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Microbial diversity in dry-cured Iberian ham: an approach to the concept of microbial terroir

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Abstract

Microbial *terroir* has been scarcely studied in the dry-cured ham industry. The microbial population and ochratoxin A (OTA) contamination in two Spanish facilities located in a specific geographical region (Salamanca) with differences in the environmental conditions used for the ham processing were evaluated for establishing the microbial *terroir*. Differences between the levels of Gram-positive catalase-positive cocci (GCC+), lactic acid bacteria (LAB), yeasts and moulds were found at the processing stage (post-salting, drying and cellar) and facility levels. Such variations could be responsible for the development of different flavour profiles, which indicate their potential functionality for determining the microbial *terroir* in the artisan dry-cured Iberian ham industry, despite the fact that this work constitutes a preliminary study. Furthermore, it has been demonstrated that the differences in the microbial population throughout the processing of both sampled facilities could directly impact on one of the main concerns of the meat industry from the food safety point of view consisting of the accumulation of OTA, since differences in its levels were detected between both checked facilities. These findings could lead to the enforcement of the *terroir* concept in artisan dry-cured Iberian ham elaboration, resulting in a value enhancement that might be reflected in economic and social positive results for the territory. Nevertheless, microbial *terroir* in the dry-cured Iberian ham must be further explored by the increase of the number of studied facilities.

Keywords: dry-cured Iberian ham; microbial *terroir*; tradition; territory; ochratoxin A.

1. Introduction

Terroir is the term that refers, in the agri-food sector, to the unique character achieved by the combination of environmental and cultural factors specific to a given place (Trubek, 2009). The concept of *terroir* generates an association with quality-origin in consumers (Aurier et al., 2005; Charters et al., 2017), being able to produce an advantage when selling a product (Bowen, 2010; Josling, 2006). However, the breadth of such term goes beyond the food field, reaching the anthropological and cultural ones (Castelló, 2021; Hammer, 2011; Tholstrup, 2012). *Terroir* adds value to traditional elements and is ultimately a social construction generated by the agents involved in the development of the value of a region (Faure, 1999). It indicates a social awareness and action (Barham, 2016; Leedon et al., 2021), increases the rootedness of the inhabitants to an area (Hinojosa et al., 2016), their sense of community (Charters et al., 2017) and the connection of visitors to it (Silva et al., 2021).

The concept of *terroir* has reached its maximum extension in the wine industry, where it is able to communicate the regional identity, encouraging the development of rural places (Marlowe & Lee, 2018; Prévost et al., 2014). The impact generated by the wine industry is also intended to be built in other food industries, for which studies that affirm the uniqueness of products made in different places are being developed (Peršurić et al., 2018; Povolo et al., 2013; Scholz et al., 2018). The concept of *terroir* is thus beginning to spread in the field of cheese (Turbes et al., 2016), chocolate (Engeseth & Ac Pangan, 2018), tea (Besky, 2014) and in a wide variety of crops (Lucini et al., 2020).

Regarding the dry-cured ham industry, several studies have confirmed the specific sensorial properties of dry-cured Iberian ham depending on different factors, such as breed, feeding and processing (Cava et al., 1997; Martín et al., 2001; Ventanas et al., 2007). The concept of *terroir* is being applied in this industry to defend the manufacturing method linked to the traditions (Augustin-Jean et al., 2012; Edwards, 2011).

Additionally, microbial *terroir* is a concept within *terroir* (Belda et al., 2021), which refers to the unique characteristics generated by the action of the microorganisms involved in the processing of foodstuff (Bokulich et al., 2016) and depends on both environmental conditions (geographical, climatic, etc) and production management (Gobbi et al., 2022). The microbial *terroir* is used and researched mainly in the wine industry (Gilbert et al., 2014), where the best practices to crop management and vinification are being discovered (Burns et al., 2016; Capozzi et al., 2015; de Celis et al.,

2019). Similarly, it has been explored in fermented food products cooked in a professional kitchen (Peraza & Perron, 2022). However, there is no research on the microbial *terroir* in the dry-cured Iberian ham industry, although the genetic and volatile compound profiles of yeasts have been associated with the Spanish Protected Designations of Origin of such ham (Andrade et al., 2010) and consequently with specific geographic areas of ripening. Regarding the microbial population of dry-cured Iberian ham, Gram-positive catalase-positive cocci (GCC+), yeasts and moulds consist of the predominant groups for most of the ripening time (Martín et al., 2006; Núñez, Rodriguez, et al., 1996; M. Rodríguez et al., 1994). The microorganisms' influence in the typical sensorial characteristics of the final product has been extensively reported (Gong et al., 2023; Martín et al., 2006; Sánchez-Molinero & Arnau, 2008; Toledano et al., 2019; Zhou et al., 2022). One of the main Spanish areas producing dry-cured Iberian ham is the province of Salamanca, located in the west of the country. In fact, Salamanca is the municipality which has the highest number of meat companies in Spain (Agencia Española de Seguridad Alimentaria y Nutrición, 2023). The roots of this industry in Salamanca can be explained by its extensive pastures and its Mediterranean climate with cold winters, which enables the dry-curing process without artificial cold rooms. Furthermore, the "chacinera" tradition of the meat industry in this area has been transmitted from generation to generation as nourishment and various artistic expressions (Hortelano et al., 2019). The artisanal nature of the process promotes the growth of a unique microbial population, which may allow the application of microbial *terroir* for dry-cured Iberian ham production. Nonetheless, this concept is not only applicable to sensorially beneficial microorganisms but also to those pose a hazard from a food safety point of view. Ochratoxigenic moulds are one of the main hazards throughout the ripening of dry-cured Iberian ham due to their ochratoxin A (OTA) production, being *Penicillium nordicum*, *Aspergillus westerdijkiae* and *P. verrucosum* some of the most commonly found in dry-cured meat products (Núñez, Rodríguez, et al., 1996; Schrenk et al., 2020). In 2020, the European Food Safety Authority (EFSA) proclaimed the dry-cured meat products among the largest contributor to chronic dietary exposure of consumers to OTA in most of the European Union countries (Schrenk et al., 2020). OTA is incorporated in the organism through the diet, accumulating over time and showing dangerous health effects, such as the genotoxic, carcinogenic, immunotoxic, neurotoxic and teratogenic ones (Klingelhöfer et al., 2020).

Accordingly, the aim of this work was to evaluate the microbial population in two facilities located in Salamanca with differences in the environmental conditions used for the ham processing, intended for establishing the microbial *terroir*. Moreover, it was evaluated how the unique microbial signature of hams can influence the OTA accumulation.

2. Materials and methods

2.1. Sample collection

Samples were taken in two different facilities (A and B) manufacturing dry-cured Iberian ham located in the province of Salamanca (Castilla y León, Spain), with 18 km of distance between them. The facility A uses a temperature and humidity-controlled room for post-salting and two traditional rooms, one for drying and one as a cellar, in which windows are used to regulate temperature and relative humidity. The facility B performs the post-salting and drying in rooms with controlled temperature and relative humidity, apart from having two traditional rooms used as cellar.

Individual protection equipment was used for sampling a 25 cm^2 surface of dry-cured Iberian hams with sterile scalpels and bags (Scharlau Chemie S.A., Barcelona, Spain). After transporting the samples to the laboratory in a container, they were maintained at 4 ºC for a maximum of 24 h for the microbiological analysis and at -20 ºC for the OTA quantification.

A total of 20 surface samples, coded as "A" or "B" according to the checked facility followed by the number of the sampled ham piece (from 1 to 20), were taken for both analyses (microbiological and toxigenic ones). In the case of the facility A, the samples taken during the post-salting phase of hams corresponded to the codes from A1 to A4, the ones from the drying stage were coded as A5-A10 and the A11-A20 were from the cellar phase. For the facility B, the post-salting ham samples were B1-B6, the samples coded as B7-B15 belonged to hams during the drying stage and the ones sampled in the cellar were B16-B20. The differences in the number of samples were due to the different sizes of the processing rooms in each facility.

Additionally, sampling to analyse the microbial load in the environment of each facility was carried out on Plate Count Agar plates (PCA; Conda Pronadisa, Madrid, Spain) by means of a single stage portable air sampler following the manufacturer's

instructions (Sampl'air Lite AES Laboratoire, Bruz, France). Air volumes of 100 L or 500 L were taken in each facility room.

2.2. Microbial counts

Counts of the main microbial groups found during the processing of dry-cured Iberian ham (GCC+, LAB, yeasts and moulds) were performed by using five ten-fold serial dilutions in 1% (w/v) peptone water (Conda Pronadisa). Plates were incubated under specific conditions for each microbial group. Concretely, PCA was used for the enumeration of mesophilic aerobic bacteria after 3 days of incubation. Mannitol Salt Agar (MSA; Conda Pronadisa) containing 0.05 g/L cycloheximide (Sigma Aldrich Co., St. Louis, MO, USA) was used for counting GCC+ after incubating for 2 days at 30 ºC. Man Rogosa and Sharpe agar (MRS; Conda Pronadisa) was used for the LAB growth during 4-5 days at 30 ºC in microaerophilic conditions. Dichloran-Glycerol Agar (DG18; Conda Pronadisa) was used for yeasts and moulds counts after 4-7 days of incubation at 25 ºC. Plates with more than 10 colonies and less than 300 colonies were considered after their incubation (International Organization for Standarization (ISO), 2007). Results were expressed as CFU/cm^2.

The air samples were incubated at the same conditions previously mentioned for PCA and the results were expressed as CFU/L.

2.3. Isolation of microorganisms

After counting, 5 representative colonies of GCC+, LAB, yeasts and moulds were selected from each ham. The selection for yeasts was performed considering the colour and morphology of their colonies, while the phenotypic characteristics were used for bacteria and moulds. LAB were isolated in MRS with cycloheximide after incubating at 30 ºC for 4 days. GCC+ were isolated in MSA without cycloheximide after incubating at 30 °C for 2 days. Yeasts and moulds were isolated in Malt Extract Agar (MEA; 20 g of malt extract (Conda Pronadisa), 20 g of glucose (Labbox Labware S.L., Barcelona, Spain), 1 g of bacteriological peptone (Conda Pronadisa), 20 g of bacteriological agar (Conda Pronadisa), 1 L of distilled water) and incubated at 25 ºC for 3 days.

The pure culture of bacteria and yeasts were kept on agar plates and conserved at 4 ºC for short periods and at -80 ºC with 10% (v/v) glycerol for extended periods. The mould spores were kept at -80 ºC using Phosphate Buffered Saline (PBS; 0.32 g of sodium dihydrogen phosphate (Scharlau Chemie S.A.), 1.09 g of disodium hydrogen phosphate

(Scharlau Chemie S.A.), 9 g of NaCl (Thermo Fisher Scientific, Waltham, MA, USA), 1 L of distilled water) with 10% (v/v) glycerol.

2.4. Phenotypic and genotypic identification

Phenotypical tests were firstly carried out to the bacterial isolates according to their similarity. Concretely, gram staining as well as the catalase and oxidase tests were performed by the widely used methodology described by the American Society for Microbiology (2016). Yeasts were individually observed under the microscope Eclipse E200 (Nikon Instruments Inc., Tokyo, Japan), looking for a cell morphology diversity (Iris et al., 2020). Moulds were visually differentiated depending on the colour sporulation (Robert et al., 2020).

The isolate selection was then made based on their microbial phenotypic characteristics, processing phase of isolation and facility of origin for performing the subsequent genotypic identification.

The selected isolates of bacteria (7), yeasts (2) and moulds (7) were identified by 16S rDNA, 18S rDNA and ITS sequencing, respectively (Alía et al., 2016; Andrade et al., 2010; Cebrián et al., 2020). For the extraction of bacterial DNA, the "MasterPure DNA and RNA purification" kit (Lucigen, Middleton, WI, USA) was used. Yeast and mould DNA was extracted following the methodology previously described by Andrade et al., (2006, 2017).

The 16S rDNA was amplified using the primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3'; Metabion International AG, Planegg, Germany) and 1492R (5'-TACGGTTACCTTGTTACGACTT-3'; Metabion International AG) as described by Cebrián et al. (2020).

The 18S rDNA was amplified using the primers P108 and M3989 as described by Andrade et al. (2010), with slight modifications. The reaction was carried out using the EmeraldAmp GT PCR Master Mix commercial kit (Takara Bio Inc., Shiga, Japan) in a 50 µL final volume containing 25 µL of Emerald (Takara Bio Inc.), 1 µL of P108 (100 ng/µL; 5'-ACCTGGTTGATCCTGCCAGT-3'; Metabion International AG), 1 µL of M3989 (100 ng/µL; 5'-CTACGCAAACCTCTACGGAAACCTTGTTACGACT-3' Metabion International AG), 18 µL of Emerald water (Takara Bio Inc.) and 5 µL of DNA (10 ng/µL). The amplification program consisted of an initial cycle at 94 ºC for 3 min, 29

cycles at 92 ºC for 1 min, 47 ºC for 1 min and 58 ºC for 5 min and one last cycle at 58 ºC for 5 min.

The ITS region was amplified using the primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3'; Metabion International AG) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'; Metabion International AG) as described by Alía et al. (2016).

After carrying out the PCR reactions in the thermocycler (Eppendorf AG, Hamburg, Germany), the resulting products were revealed by electrophoresis on 1% (w/v) agarose gels using TAE1x (98% distilled water, 2% (v/v) TAE50x (242 g of 121.14 MW Tris Base (Scharlau Chemie S.A.), 57.1 mL of 17.5 M glacial acetic acid (Scharlau Chemie S.A.), 100 mL of 0.5M EDTA pH 8 (Scharlau Chemie S.A.), 1 L of milliQ water, pH 8)) and stained with SYBR Safe DNA Gel Stain (Thermo Fisher Scientific) at 90 V for 1 h.

The PCR product sequencing was performed by the Service of Applied Techniques to Bioscience (STAB, Universidad de Extremadura, Spain). The obtained sequences were then aligned with the National Center for Biotechnology Information (NCBI, Bethesda MD, USA) database. The identity of the evaluated isolates was established by the basis of the highest score.

Pulsed-field Gel Electrophoresis (PFGE) following the indications of the chromosomal preparation of *Hansenula wingei* (Bio-Rad laboratories, Hercules, CA, USA) utilised as a marker was carried out for the differentiation of the selected yeast isolates at strain level. A 0.8% (w/v) agarose gel was made using SeaKem Gold Agarose (Lonza, Morristown, NJ, USA) according to Corredor et al. (2003). The PFGE was carried out with TAE1x in the PFGE chamber (Bio-Rad laboratories) at 14 ºC, with a reorientation angle of 106º, 3 V/cm, 500 sec, 30 h (Cebrián et al., 2022). The gel was visualised in a UV transilluminator (Labnet International Inc., Edison, NJ, USA) after staining in a 400 mL water bath with 100 µL of SYBR Gold nucleic acid gel stain (Thermo Fisher Scientific) for 45-60 min while shaking. The obtained PFGE profiles were visually analysed to differentiate the yeasts at strain level.

2.5. Ochratoxin A extraction

OTA extraction was performed using the QuEChERs procedure previously described by Delgado et al. (2018). Briefly, the method involved the extraction of components using water and acetonitrile (Scharlau Chemie S.A.) acidified with 0.1%

(v/v) acetic acid (Thermo Fisher Scientific) before phase partitioning with NaCl (Scharlau Chemie S.A.) and anhydrous $MgSO_4$ (Scharlau Chemie S.A.). The resulting mixture was promptly and vigorously shaken manually, followed by centrifugation for 5 minutes at 4 °C at 5300 rpm (Diglicen 21R, Ortoalresa, Spain). Subsequently, a 0.75 mL aliquot from the supernatants was evaporated. The resulting dry extracts were then redissolved in 0.75 mL of HPLC-grade methanol (Scharlau Chemie S.A.), filtered through a 0.22 μm pore size nylon membrane (Jet Bio-Filtration Co., Ltd., China), and stored at -20 °C until use. The analysis was carried out in a Dionex Ultimate 3000 UHPLC (Thermo Fisher Scientific), having a degassing system, a Quaternary HPLC pump, an autosampler device and a thermostated (45 °C) Thermo Fisher Accucore Aq C18 (150 × 2.1 mm) column 2.6 μm particle size (Cebrián et al., 2020). The results were expressed in ng/cm². The limit of detection (LOD) obtained in this study was 0.05 μg/kg, and the limit of quantification (LOQ) was 0.15 μg/kg. They were calculated through the same matrix from untreated samples, being the lowest evaluable concentration level at which the qualifier ion signal exceeds the noise level by a factor of 3.5 and 10, respectively (Delgado et al., 2018).

2.6. Statistical analysis

Statistical analysis was performed using the IBM SPSS Statistics v. 22.0 software (IBM, Armonk, NY, USA). To check the normality of the data set, the Shapiro-Wilk test was performed. The homogeneity of variances was checked by means of the Levene statistic. For the group of data normally distributed and with homogeneity of variance (mould and yeast counts), a one-way ANOVA was used to determine the groups between which there were significant differences ($p \leq 0.05$). When the data set failed the normality test (almost all bacterial counts and OTA results), the Kruskal-Wallis H test (for more than two independent samples) or the U of Mann-Whitney test (for two independent samples) were performed to reveal whether significant differences ($p \leq 0.05$) between the groups were noticed.

3. Results and discussion

3.1. Microbial population

The composition of the microbial population growing in dry-cured ham during their processing and its contribution to their outstanding organoleptic characteristics have been long studied (Andrade et al., 2009; Gong et al., 2023; Martínez-Onandi et al., 2019; Toledano et al., 2011). When the counts of GCC+, LAB, yeasts and moulds in the 40

surface ham samples obtained from the two facilities were established, differences between them were found depending on the processing stage, as expected (Table 1).

Table 1. Gram-positive catalase-positive cocci (GCC+), lactic acid bacteria (LAB) yeast and mould levels on the surface of dry-cured Iberian hams analysed in two facilities (A and B) at the post-salting, drying and cellar stages.

Facility	Microbial group	Post-salting counts (log CFU/cm^2)	Drying counts (log CFU/cm^2)	Cellar counts (log CFU/cm^2)
A	GCC+	4.96 ± 0.15	nd*	3.21 ± 0.56
	LAB	4.77 ± 0.37	4.57 ± 0.51	3.48 ± 0.49*
	Yeasts	6.39 ± 0.25	6.39 ± 0.56	nd*
	Moulds	nd[a]	5.76 ± 0.22	5.64 ± 0.00[b]
B	GCC+	4.22 ± 0.75	4.93 ± 0.21*	3.72 ± 0.70
	LAB	3.42 ± 1.09	4.95 ± 0.16	4.93 ± 0.24*
	Yeasts	nd	6.36 ± 0.45	5.90 ± 0.27*
	Moulds	nd	6.03 ± 0.34	nd

The results are presented as mean of the counts of each microbial group sampled as explained in section 2.1. ± standard deviation.

[a] nd: counts lower than the limit of quantification (5 log CFU/cm^2 in the case of yeasts and moulds and 2 log CFU/cm^2 in the case of GCC+).

[b] Standard deviation is equal to zero because only one count value was obtained.

* Indicates statistical differences ($p \leq 0.05$) between the counts of the same microbial group in both facilities.

Regarding GCC+, the decrease of their counts was observed from post-salting (about 5 log CFU/cm^2) to cellar in the facility A, despite their low presence in drying stage. On the contrary, their highest level in the facility B (about 5 log CFU/cm^2) was obtained in drying, while cellar showed the lowest one (about 3 log CFU/cm^2). Such GCC+ counts differ from the ones reported by Rodríguez et al. (1994) in dry-cured Iberian ham, since the post-salting quantities found by these authors were 3 log higher than the ones from the rest of the processing phases. These differences in the levels of GCC+ could be the responsible for variations in the compounds derived from reactions during

ripening, such as proteolysis and lipolysis (Johansson et al., 1994; Olesen et al., 2004; Søndergaard & Stahnke, 2002). The highest proportion of GCC+ in the drying stage of the facility B is remarkable and might result in a higher content of peptides and amino acids than in the facility A that will be transformed into volatile compounds by microbial enzymatic reactions due to their proteolytic and lipolytic activities (Rodríguez et al., 1998). This fact could also lead to less rancidity and more intense red colour in hams from the facility B than from the facility A due to the catalase and nitrate and nitrite reductase activities inherent to such microbial group (Martínez-Onandi et al., 2019; M. Rodríguez et al., 1994). The sensory impact of GCC+ on the previously mentioned characteristics has been proved in meat products such as dry cured sausages inoculated with them (Álvarez et al., 2023).

A different tendency in the LAB counts was observed between both facilities too, being the microbial group with the largest variations. Concretely, they decreased throughout the maturation process in the facility A (from about 5 to 3.5 log CFU/cm^2), while an increase was detected in the facility B with similar counts in drying and cellar stages (about 5 log CFU/cm^2). These differences could be responsible for the development of different flavour profiles due to their participation in the formation of volatile compounds (Johansson et al., 1994; Ozogul & Hamed, 2018; Toledano et al., 2011). When compared with previously reported LAB counts, their cellar levels in the facility B were higher than those previously reported by other authors for Spanish dry-cured ham (Huerta et al., 1988; Toledano et al., 2019), while those from the facility A were lower.

Yeasts consisted of the microbial group showing the highest counts in both facilities. Their presence can result in dry-cured Iberian hams with a high presence of alcohols, aldehydes, esters or ketones (Andrade et al., 2009). Levels around 6 log CFU/cm^2 were detected in the two first stages of the facility A, although this microbial group had lower levels in the cellar. Similar results were found for them in the facility B, but their lowest levels were detected in the post-salting. The counts of this microbial group were found within the usual range according to other authors (Núñez, Rodriguez, et al., 1996).

Similar mould loads (about 6 log CFU/cm^2) were found in drying of both facilities and cellar in the facility A, despite their lower levels in post-salting of facilities A and B and cellar in the facility B. These counts match those expected in dry-cured Iberian ham

304 (Rodríguez et al., 2012), and the absence of moulds during the post-salting phase (Asefa
305 et al., 2010).

306 A total of 6 environmental samples were taken from the facility A and 14 were
307 taken from the facility B to evaluate the influence of environmental microorganisms in
308 shaping the final microbial *terroir* of dry-cured Iberian ham. The facility A's
309 environmental samples showed different loads in the post-salting (18 CFU/m^3), drying
310 (117 CFU/m^3) and cellar phases (549 CFU/m^3). In the facility B the counts were
311 significantly higher ($p \leq 0.05$) than in the facility A's post-salting (108 CFU/m^3) and
312 cellar phases (1302 CFU/m^3), but not in the drying one (384 CFU/m^3). These differences
313 were not correlated with those achieved on the ham counts, except in the case of cellar's
314 LAB, which were significantly higher in the facility B than in the facility A. Although it
315 was not carried out an identification of the environmental microorganisms, their
316 implantation on the surface of dry-cured meat products has been studied by other authors,
317 who reported lower environmental counts that the ones found in this work (Asefa et al.,
318 2010; Comi & Iacumin, 2013).

319 **3.2. Microorganisms' identification**

320 After counting and selecting 5 representative colonies of each studied microbial
321 groups, the phenotypical characteristics of the 280 isolates were evaluated (Table 2).
322 Considering the obtained results, a total of 6 isolates from the facility A and 7 isolates
323 from the facility B were selected for being identified by sequencing (Table 2) and related
324 to the microbial *terroir* of each sampled facility.

325 **Table 2.** Number of bacteria, yeast and mould isolates obtained from the surface of
326 dry-cured Iberian ham analysed in two facilities (A and B) at the post-salting, drying and
327 cellar stages. The number in parentheses refers to the number of isolates selected for being
328 identified by sequencing.

Facility	Microorganism	Post-salting	Drying	Cellar
A	Bacteria	16 (1)	1	18 (2)
	Yeasts	17	25 (1)	43
	Moulds	1 (1)	13 (1)	17 (3)
B	Bacteria	7 (1)	45 (1)	10 (2)
	Yeasts	24 (1)	13	25
	Moulds	0	5 (2)	0

329

330 A total of 7 bacterial isolates were identified by sequencing of the 16S rDNA
331 region, resulting 5 in *Staphylococcus equorum*, 1 in *Brevibacterium sanguinis* and 1 in
332 *Pseudomonas sp*, (Table 3). *S. equorum* was the GCC+ most frequently identified after
333 sequencing in both facilities (Table 3). On the contrary, the majority GCC+ found in
334 previous studies focused on dry-cured Iberian hams was *Staphylococcus xylosus*
335 (Rodríguez et al., 1994, 1996). Both GCC+ species (*S. xylosus* and *S. equorum*) generate
336 different aromas, and their participation will thus be responsible for different attributes in
337 the final products (Søndergaard & Stahnke, 2002). The Gram-positive *B. sanguinis* was
338 also identified in the facility A (Table 3). Although this species has not been reported for
339 meat products, *Brevibacterium* sp. has been found in other ripened products, such as
340 cheese (Anast et al., 2019). A black colony-forming bacteria was isolated in MEA from
341 the facility B, identified as *Pseudomonas sp.* (Table 3), which is believed to be *P.*
342 *fluorescens* because this specie is known to produce black spots on the surface of hams
343 during the post-salting phase (Andrade et al., 2012), coinciding with that observed in the
344 piece from which the sample was taken.

345 **Table 3.** Molecular identification of microbial isolated from dry-cured Iberian ham
346 in two facilities (A and B).

Bacteria					
Facility	**Isolate code**	**Identities GenBank**	**Homology to GenBank (%)**	**GenBank accession number[a]**	**Isolation phase**
A	A2.1	*Staphylococcus equorum*	100	KY940339.1	Post-salting
	A5.5	*S. equorum*	99.93	KY940339.1	Cellar
	A13.3	*Brevibacterium sanguinis*	99.85	KU904403.1	Cellar
B	B11.3	*S. equorum*	99.93	KY940339.1	Drying
	B19.4	*S. equorum*	99.79	KY940339.1	Cellar
	B21.4	*S. equorum*	100	MN229551.1	Cellar
	B1P1	*Pseudomonas gessardii*	99.71	MN069032.1	Post-salting

		Pseudomonas paralactis	99.71	MT071746.1	
		Pseudomonas fluorescens	99.71	MN685247.1	
		Pseudomonas azotoformans	99.71	MN173418.1	
		Pseudomonas lactis	99.71	MN062078.1	
Yeasts					
A	A16L5	*Debaryomyces hansenii*	99.81	HQ717147.1	Drying
		Candida sp.	99.81	AY520242.1	
		Kluyveromyces marxianus	99.81	KU058154.1	
B	B1L3	*D. hansenii*	99.81	HQ717147.1	Post-salting
		Candida sp.	99.81	AY520242.1	
		K. marxianus	99.81	KU058154.1	
Moulds					
A	A1M1	*Alternaria leptinellae*	100	AY278845.1	Post-salting
	A5M3	*Cladosporium floccosum*	100	MK460809.1	Cellar
		Cladosporium herbarum	100	MH399459.1	
		Cladosporium allicinum	100	MF472917.1	
		Cladosporium aerium	100	MF472899.1	
		Cladosporium macrocarpum	100	KC311478.1	
	A12M2	*Penicillium discolor*	99.83	MT832027.1	Cellar

		Penicillium solitum	99.83	NR_119494.1	
		Penicillium commune	99.83	AF348419.1	
	A13M1	*Penicillium verrucosum*	100	KC009832.1	Cellar
		Penicillium albocoremium	100	MH861768.1	
	A17M5	*P. solitum*	99.83	MH860945.1	Drying
		Penicillium cavernicola	99.66	MN413150.1	
		Penicillium speluncae	99.66	MG490866.1	
		P. commune	99.66	MT626053.1	
B	B10M1	*Aspergillus pseudoglaucus*	100	MT316341.1	Drying
		Aspergillus cibarius	100	MK267410.1	
		Aspergillus niveoglaucus	100	AF459728.1	
		Aspergillus ruber	100	MH855375.1	
	B15M4	*A. ruber*	99.82	MH854663.1	Drying

[a] Identification by sequencing and alignment in GenBank as reported in Section 2.4.

None of the 2 yeasts isolates sequenced showed a reliable identification (Table 3). Nonetheless, since *D. hansenii* is the most frequently isolated yeast species from dry-cured Iberian ham (Andrade et al., 2010; Martínez-Onandi et al., 2019; Núñez, Rodríguez, et al., 1996) and that *Kluyveromyces marxianus* has not been previously identified in meat products, it is expected that both isolates belong to the former species.

With the aim to characterise the identified yeast isolates at strain level, PFGE was performed being obtained different patterns for each of them (Figure 1). Since different *D. hansenii*'s technological characteristics have been related to different chromosomal polymorphism (Huang et al., 2021), it is assumed that the diverse biotypes of yeasts may develop distinctive aromatic profiles due to the differences on their enzymatic activity

and modifications of the flavour enhancement and volatile compounds (Andrade et al., 2009; Gong et al., 2023; Simoncini et al., 2015). This characteristic could relate the dry-cured Iberian ham microbial population to microbial *terroir* as it is done within the wine industry, in which yeasts comprise the main microbial group associated with the generation of a different product (Capozzi et al., 2015; Gilbert et al., 2014; Gobbi et al., 2022).

Regarding moulds, the presence of *Aspergillus pseudoglaucus* and *Aspergillus ruber* was confirmed in the drying stage of the facility B (Table 3). The later *Aspergillus* species has been frequently isolated from dry-cured ham in such processing phase as well as from other meat products and has been studied due to their miticidal activity (Ortiz-Lemus et al., 2021). However, they have been *in vitro* characterised by their mycotoxins production (Chen et al., 2017). Additionally, two mould isolates from the facility A showed black colour, being identified as *Cladosporium* (isolate from cellar), what is congruent with that reported by Alía et al. (2016), and *Alternaria leptinellae* (isolate from post-salting). The negative effect on the organoleptic characteristics and safety of the final product has been described for both of them (Alía et al., 2016; Pavón et al., 2012). Different *Penicillium* species that could affect the safety of the final product because of the potential ability of some of the species to produce mycotoxins were also found in the facility A (Table 3). Concretely, *Penicillium verrucosum* could have an impact on the OTA levels (Rodríguez et al., 2012). On the other hand, *Penicillium solitum*, that has been found in other meat products (Asefa et al., 2009), has not been described as toxigenic.

3.3. Ochratoxin A quantification

Mould activity is essential for the development of the characteristics of dry-cured Iberian ham, though its activity could generate a negative effect on the safety of the final product too (Martín et al., 2004). Mycotoxins, and specifically OTA, constitute a problem in the dry-cured Iberian ham industry because of their accumulation along the processing in both the surface and the meat (Dall'Asta et al., 2010; A. Rodríguez et al., 2012, 2015). The EFSA considers OTA as a potential hazard, with diet being the main way of incorporation into the human body and paying attention to the consumption of dry-cured meat products, in which is non-regulated except for Italy (della Sanità, 1999; Schrenk et al., 2020).

In this work, OTA levels found in the 20 surface ham samples of each of the facilities were studied (Table 4) since potentially ochratoxigenic moulds were identified

391 (Table 3). OTA contamination ranged from 0.186 to 78.792 ng/cm^2 in the facility A and
392 from 0.181 to 0.673 ng/cm^2 in the facility B. Statistical analyses were carried out by
393 grouping the samples collected from the same industry and stage of processing.
394 Significant differences were only found between the drying and cellar samples and the
395 ones from the post-salting stage within the facility A. Since the facility A has a traditional
396 room for drying, the environmental conditions to which the product is subjected are more
397 climate dependent than those for dry-cured hams from the facility B. Thus, it seems that
398 the technology greatly impacts on the ranges of OTA in the facility A. These findings are
399 related to those previously reporting lower OTA quantification under conditions of
400 controlled temperature at different water activities (Battilani et al., 2010).

401 **Table 4.** Levels of ochratoxin A (OTA) in superficial samples taken in the different
402 phases of curing Iberian hams for both facilities (A and B).

Facility A		**Facility B**	
Post-salting samples	OTA (ng/cm^2)	Post-salting samples	OTA (ng/cm^2)
A1	0.186	B1	0.183
A2	0.196	B2	0.183
A3	0.186	B3	0.184
A4	0.187	B4	0.184
		B5	0.183
		B6	0.183
Drying and cellar samples	OTA (ng/cm^2)	Drying and cellar samples	OTA (ng/cm^2)
A5	0.199	B7	0.183
A6	0.222	B8	0.183
A7	0.200	B9	0.183
A8	78.792	B10	0.181
A9	0.216	B11	0.182
A10	0.247	B12	0.182
A11	1.236	B13	0.357
A12	0.348	B14	0.287
A13	21.608	B15	0.183

A14	13.197	B16	0.214
A15	0.646	B17	0.673
A16	0.296	B18	0.195
A17	0.480	B19	0.195
A18	0.452	B20	0.254
A19	10.571		
A20	0.314		

403

404 All samples showed the presence of OTA, though the comparison of these levels
405 with the maximum allowed in some countries with legal limits for commercialization is
406 quite complicate. Extrapolation of legislation's data in µg/kg is not comparable with the
407 measure of this study carried out by superficial scratches, expressing the following results
408 in ng/cm^2. If the comparison were to be made on the assumption that 1 µg/kg is equal to
409 1 ng/cm^2, the hams analysed in this study with the highest OTA results are not likely to
410 meet the criteria established by the current Italian legislation of 1 µg/kg (della Sanità,
411 1999). Similarly, most of the dry-cured Iberian hams tested in other studies exceed this
412 level, especially in the superficial analyses where levels up to 160.9 µg/kg have been
413 described (Rodríguez et al., 2012).

414 The presence of toxigenic moulds in the surface of dry-cured hams is related to the
415 amount of OTA detected in the final product (Rodríguez et al., 2012), which has to be
416 also considered for establishing their microbial *terroir*. Congruently, the presence of
417 moulds able to produce OTA in some samples with outstanding levels of OTA, such as
418 the A13 sample, has been proved by the genotypic mould identification. Concretely, the
419 OTA-producing species *P. verrucosum* was identified.

420 The microbial population control using *D. hansenii* is one of the most studies
421 strategies in order to reduce OTA levels in dry-cured meat products (Andrade et al., 2014;
422 Cebrián et al., 2020; Meftah et al., 2018; Peromingo et al., 2018; Virgili et al., 2012). This
423 feature could be related to the lowest OTA level detected in sample A16, in which the
424 presence of *D. hansenii* was found.

425 Though the microbial *terroir* of the evaluated facilities has been confirmed through
426 several methods described along this work, focusing on culturable microorganisms might
427 not provide the full picture of the microbial population of dry-cured Iberian ham. Thus,

this study corresponds to a preliminary study to the concept of microbial *terroir* in the industry and further research should be carried out in order to characterise in depth the microorganisms that define the final technological, sensory and safety characteristics of the product.

4. Conclusions

The differences found in the tested microbial groups between the two facilities indicate their potential utility for establishing the microbial *terroir* in the artisan dry-cured Iberian ham industry, despite the fact that this work constitutes a preliminary study. Furthermore, such variations could directly impact on one of the main concerns of the meat industry from the food safety point of view consisting of the accumulation of OTA. Nevertheless, microbial *terroir* in the dry-cured Iberian ham must be further explored by the increase of the number of studied industries of its different production zones in Spain.

CRediT authorship contribution statement

José María Martín-Miguélez: conceptualisation, formal analysis, investigation, writing - original draft, visualisation. Laura Perezábad: writing - original draft, writing - review & editing, supervision. Josué Delgado: writing - review & editing. Eva Cebrián: formal analysis, writing - review & editing. María J. Andrade: conceptualisation, resources, writing - original draft, writing - review & editing, supervision.

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Conflicts of Interest

The authors declare no conflict of interest.

References

Agencia Española de Seguridad Alimentaria y Nutrición. (2023). *Buscador de Empresas Alimentarias Inscritas (RGSEAA), Ministerio de Sanidad. Date accessed: 2023-06-16*. http://www.aesan.gob.es/AECOSAN/web/seguridad_alimentaria/subseccion/buscadores.htm

Alía, A., Andrade, M. J., Rodríguez, A., Reyes-Prieto, M., Bernáldez, V., & Córdoba, J. J. (2016). Identification and control of moulds responsible for black spot spoilage in dry-cured ham. *Meat Science*, *122*, 16–24. https://doi.org/10.1016/j.meatsci.2016.07.007

Álvarez, M., Núñez, F., Cebrián, E., Roncero, E., & Andrade, M. J. (2023). 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

American Society for Microbiology. Date accessed: 2021-05-15. (2016). American Society for Microbiology. https://asm.org/browse-by-content-type/protocols

Anast, J. M., Dzieciol, M., Schultz, D. L., Wagner, M., Mann, E., & Schmitz-Esser, S. (2019). *Brevibacterium* from Austrian hard cheese harbor a putative histamine catabolism pathway and a plasmid for adaptation to the cheese environment. *Scientific Reports*, *9*(1), 1–12. https://doi.org/10.1038/s41598-019-42525-y

Andrade, M. J., Córdoba, J. J., Sánchez, B., Casado, E. M., & Rodríguez, M. (2009). Evaluation and selection of yeasts isolated from dry-cured Iberian ham by their volatile compound production. *Food Chemistry*, *113*(2), 457–463. https://doi.org/10.1016/j.foodchem.2008.07.080

Andrade, M. J., Rodas, E., Durbán, A., Moya, A., & Córdoba, J. J. (2012). Characterization and control of microbial black spot spoilage in dry-cured Iberian ham. *Food Control*, *23*(1), 128–136. https://doi.org/10.1016/j.foodcont.2011.06.023

Andrade, M. J., Rodríguez, M., Casado, E., & Córdoba, J. J. (2010). Efficiency of mitochondrial DNA restriction analysis and RAPD-PCR to characterize yeasts

growing on dry-cured Iberian ham at the different geographic areas of ripening. *Meat Science*, *84*(3), 377–383. https://doi.org/10.1016/J.MEATSCI.2009.09.005

Andrade, M. J., Rodríguez, M., Córdoba, J. J., & Rodríguez, A. (2017). Targeting other mycotoxin biosynthetic genes. In *Methods in molecular Biology* (Vol. 1542, pp. 215–235). Springer Nature. https://doi.org/10.1007/978-1-4939-6707-0

Andrade, M. J., Rodríguez, M., Sánchez, B., Aranda, E., & Córdoba, J. J. (2006). DNA typing methods for differentiation of yeasts related to dry-cured meat products. *International Journal of Food Microbiology*, *107*(1), 48–58. https://doi.org/10.1016/J.IJFOODMICRO.2005.08.011

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

Asefa, D. T., Gjerde, R. O., Sidhu, M. S., Langsrud, S., Kure, C. F., Nesbakken, T., & Skaar, I. (2009). Moulds contaminants on Norwegian dry-cured meat products. *International Journal of Food Microbiology*, *128*(3), 435–439. https://doi.org/10.1016/j.ijfoodmicro.2008.09.024

Asefa, D. T., Kure, C. F., Gjerde, R. O., Omer, M. K., Langsrud, S., Nesbakken, T., & Skaar, I. (2010). Fungal growth pattern, sources and factors of mould contamination in a dry-cured meat production facility. *International Journal of Food Microbiology*, *140*(2–3), 131–135. https://doi.org/10.1016/J.IJFOODMICRO.2010.04.008

Augustin-Jean, L., Ilbert, H., & Saavedra-Rivano, N. (2012). Geographical indications and international agricultural trade: The challenge for Asia. *Geographical Indications and International Agricultural Trade: The Challenge for Asia*, 1–263. https://doi.org/10.1057/9781137031907

Aurier, P., Fort, F., & Sirieix, L. (2005). Exploring *terroir* product meanings for the consumer. *Anthropology of Food*, *4*, 1–17. https://doi.org/10.4000/aof.187

Barham, E. (2016). "Translating terroir" revisited: The global challenge of French AOC labeling. *Research Handbook on Intellectual Property and Geographical Indications*, *19*, 46–71. https://doi.org/10.4337/9781784719470.00012

Battilani, P., Formenti, S., Toscani, T., & Virgili, R. (2010). Influence of abiotic parameters on ochratoxin A production by a *Penicillium nordicum* strain in dry-cured meat model systems. *Food Control*, *21*(12), 1739–1744. https://doi.org/10.1016/J.FOODCONT.2010.08.003

Belda, I., Gobbi, A., Ruiz, J., de Celis, M., Ortiz-Alvarez, R., Acedo, A., & Santos, A. (2021). Microbiomics to define wine terroir. *Comprehensive Foodomics*, *3*, 438–451. https://doi.org/10.1016/B978-0-08-100596-5.22875-8

Besky, S. (2014). The labor of terroir and the terroir of labor: Geographical indication and Darjeeling tea plantations. *Agriculture and Human Values*, *31*(1), 83–96. https://doi.org/10.1007/s10460-013-9452-8

Bokulich, N. A., Lewis, Z. T., Boundy-Mills, K., & Mills, D. A. (2016). A new perspective on microbial landscapes within food production. *Current Opinion in Biotechnology*, *37*, 182–189. https://doi.org/10.1016/J.COPBIO.2015.12.008

Bowen, S. (2010). Embedding local places in global spaces: geographical indications as a territorial development strategy. *Rural Sociology*, *75*(2), 209–243. https://doi.org/10.1111/j.1549-0831.2009.00007.x

Burns, K. N., Bokulich, N. A., Cantu, D., Greenhut, R. F., Kluepfel, D. A., O'Geen, A. T., Strauss, S. L., & Steenwerth, K. L. (2016). Vineyard soil bacterial diversity and composition revealed by 16S rRNA genes: Differentiation by vineyard management. *Soil Biology and Biochemistry*, *103*, 337–348. https://doi.org/10.1016/j.soilbio.2016.09.007

Capozzi, V., Garofalo, C., Chiriatti, M. A., Grieco, F., & Spano, G. (2015). Microbial terroir and food innovation: The case of yeast biodiversity in wine. *Microbiological Research*, *181*, 75–83. https://doi.org/10.1016/j.micres.2015.10.005

Castelló, E. (2021). The will for terroir: A communicative approach. *Journal of Rural Studies*, *86*, 386–397. https://doi.org/10.1016/j.jrurstud.2021.06.007

Cava, R., Ruiz, J., López-Bote, C., Martín, L., García, C., Ventanas, J., & Antequera, T. (1997). Influence of finishing diet on fatty acid profiles. *Meat Science*, *45*(2), 263–270. https://doi.org/10.1016/S0309-1740(96)00102-7

548 Cebrián, E., Fernando, J. G., Delgado, J., Bermúdez, E., & Rodríguez, M. (2020).
549 Selection and evaluation of *Staphylococcus xylosus* as a biocontrol agent against
550 toxigenic moulds in a dry-cured ham model system. *Microorganisms*, *8*(6), 793.
551 https://doi.org/10.3390/microorganisms8060793

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

557 Charters, S., Spielmann, N., & Babin, B. J. (2017). The nature and value of terroir
558 products. *European Journal of Marketing*, *51*(4), 748–771.
559 https://doi.org/10.1108/EJM-06-2015-0330

560 Chen, A. J., Hubka, V., Frisvad, J. C., Visagie, C. M., Houbraken, J., Meijer, M., Varga,
561 J., Demirel, R., Jurjević, Kubátová, A., Sklenář, F., Zhou, Y. G., & Samson, R. A.
562 (2017). Polyphasic taxonomy of *Aspergillus* section *Aspergillus* (formerly
563 *Eurotium*), and its occurrence in indoor environments and food. *Studies in*
564 *Mycology*, *88*, 37–135. https://doi.org/10.1016/J.SIMYCO.2017.07.001

565 Comi, G., & Iacumin, L. (2013). Ecology of moulds during the pre-ripening and
566 ripening of San Daniele dry cured ham. *Food Research International*, *54*(1), 1113–
567 1119. https://doi.org/10.1016/J.FOODRES.2013.01.031

568 Corredor, M., Davila, A. M., Casarégola, S., & Gaillardin, C. (2003). Chromosomal
569 polymorphism in the yeast species *Debaryomyces hansenii*. *Antonie van*
570 *Leeuwenhoek*, *84*(2), 81–88. https://doi.org/10.1023/A:1025432721866

571 Dall'Asta, C., Galaverna, G., Bertuzzi, T., Moseriti, A., Pietri, A., Dossena, A., &
572 Marchelli, R. (2010). Occurrence of ochratoxin A in raw ham muscle, salami and
573 dry-cured ham from pigs fed with contaminated diet. *Food Chemistry*, *120*(4),
574 978–983. https://doi.org/10.1016/j.foodchem.2009.11.036

575 de Celis, M., Ruiz, J., Martín-Santamaría, M., Alonso, A., Marquina, D., Navascués, E.,
576 Gómez-Flechoso, M., Belda, I., & Santos, A. (2019). Diversity of *Saccharomyces*
577 *cerevisiae* yeasts associated to spontaneous and inoculated fermenting grapes from

Spanish vineyards. *Letters in Applied Microbiology*, *68*(6), 580–588. https://doi.org/10.1111/lam.13155

Delgado, J., da Cruz, L., Rodríguez, M., & Rodríguez, A. (2018a). 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, J., da Cruz, L., Rodríguez, M., & Rodríguez, A. (2018b). 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

della Sanità, M. (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).

Edwards, M. E. (2011). Virginia ham: The local and global of colonial foodways. *Food and Foodways*, *19*(1–2), 56–73. https://doi.org/10.1080/07409710.2011.544175

Engeseth, N. J., & Ac Pangan, M. F. (2018). Current context on chocolate flavor development — a review. *Current Opinion in Food Science*, *21*, 84–91. https://doi.org/10.1016/j.cofs.2018.07.002

Faure, M. (1999). Un produit agricole « affiné » en objet culturel. *Terrain*, *33*, 81–92. https://doi.org/10.4000/terrain.2703

Gilbert, J. A., Lelie, D. V. D., & Zarraonaindia, I. (2014). Microbial terroir for wine grapes. *Proceedings of the National Academy of Sciences*, *111*(1), 5–6. https://doi.org/10.1073/pnas.1320471110

Gobbi, A., Acedo, A., Imam, N., Santini, R. G., Ortiz-Álvarez, R., Ellegaard-Jensen, L., Belda, I., & Hansen, L. H. (2022). A global microbiome survey of vineyard soils highlights the microbial dimension of viticultural *terroirs*. *Communications Biology*, *5*(1), 1–9. https://doi.org/10.1038/s42003-022-03202-5

Gong, X., Mi, R., Chen, X., Zhu, Q., Xiong, S., Qi, B., & Wang, S. (2023). Evaluation and selection of yeasts as potential aroma enhancers for the production of dry-

cured ham. *Food Science and Human Wellness*, *12*(1), 324–335. https://doi.org/10.1016/J.FSHW.2022.07.022

Hammer, B. A. (2011). Terroir and cultural identity. *Diversipede*, *1*(1), 22–34. https://doi.org/10.29173/comp34

Hinojosa, L., Lambin, E. F., Mzoughi, N., & Napoléone, C. (2016). Place attachment as a factor of mountain farming permanence: A survey in the French Southern Alps. *Ecological Economics*, *130*, 308–315. https://doi.org/10.1016/j.ecolecon.2016.08.004

Hortelano, L. A., Azofra, E., Martín, M. I., & Izquierdo, J. I. (2019). Patrimonio cultural y turismo en torno al cerdo ibérico en Salamanca. *Cuadernos de Turismo*, *44*, 193–218. https://doi.org/10.6018/turismo.44.404811

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*, 1–14. https://doi.org/10.3389/FMICB.2021.662785/FULL

Huerta, T., Hernandez, J., Guamis, B., & Hernandez, E. (1988). Microbiological and physico-chemical aspects in dry-salted Spanish ham. *Zentralbl Mikrobiol*, *143*(6), 475–482. https://doi.org/10.1016/S0232-4393(88)80062-3

International Organization for Standarization (ISO). (2007). *ISO 7218:2007; Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations* (p. 66).

Iris, L., Antonio, M., Antonia, B. M., & Antonio, S. L. J. (2020). Isolation, Selection, and Identification Techniques for Non-Saccharomyces Yeasts of Oenological Interest. *Biotechnological Progress and Beverage Consumption: Volume 19: The Science of Beverages*, 467–508. https://doi.org/10.1016/B978-0-12-816678-9.00015-1

Johansson, G., Berdagué, J. L., Larsson, M., Tran, N., & Borch, E. (1994). Lipolysis, proteolysis and formation of volatile components during ripening of a fermented sausage with *Pediococcus pentosaceus* and *Staphylococcus xylosus* as starter

cultures. *Meat Science*, *38*(2), 203–218. https://doi.org/10.1016/0309-1740(94)90110-4

Josling, T. (2006). The war on terroir: geographical indications as a transatlantic trade conflict. *Journal of Agricultural Economics*, *57*(3), 337–363. https://doi.org/10.1111/j.1477-9552.2006.00075.x

Klingelhöfer, D., Braun, M., Schöffel, N., Oremek, G. M., Brüggmann, D., & Groneberg, D. A. (2020). Ochratoxin – Characteristics, influences and challenges of global research. *Food Control*, *114*, 107230.

Leedon, G., L'Espoir, J. N. P., Buttriss, G., & Lu, V. N. (2021). Consuming the earth? Terroir and rural sustainability. *Journal of Rural Studies*, *87*, 415–422. https://doi.org/10.1016/j.jrurstud.2021.09.030

Lucini, L., Rocchetti, G., & Trevisan, M. (2020). Extending the concept of terroir from grapes to other agricultural commodities: an overview. *Current Opinion in Food Science*, *31*, 88–95. https://doi.org/10.1016/j.cofs.2020.03.007

Marlowe, B., & Lee, S. (2018). Conceptualizing terroir wine tourism. *Tourism Review International*, *22*(2), 143–151. https://doi.org/10.3727/154427218X15319286372298

Martín, A., Antequera, T., Ventanas, J., Benítez-Donoso, R., & Córdoba, J. J. (2001). Free amino acids and other non-volatile compounds formed during processing of Iberian ham. *Meat Science*, *59*, 363–368. https://doi.org/10.1016/s0309-1740(01)00088-2

Martín, A., Córdoba, J. J., Aranda, E., Córdoba, M. G., & Asensio, M. A. (2006). Contribution of a selected fungal population to the volatile compounds on dry-cured ham. *International Journal of Food Microbiology*, *110*(1), 8–18. https://doi.org/10.1016/j.ijfoodmicro.2006.01.031

Martín, A., Córdoba, J. J., Núñez, F., Benito, M. J., & Asensio, M. A. (2004). Contribution of a selected fungal population to proteolysis on dry-cured ham. *International Journal of Food Microbiology*, *94*(1), 55–66. https://doi.org/10.1016/j.ijfoodmicro.2003.12.018

667 Martínez-Onandi, N., Sánchez, C., Nuñez, M., & Picon, A. (2019). Microbiota of
668 Iberian dry-cured ham as influenced by chemical composition, high pressure
669 processing and prolonged refrigerated storage. *Food Microbiology*, *80*, 62–69.
670 https://doi.org/10.1016/j.fm.2019.01.002

671 Meftah, S., Abid, S., Dias, T., & Rodrigues, P. (2018). Effect of dry-sausage starter
672 culture and endogenous yeasts on *Aspergillus westerdijkiae* and *Penicillium*
673 *nordicum* growth and OTA production. *LWT - Food Science and Technology*, *87*,
674 250–258. https://doi.org/10.1016/j.lwt.2017.08.090

675 Núñez, F., Rodríguez, M. M., Bermúdez, M. E., Córdoba, J. J., & Asensio, M. A.
676 (1996). Composition and toxigenic potential of the mould population on dry-cured
677 Iberian ham. *International Journal of Food Microbiology*, *32*(1–2), 185–197.
678 https://doi.org/10.1016/0168-1605(96)01126-9

679 Núñez, F., Rodriguez, M. M., Córdoba, J. J., Bermúdez, M. E., & Asensio, M. A.
680 (1996). Yeast population during ripening of dry-cured Iberian ham. *International*
681 *Journal of Food Microbiology*, *29*, 271–280. https://doi.org/10.1016/0168-
682 1605(95)00037-2

683 Olesen, P. T., Meyer, A. S., & Stahnke, L. H. (2004). Generation of flavour compounds
684 in fermented sausages - The influence of curing ingredients, *Staphylococcus* starter
685 culture and ripening time. *Meat Science*, *66*(3), 675–687.
686 https://doi.org/10.1016/S0309-1740(03)00189-X

687 Ortiz-Lemus, J. F., Campoy, S., & Martín, J. F. (2021). Biological control of mites by
688 xerophile *Eurotium* species isolated from the surface of dry cured ham and dry
689 beef cecina. *Journal of Applied Microbiology*, *130*(3), 665–676.
690 https://doi.org/10.1111/jam.14839

691 Ozogul, F., & Hamed, I. (2018). The importance of lactic acid bacteria for the
692 prevention of bacterial growth and their biogenic amines formation. A review.
693 *Critical Reviews in Food Science and Nutrition*, *58*(10), 1660–1670.

694 Pavón, M. Á., González, I., Martín, R., & García, T. (2012). Importancia del género
695 Alternaria como productor de micotoxinas y agente causal de enfermedades
696 humanas. *Nutrición Hospitalaria*, *27*(6), 1772–1781.
697 https://doi.org/10.3305/nh.2012.27.6.6017

698 Peraza, R., & Perron, G. G. (2022). Investigating the microbial terroir of fermented
699 foods produced in a professional kitchen. *International Journal of Gastronomy and*
700 *Food Science*, *28*, 1–11. https://doi.org/10.1016/j.ijgfs.2022.100509

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

705 Peršurić, Ž., Saftić, L., Mašek, T., & Kraljević Pavelić, S. (2018). Comparison of
706 triacylglycerol analysis by MALDI-TOF/MS, fatty acid analysis by GC-MS and
707 non-selective analysis by NIRS in combination with chemometrics for
708 determination of extra virgin olive oil geographical origin. A case study. *LWT -*
709 *Food Science and Technology*, *95*, 326–332.
710 https://doi.org/10.1016/j.lwt.2018.04.072

711 Povolo, M., Pelizzola, V., Passolungo, L., Biazzi, E., Tava, A., & Contarini, G. (2013).
712 Characterization of two agrostis–festuca alpine pastures and their influence on
713 cheese composition. *Journal of Agricultural and Food Chemistry*, *61*(2), 447–455.
714 https://doi.org/10.1021/jf304756w

715 Prévost, P., Capitaine, M., Gautier-Pelissier, F., Michelin, Y., Jeanneaux, P., Fort, F.,
716 Javelle, A., Moïti-Maïzi, P., Lériche, F., Brunschwig, G., Fournier, S., Lapeyronie,
717 P., & Josien, É. (2014). Le terroir, un concept pour l'action dans le développement
718 des territoires. *Vertigo*, *14*(1), 1–20. https://doi.org/10.4000/vertigo.14807

719 Robert, M. G., Romero, C., Dard, C., Garnaud, C., Cognet, O., Girard, T., Rasamoelin,
720 T., Cornet, M., & Maubon, D. (2020). Evaluation of ID fungi plates medium for
721 identification of molds by MALDI biotyper. *Journal of Clinical Microbiology*,
722 *58*(5). https://doi.org/10.1128/JCM.01687-19/ASSET/A61F0CFF-F5DC-410F-
723 BD25-CC93538C66A4/ASSETS/GRAPHIC/JCM.01687-19-F0002.JPEG

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

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

Rodríguez, M., Núñez, F., Córdoba, J. J., Bermúdez, E., & Asensio, M. A. (1996). Gram-positive, catalase-positive cocci from dry cured Iberian ham and their enterotoxigenic potential. *Applied and Environmental Microbiology*, *62*(6), 1897–1902. https://doi.org/10.1128/aem.62.6.1897-1902.1996

Rodríguez, M., Núñez, F., Córdoba, J. J., Bermúdez, M. E., & Asensio, M. A. (1998). Evaluation of proteolytic activity of micro-organisms isolated from dry cured ham. *Journal of Applied Microbiology*, *85*(5), 905–912. https://doi.org/10.1046/j.1365-2672.1998.00610.x

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. https://doi.org/10.1016/0168-1605(94)90131-7

Sánchez-Molinero, F., & Arnau, J. (2008). Effect of the inoculation of a starter culture and vacuum packaging during the resting stage on sensory traits of dry-cured ham. *Meat Science*, *80*(4), 1074–1080. https://doi.org/10.1016/J.MEATSCI.2008.04.029

Scholz, M. B. dos S., Kitzberger, C. S. G., Prudencio, S. H., & Silva, R. S. dos S. F. da. (2018). The typicity of coffees from different terroirs determined by groups of physico-chemical and sensory variables and multiple factor analysis. *Food Research International*, *114*, 72–80. https://doi.org/10.1016/j.foodres.2018.07.058

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). Scientific Opinion on the risk assessment of ochratoxin A in food. *EFSA Journal 2020*, *18*(5), 150. https://doi.org/10.2903/j.efsa.2020.6113

Silva, C., Kastenholz, E., & Marques, C. P. (2021). Place attachment and involvement with rural wine tourism. *Revista Turismo & Desenvolvimento*, *36*(1), 113–123. https://doi.org/10.34624/rtd.v1i36.11365

Simoncini, N., Pinna, A., Toscani, T., & Virgili, R. (2015). Effect of added autochthonous yeasts on the volatile compounds of dry-cured hams. *International Journal of Food Microbiology*, *212*, 25–33. https://doi.org/10.1016/j.ijfoodmicro.2015.06.024

Søndergaard, A. K., & Stahnke, L. H. (2002). Growth and aroma production by *Staphylococcus xylosus*, *S. carnosus* and *S. equorum* — a comparative study in model systems. *International Journal of Food Microbiology*, *75*(1–2), 99–109.

Tholstrup, M. E. (2012). Creating terroir. An anthropological perspective on new Nordic cuisine as an expression of Nordic identity. *Anthropology of Food*, *7*, 1–17. https://doi.org/10.4000/aof.7249

Toledano, A., Jordano, R., López, C., & Medina, L. M. (2011). Proteolytic activity of lactic acid bacteria strains and fungal biota for potential use as starter cultures in dry-cured ham. *Journal of Food Protection*, *74*(5), 826–829. https://doi.org/10.4315/0362-028X.JFP-10-471

Toledano, A., Jordano, R., Medina, L. M., & López-Mendoza, M. C. (2019). Behavior and effect of combined starter cultures on microbiological and physicochemical characteristics of dry-cured ham. *Journal of Food Science and Technology*, *56*(1), 122–131. https://doi.org/10.1007/s13197-018-3465-7

Trubek, A. (2009). The taste of place: a cultural journal into terroir. *Journal of Regional Science*, *49*(5), 1010–1014. https://doi.org/10.1111/j.1467-9787.2009.00652.x

Turbes, G., Linscott, T. D., Tomasino, E., Waite-Cusic, J., Lim, J., & Meunier-Goddik, L. (2016). Evidence of terroir in milk sourcing and its influence on Cheddar cheese. *Journal of Dairy Science*, *99*(7), 5093–5103. https://doi.org/10.3168/jds.2015-10287

Ventanas, S., Ruiz, J., García, C., & Ventanas, J. (2007). Preference and juiciness of Iberian dry-cured loin as affected by intramuscular fat content, crossbreeding and rearing system. *Meat Science*, *77*(3), 324–330. https://doi.org/10.1016/j.meatsci.2007.04.001

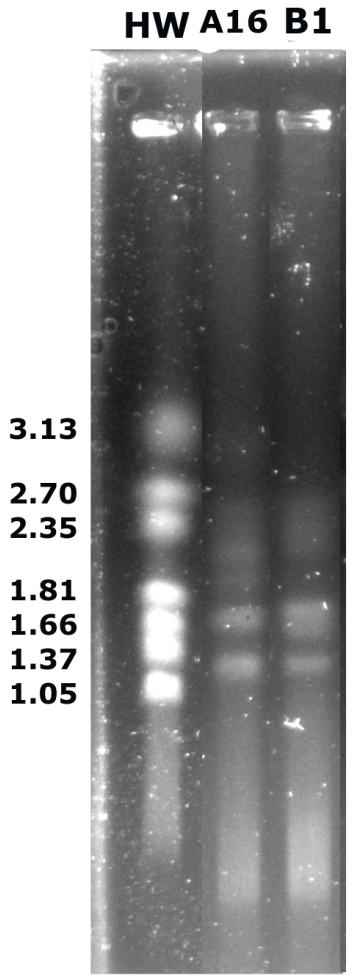
Virgili, R., Simoncini, N., Toscani, T., Leggieri, M. C., Formenti, S., & Battilani, P. (2012). Biocontrol of Penicillium nordicum growth and ochratoxin A production by native yeasts of dry-cured ham. *Toxins*, *4*(2), 68–82. https://doi.org/10.3390/TOXINS4020068

Zhou, C., Xia, Q., Du, L., He, J., Sun, Y., Dang, Y., Geng, F., Pan, D., Cao, J., & Zhou, G. (2022). Recent developments in off-odor formation mechanism and the potential regulation by starter cultures in dry-cured ham. *Critical Reviews in Food Science and Nutrition*, *1*, 1–15. https://doi.org/10.1080/10408398.2022.2057418

Figure legend

Figure 1. PFGE profiles of the yeasts isolates from two facilities processing dry-cured Iberian ham. Lane HW: *Hansenula wingei* standard (sizes of bands in megabases). Lane A16: yeast isolate from the facility A during the drying phase (isolation code A16L5). Lane B1: yeast isolate from the facility B during the post-salting phase (isolation code B1L3).

Martín et al. - Figure 1



Implications for gastronomy

Microbial *terroir* defines the unique characteristics of a product generated by the microbial action of its processing, a term widely used in the wine industry that generates value and ~~standardizes~~ standardises production.

Although the microbial participation throughout the processing of dry-cured Iberian ham has been studied for years, the microbial *terroir* has not been noticed.

The present study reveals the microbial differences that exist between various facilities and even the hams of each one of them, demonstrating the existence of a microbial *terroir* implemented in the artisanal facilities that can be modified according to the use of temperature control along the process. Besides, this work has established the importance of the microbial *terroir* in identifying one of the main hazards of the cured meat products consisting of the ochratoxin A. This manuscript thus opens the field of studies focused on microbial *terroir* on cured meat products, leading to greater professionalization of the industry, the production of more valuable products, the ampliation of knowledge in gastronomy, the differentiation of dry-cured production zones and the elaboration of hams in a more efficient way, with a greater food safety and controlled organoleptic characteristics.

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

The informed consent was not necessary in this work since it was not performed with human subjects.