2016). Assessment of possible human exposure to ochratoxin A in Croatia due to the consumption of dry-cured and fermented meat products. *Food Additives & Contaminants: Part A, 33*(9), 1428–1434. https://doi.org/10.1080/19440049.2016.1213428

Zhang, X., Cheng, Z., Ma, L., & Li, J. (2017). A study on accumulation of volatile organic compounds during ochratoxin A biosynthesis and characterization of the correlation in *Aspergillus carbonarius* isolated from grape and dried vine fruit. *Food Chemistry, 227*, 55–63. https://doi.org/10.1016/j.foodchem.2016.12.061

Zhu, Z., Zhou, Y. J., Kang, M. K., Krivoruchko, A., Buijs, N. A., & Nielsen, J. (2017). Enabling the synthesis of medium chain alkanes and 1-alkenes in yeast. *Metabolic Engineering, 44*, 81–88. https://doi.org/10.1016/J.YMBEN.2017.09.007