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The Influence of *Debaryomyces hansenii*, *Candida deformans* and *Candida zeylanoides* on the aroma formation of dry-cured "lacón"

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ABSTRACT

The volatile profile of dry-cured "lacón" that has been inoculated with three different yeasts were determined and compared with a non-inoculated dry-cured "lacón". Yeasts (*Debaryomyces hansenii*, *Candida deformans* and *Candida zeylanoides*) that were used as starter cultures in the present study were selected among yeasts that were isolated from native dry-cured "lacón" at different stages of ripening process. These starters were spread on dry-cured "lacón" surface in order to test their capacity to contribute on the generation of volatile compounds. A total of forty two volatile compounds were detected by dynamic headspace sampling followed by gas chromatography–mass spectrometry analysis. Significant differences ($P<0.001$) on the volatile profiles of different batches were found in comparison with non-inoculated samples, showing the highest total area values for the inoculated ones. Esters were the most abundant chemical family in all batches studied except for *C. zeylanoides* batch, which showed greater amount of hydrocarbons than esters. The second more abundant family was hydrocarbons for control and *C. deformans* batches (147.6 and 445.24×10^6 area units, respectively), alcohols for *D. hansenii* (363.77×10^6 area units) and esters for *C. zeylanoides* (248.33×10^6 area units). However, the aldehyde compound group in control batch samples was found to be significantly higher than in the inoculated ones ($P<0.001$). Among inoculated batches, *D. hansenii* batch showed the lowest hexanal content (14.42×10^6 area units) in comparison with non-inoculated batch (105.99×10^6 area units). Among all batches studied, *D. hansenii* batch presented the highest area values for esters, alcohols, linear hydrocarbons, ketones, acids and furans; control batch for aldehydes and *C. zeylanoides* batch for branched hydrocarbons. Therefore, the study showed that every yeast strain produced a specific volatile profile which was also different from that of the control dry-cured "lacón".

1. Introduction

Dry-cured "lacón" is a traditional dry-cured meat product made in the north-west of Spain from the pig foreleg that is cut at the shoulder blade–humerus joint, following very similar manufacturing processes to those used in the production of dry-cured ham as described by Marra, Salgado, Prieto, and Carballo (1999). In Galicia, this product has been awarded a Geographically Protected Identity (Official Journal of the European Communities, 2001).

Dry-cured meat products are appreciated because of their unique flavor. It is important to know the factors influencing meat flavor in order to produce quality meat products due to the relationship between flavor and consumer acceptability. The aroma is perhaps the most important quality parameter of dry-cured meat products, and it is markedly affected by raw material, processing techniques and aging time (Sánchez-Peña, Luna, García-González, & Aparicio-Ruiz, 2005). Its perception depends on the concentration and odor threshold of volatile compounds and on their interactions with other food components that will affect its gas phase concentration (Guichard, 2002). The compounds that are implicated in flavor generation arise from many sources, such as spices, sugar metabolism, lipolysis and lipid oxidation, proteolysis and amino acid degradation (Toldrá, Sanz, & Flores, 2001). The main enzymatic reactions related to formation of flavor precursors are proteolysis and lipolysis. Both reactions take place by the contribution of endogenous proteases and lipases, enzymes that are either naturally originated by microbes or added during the manufacturing process. The role of microorganisms in the generation of volatile compounds is well documented for dry-fermented sausages (Bruna et al., 2001; Zapelena, Astiasarán, & Bello, 1999). In the case of dry-cured ham the role of microorganisms is considered to be less important because the microbial population inside the ham is relatively low (Silla, Molina, Flores, & Silvestre, 1989).

Yeasts are one of the predominant microbial groups during the ripening period of dry-cured meat products (Cocolin, Urso, Rantsiou, Cantoni, & Comi, 2006; Núñez, Rodríguez, Córdoba, Bermúdez, & Asensio, 1996). Several studies carried out with different yeast strains have shown that they contribute to the development of the characteristic flavor of these

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products (Andrade, Córdoba, Casado, Córdoba, & Rodríguez, 2010; Martín, Córdoba, Benito, Aranda, & Asensio, 2003; Patrignani et al., 2007). Moreover, some authors (Andrade, Córdoba, Sánchez, Casado, & Rodríguez, 2009; Regodon, Perez, & Ramirez, 2006) have reported differences in flavor development related to the particular yeast species and the particular strain on the species. Among yeasts, *Debaryomyces* spp. have showed to have a positive impact on the volatile compounds that are involved in flavor development of dry cured meat products (Martín, Córdoba, Aranda, Córdoba, & Asensio, 2006). In the same line, Flores, Dura, Marco, and Toldrá (2004) observed that an appropriate inoculum level of *Debaryomyces* spp. influenced volatile production by inhibiting lipid oxidation due to its antioxidative effect and by promoting the generation of ethyl esters. This information about the effect of yeasts on volatile compounds formation is increasing the interest in developing and using yeast starter in dry cured meat products (Andrade, Rodríguez, Sánchez, Aranda, & Córdoba, 2006; Bolumar et al., 2006) to improve some of their sensorial characteristics. The evaluation of yeast strains ability to produce volatile compounds can be done using a meat model system (Martín et al., 2003) or directly on dry-cured meat products (Andrade et al., 2010; Martín et al., 2006; Patrignani et al., 2007).

Given that it is not possible to keep dry-cured "lacón" as sterile control without interfering with manufacturing process, the contribution of the tested strains on the volatile compounds has to be tested against "lacón" with a wild microbial population. Thus, the aim of this study was to evaluate the contribution of selected strains in the production of dry-cured "lacón" volatile compounds.

2. Material and methods

2.1. Samples

2.1.1. Yeast strains

Yeast strains tested in this study were obtained from the indigenous flora of dry cured "lacón" at different ripening stages. Each sample was homogenized in masticator IUL (IUL Instruments, Barcelona, Spain) using 0.1% w/v peptone water as diluent. Further decimal dilutions were prepared by mixing 1 mL of the previous dilution with 9 mL of the same diluent and 0.1 mL portions were spread onto the surface of OGYEA (OGYE agar, Base, Merck KGaA, Darmstadt, Germany) agar plates. They were incubated at 25 °C for 5 days. All the yeasts were purified on repeat cultivation on MEB (malt extract broth, Merck KGaA, Darmstadt, Germany) and maintained at −80 °C in MEB containing 30% v/v glycerol until analysis. Identification of these strains has been described by molecular techniques in a pending work yet to be published. Three different yeast strains (*Debaryomyces hansenii* (DH), *Candida deformans* (CD) and *zeylanoides zeylanoides* (CZ)) were chosen to be inoculated in the present study.

2.1.2. Preparation of yeast inocula

The cultures were successively subcultured on MEA (malt extract agar, Merck KGaA, Darmstadt, Germany). The cell concentration was calculated by comparison with the absorbance at 625 nm in a spectrophotometer 8453 (Agilent Technologies Spain, S.L., Madrid, Spain). The cells were obtained by centrifugation (4500 rpm for 20 min at 4 °C), washed with sterile distilled water and centrifuged again. The pellet cells were concentrated in 15 ml of sterile distilled water and inoculated by spraying on the surface of the dry cured "lacón". The starters were inoculated at 10^6 CFU/cm^2.

2.1.3. Preparation of dry-cured "lacón"

In order to carry out this study, four batches of "lacón" were manufactured. Each batch consisted of eight "lacón" pieces weighing around 4 kg in the green stage (fresh pieces). Raw pieces were salted using an excess of coarse salt. A heap was formed consisting of alternating layers of "lacón" pieces and layers of salt. Pieces were totally covered in salt following this structure, remaining this way for four days (a day per kg) in salting room (temperature between 2 and 5 °C; relative humidity between 80 and 90%). After the salting stage the pieces were taken from the heap, brushed and washed. Then, all pieces were divided into four batches, three of them were inoculated with the different yeast strains and the remaining one was used as control batch (without starter culture). Then, batches were transferred to a post-salting room and kept for 14 days at 2–5 °C and around 85–90% relative humidity. After the post-salting stage the pieces were transferred to a room at 12 °C and 74–78% relative humidity where a drying–ripening process took place for 84 days. The air convection in the drying room was intermittent and the air velocity around the pieces when the fan was running ranged between 0.3 and 0.6 m/s. Each sample consisted of one whole "lacón" piece. Samples were transported to the laboratory under refrigerated conditions (<4 °C) and analyzed at this point. Once in the laboratory, the entire pieces were skinned, deboned, and *Triceps brachii* muscle was extracted and finally minced in a high-capacity mincer. The samples were stored in air-tight bottles, frozen at −80 °C in dark, for no longer than 4 weeks until analysis.

2.2. Analytical methods

2.2.1. Analysis of volatile compounds

Samples were ground in a domestic blender, and a 10 g sample was put into a dynamic headspace vial. The volatile compounds were extracted and concentrated in a purge and trap concentrator that was coupled with a cryofocusing module (Teledyne Tekmar, Mason, OH, USA).

2.2.1.1. Dynamic headspace volatile concentration. Samples were transferred into headspace vials and concentrated in a purge-and-trap concentrator (Stratum, Teledyne Tekmar, Mason, OH, USA) that was equipped with a cryofocusing module in connection with an autosampler (Solatek 72 Multimatrix Vial Autosampler, Teledyne Tekmar, Mason, OH, USA). They were kept at 60 °C for 5 min and then flushed with helium at a flow rate of 60 mL/min for 20 min. Volatile compounds were adsorbed on a Tenax Trap (Strat trap, 30.48 cm, Agilent Technologies Spain, S.L., Madrid, Spain) and thermally desorbed from the Tenax trap at 225 °C for 4 min with a helium flow rate of 300 mL/min. The desorbed compounds were cryofocused at −30 °C using liquid nitrogen at the entrance of a DB-624 capillary column (J&W scientific, Folsom, CA, USA).

2.2.1.2. Gas chromatography/mass spectrometry (GC/MS). A gas chromatograph 6890 N (Agilent Technologies Spain, S.L., Madrid, Spain) equipped with mass detector 5973 N (Agilent Technologies Spain, S.L., Madrid, Spain) was used with a DB-624 capillary column (J&W scientific: 30 m × 0.25 mm id, 1.4 μm film thickness). The sample was injected in split mode (1:20). Helium was used as a carrier gas with a linear velocity of 36 cm/s. The temperature program used was as follows: we started at 40 °C for 2 min and then temperature was raised from 40 to 100 °C at 3 °C/min, then from 100 to 180 °C at 5 °C/min, and from 180 to 250 °C at 9 °C/min with a final holding time of 5 min; total run time 50.8 min. Injector and detector temperatures were set at 220 and 260 °C, respectively.

The mass spectra were obtained by means of a mass selective detector working in electronic impact at 70 eV, with a multiplier voltage of 1953 V and collecting data at a rate of 6.34 scans/s over the range m/z 40–300. Compounds were identified by comparing their mass spectra with those contained in the NIST05 (National Institute of Standards and Technology, Gaithersburg) library and/or by calculating retention index in relation to a series of standard alkanes (C_5–C_{14}) (to obtain Kovats indexes, Supelco 44585-U, Bellefonte, PA, USA) and matching them with literature reported data.

A total of 32 units of dry-cured "lacón" samples were analyzed in triplicate. Results were expressed in relative abundance units and measured as total area counts (AU × 10^6).

2.3. Statistical analysis

For the statistical analysis of the results, data were analyzed using the SPSS 19.0 for Windows (SPSS, Chicago, IL, USA) software package. One way analysis of variance was used to analyze the effect of yeast strains on volatile compounds composition. The least squares mean were separated using Duncan's *t*-test. All statistical tests of LSM were performed for $\alpha < 0.05$ significance level.

The principal component analysis (PCA) was conducted in order to identify the most important factors involved on volatile compounds at the end of process of dry-cured "lacón" from four different batches (Control, DH, CD and CZ). The PCA was performed on the correlation matrix. All the statistical analyses were carried out using the SPSS 19.0 for Windows (SPSS, Chicago, IL, USA) software package.

3. Results and discussion

Over 42 volatile compounds were identified in samples corresponding to "lacón" at the end of process. Table 1 shows mean values of chromatographic areas ($AU \times 10^6$) grouped by chemical families. Overall, from the qualitative point of view, the global volatile profile was very similar among batches. Nevertheless, significant quantitative differences ($P < 0.001$) in the total area of volatile profiles were detected. The total area of volatile compounds was significantly higher in the inoculated batches (1395, 774 and 886×10^6 area units, for DH, CD and CZ groups, respectively) than in control batch (701×10^6 area units). The predominant volatile compound family was esters for all batches studied, with the exception of CZ batch, which showed higher values of hydrocarbons than esters (see Fig. 1). The second more abundant families

Table 1
Volatile compounds of dry-cured "lacón", expressed as total area counts ($AU \times 10^6$) (mean area ± standard deviation).

Volatile compounds	IK	R	Control	Inoculated yeast strains[1]			P value
				DH	CD	CZ	
Linear hydrocarbons							
Pentane	467	k,m	0.00 ± 0.00^{a}	2.76 ± 1.55^{b}	0.00 ± 0.00^{a}	1.18 ± 0.31^{a}	0.000
Hexane	595	k,m	37.38 ± 6.85^{a}	57.54 ± 6.36^{b}	60.20 ± 6.97^{b}	54.34 ± 9.32^{b}	0.002
Heptane	698	k,m	5.78 ± 1.72^{a}	80.96 ± 7.73^{b}	11.73 ± 2.77^{a}	7.32 ± 3.21^{a}	0.000
Octane	799	k,m	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	9.70 ± 1.38^{b}	0.000
Branched hydrocarbons							
Heptane, 2,2,3,5-tetramethyl	1006	k,m	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	2.53 ± 0.50^{b}	0.00 ± 0.00^{a}	0.000
Heptane, 2,5-dimethyl	1052	k,m	0.00 ± 0.00^{a}	37.17 ± 4.24^{b}	0.00 ± 0.00^{a}	78.15 ± 8.75^{c}	0.000
Octane, 2,5,6-trimethyl	1056	k,m	30.50 ± 0.79^{c}	8.62 ± 0.83^{a}	20.69 ± 4.56^{b}	36.19 ± 4.85^{d}	0.000
2,2,7,7-Tetramethyloctane	1065	k,t	9.43 ± 3.07^{c}	0.00 ± 0.00^{a}	5.48 ± 1.47^{b}	0.00 ± 0.00^{a}	0.000
Nonane, 3,7-dimethyl	1088	k,m	4.71 ± 0.30^{a}	15.74 ± 2.83^{b}	3.66 ± 0.75^{a}	33.85 ± 5.50^{c}	0.000
Hexane, 2,2,5-trimethyl	1092	k,m	23.46 ± 1.19^{b}	15.06 ± 5.96^{a}	17.02 ± 3.84^{a}	12.79 ± 1.47^{a}	0.000
Octane, 2,6-dimethyl	1097	k,m	16.14 ± 0.62^{b}	10.93 ± 1.77^{a}	11.75 ± 1.97^{ab}	94.12 ± 6.03^{c}	0.005
Undecane, 2,8-dimethyl	1111	k,m	16.16 ± 0.73^{a}	12.81 ± 3.91^{a}	10.47 ± 2.50^{a}	96.36 ± 14.54^{b}	0.000
Undecane, 4,8-dimethyl	1119	k,m	4.03 ± 1.05^{a}	11.08 ± 2.62^{b}	2.59 ± 0.25^{a}	21.25 ± 5.48^{c}	0.000
Alcohols							
2-Methylpropanol	636	k,m	0.00 ± 0.00^{a}	3.54 ± 0.56^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.000
1-Penten-3-ol	734	k,m	0.00 ± 0.00^{a}	3.63 ± 0.81^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.000
3-Methylbutanol	768	k,m	9.18 ± 0.92^{a}	320.14 ± 63.58^{c}	125.38 ± 31.45^{b}	7.82 ± 1.81^{a}	0.000
1-Hexanol	921	k,m	26.71 ± 6.75^{b}	36.46 ± 7.75^{c}	9.66 ± 1.75^{a}	12.97 ± 4.64^{a}	0.000
Ketones							
2,3-Butanedione	633	k,t	13.26 ± 1.87^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	15.24 ± 1.35^{c}	0.000
Methyl vinyl ketone	597	m	0.00 ± 0.00^{a}	58.05 ± 13.59^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.000
2-Pentanone	731	k,t	12.03 ± 2.46^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.000
2-Heptanone	936	k,m	16.32 ± 4.22^{b}	11.85 ± 1.71^{b}	12.28 ± 5.21^{b}	6.35 ± 2.05^{a}	0.007
2-Nonanone	1159	k,m	6.91 ± 0.94^{c}	0.00 ± 0.00^{a}	3.68 ± 1.12^{b}	0.00 ± 0.00^{a}	0.000
Aldehydes							
3-Methylbutanal	692	k,m	10.99 ± 3.12^{b}	27.81 ± 6.59^{c}	14.21 ± 3.22^{b}	0.00 ± 0.00^{a}	0.000
Hexanal	842	k,m	105.99 ± 4.88^{c}	14.42 ± 3.63^{a}	22.60 ± 9.62^{a}	61.34 ± 9.18^{b}	0.000
Nonanal	1077	k,m	4.06 ± 0.85^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	4.24 ± 1.54^{b}	0.000
3-Cyclopentene-1-acetaldehyde, 3-trimethyl	1199	m	5.01 ± 1.02^{b}	1.77 ± 1.09^{a}	4.30 ± 1.00^{ab}	9.37 ± 3.35^{c}	0.000
Esters							
Methyl isobutanoate	670	k,m	14.23 ± 2.85^{a}	54.87 ± 11.46^{c}	29.26 ± 4.92^{b}	12.25 ± 4.67^{a}	0.000
Methyl butanoate	713	k,m	51.56 ± 6.05^{a}	50.21 ± 6.25^{a}	50.81 ± 19.38^{a}	59.85 ± 16.40^{a}	0.665
Methyl isopentanoate	781	k,m	96.39 ± 14.79^{b}	336.67 ± 11.03^{d}	174.09 ± 20.97^{c}	50.36 ± 17.74^{a}	0.000
Methyl pentanoate	844	k,m	7.73 ± 1.39^{a}	14.70 ± 2.52^{b}	13.61 ± 3.31^{b}	6.82 ± 3.27^{a}	0.001
Methyl hexanoate	936	k,m	99.37 ± 12.05^{b}	118.23 ± 17.28^{b}	106.32 ± 33.81^{b}	68.07 ± 16.51^{a}	0.016
Methyl heptanoate	1080	k,m	0.00 ± 0.00^{a}	8.24 ± 1.96^{b}	0.00 ± 0.00^{a}	15.63 ± 3.53^{c}	0.000
Methyl octanoate	1170	k,m	4.65 ± 1.08^{a}	6.98 ± 1.44^{b}	7.08 ± 1.68^{b}	5.74 ± 0.44^{ab}	0.036
3-Hidroximandelic acid, ethyl ester, di-TMS	1164	m	24.96 ± 1.91^{ab}	26.16 ± 2.29^{ab}	22.55 ± 1.84^{a}	29.62 ± 5.25^{b}	0.025
Methyl propionate	618	k,m	0.00 ± 0.00^{a}	11.80 ± 3.06^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.000
Acids							
Acetic acid	664	k,m	0.00 ± 0.00^{a}	12.82 ± 1.72^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.000
Malonic acid	1292	m	3.67 ± 0.27^{b}	3.48 ± 0.74^{ab}	2.74 ± 0.31^{a}	4.71 ± 0.85^{c}	0.001
Furans							
Furan, 2-pentyl	1003	k,m	0.00 ± 0.00^{a}	5.32 ± 0.95^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.000
Other compounds							
Camphene	991	k,m	13.23 ± 1.84^{a}	9.07 ± 2.10^{a}	9.76 ± 2.29^{a}	62.51 ± 16.94^{b}	0.000
Octadecane, 1-chloro	1073	t	18.83 ± 1.29^{c}	0.00 ± 0.00^{a}	12.44 ± 2.90^{b}	0.00 ± 0.00^{a}	0.000
Camphor	1224	m	9.02 ± 0.94^{b}	6.29 ± 0.66^{a}	7.68 ± 1.47^{ab}	8.67 ± 3.03^{ab}	0.139

[a–c]Means with different superscripts were significantly different for $P < 0.05$.
KI: Kovats index calculated for DB-624 capillary column (J&W scientific: 30 m × 0.25 mm id, 1.4 μm film thickness) installed on a gas chromatograph equipped with a mass selective detector.
R: Reliability of identification: k: Kovats index in agreement with literature (Flores et al., 1997; Jurado, Carrapiso, Ventanas, & García, 2009; Liu, Xu, & Zhou, 2007; Ruiz, Ventanas, Cava, Andrés and García, 1999); m: mass spectrum agreed with mass database (NIST05); and t: tentative identification by mass spectrum.
[1] "Lacón" samples inoculated with yeast strains: *D. hansenii* (DH), *C. deformans* (CD) and *C. zeylanoides* (CZ).

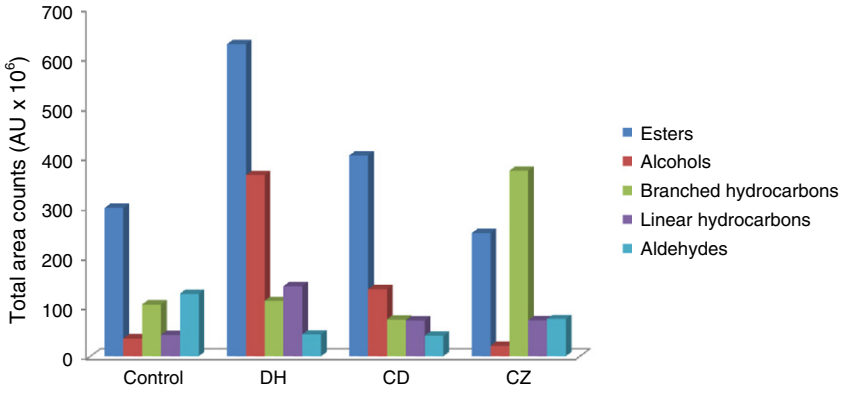

Fig. 1. Majority chemical families of volatile compounds from dry-cured "lacón" "Lacón" samples inoculated with yeast strains: *D. hansenii* (DH), *C. deformans* (CD) and *C. zeylanoides* (CZ).

were hydrocarbons (for control and CD batch), alcohols (for DH group) and esters (for CZ group) (see Fig. 1). Acids and furans showed the lowest values in all batches studied. Taking into account that all the batches were produced under the same technological conditions, it seems that the different inoculated yeasts were responsible for these different volatile profiles.

Formation of hydrocarbons can be attributed to lipid autoxidation of fatty acids that are released from triglycerides (Ordóñez, Hierro, Bruna & de la Hoz, 1999), playing an important role on both linear and branched hydrocarbon formations in dry-cured meat (Ruiz, Ventanas, Cava, Andrés & García, 1999). Linear hydrocarbons were approximately 6–10% of volatile compounds total area. The profile of these CD batch-obtained compounds was similar to that displayed by control group and different to those of the other ones. Significant differences ($P<0.001$) were found for the sum of all linear hydrocarbons for each batch. In general, the observed values were significantly higher in the inoculated batches (141, 72 and 73×10^6 area units, for DH, CD and CZ groups, respectively) than in the control group (43×10^6 area units) (see Fig. 1). Among these compounds, hexane was the most abundant, showing CD treatment the highest content (60.20×10^6 area units) while control group showed the lowest one (37.38×10^6 area units). In the case of heptane, the amount found in control batch (5.78×10^6 area units) was also lower than in DH group (80.96×10^6 area units). On the other hand, pentane and octane only appeared in some inoculated batches (DH and CZ). These results are not relevant given that linear hydrocarbons are not important contributors to the meat product flavor because of their high odor thresholds (Ansorena, Gimeno, Astiasarán, & Bello, 2001; Bianchi et al., 2007).

The group of branched hydrocarbons in CZ batch (372.71×10^6 area units) was found to be significantly higher than in the control one (104×10^6 area units, $P<0.001$) (see Fig. 1). The batch inoculated with *C. zeylanoides* showed the highest value compared to the rest of batches studied due mainly to the high values of octane, 2,6-dimethyl (94.12×10^6 area units) and undecane, 2,8-dimethyl (96.36×10^6 area units). On the other hand, CD batch showed lower values than control group (74.19×10^6 area units). The most abundant branched hydrocarbon in control and CD batches was octane, 2,5,6-trimethyl (30.50 and 20.69×10^6 area units, respectively) while undecane, 2,8-dimethyl was more common in CZ group (96.36×10^6 area units, respectively) and heptane, 2,5-dimethyl was prevalent in DH batch (37.17×10^6 area units). The profile of these volatile compound groups was quite similar except for DH and CZ batches, which showed high values for one specific volatile compound (heptane, 2,5-dimethyl) (see Table 1). Total amount of hydrocarbons in CZ batch was remarkably high, representing around 50% of total volatile compounds area. These results are in agreement with those reported by Tejeda, Antequera, Martín, Ventanas, and García (2001) who observed that some microorganisms may contribute to the differences in n-alkanes found in dry cured ham, while other authors (Martín et al., 2006; Ruiz et al., 1999) have observed that the increase of linear and branched hydrocarbons can be attributed to a microbial lipolytic activity. Regardless of their origin, linear and branched hydrocarbons have been considered as non-contributors to meat flavor (Ansorena et al., 2001), and they are not among the most odor active compounds described for dry-cured ham (Carrapiso, Ventanas & Garcia, 2002).

Significant differences ($P<0.001$) in volatile aldehydes were detected in all batches studied, they are known as the major contributors to the unique flavor of dry-cured ham due to their rapid formation in lipid oxidation and their low odor thresholds (Dirinck, Opstaele, & Vandendriessche, 1997). In general, total aldehydes showed lower levels in inoculated "lacón" samples (44, 41 and 75×10^6 area units, for DH, CD and CZ groups, respectively) than in the control group (126×10^6 area units) (see Fig. 1). Among the linear aldehydes, hexanal was detected in all batches studied while nonanal was only found in control and CZ groups. The level of hexanal was markedly ($P<0.001$) higher in the control (106×10^6 area units), which surface was not inoculated, than in the other batches studied (14, 23 and 61×10^6 area units, for DH, CD and CZ groups, respectively) (see Table 1). The presence of linear aldehydes is related to the oxidation (Ansorena et al., 2001; Ordóñez, Hierro, Bruna, & de la Hoz, 1999). This result suggests a smaller fat oxidation in the inoculated samples, probably due to a lower lipolytic activity of the strains used and a positive effect on flavor by inhibiting the development of rancidity. This antioxidative effect of yeast has been reported previously by Lücke (1985). These results are in agreement with those reported by Flores et al. (2004) who found a reduction on heptanal, octanal and nonanal contents in fermented sausage samples that were inoculated *D. hansenii*. However, other authors (Andrade et al., 2010; Carrapiso, Ventanas, & Garcia, 2002) observed higher values for these aldehydes in the inoculated sausages than in the control. The branched aldehyde 3-methylbutanal derives from amino acids by Strecker degradation (Barbieri et al., 1992; Ventanas et al., 1992) but it can also be generated by microbial metabolism (Durá, Flores, & Toldra, 2004; Martín et al., 2006); it was detected in all batches with the exception of CZ batch. This aldehyde was found in lower amounts in control and CD group (10.99 and 14.21×10^6 area units, respectively), while "lacón" samples inoculated with *D. hansenii* showed the higher values (27.81×10^6 area units) (see Table 1). This compound has been reported to contribute considerably to the overall flavor of dry cured ham (Carrapiso et al., 2002; Martín et al., 2006).

Alcohols showed significant differences ($P<0.001$) among batches studied (36, 364, 135 and 21×10^6 area units, for control, DH, CD and CZ groups, respectively) (see Fig. 1). The yeast ability to produce alcohols from both carbohydrate metabolism and branched amino acids is well known (Olesen & Stahnke, 2000). The branched alcohols 2-methylpropanol and 3-methylbutanol are formed by reduction of the corresponding aldehydes (Stahnke, 1994). The production of 2-methylpropanol associated to the absence of the corresponding precursor can be attributed to the conversion of branched amino acids through

the Ehrlich pathway used by yeasts (Schoondermark-Stolk et al., 2006). The most abundant alcohol was 3-methylbutanol for DH and CD batches (320.14 and 125.38×10^6 area units, respectively) while 1-hexanol was more extensive for control and CZ groups (26.71 and 12.97×10^6 area units, respectively) (see Table 1). On the other hand, 2-methylpropanol and 1-penten-3-ol were only detected in "lacón" sample inoculated with *D. hansenii*. Alcohols are considered important contributors to the aroma of dry-cured meat products due to their low odor threshold (Sabio, Vidal-Aragon, Bernalte, & Gata, 1998).

Ester compounds are formed by the esterification of carboxylic acids and alcohols. The enzymes involved in this reaction are esterase enzymes which are produced by different microorganisms (yeasts, molds and bacteria) (Jelén & Wasowicz, 1998; Stahnke, Holck, Jensen, Nilsen, & Zanardi, 2002). They can modulate the global flavor due to their low odor thresholds, imparting fruity qualities (Marco, Navarro, & Flores, 2006; Stahnke, 1994). Their presence, together with branched aldehydes, has been associated with a "ripened flavor" in cured meat products (Barbieri et al., 1992; Careri et al., 1993; Montel, Reitz, Talon, Berdagué, & Rousset-Akrim, 1996). In general esters were the majority volatile compounds group for all batches studied except for the one inoculated with *C. zeylanoides*. In dry-cured "lacón" samples, they reached high values showing statistically significant differences ($P<0.001$) among batches. The amount of esters was higher in inoculated "lacón" samples (628 and 404×10^6 area units, for DH and CD groups, respectively) than in control and CZ batch (299 and 248×10^6 area units, respectively) (see Fig. 1) and it could be related to microbial esterase activity (Stahnke, 1995). The most numerous esters were methyl isopentanoate and methyl hexanoate. The content of these compounds was significantly higher ($P<0.001$) in "lacón" samples inoculated with *D. hansenii* and *C. deformans* than in the other ones. Methyl heptanoate only appeared in DH and CZ batches (8.24 and 15.63×10^6 area units, respectively), while methyl propanoate only appeared in "lacón" samples inoculated with *D. hansenii* (11.80×10^6 area units) (see Table 1). Methyl isobutanoate, methyl butanoate, methyl pentanoate, methyl octanoate and 3-hidroximandelic acid, ethyl ester, di-TMS appeared in all batches studied. For methyl isobutanoate the lowest amounts were found in control and CZ batches while for methyl butanoate the highest amounts were observed in "lacón" samples inoculated with *C. zeylanoides*. On the other hand, the lowest amounts of methyl pentanoate were detected in control and CZ groups, while the lowest contents of methyl octanoate were observed in control samples. For 3-hidroximandelic, acid, ethyl ester, di-TMS from "lacón" samples inoculated with *C. zeylanoides* showed the highest value (29.62×10^6 area units) while "lacón" samples inoculated with *C. deformans* presented the lowest (22.55×10^6 area units).

Ketones are mainly formed by catabolism or fermentation of carbohydrate chains and by β-oxidation of fatty acids (Flores, Grimm, Toldrá, & Spanier, 1997; Ordóñez et al., 1999; Sondergaard & Stahnke, 2002). Total ketones showed significantly higher contents in "lacón" samples inoculated with *D. hansenii* (69.90×10^6 area units) than in the other ones (48.53, 15.98 and 21.59×10^6 area units, for control, CD and CZ groups, respectively) (see Fig. 2). Among them, especially 2-ketones are considered to have a great influence on the aroma of meat and meat products. 2-pentanone, 2-heptanone and 2-decanone present a peculiar aroma: ethereal, butter, spicy notes or blue cheese notes (Novelli, Gandemer, Meynier, Zanardi, & Chizzolini, 1995). In our study, 2-heptanone presented higher levels in control and DH and CD groups (16.3, 11.9 and 12.3×10^6 area units, respectively) than CZ batch (6.4×10^6 area units). On the other hand, 2-pentanone was only detected in control group.

Furans (furan, 2-pentylfuran) have been detected in "lacón" samples inoculated with *D. hansenii*, so the inoculated strains contribution to its production could be considered. Furans are commonly found in dry-cured hams (Ruiz, Ventanas, Cava, Andrés, & García, 1999), nevertheless they are not among the most odor active compounds described for dry-cured ham (Carrapiso et al., 2002). On the other hand, Martín et al. (2003) found that *Penicillium chryso* and *D. hansenii* did not affect furan formation in dry-cured pork loins.

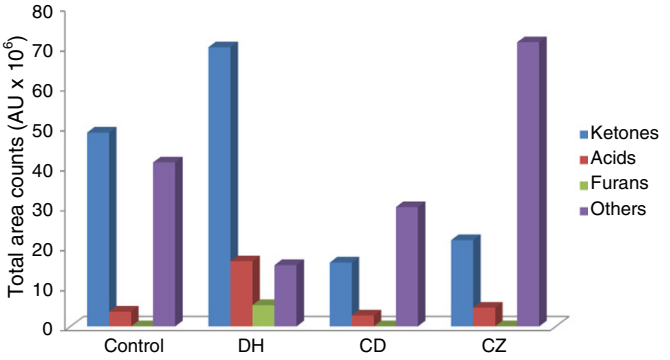

Fig. 2. Minority chemical families of volatile compounds from dry-cured "lacón" samples inoculated with yeast strains: *D. hansenii* (DH), *C. deformans* (CD) and *C. zeylanoides* (CZ).

3.1. Principal components analysis

A principal component analysis (PCA) was carried out to determine the relationship between the volatile compounds and to discriminate dry-cured "lacón" by their inoculated yeast strains. PCA results for mean values of the parameters are summarized in Fig. 3 (a) and (b). PCA showed that about 86.42% of the variability was explained by the first three principal components. The correlation matrix for this model had a close to zero determinant indicating significant correlations among variables and suitability for PCA (Table 2). Principal component 1 (PC1) was the most important variable in terms of differences among inoculated yeast strains as it accounted for 54.91% of the total variability. PC1 was positively related to linear hydrocarbons (heptane), alcohols (3-methylbutanol and 1-hexanol) and esters (methyl isobutanoate, methyl pentanoate and methyl hexanoate) and negatively correlated to branched hydrocarbons (octane, 2,5,6-trimethyl) and aldehydes (3-cyclopentene-1-acetaldehyde). Dry-cured "lacón" that was inoculated with *D. hansenii* had the greatest component 1 value; this was related to heptane, 1-hexanol, 3-methylbutanol, methyl isobutanoate and methyl isopentanoate. As shown in Fig. 3 (a), DH group is in the positive side of both PC1 and PC2; control and CD batches were on the negative side of PC1 and PC2, while CZ group was on the negative side of PC1 and on the positive side of PC2.

On the other hand, principal component 2 (21.53%) was positively related to branched hydrocarbons (nonane, 3,7-dimethyl, octane, 2,6-dimethyl, undecane, 2,8-dimethyl and undecane, 4,8-dimethyl) and acids (malonic acid) and negatively correlated with ketones (2-heptanone) and esters (methyl hexanoate). Control and CD batches were on the negative PC2 axis while DH and CZ groups were on the positive PC2 axis. Finally, PC3 (9.98%) was positively related to aldehydes (hexanal) and negatively correlated to linear hydrocarbons (hexane). *C. deformans* inoculated dry-cured "lacón" had the greatest component 3 value; this was related to hexane content. CZ, CD and DH batches were on the negative PC3 axis while control group was on the positive PC3 axis.

In conclusion, PC1 differentiated the dry-cured "lacón" that was inoculated with *D. hansenii* from the other ones. This result were related to heptane, 3-methylbutanol, 1-hexanol, methyl isobutanoate and methyl isopentanoate contents, which were more abundant in "lacón" samples that were inoculated with *D. hansenii* and they had a positive effect on final flavor.

4. Conclusions

Results from this research showed that the tested yeast strains that were inoculated remarkably alter the total area of volatile compounds when compared to the wild yeast population. This study demonstrated

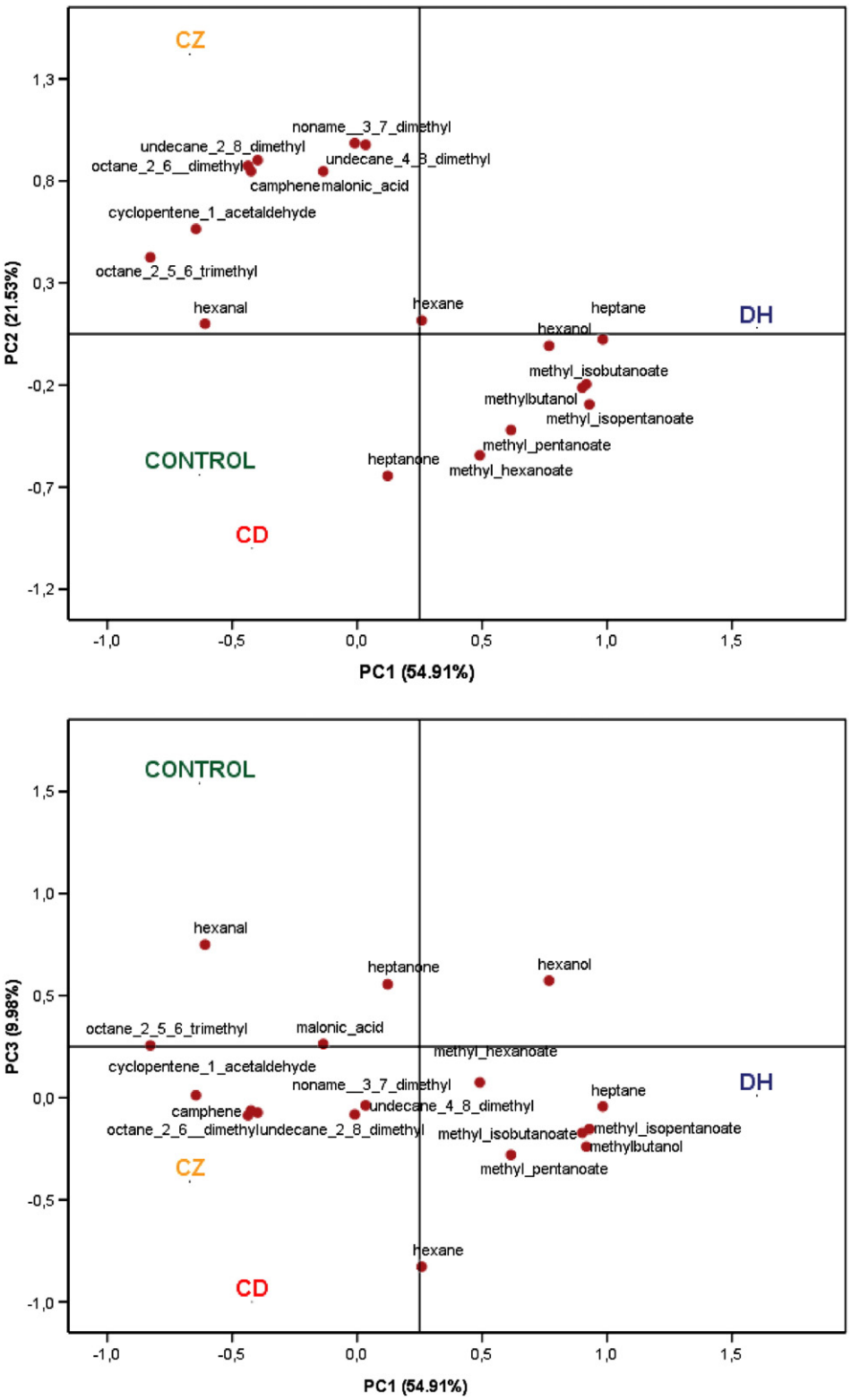

Fig. 3. Relationships among dry-cured "lacón" inoculated with three different yeast strains (*D. hansenii* (DH), *C. deformans* (CD) and *C. zeylanoides* (CZ)) and one control batch and volatile compounds obtained by PCA. a) Projection of the variables and dry-cured "lacón" groups in the plane defined by the first two principal components. b) Projection of the variables in the plane defined by PCA first and three.

that the inoculated yeasts had an important impact on volatile compounds generation. The reduction of total aldehyde compound values that is caused by the inoculation suggested a smaller fat oxidation of dry-cured "lacón". In particular, hexanal contents decrease could be related to a positive effect on flavor by inhibiting rancid odor. However, the production of volatile compounds clearly differed among inoculated batches. In fact, the use of *D. hansenii* was able to produced high contents of esters, alcohols and ketones which play an important role in the aroma due to their low odor threshold. Based on these results, we could conclude that appropriate selection of yeast starter cultures could result in a positive impact on the volatile compounds that are involved on aroma development of dry-cured "lacón".

Table 2
Factor loadings for each parameter studied on the first three principal components obtained.

Compounds	PC1	PC2	PC3	Communality
Hexane	0.259	0.117	−0.828	0.766
Heptane	0.983	0.024	−0.043	0.969
Octane, 2,5,6-trimethyl	−0.827	0.425	0.256	0.931
Nonane, 3,7-dimethyl	−0.009	0.985	−0.082	0.977
Octane, 2,6-dimethyl	−0.437	0.874	−0.087	0.962
Undecane, 2,8-dimethyl	−0.398	0.902	−0.073	0.977
Undecane, 4,8-dimethyl	0.034	0.977	−0.038	0.957
3-Methylbutanol	0.917	−0.196	−0.240	0.937
1-Hexanol	0.768	−0.008	0.573	0.919
2-Heptanone	0.122	−0.645	0.556	0.740
Hexanal	−0.608	0.100	0.750	0.942
3-Cyclopentene-1-acetaldehyde	−0.645	0.564	0.012	0.734
Methyl isobutanoate	0.901	−0.213	−0.172	0.887
Methyl isopentanoate	0.930	−0.295	−0.153	0.974
Methyl pentanoate	0.616	−0.420	−0.280	0.634
Methyl hexanoate	0.491	−0.544	0.075	0.543
Malonic acid	−0.135	0.847	0.263	0.805
Camphene	−0.425	0.847	−0.062	0.902
Eigenvalue	9.883	3.876	1.797	
Percent of variance	54.905	21.533	9.984	
Accumulative percentage	54.905	76.438	86.421	

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