Food Research International 153 (2022) 110977

Contents lists available at ScienceDirect

Food Research International

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

Influence of the ripening chamber's geographical location on dry-cured Iberian ham's key odorants

MP. Segura-Borrego [a], R. Ríos-Reina [a], H. Galán-Soldevilla [b], FJ. Forero [c], M. Venegas [c], P. Ruiz Pérez-Cacho [b], ML. Morales [a,*], RM. Callejón [a]

[a] Área de Nutrición y Bromatología, Dpto. de Nutrición y Bromatología, Toxicología y Medicina Legal, Facultad de Farmacia, Universidad de Sevilla, C/P. García González n°2, E-41012 Sevilla, España
[b] Laboratorio de Estudios Sensoriales (GrupoSens), Departamento de de Bromatología y Tecnología de Alimentos, Universidad de Córdoba, Edificio Darwin, Campus de Rabanales, 14071 Córdoba, España
[c] Diputación Provincial de Huelva, Servicio de Investigación Agrícola y Ganadera, Ctra. Huelva-San Juan del Puerto A-5000 Km 1.8, 21007 Huelva, España

ARTICLE INFO

Keywords:
Dry-cured Iberian ham
Ripening chamber, Odour-active compound
Olfactometric analysis
Quantitative descriptive profile

ABSTRACT

Olfactometric and sensory analyses have been applied to study the possible influence of the ripening chamber's geographical location on the aroma sensory profiles and key odorants of Iberian ham. Dry-cured Iberian ham was obtained from 3 acorn-fed pigs and, for the first time, both of the participating production facilities, located in two different Andalusian municipalities with different altitudes above mean sea level, processed one of the two hind legs from each pig. The descriptive sensory profile of orthonasal and retronasal odours was determined by trained panellists, while odour-active compounds were determined by gas chromatography/mass spectrometry-olfactometry (GC/MS-O). The results obtained showed that, separately, both techniques enable Iberian ham samples to be differentiated by their ripening chamber's geographical location. For sensory analysis, retronasal sensory analysis appeared to be the most suitable for this goal, highlighting the "meat broth odour" and "roasted nuts odour" descriptors which presented significant differences between geographical locations for samples from all pigs. Moreover, ripening chamber's geographical location characteristics and the initial composition of the raw material seemed to influence the content of some odour-active compounds. The odour-active compound identified as octane/acetone and isobutanol were conditioned by the ripening chamber's geographical location, while decanal/2-ethyl-1-hexanol, 1-undecanol, 2-furanmethanol and *cis*-2-nonenal were also influenced by the individual pig itself. This study showed that slight climatological differences due to the location of the ripening chamber seem to have somewhat of an influence on the aromatic profile.

1. Introduction

Iberian ham is one of gastronomy's most highly-appreciated meat products, its sensory uniqueness being one of the features most appreciated by consumers. The sensory quality of Iberian ham is mainly influenced by meat quality (pig genotype, feed, and rearing system) and processing method (salt content, length of process and processing conditions). Traditional Iberian ham dry-curing is a long process (Narváez-Rivas, Gallardo, & León-Camacho, 2012). The process takes over 24–36 months in humidity- and temperature-controlled conditions. During this process, raw hams undergo three stages: salting with dry salt (0–5 °C with a 70 to 90% relative humidity), post-salting for salt equalisation (1–3 °C, 70–90% Hr) in humidity- and temperature-controlled chambers, and drying-maturing or ripening stage in a chamber where the hams mature under ambient conditions, temperatures ranging from 20 to 35 °C. Ambient conditions are controlled by opening and closing windows.

Perhaps the most important quality parameter of Iberian hams is their aroma. This aroma is due to the presence of many volatile compounds. Most are produced by reactions such as lipolysis (chemical or enzymatic oxidation), proteolysis, Strecker degradation and Maillard reactions, as well as the chemical and enzymatic mechanisms that take place during the dry-curing process (Narváez-Rivas et al., 2012). Research undertaken by several authors (Andrés, Cava, Ventanas, Thovar, & Ruiz, 2004a; Domínguez-Gómez, 2020) has shown the important role of ambient conditions on organoleptic characteristic of this product,

* Corresponding author.
E-mail address: mlmorales@us.es (ML. Morales).

https://doi.org/10.1016/j.foodres.2022.110977
Received 2 July 2021; Received in revised form 29 December 2021; Accepted 30 January 2022
Available online 5 February 2022

thus emphasising the importance of the drying and curing stages on the quality of Iberian ham.

Aroma profile can be determined by sensory or instrumental analysis. Sensory descriptive analysis consists of a group of trained panellists measuring the specific attributes of a food product by using intensity scales in order to define said product's sensory profile.

The instrumental technique of gas chromatography, coupled to mass spectrometry (GC/MS), together with odour activity values (OAVs) enable researchers to identify the volatile compounds that generate a food's aromatic profile. GC/MS enables the volatile compounds to be identified, while OAV, calculated using detection threshold values, establishes the compounds' importance on aromatic profile (Ríos-Reina, Segura-Borrego, Morales, & Callejón, 2020).

GC/MS coupled to olfactometric analysis (GC/MS-O) can, moreover, be a powerful tool that makes use of the human nose as a highly selective detector. In contrast to conventional instrumental detectors, the human nose is able to detect low levels of aroma compounds. GC/MS-O is an analytical technique that enables eluted volatile compounds to be perceived simultaneously by two detectors: human olfaction and MS. This makes it possible to detect and identify the volatile compounds – the odorants – that contribute to the aroma of the product under study. This methodology, therefore, evaluates the contribution of the individual odorants, enabling a better understanding of odorant composition and showing a possible correlation between the smell perceived and the chemical nature of an odorant (Brattoli et al., 2013).

Dry-cured Iberian ham is a complex product whose sensory attributes and volatile profile have been studied by several authors with different scientific aims. The intention of Ruiz, Ventanas, Cava, Timón, & García (1998) was to determine, by sensory analysis, the differences in the organoleptic characteristics of dry-cured Iberian hams by comparing the different processing times allowed. Andrés et al. (2004a) evaluated the effect of two different salt levels and different dry-cured Iberian ham processing conditions on sensory attributes. Other authors have identified and characterised the aromatic compounds of dry-cured ham by GC/Olfactometric analysis (Carrapiso & García, 2004; Sánchez del Pulgar, García, Reina, & Carrapiso, 2013). Moreover, odour-active compounds have been used to compare different types of Iberian ham (Carrapiso, Jurado, Timón, & García, 2002a; Narváez-Rivas et al., 2012), and also to study the aroma compounds related to bone-taint defect in Iberian ham (Carrapiso, Martí, Jurado, & García, 2010). Some researchers have even tried to determine the relationship between odour and flavour sensory attributes and volatile compounds in hams, or to combine methods such as GC/olfactometry and sensorial descriptive analysis (Flores, Grimm, Toldrá, & Spanier, 1997; García-González, Tena, Aparicio-Ruiz, & Morales, 2008; García-González, Aparicio, & Aparicio-Ruiz, 2013). Notwithstanding the above, studies into the effects of the processing methods employed to produce dry-cured Iberian ham on its odour-active compounds are scarce.

Several authors (Ruiz et al., 2002; García-González et al., 2013) have pointed out the influence of the raw material on the dry-cured ham's composition. Variability between samples is inevitable, even though the samples studied came from pigs of the same breed which had undergone the same kind of rearing and feeding.

The aim of this work was to study the influence of the ripening chamber's geographical location on the aroma of dry-cured Iberian ham by determining their key odorants and sensory profile by using olfactometric and sensory analysis. Moreover, we tried to detect possible correlations between sensory attributes and key odorants. For this purpose, and for the first time, both of the participating production facilities, located in two different Andalusian municipalities, processed one of the two hind legs from each pig. The reason for this action was to know the effects of environmental conditions, regardless of the variability between specimens. With respect to previous research works, this is an innovative aspect in experimental design.

2. Materials and methods

2.1. Samples

The dry-cured Iberian ham samples used in this study were obtained from 3 pigs (PIG1, PIG2 and PIG3) from *Retinto del Andévalo* Iberian acorn-fed pigs. Both hind legs from the same pig were processed according to the traditional dry-curing method, each in one of two different Andalusian production facilities. Both production facilities were in the province of Huelva, Spain. One was located in Andévalo, 260 m a.s.l. (A), while the other (B) was in Corteconcepción, 673 m a.s.l. The geographical location B presented lower minimum temperatures and higher rainfall than A. The stages previous to ripening were salting with dry salt (0–5 °C with 70 to 90% relative humidity, followed by washing and post-salting for salt equalisation (1–3 °C, 70–90% Hr) in temperature- and humidity-controlled chambers. These conditions were equal in both production facilities. Then, hams were submitted to ripening stage, including dry and maturation processes, in a ripening chamber under ambient conditions controlled by the traditional method of opening and closing windows. The hams were processed for 3 years until they reached a weight of 7–8 Kg (Table S1, supplementary material). All hams had pH values of between 5.4 and 5.7.

Thin slices of Iberian ham were hand-cut by a professional ham cutter following the traditional technique. One thin slice of approx. 2 mm. thick, weighing about 5 g and containing a balanced amount of lean and fat was served to a taster. In order to guarantee the representativeness of the samples, the central part of the ham, containing mainly Semimembranosus, Biceps femoris and Semitendinosus muscles and known in Spanish as the *maza*, was sampled at different depths and each slice was cut without distinguishing between the muscles included therein.

2.2. Chemicals and materials

Sigma-Aldrich Química S.l. (Madrid, Spain) supplied the standards of aromatic compounds used for identifying the compounds usually found in dry-cured Iberian ham. These compounds are: 1-hexanol, 1-octen-3-ol, 1-octen-3-one, 1-pentanol, 1-penten-3-one, 2-nonanone, 2-octanone, 2, 6-dimethylpyrazine, 2-butanone, 2-heptanol, 2-heptanona, 2-hexanone, 2-methyl-1-butanol, 2-methyl-3-furanthiol, 2-methylbutyraldehyde, 2-pentanone, 3-methyl-1-butanol, benzaldehyde, *cis*-2-penten-1-ol, decanal, diacetyl, ethyl isovalerate, ethylbenzene, ethyl butyrate, heptanal, hexanal, isobutyraldehyde, limonene *r (+), m*-xylene, nonanal, octanal, octane, pentanal, propionic acid liquid, *trans*-2-heptenal, *trans*-2-hexen-1-ol, *trans*-2-hexenal, *trans*-2-nonenal, *trans*-2-octenal, ethanol, acetone, acetic acid, toluene, 6-methyl-5-hepten-2-one, acetoin, ethyl hexanoate, methional, 1-propanol, dimethyl disulphide and 2-propanol.

A neutralised and deodorised olive oil was supplied by Sovena España S.A. (Brenes, Spain) and was used to prepare a mix of standard aromatic compounds for training the sensory panel prior to the GC/MS-O analysis.

An alkane standard mixture C_{10}-C_{40} was purchased from Fluka (Madrid, Spain) and was used to calculate the Linear Retention Index (LRI).

Twisters® of polydimethylsiloxane (PDMS) (Gerstel, Müllheim an der Ruhr, Germany) were used as microextraction devices for extracting the volatile fraction. The Twisters® were 10 mm long, had a 2-μL coating, and were pre-conditioned following the supplier's instructions.

2.3. Sensory descriptive profile

2.3.1. Sample preparation

Samples, wrapped in aluminium foil and labelled with random three-digit numbers, were presented to the tasters. In each session three or four samples were served singly at room temperature. Mineral water was

used to cleanse the palate between samples.

2.3.2. Assessors

Eight highly-trained panellists from the Sensory Laboratory at Universidad de Córdoba (Spain) participated in this study. The three male and five female members whose ages ranged from 27 to 60, were selected, based on detection, recognition, and discrimination tests, and on their ability to memorise and communicate sensory impressions. Once selected, the panellists were then trained following international ISO standards 5492, 8586 and 13299. All had prior experience in the sensory evaluation of different products (Araujo, Pérez-Cacho, Serrano, Dios-Palomares, & Galán-Soldevilla, 2020; Guzmán et al., 2020; Ruiz Pérez-Cacho, De la Haba Ruiz, Dios-Palomares, & Galán-Soldevilla, 2019) and had undergone specific training in Iberian ham (Martín-Gómez et al., 2019). Testing was carried out in a sensory laboratory equipped with both a round table for training sessions and individual booths, in accordance with ISO 8589. All analyses took place in the morning.

2.3.3. Quantitative descriptive profile

The methodology followed was based on ISO standards 5492 and 13299 and the aroma/odour profile was performed following the method described by Galán-Soldevilla et al. (2005). In this earlier work, a group of assessors with previous experience in the sensory analysis of several foods participated in the aroma/odour lexicon generation using the unguided free selection technique (ISO 11035). In order to obtain a comprehensive set of descriptors, they were exposed in panel booths to a variety of dry-cured commercial hams from both white and Iberian pigs. After all of the assessors had separately generated the vocabulary in their tasting booths, the panel leader led a discussion to select the descriptors which best characterised the orthonasal aroma and retronasal odour of the cured hams. A total of 37 aroma/odour attributes were defined and referenced: rancid aroma/odour, fat/grease aroma/odour, soap aroma/odour, nutty aroma/odour, acorn aroma/odour, bread crust aroma/odour, roasted nuts aroma/odour, caramel aroma/odour, burnt sugar aroma/odour, meat broth aroma/odour, leather aroma/odour, sexual aroma/odour, stable aroma, sweat aroma, blood aroma, fungus/truffe aroma/odour, mould aroma/odour, musty aroma/odour, wet earth aroma/odour and medicine/drugs aroma/odour. They were then classified by odour similarities into seven families: fat, nutty, toast, animal, vegetable, humidity, and others. Using this set of sensory attributes, the tasters received a specific 10-h period of training in Iberian hams. Five aroma-orthonasal attributes (overall intensity, rancid, meat broth, bread crust and roasted nuts), and 6 odour-retronasal attributes (overall intensity, rancid, meat broth, acorn, bread crust and roasted nuts) were then evaluated on a non-structured scale of 10 cm from not noticeable to very strong. All evaluations were performed in duplicate.

2.4. Volatile fraction extraction methods

The extraction procedure was performed by headspace sorptive extraction (HSSE) in duplicate. In all cases, 2.5 g of dry-cured Iberian ham was cut into small pieces and put into a special 20 mL headspace vial (Gerstel, Müllheim an der Ruhr, Germany). A PDMS Twister® was placed inside the vial in an open glass adapter provided by Gerstel (Müllheim an der Ruhr, Germany). The vial was tightly capped for extraction and then heated for 60 min at 60 °C in a thermostatic bath (BÜCHI Heating Bath B-490, Hamptons, US). After the vial had been at room temperature for five minutes, the Twister® was rinsed with Milli-Q water and dried with lint-free tissue paper. Finally, it was transferred into a glass tube 60 mm long, 6 mm o.d. and 4 mm i.d., which then was placed in the autosampler tray for thermal desorption and GC/MS-O analysis.

2.5. Gas Chromatography/Mass Spectrometry-Olfactometry (GC/MS-O) analysis

The gas chromatography analyses were performed using an Agilent 6890 GC system coupled to an Agilent 5975 inert quadrupole mass spectrometer (Agilent, Santa Clara, CA, US) and an olfactory detection port (OPD3, Gerstel, Müllheim an der Ruhr, Germany). The system was equipped with a Gerstel Thermo Desorption System (TDS2) and a CIS-4 PTV inlet Cooling Injector System (Gerstel, Müllheim an der Ruhr, Germany). The desorption stage was performed in splitless mode, and the temperature programme was as follows: the temperature was held at 35 °C for 0.1 min, and then ramped at a rate of 60 °C/min to 220 °C, where it was held for 4 min. The temperature of the CIS-4 PTV injector, with Tenax TA^{TM} (Gerstel, Müllheim an der Ruhr, Germany) inlet liner, was held at −35 °C, using liquid nitrogen for the total desorption time and was then raised to 260 °C at a rate of 10 °C/s and held for 4 min. Solvent vent mode was used to transfer the sample to the analytical column. A 50 m × 0.25 mm J&W CPWax-57CB column and a 0.20 μm film thickness (Agilent, Santa Clara, CA, US) was used. The carrier gas was He at a 1.0 mL/min flow rate. The oven temperature programme was as follows: 35 °C for 4 min and then raised to 220 °C at a rate of 2.5 °C/min and held for 7 min.

The column effluent was split 1:2 into MS and ODP detectors by means of a GRAPHPACK 3D/2 crosspieces Sulfinert® (Gerstel, Müllheim an der Ruhr, Germany). The OPD transfer line and mixing chamber were heated to 250 °C. The MS quadrupole, source and transfer line temperatures were maintained at 150 °C, 230 °C and 280 °C, respectively. The electron ionisation mass spectra in the full-scan mode were recorded at 70 eV with the electron energy in the range of 29 to 300 *m/z*.

A total of six olfactometric analyses per sample were performed by a trained panel of three panellists. The panellists smelled the odours coming from the ODP and gave a score of the odour intensity ranging from 1 to 3, 1 being the lowest and 3 being the highest intensity. Panellists also provided a verbal description of each perceived odour.

2.6. Data processing and aroma compounds identification

The data obtained from olfactometric analysis, frequency of odour occurrence (F) and intensity (I) were used to calculate the "modified frequency" (MF) of each odorant by the following equation: $MF\ (\%) = \sqrt{F(\%)xI(\%)}$, where F(%) is the detection frequency of an odorant expressed as a percentage of the maximum frequency and I(%) is the average intensity expressed as a percentage of the maximum intensity (Dravnieks, 1985).

Compound identification was performed using mass spectrum, LRI and odour description. The NIST/EPA/NIH Mass Spectral Library was used for identifying the odorants by mass spectrum matching in the standard NIST MS Search program (v.2.0) (Gaithersburg, MD, US). The odour LRI was calculated using the retention time of a series of n-alkanes analysed under the same conditions of the samples and compared with LRIs of standards. The odour descriptors of each odorant were selected by panellists' frequency of citations. Finally, these experimental odour descriptions were compared with those reported in the literature and in databases. A tentative identification (TI) was considered when the odour description and LRI agreed with the literature without confirmation by standards.

2.7. Statistical analysis

In order to study the significant differences between data on dry-cured Iberian ham samples from two ripening chambers with different geographical locations (DA and DB), ANOVA analysis of variance, followed by a *post hoc* comparison test (LSD Fisher test) were performed using INFOSTAT software (FCA, Universidad Nacional de Córdoba, Argentina). A statistically significant difference was considered when

the p-value was<0.05. Moreover, two-way ANOVA was performed with sensory data in order to ascertain the interaction between independent variables. Also, correlation analysis and principal component analysis (PCA) were undertaken using Statsoft Statistical, version 7.0 (Statsoft, Tulsa, OK). PCA was performed as an unsupervised method in order to ascertain the degree of differentiation among samples according to the ripening chamber's geographical location or the pig of origin. The correlation analysis was undertaken in order to try to ascertain the possible relationship between sensory attributes and key odorants.

3. Results and discussion

3.1. Odour-active profile of dry-cured Iberian ham

Among all the odorant detected in the GC/MS-O analyses in this study, and following the criteria of other authors (Márquez et al., 2013; Vera, Uliaque, Canellas, Escudero, & Nerín, 2012), we considered as odour-active those compounds detected in at least half of the total sniffing analyses and which reached an MF value ≥ 70%. A total of 40 odour-active compounds were, therefore, detected, with a range of between 20 and 29 aroma active compounds in each sample. Their corresponding odour description and identification are listed in Table 1. Moreover, those compounds with MF values ≥ 80% were considered as key odorants, also known as key odorants. In other words, these were compounds that lent a major contribution to the product aroma (Ríos-Reina et al., 2020). Employing the above MF ≥ 80% value criterion for key odorants, we found a total of 34 key odorants which varied from 15 to 23, depending on the sample.

There were some compounds that reached the highest MF values in all samples analysed, and were, therefore, key odorants in all of them. The most important key odorant was 2,6-dimethylpyrazine. Indeed, it was the only one that reached the maximum MF value (100%) in all samples (Table 1). This result agreed with other authors who found this compound as one of the most impactful odorants in dry-cured ham (Jurado, Carrapiso, Ventanas, & García, 2009). The compound 2,6-dimethylpyrazine provides different notes between toasted and nutty aromatic characters (Jurado, García, Timón, & Carrapiso, 2007), possibly from amino acids generated through Strecker and Maillard reactions, and it could be used as an indicator of the feeding system employed (Jurado et al., 2009). The second key odorant to highlight is 1-octen-3-one, which presented the maximum MF value (100%) in most cases and slightly lower one of 91% in the remainder. 1-octen-3-one probably originated in linoleic and linolenic catabolism by moulds or from methyl linoleate autooxidation (Rivas-Cañedo, Fernández-García, & Nuñez, 2009). This compound was described as mushroom odour (Table 1) by other authors (Carrapiso, Ventanas, & García, 2002b; Sánchez-Peña, Luna, García-González, & Aparicio, 2005).

The odorant identified as hexanal, 2-acetylfuran, 2(5H)-furanone, and 2-acetyl-5-methylfuran, were also key odorants in all samples (Table 1). The first two compounds reached the maximum MF values in three samples, two of them corresponding to samples from the same pig, hexanal in PIG 2 and 2-acetylfuran PIG 1, respectively. This was also the first time the 2-acetylfuran has been detected in Iberian ham. Hexanal content has been widely used for monitoring oxidative stability in meat and meat products (Andrés, Cava, Ventanas, Muriel, & Ruiz, 2004b). This result may be related to the fact that hexanal is formed by the oxidation of either esterified or free linoleic acid, and that the percentage of this acid is higher in subcutaneous fat (Sánchez-Peña et al., 2005).

2(5H)-Furanone, also called gamma-crotonolactone, was a key odorant with a toasted aroma. Its presence was possibly due to the Maillard and Strecker reactions on amino acids (Ventanas et al., 1992). Previously, this key odorant has been described by other authors in dry-cured Chinese and Italian ham (Sánchez del Pulgar et al., 2014; Wang et al., 2018) but not in dry-cured Iberian ham. It is, therefore, the first time that the compound has been described in this particular ham.

Although they were not key odorants in all of them (Table 1), other odour-active compounds, such as 1-octen-3-ol, *cis*-2-penten-1-ol, diacetyl, *cis*-2-nonenal (TI) and LRI 1472 described as plastic, with high MF values, were found in most of the samples. These compounds could also make an important contribution to the aroma of dry-cured ham. *Cis*-2-penten-1-ol was a key odorant in all samples except for sample PIG1DA. This compound is generally derived from chemical degradation and can be formed following the decomposition of the secondary hydroperoxides of fatty acids by the action of lipoxygenases (Paleari, Moretti, Beretta, & Caprino, 2008). It is the first time that this compound has been determined in this type of sample. 1-Octen-ol is an important odour-active compound identified by other authors as a dry-cured Iberian ham odorant (Jurado et al., 2009). 1-Octen-ol could arise from lipid oxidation reactions and, according to authors, it may be used as an indicator of the feeding system (Jurado et al., 2009; Paleari et al., 2008). Diacetyl is a ketone present in dry-cured ham and its content may be related to the amount of salt used in the curing process (Wang, Jin, Zhang, Ahn, & Zhang, 2012), or with the metabolism of microorganisms (Berdagué, Monteil, Montel, & Talon, 1993).

Trans-geranylacetone was a key odorant except in PIG 1 samples. Indeed, in the sample from ripening chamber DB (PIG1DB) (Table 1), it was not even an odour-active compound. Isobutanol and odour with LRI 1629, providing plastic and potato peel aromas, respectively, were key odorants in three samples: PIG1DB, PIG2DB and PIG3DB in the case of isobutanol, and PIG1DA, PIG2DB and PIG3DA in the case of odour with LRI 1629. Isobutanol, a compound described in Italian dry-cured ham by other authors (Gaspardo, Procida, Toso, & Stefanon, 2008), showed a significant difference between ripening chamber's geographical locations, only being a key odorant in the samples from ripening chamber DB. In general terms, the greater the degree of lipid oxidation, greater is the production of this alcohol (Marušić, Vidaček, Janči, Petrak, & Medić, 2014). Moreover, 2-octanone was a key odorant in the PIG1DB and PIG2D samples. Indeed, all other samples, except PIG1DA, reached 2-octanone values of MF > 70%, indicating that this compound possibly exerted an influence on the aroma of most samples (Table 1). This compound is usually found in semitendinosus muscle and formed by a chemical process if the microbial population is low. As this compound has been reported as being responsible for blue cheese aroma, if its sensory perception is intense, it can be a symptom of bad quality hams. This is due to the fact that it can also be formed by the action of microorganisms (Sánchez-Peña et al., 2005).

Of immediate concern to the aim of this work, were the ambient conditions in both ripening chambers, conditions controlled by the traditional method of opening and closing windows. The main difference between the ripening chambers was their location at different altitudes with respect to sea level, leading to climatological differences in both temperature and rainfall. In ripening chamber DB's location, the lower minimum temperatures are slightly lower and rainfall is higher than in the case of ripening chamber DA. During the drying and maturing process of Iberian ham, water loss occurs and numerous chemical and enzymatic reactions take place. This leads to a large number of aroma compounds developing, phenomena influenced by humidity and ambient temperature (Domínguez-Gómez, 2020). In this context, we have observed that there were several compounds showing differences in MF values that depended on the chamber's geographical location where the samples were processed. Hence, octane/acetone, ethyl isovalerate, isobutanol and *cis*-2-nonenal (TI), reached higher MF values in the samples from ripening chamber DB than those from ripening chamber DA. These differences were only statistically significant for octane/acetone and isobutanol, both of which contribute to chemical aromatic notes.

In some cases, the influence of the ripening chamber's geographical location affected two pigs only. Thus, decanal/2-ethyl-1-hexanol and 1-undecanol showed significant differences between both ripening chambers only when samples from PIG 2 and 3 were considered; 2-furanmethanol in the case of PIG 1 and 3; and *cis*-2-nonenal (TI) in PIG 1 and 2 (Table 1). Sánchez del Pulgar et al. (2014) observed that in the

Table 1
Odour-active compounds in Dry-cured Iberian ham samples.

					PIG 1				PIG 2				PIG 3			
					DA		DB		DA		DB		DA		DB	
LRIexp	Odour Descriptor	Aroma Category	Odorant	ID	I	MF	I	MF	I	MF	I	MF	I	MF	I	MF
750	Nasty, rotten	Animal	Methanethiol	ST, MS, OD	2	75	1.5	65	1.5	58	2	82	1.5	58	2	67
796	Chemical, stale	Chemical	Octane/Acetone*	ST, MS, OD	1.5	50[a]	2	67[b]	1	47[a]	1.5	71[b]	1	47[a]	1	58[b]
883	Chemical, stale	Chemical	2-Methylbutyraldehyde/3-Methylbutyraldehyde	ST, MS, OD	2	75	2.5	65	2	67	2	67	2	82	2	67
945	Butter	Lactic	Diacetyl	ST, MS, OD	2	82	2	82	2	82	2	82	2	47	2	82
1052	Fruit, sweet	Fruity	Ethyl isovalerate	ST, MS, OD	0	0	1.5	41	1	33	2	82	1	33	2	47
1065	Green, grass	Vegetable	Hexanal	ST, MS, OD	2.5	91	2	82	3	100	3	100	2	82	3	100
1082	Plastic, glue	Chemical	Isobutanol*	ST, OD	2	75[a]	2	82[b]	2	67[a]	2	82[b]	1.5	71[a]	2	82[b]
1226	Green, grass, plastic	Vegetable	2-Hexenal	LRI, OD	2.5	53	2.5	75	3	58	2	47	1.5	58	1.5	58
1278	Citric	Fruity	2-Octanone	ST, MS, OD	2	67	2	82	3	100	2.5	75	2.5	75	2.5	75
1291	Mushroom, metallic	Humidity	1-Octen-3-one	ST, MS, OD	3	91	3	100	3	100	3	100	3	100	3	100
1297	Potato peel	Humidity	Hydroxyacetone	MS, LRI	3	71	3	41	2	47	2	47	2	47	2	47
1335	Toasted corn	Toasted	2,6-Dimethylpyrazine	ST, MS, OD	3	100	3	100	3	100	3	100	3	100	3	100
1361	Vegetable, metallic	Vegetable	1-Hexanol	ST, MS, OD	1.5	41	2	82	2	67	2	47	2	47	2	47
1366	Vegetable	Vegetable	*cis*-2-Penten-1-ol	ST, MS, OD	2.5	65	2	82	3	100	2	82	2	82	3	100
1423	Toasted corn	Toasted	*trans*-2-Octenal	ST, MS, OD	1.5	65	2	75	2	82	1.5	58	1.5	58	1.5	58
1446	Cooked vegetable	Vegetable	1-Octen-3-ol	ST, MS, OD	3	82	3	82	3	82	2.5	75	2.5	75	3	82
1468	Vegetable	Vegetable	Dihydromyrcenol*	MS, LRI, OD	0	0[a]	0	0[a]	1	47[b]	3	82[b]	1.5	58[b]	2	67[b]
1472	Plastic, glue	Chemical	–	–	2.5	91	2.5	75	3	100	2	82	2	82	2	82
1486	Potato peel	Humidity	Decanal/2-Ethyl-1-hexanol	ST, MS, OD	2	82	2	75	1.5	58	2.5	91	2	47	2	82
1500	River water	Others	2-Acetylfuran	MS, LRI, OD	3	100	3	100	2.5	91	2	82	2	82	3	100
1506	Potato peel	Humidity	*cis*-2-Nonenal	LRI, OD	2	75	3	91	2.5	75	3	100	3	100	3	100
1530	Plastic, chemical	Chemical	*trans*-2-Nonenal	ST, MS, OD	2.5	91	2.5	65	2	82	3	100	3	100	2	47
1564	Potato peel	Humidity	5-Methyl-2-furfuraldehyde	MS, LRI, OD	2	75	2	47	2	67	2	82	2	82	2	67
1581	Grass	Vegetable	*trans*-2, *cis*-6-Nonadienal	LRI, OD	3	100	2	82	1.5	71	1.5	71	1.5	71	1.5	71
1606	Plastic, river water	Others	2-Acetyl-5-methylfuran	LRI, OD	2	82	2	82	2	82	2	82	2	82	2	82
1629	Potato peel	Humidity	–	–	3	100	2.5	75	1.5	58	3	82	2	82	2.5	75
1657	Toasted corn	Toasted	2-Furanmethanol	MS, LRI, OD	2.5	83	1.5	50	2	47	2	82	2	82	1	33

(*continued on next page*)

Table 1 (*continued*)

LRIexp	Odour Descriptor	Aroma Category	Odorant	ID	PIG 1				PIG 2				PIG 3			
					DA		DB		DA		DB		DA		DB	
					I	MF	I	MF	I	MF	I	MF	I	MF	I	MF
1700	River water, plastic	Others	2,4-Nonadienal	MS, LRI, OD	2	33	2	82	2	82	2	67	1	47	2	47
1717	Citric	Fruity	*cis-cis*-2,6-Nonadienal	LRI, OD	1	33	1.5	71	0	0	2	82	2	82	2	67
1760	Toasted corn	Toasted	2(5H)-Furanone	MS, LRI, OD	2.5	91	2	82	2	82	2	82	2	82	2	82
1765	Sweet	Spicy	–	–	2	33	2.5	53	2	47	2	47	2	82	2	47
1811	Vegetable	Vegetable	2,4-Decadienal	MS, LRI, OD	2	33	2	82	1	58	1.5	58	2	67	2	82
1850	Vegetable	Vegetable	*trans*-Geranylacetone	MS, LRI, OD	2	75	1	41	2	82	2	82	2	82	2	82
1858	Liquorice, curry	Spicy	Guaiacol	MS, LRI, OD	2	75	1	24	2	47	0	0	2	47	3	58
1863	Medicinal	Chemical	1-Undecanol	MS, LRI, OD	2	47	3	58	3	100	2.5	75	3	100	2	67
1925	Chemical, floral	Chemical	2-Phenylethanol	ST, MS, OD	0	0	1.5	41	1.5	58	1.5	71	2.5	75	2.5	75
2039	Spicy	Spicy	γ-Nonalactone	MS, LRI, OD	2	75	2	58	2	47	2	47	0	0	2	47
2067	Sweet	Spicy	4-Ethylguaiacol	LRI, OD	2.5	91	2	67	1.5	71	1	33	2	47	2	82
2087	Sweet	Spicy	4-Propylguaiacol	LRI, OD	1	24	1.5	41	2	67	2	82	0	0	2	47
2096	Nasty, stable	Animal	*m*-Cresol/*p*-Cresol*	LRI, OD	2.5	53[a]	2.5	53[a]	3	82[b]	3	82[b]	3	100[c]	3	100[c]

ID (identification): ST: LRI value matches with LRI of real standard; MS: Mass spectrum matches with that from the NIST98library; LRI: LRI value matches with that reported in literature or online database; OD: odour description matches with that reported in literature. I: odour intensity value. MF: Modified frequency value. *: Significant differences. A different superscript letter in the same row indicates statistically significant differences ($p < 0.05$).

case of 1-undecanol, a fatty alcohol present in the intermuscular fat of dry-cured Iberian ham (Timón, Ventanas, Carrapiso, Jurado, & García, 2001), its greater or lesser presence could be related to the genotype of the Iberian pig.

It could be also observed that some compounds were characteristic of each pig. Hence, m-cresol/p-cresol showed significant statistical differences among the three pigs. This compound provided an off-flavour (nasty and stable) and presented the highest MF values in samples from PIG 3. Another example was the odour compounds identified as dihydromyrcenol, which was not detected in samples from PIG 1.

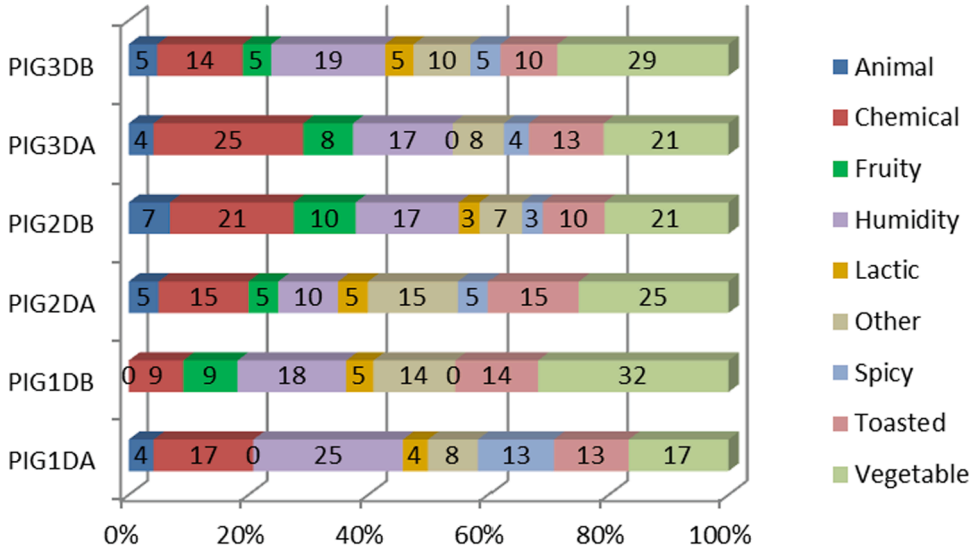

Fig. 1. Bar graph of the contribution of each aroma category as a percentage of the number of odour-active compounds in each sample (DA: ripening chamber DA, DB: ripening chamber DB).

In order to understand better the aromatic profile of each sample, odour-active compounds were grouped into 9 categories based on their aromatic characteristics: animal, spicy, fruity, humidity, lactic, chemical, toasted, vegetable and others (Table 1). This last group included odorant that were described as "river water" and others. Fig. 1 shows the contribution of each aroma category to the aroma profile of ham samples as a percentage of the number of odour-active compounds present in each sample. As can be seen, the vegetable aroma group predominated in most of the samples, followed by the chemical aroma and humidity aroma groups. In the vegetable aroma group, compounds such as the

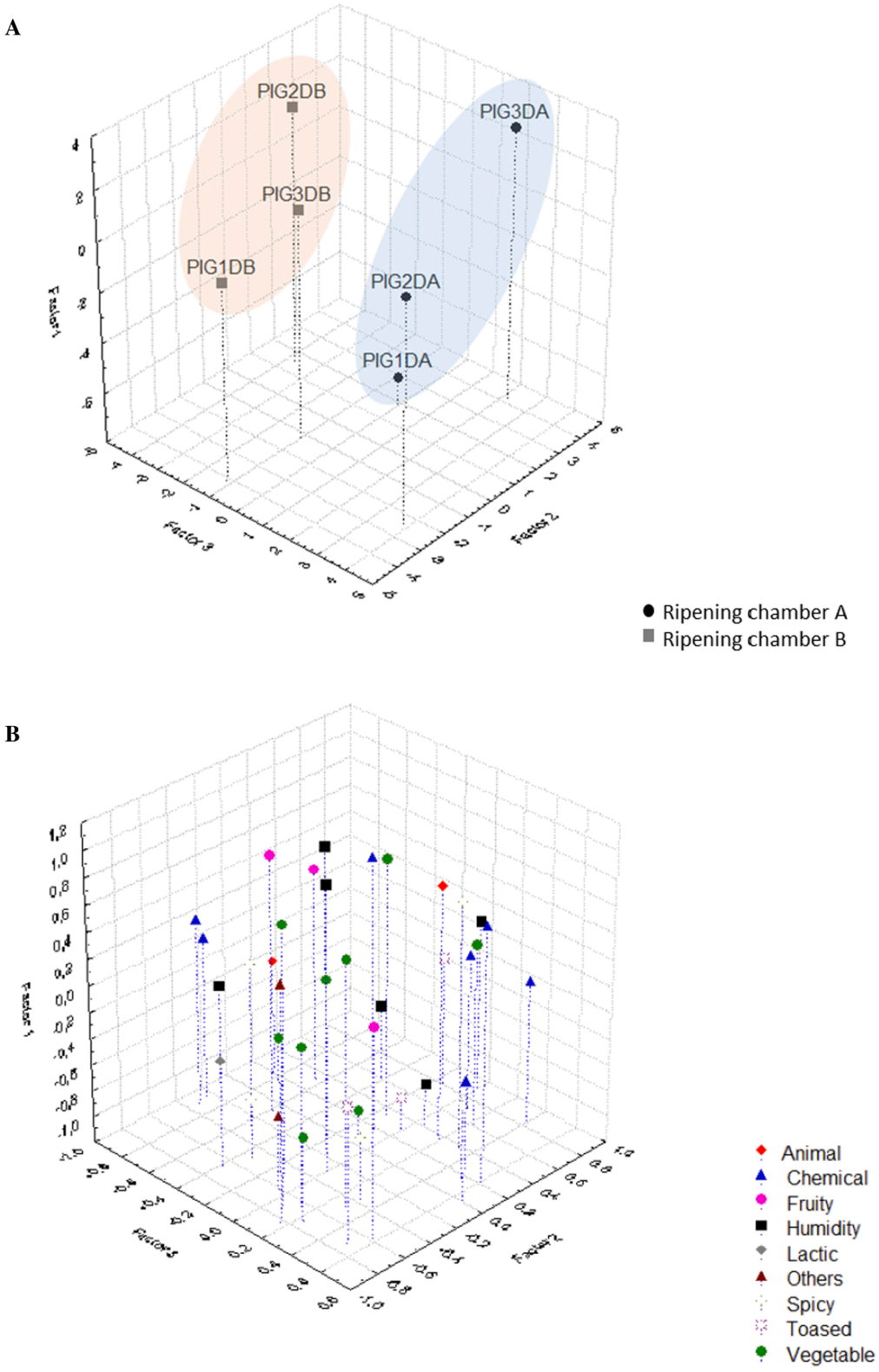

Fig. 2. Score (A) and Loading (B) values obtained in principal component analysis of the odour-active compounds obtained by gas chromatography/mass spectrometry-olfactometry.

above mentioned hexanal, *cis*-2-penten-1-ol, *trans*-geranylacetone and 1-octen-3-ol, among others, were included. However, the aroma category percentage did not show a clear trend related either to the ripening chamber's geographical location or to the pig whence the samples originated.

Principal component analysis (PCA) was performed by using MF values of all odour-active compounds as variables, the first three principal components explaining 76.5% of the total variance. In Fig. 2A, the samples appear as separate, depending on the geographical location where the hams were processed, taking into consideration the second and third principal components (PCs). The loading plot (Fig. 2B) showed that the variables primarily correlated to samples from ripening chamber DA were 1-undecanol, 2-octanone, *trans*-2-octenal, guaiacol and LRI 1472. In the case of samples from ripening chamber DB, located on the negative side of PC3, these were highly correlated with octane/acetone, decanal/2-ethyl-1-hexanol, methanethiol and isobutanol. In general, a greater number of odour-active compounds with vegetable and chemical notes were correlated with samples from ripening chamber DA, and humidity notes with samples from ripening chamber DB. The compounds with vegetable and chemical aromas were mainly aldehydes and linear alcohols, while those providing a humidity aroma were aldehydes and ketones. In dry-cured Iberian ham, alcohols are mainly formed by lipid oxidation while aldehyde and ketones originate in Maillard and Strecker reactions, as well as by lipid oxidation (Narváez-Rivas et al., 2012; Domínguez-Gómez, 2020). According to PCA results, therefore, ripening chamber DA's ambient conditions seem slightly to favour lipid oxidation.

3.2. Quantitative descriptive profile of dry-cured Iberian ham

Table 2 presents the mean values, standard deviations and the ANOVA analysis results between both pigs and ripening chamber's geographical locations for the sensory attributes studied. The results showed that there was a single qualitative descriptive profile for the samples analysed; all the Iberian hams presented rancid, meat broth, bread crust, roasted nuts, and acorn olfactory notes. Other authors (Cava et al., 2000; García-González et al., 2008; García-González, Tena, & Aparicio, 2009) have habitually employed most of these sensory attributes to describe the Iberian ham sensory profile.

ANOVA results showed that there were significant differences ($p < 0.05$) between ham samples from different pigs for the attributes of rancid aroma, bread crust aroma and odour, and acorn odour. We can highlight that the main differences between samples were due to the toasted olfactory notes (bread crust), PIG 2 giving the highest statistically significant values for the samples (Table 2 and Fig. 3A).

In terms of differences between the two ripening chamber's geographical locations, meat broth odour and roasted nuts odour were the only descriptors that presented significant differences ($p < 0.05$) between them for samples of all pigs. Ripening chamber DA showed the higher statistically significant values for meat broth odour, while the same was true for ripening chamber DB in terms of roasted nuts odour. We also observed that PIG 3 samples were those most affected by the ripening chamber. The individual ripening chamber also accounted for significant differences related to meat broth aroma/odour and roasted nuts aroma/odour (Table 2 and Fig. 3B).

The two-way ANOVA showed significant differences among ham samples regarding bread crust aroma/odour and roasted nut aroma/odour attributes. In these cases, both variables, pig, and ripening chamber, influenced toasted notes as perceived by orthonasal and retronasal pathways.

Three PCA were carried out: one considered all sensory attributes as variables, another considered orthonasal attributes only and finally one that considered retronasal attributes only. The last of these three PCA models, where the first three principal components (PCs) explained 90.1% of the total variance, was the only one in which it was possible to observe, thanks to PC2, a clear separation of samples by ripening chamber's geographical location (Fig. 4A). The variables correlated with samples from ripening chamber DA were meat broth odour, rancid odour, acorn odour and overall odour intensity, while samples from ripening chamber DB (Fig. 4B) correlated with bread crust and roasted nuts odour. Sensory attributes acorn and rancid has been related to aldehydes such as hexanal, 2-methylbutanal, etc., from lipid oxidation while toasted notes seem to be provided by ketones and pyrazines, compounds mainly originated in Maillar and Strecker reactions (Narváez-Rivas et al., 2012). This fact supports the hypothesis, indicated in the previous section, about the possible favouring of lipid oxidation by ripening chamber DA's ambient conditions. Climatological differences, therefore, seem to influence the different chemical and enzymatic reactions that take place during the curing process, resulting in sensory profile differences.

3.3. Relationship between sensory attributes and odour-active compounds

Sometimes, the attempt to relate sensory attributes and volatile compounds that contribute to the aroma of a food product can be challenging. Furthermore, since the food aroma perception in the sensory analysis is induced by a complex mixture of aroma compounds, neither is there an easy solution. Although it shows the individual contribution of each aromatic compound, the best analytical technique for solving this issue is olfactometric analysis. In order, therefore, to try to relate the results of the both analyses (olfactometric analysis and sensory profile), a correlation analysis and PCA were performed. When

Table 2
Results of mean intensities of the sensory attributes obtained by quantitative descriptive analysis of Dry-cured Iberian ham and two-way ANOVA results.

Attributes	PIG 1		PIG 2		PIG 3		*p*-values		
	DA	DB	DA	DB	DA	DB	Pig	D-M chamber	Pig*D-M chamber
Overall aroma intensity	5.9 ± 0.3	6.3 ± 0.3	6.1 ± 0.1	6.7 ± 1.0	6.3 ± 0.6	6.3 ± 0.4	0.78	0.38	0.73
Rancid aroma	4.8 ± 1.0^{a}	3.9 ± 0.3^{a}	$3.6 \pm 0.2^{a,b}$	$3.3 \pm 0.3^{a,b}$	3.0 ± 0.7^{b}	2.1 ± 0.2^{b}	**0.01**	0.06	0.65
Meat broth aroma	4.1 ± 0.5	4.3 ± 0.5	4.4 ± 1.1	3.0 ± 0.8	4.5 ± 0.0^{A}	3.0 ± 0.0^{B}	0.47	0.05	0.16
Bread crust aroma	3.4 ± 0.5^{a}	4.3 ± 0.1^{a}	6.4 ± 0.7^{b}	6.6 ± 0.1^{b}	4.5 ± 0.7^{a}	2.9 ± 0.1^{a}	**0.0002**	0.50	**0.017**
Roasted nuts aroma	3.8 ± 0.3	4.8 ± 0.2	4.5 ± 1.4	4.3 ± 0.1	4.5 ± 0.3^{A}	2.1 ± 0.6^{B}	0.09	0.22	**0.027**
Overall odour intensity	5.9 ± 0.2	5.9 ± 0.1	6.5 ± 0.3	6.1 ± 0.7	5.8 ± 0.6	6.2 ± 0.4	0.41	0.97	0.55
Rancid odour	3.8 ± 0.4	3.4 ± 0.2	4.6 ± 0.6	3.6 ± 0.0	3.7 ± 0.4	3.0 ± 1.2	0.29	0.09	0.81
Meat broth odour[1]	4.7 ± 0.4	4.7 ± 0.9	4.8 ± 1.0	3.3 ± 0.9	6.4 ± 0.2^{A}	3.9 ± 0.3^{B}	0.16	**0.01**	0.11
Acorn odour	$3.6 \pm 0.3^{a,b}$	$4.0 \pm 0.1^{a,b}$	4.5 ± 0.3^{a}	3.7 ± 0.3^{a}	2.7 ± 1.5^{b}	2.9 ± 0.3^{b}	0.07	0.9	0.43
Bread crust odour	$0^{a, A}$	$3.0 \pm 0.3^{a, B}$	2.8 ± 1.3^{b}	4.7 ± 0.2^{b}	$2.9 \pm 0.4^{a,b}$	$2.3 \pm 0.3^{a,b}$	**0.006**	**0.006**	**0.013**
Roasted nuts odour[1]	0^{A}	3.5 ± 0.8^{B}	3.5 ± 0.2	3.7 ± 1.1	0^{A}	2.7 ± 0.1^{B}	**0.003**	**0.001**	**0.018**

Superscript: different lowercase letters in the same row indicate statistically significant differences ($p < 0.05$) between samples from different pigs; different capital letters in the same row indicate statistically significant differences ($p < 0.05$) between samples from different ripening chamber geographical locations but from the same pig.

p-values in bold were significant ($p < 0.05$).

[1] There were statistically significant differences ($p < 0.05$) between ripening chamber geographical locations for samples from all pigs.

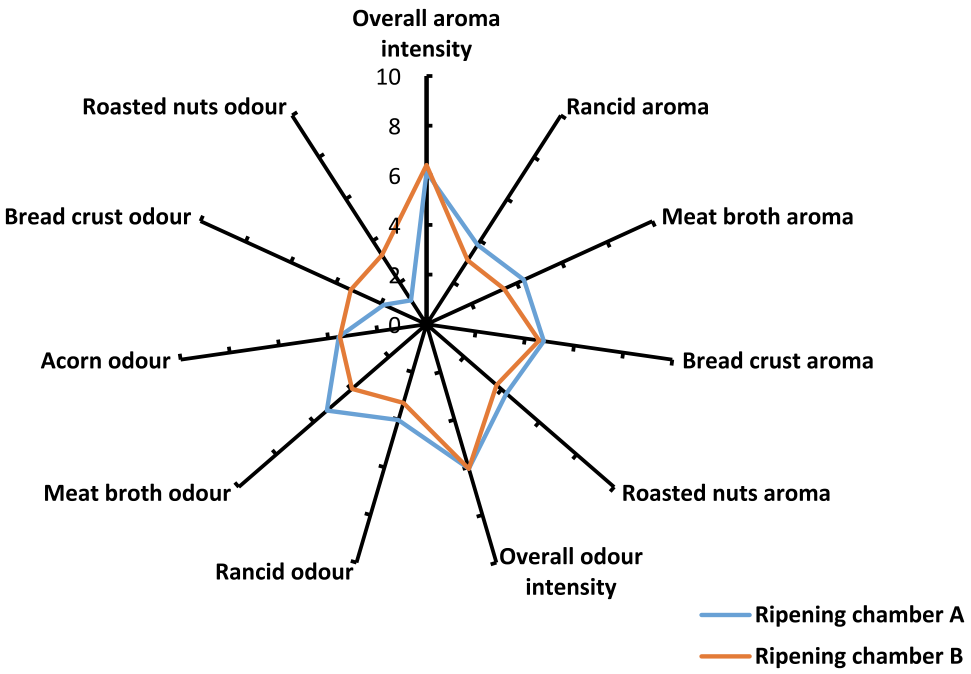

Fig. 3. Quantitative descriptive profile of Iberian ham samples produced in ripening chamber located in different geographical locations.

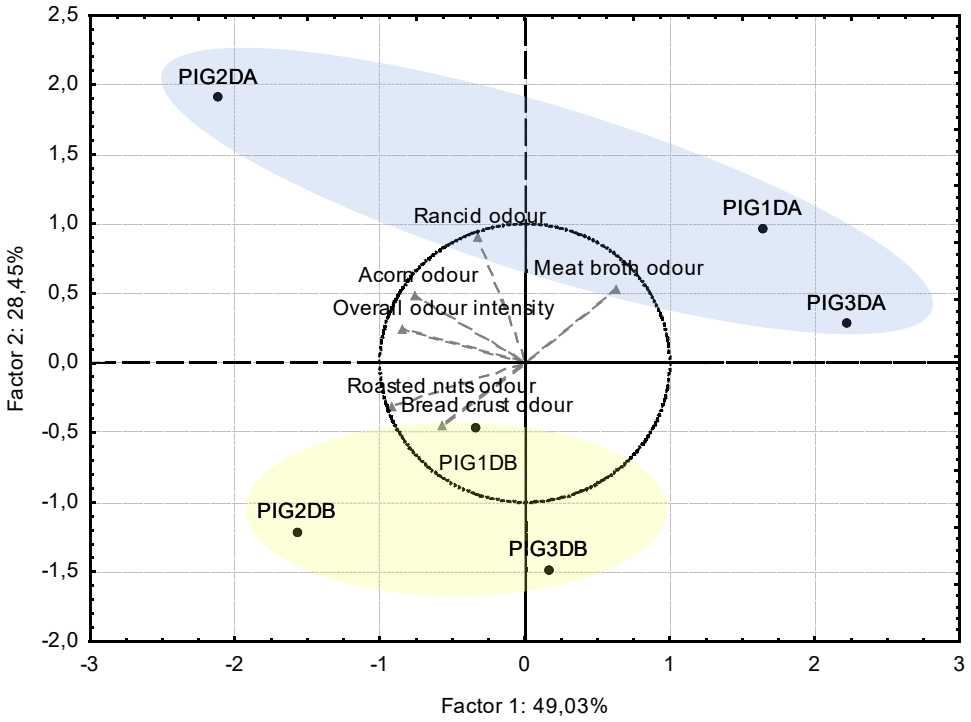

Fig. 4. Plot of principal component analysis results obtained using retronasal attributes as variables: Score (DA: ripening chamber DA, DB: ripening chamber DB) and Loading values.

the total MF values of odour-active compounds grouped by aromatic characteristics were considered together with sensory attributes, a direct correlation between bread crust odour descriptor and the fruity category ($r = 0.96$) can be observed. Furthermore, rancid aroma was directly correlated with the toasted group, which included all compounds with the "toasted corn" odour descriptor. In contrast, this sensory attribute was inversely correlated with the vegetable group (-0.88). Moreover, when individual odour-active compounds were considered as variables, some sensory attributes were also correlated. The results regarding the bread crust odour can therefore be highlighted. It was correlated with five odorants, showing a direct correlation with ethyl isovalerate (fruit odour description) and 1-octen-3-one (mushroom odour), while there was an inverse correlation with guaiacol (toasted corn odour), isoamyl laureate (sweet odour) and 2(5H)-furanone (liquorice odour). 4-Propyl-guaiacol (sweet odour) and 2,4-nonadienal (river water odour) were directly correlated with the roasted nuts odour. Odorants related to orthonasal and retronasal rancid perception were correlated differently. In the first case, a direct correlation with 2,3-butanediol, described as

grass odour, was observed, while the rancid odour was correlated with an LRI 1472 odorant with chemical aromatic notes. Finally, the acorn odour was directly correlated with the odorant identified as *trans*-2-octenal, which, in our case, provided the toasted corn aroma. This odour-active compound (*trans*-2-octenal) was quantified in concentrations higher than those that had been found in other studies on Iberian hams (García-González et al., 2008). Therefore, a higher number of retronasal sensory attributes than orthonasal sensory attributes were correlated with odour-active compounds. This may be due to the experimental conditions of aroma extraction used in the olfactometric

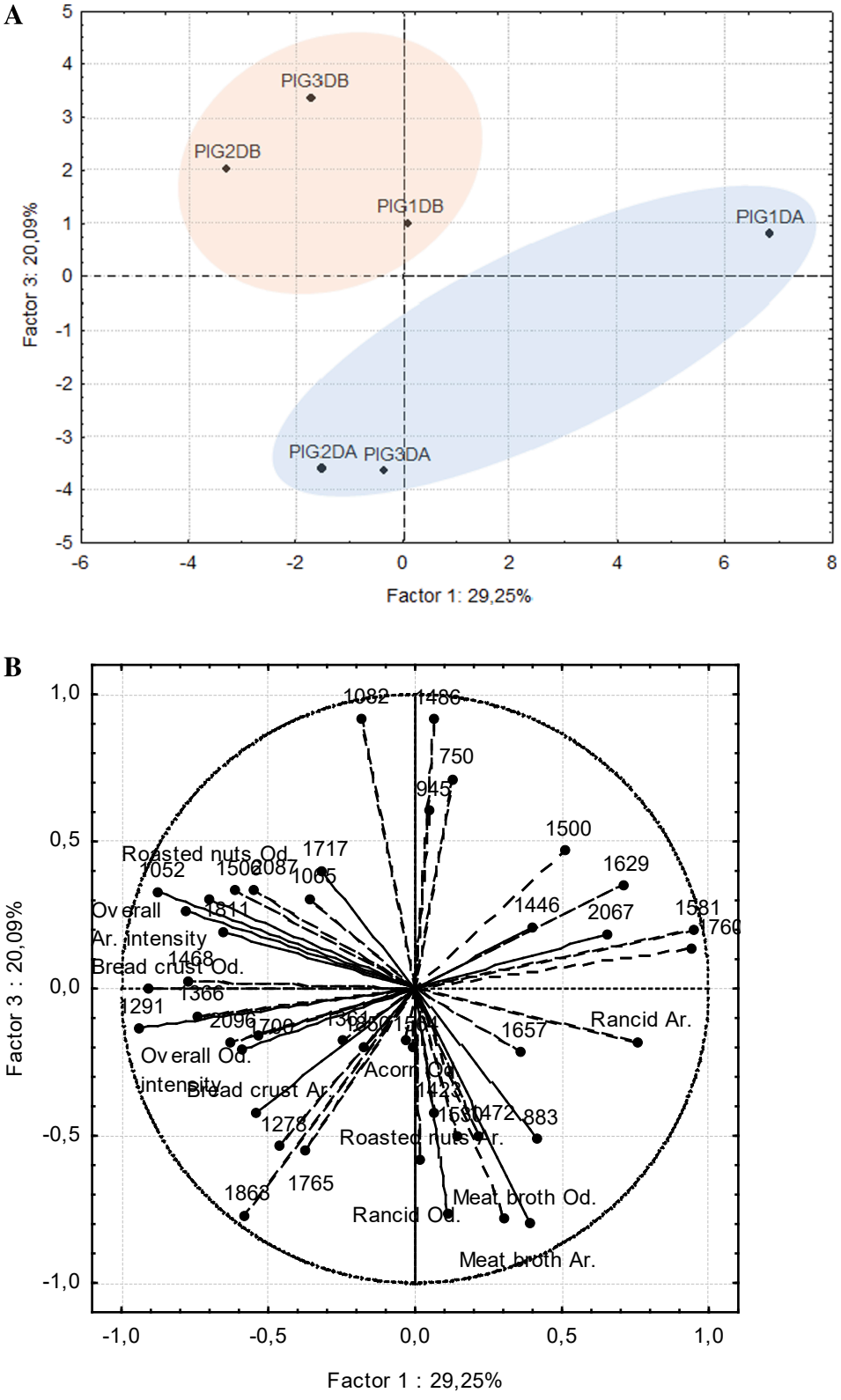

Fig. 5. (A) Score (DA: ripening chamber DA, DB: ripening chamber DB) and (B) Loading values obtained in principal component analysis using key odorants and all sensory attributes as variables.

analyses in which the samples were cut into small pieces. This condition was more similar to those in which the aromas were released and perceived during the retronasal (buccal cavity conditions) than during the orthonasal sensory analyses.

PCA was performed using key odorant MF values and sensory attributes values as variables. The MF values were previously divided by 100 to normalise them with the sensory variables' values, whose data ranged from 0 to 10. The first three components explained 73.5% of the total variance. The ham samples were separated by their ripening chamber's geographical locations on the plane formed by the first (PC1) and the third (PC3) principal components, specifically by PC3 (Fig. 5A). The ham samples from ripening chamber DB were located on the positive side of PC3. Most of the samples from ripening chamber DA were on the negative side of PC3, except for the PIG 1 sample which was located in the right upper quadrant, far away from the rest. Regarding loading values (Fig. 5B), variables highly correlated with ham samples from ripening chamber DB were the key odorants isobutanol, decanal/2-ethyl-1-hexanol and *cis-cis*-2,6-nonadienal and the sensory attribute roasted nuts odour. On the other hand, most of the sensory attributes, such as bread crust aroma, rancid odour, meat broth odour and aroma and roasted nuts aroma, appeared on the negative side of PC3 (Fig. 5B), where most samples from ripening chamber DA were located.

As can be seen in Fig. 5A, the PIG 1 ham samples were separated from the other samples, primarily by PC1, revealing that samples from PIG 1 seemed to be quite different to those from PIG 2 and PIG 3, a difference which prevailed over the effect exercised by the ripening chamber's geographical locations. Variables correlated with samples from PIG 1 were 2,3-butanediol, 2(5H)-furanone, 2-acetylfuran, 1-octen-3-ol and *m*-cresol/*p*-cresol and the odorant with LRI 1629 (Fig. 5A).

4. Conclusions

In this work, the influence of the ripening chamber's geographical location on the aroma of the hams cured therein was studied by using olfactometric and sensory analyses. One of the novelties of this work was the experimental design, in which both participating production facilities processed one of the two hind legs from each pig.

Moreover, this work has, for the first time, determined the compounds 2(5H)-furanone, *cis*-2-penten-1-ol and 2-acetylfuran in dry-cured Iberian hams.

Both kind of analyses (olfactometric analysis and quantitative descriptive profile) enabled the ham samples to be differentiated by their ripening chamber's geographical location. In the case of the sensory profile, the best results were obtained when retronasal sensory analysis was performed.

Moreover, the results obtained showed that the odour-active compounds and key odorants were not the same for the samples from the same pig processed in the two different ripening chamber's geographical locations. This preliminary study showed that slight climatological differences due to location of the ripening chamber seem to exercise a certain influence on the aromatic profile. This knowledge could be useful to produce hams with different organoleptic characteristics, or even to know how to enhance certain sensory attributes in order to cater to consumer tastes, as well as to ensure the authenticity of the hams' geographical origin. Nevertheless, further studies with a higher number of samples and production facilities' geographical locations would be necessary to confirm these conclusions and their relevance to Iberian ham quality. In addition, it would also be of great interest to study how volatile compounds evolve during the drying process of ham, using non-invasive sampling in order to explain and understand in greater depth the results obtained in this work.

CRediT authorship contribution statement

MP. Segura-Borrego: Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **R. Ríos-Reina:** Data curation, Formal analysis, Writing – review & editing. **H. Galán-Soldevilla:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Writing – review & editing. **FJ. Forero:** Funding acquisition, Resources. **M. Venegas:** Funding acquisition, Resources. **P. Ruiz Pérez-Cacho:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Writing – review & editing. **ML. Morales:** Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Writing – original draft, Writing – review & editing. **RM. Callejón:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing.

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.

Acknowledgements

The authors would like to thank Universidad de Sevilla for the PIF scholarship (VIPPIT-2019-IV.3), to the Excelentísima Diputación de Huelva, Spain for their funding support and the GrupoSens panel members-Universidad de Córdoba (Spain) for their voluntary participation.

Ethical statement

The content of the sensory evaluation experiment has been with the informed consent of the candidates and has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2022.110977.

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