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Differentiation of yeasts growing on dry-cured Iberian ham by mitochondrial DNA restriction analysis, RAPD-PCR and their volatile compounds production

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ABSTRACT

The efficiency of mitochondrial DNA (mtDNA) restriction analysis, RAPD-PCR and volatile compounds analysis to differentiate yeast biotypes involved in flavour development of dry-cured Iberian ham throughout the ripening process is evaluated. For this purpose, 86 yeasts isolated from Iberian hams in the main ripening stages at different industries of the four Protected Designations of Origin of this product, were used. The combination of mtDNA restriction analysis and RAPD-PCR using the primer $(GACA)_4$ showed a higher variability in the yeast species detected than obtained using only mtDNA restriction analysis. Only two species, *Debaryomyces hansenii* and *Candida zeylanoides*, were identified throughout the whole ripening process and a wide diversity of biotypes was found in these two species, with those of *D. hansenii* predominating. Clear differences between biotypes were detected in the generation of volatile compounds, with the biotype C2-2 of *D. hansenii* showing the highest concentrations of volatiles. The combined use of mtDNA restriction analysis and RAPD-PCR distinguishes yeast biotypes with different production of volatile compounds. In addition, analysis of the production profile of volatile compounds is needed to differentiate yeast strains of the same biotype recovered at different stages of ripening. Thus, the combination of these three methods could be very useful to select or monitor yeasts as starter cultures in dry-cured meat products.

1. Introduction

Dry-cured Iberian ham is a traditional meat product obtained by a process that involves 18–24 months of ripening. During this long period, an uncontrolled microbial population proliferates on the product. Yeasts are one of the predominant groups among this microbial population throughout most of the maturation process (Núñez et al., 1996). *Debaryomyces hansenii* and *Candida zeylanoides* have been reported to be the most abundant yeast species in this product (Núñez et al., 1996). Some studies carried out with different yeast strains have shown their influence on the development of the characteristic flavour of dry-cured meat products (Jessen, 1995; Durá et al., 2004; Flores et al., 2004; Martín et al., 2006). Furthermore, differences in flavour development associated with particular yeast species and biotypes growing on hams have been recently reported (Andrade et al., 2009). Thus, the flavour of dry-cured Iberian ham can be related to particular biotypes of yeasts that participate in its maturation. Consequently, it should be of great interest to differentiate the main yeast biotypes growing on Iberian hams throughout the ripening process in relation to the production of volatile compounds involved in flavour development.

Mitochondrial DNA (mtDNA) restriction analysis and random amplification of polymorphic DNA (RAPD)-PCR have been extensively used as individual techniques to discriminate yeasts from different food products at strain level (Fernández-Espinar et al., 2001; Petersen et al., 2001; Vasdinyei and Deák, 2003; Martorell et al., 2005; Cocolin et al., 2006; Nikolaou et al., 2007; Walczak et al., 2007). The combination of both methods could be very useful for routine differentiation of yeast biotypes that usually grow on dry-cured meat products with a long time of ripening, such as dry-cured Iberian ham. Furthermore, the generation of volatile compounds may also be used to differentiate yeast biotypes that grow on hams during the ripening process.

Analysis of volatile compound production by multiple yeast strains in dry-cured Iberian ham is not feasible as a routine method because a great number of pieces of products would have to be kept as sterile samples to be inoculated. A more practical method would be to use a culture medium that emulates the composition of dry-cured ham throughout the ripening process. A medium composed of oleic acid, ribose, creatine and free amino acids has been reported to be appropriate for evaluation of the generation of volatile compounds by yeast isolates from dry-cured ham (Andrade et al., 2009).

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The aim of this work is to investigate the efficiency of mtDNA restriction analysis, RAPD-PCR and volatile compounds analysis to differentiate yeast biotypes involved in flavour development of dry-cured Iberian ham throughout the ripening process. In addition the distribution of yeast biotypes in the main ripening stages of this meat product is determined.

2. Materials and methods

2.1. Molecular differentiation of yeast isolates

Eighty-six yeast isolates collected from the surface of dry-cured Iberian hams in the main ripening stages (post-salting and drying-cellar) were used in this study. These strains were taken from hams belonging to different industries in each of the four Spanish Protected Designations of Origin (PDO) of dry-cured Iberian ham ("Guijuelo" PDO, "Jamón de Huelva" PDO, "Dehesa de Extremadura" PDO and "Los Pedroches" PDO). They were isolated by repeated cultivation on malt extract agar (MEA) (2% malt extract, 2% glucose, 0.1% peptone, 2% agar) and differentiated by mtDNA restriction analysis (Querol et al., 1992) using the enzyme *Hae*III and RAPD-PCR with primer $(GACA)_4$ at strain level (Andrade et al., 2006).

Thirty-five yeast reference strains from the Spanish Type Culture Collection (CECT), belonging to *C. zeylanoides*, *D. hansenii*, *Debaryomyces polymorphus*, *Pichia carsonii*, *Rhodotorula mucilaginosa*, *Saccharomyces cerevisiae* and *Yarrowia lipolytica*, were used for comparison with dry-cured Iberian ham isolates. Identification of representative strains of the mtDNA restriction patterns obtained were confirmed by 18S rDNA sequencing.

2.2. Differentiation of yeast isolates by their volatile compound production

2.2.1. Inoculation of culture medium

All yeast isolates were inoculated at 10^6 cfu mL^{-1} in a culture medium prepared as described previously Andrade et al. (2009). Then, the cryotubes containing the inoculated medium were incubated for 30 days at 25 °C with shaking. Three replicates for each yeast isolate were used. Uninoculated culture medium was used as control and it was grown in the same conditions as the inoculated samples.

After 30 days of incubation, microbial counts in the control samples were detected on plate count agar (PCA) and no growth was found in any of them. Regarding the inoculated samples, yeast counts were determined on MEA and levels higher than 10^7 cfu mL^{-1} were detected.

2.2.2. Extraction and analysis of volatile compounds

At the end of the incubation period, the cryotubes with the culture medium were maintained at −80 °C until analysis. Aliquots of 1 mL of culture medium were placed in a 5 mL headspace vial (Hewlett-Packard, Palo Alto, CA, USA) and sealed with a PTFE butyl septum (Perkin-Elmer, Foster City, CA, USA) in an aluminium cap. The extraction of volatile compounds was performed by solid-phase micro-extraction technique (SPME) (Ruiz et al., 1998), using a 100 μm carboxen/polydimethylsiloxane fibre (Supelco, Bellefonte, PA, USA). The SPME fibre was inserted into the headspace vial through the septum and exposed to headspace for 45 min at 42 °C in a water bath with stirring. Prior to collection of volatiles, the fibre was preconditioned at 220 °C for 50 min in the GC injection port.

GC/MS analyses were performed using a Hewlett-Packard 5890 S II gas chromatograph coupled with a Hewlett-Packard 5971A ion-trap mass spectrometer. A 5% phenyl–95% dimethyl polysiloxane column (50 m × 0.32 mm ID, 1.05 μm film thickness; Hewlett-Packard) was used for the separation of volatile compounds. Helium was used as carrier gas. The injection port was in a splitless mode. The SPME fibre was kept in the injection port at 220 °C during the whole chromatographic run. The temperature program was isothermal for 15 min at 35 °C, then increased to 150 °C at 4 °C min^{-1}, and then to 250 °C at 20 °C min^{-1}. The GC/MS transfer line temperature was 280 °C. The mass spectrometer was operated in the electron impact mode, with electron energy of 70 eV, a multiplier voltage of 1650 V and a rate of 1 scan s^{-1} over a range of m/z of 40–300 for data collection.

2.3. Data analysis

Dendrograms were built using the program NTSYS-Pc (version 2.0). The similarities between molecular profiles were determined by the Dice coefficient. Clustering analysis was based on the unweighted pair group method using the arithmetic average (UPGMA) method.

Identification of the volatile compounds was achieved by comparing their mass spectra with the NIST/EPA/NIH library. The Kovats indexes, calculated for each peak with reference to *n*-alkanes (Sigma R-8769) run under the same conditions, were used for the identification. Then, statistical analysis was performed using the software SPSS for Windows (version 15.0). ANOVA and a subsequent Tukey test were used to determine significant differences ($P < 0.05$) among the different molecular biotypes for every volatile compound.

3. Results

3.1. Differentiation of yeast isolates from dry-cured Iberian ham by mtDNA restriction analysis and RAPD-PCR

The analysis of mtDNA restriction fragments of the 86 yeast isolates, which ranged from 10.0 to 1.0 kbp approximately (data not shown), revealed 10 different patterns that were designated as B, C1, C2, D, E, F, H, K, O and S (Fig. 1). These mtDNA restriction patterns were compared with those of the yeast reference strains from the CECT. The D and E patterns showed the same profile as the reference strains of *D. hansenii* CECT 10026 and CECT 10360, respectively. Similarly, the H restriction pattern matched the reference strains of *C. zeylanoides* CECT 1441 and CECT 10128. The F and B restriction patterns showed a high similarity with *D. hansenii* CECT 10360. The remaining mtDNA restriction profiles presented a low similarity with reference yeast strains being tentatively characterized as those species (Fig. 1).

When 18S rDNA sequencing was used to confirm the characterization at species level obtained by mtDNA restriction analysis, the H, O and S profiles were identified as *C. zeylanoides* and the remaining profiles as *D. hansenii*. These results were in total accordance with the tentative characterization done with mtDNA restriction analysis (Table 1).

To discriminate among the yeast strains that showed the same mtDNA restriction pattern, RAPD-PCR with the primer $(GACA)_4$ was applied. Thus, all the yeast isolates tested by mtDNA restriction analysis were also differentiated by means of RAPD-PCR. The size of the amplification products obtained ranged from 3.5 to 0.2 kbp approximately (data not shown). Different RAPD-PCR profiles were obtained in each mtDNA restriction pattern. Thus, three different profiles were detected in the C1 mtDNA restriction pattern and only two in each of the B, C2, E and H mtDNA restriction patterns (Fig. 2). However, no different patterns were detected in the D, F, K, O and S mtDNA restriction profiles (Table 1). Consequently, a total of 16 different yeast biotypes were established after combining mtDNA restriction analysis and RAPD-PCR (Fig. 2).

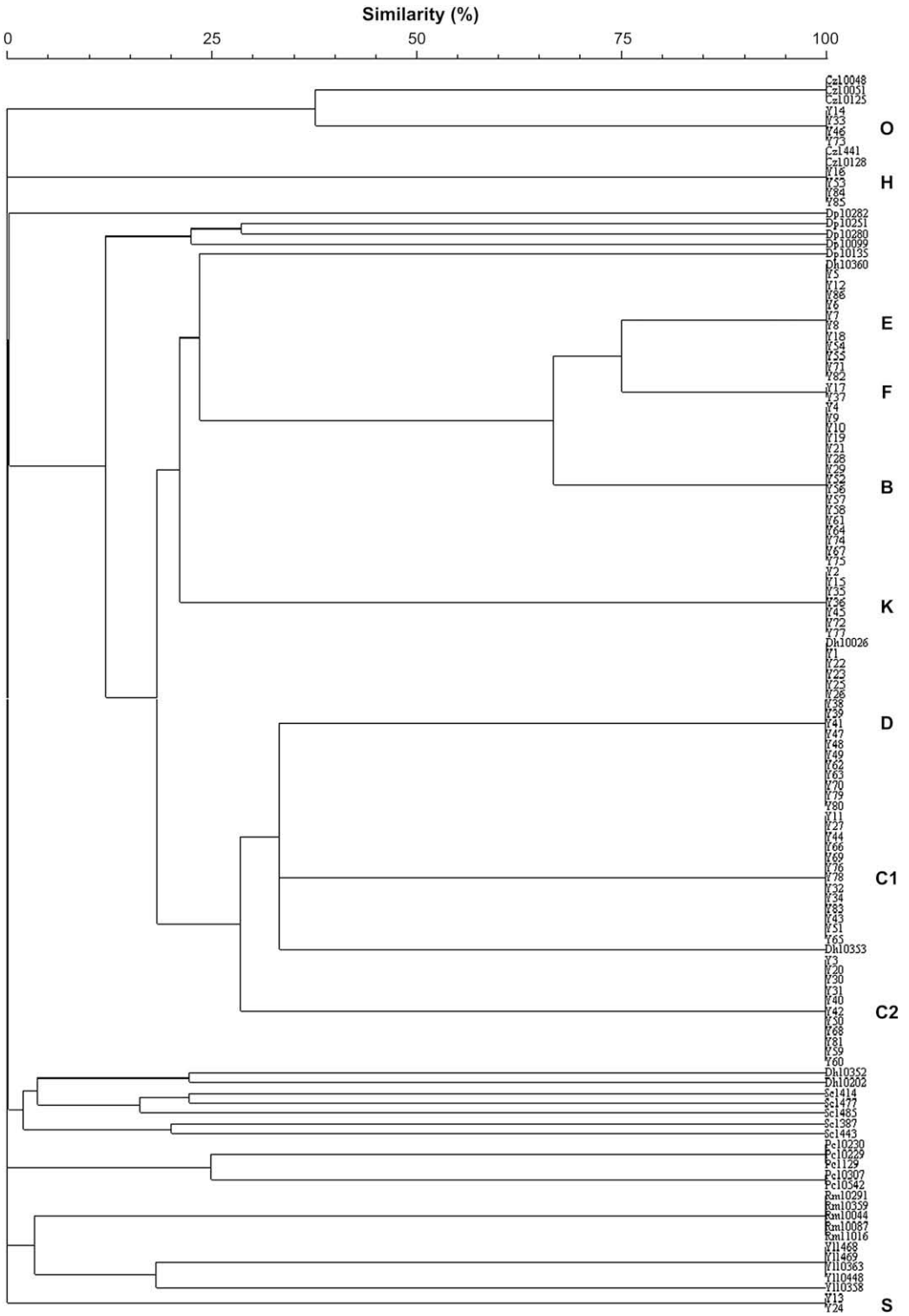

Fig. 1. Dendrogram based on the mitochondrial DNA restriction analysis of yeasts isolated from dry-cured Iberian ham throughout ripening process and yeast reference strains from the Spanish Type Culture Collection. Distance values between branches in the dendrogram are reported as percentage of similarity (0–100%). Cz, *C. zeylanoides*; Dh, *D. hansenii*; Dp, *D. polymorphus*; Pc, *P. carsonii*; Sc, *S. cerevisiae*; Yl, *Y. lipolytica*; Rm, *R. mucilaginosa*. Mitochondrial DNA restriction patterns of yeasts B, C1, C2, D, E, F, H, K, O and S. Y, yeast strain.

3.2. Distribution of yeast biotypes obtained by mtDNA restriction analysis and RAPD-PCR throughout the ripening of dry-cured Iberian ham

The distribution of yeast biotypes obtained by mtDNA restriction analysis and RAPD-PCR was analyzed to find out the evolution of the yeast population throughout the ripening of dry-cured Iberian ham (Table 1). Only 8 of the 16 biotypes detected were found in the post-salting stage, whereas 13 biotypes were present at the drying-cellar stage.

The H1, H2 and S1 biotypes of *C. zeylanoides* were present only at the post-salting stage. The *D. hansenii* B2, C1-1, C1-2, C1-3, C2-2, E1

Table 1
Distribution of yeast biotypes, obtained by mitochondrial DNA (mtDNA) restriction analysis and RAPD-PCR with primer $(GACA)_4$, throughout the ripening process of dry-cured Iberian ham; yeast identification by 18S rDNA sequencing also is reported.

Ripening stages	mtDNA restriction patterns	RAPD patterns	Yeast biotypes	Identification by 18S rDNA sequencing	Frequency of isolates (%)
Post-salting	B	1	B1	*D. hansenii*	4.7
	C2	6	C2-1	*D. hansenii*	3.5
	D	7	D1	*D. hansenii*	9.3
	E	2	E2	*D. hansenii*	3.5
	H	9	H1	*C. zeylanoides*	5.8
	H	10	H2	*C. zeylanoides*	2.3
	K	2	K1	*D. hansenii*	2.3
	S	6	S1	*C. zeylanoides*	2.3
Drying-cellar	B	1	B1	*D. hansenii*	11.6
	B	2	B2	*D. hansenii*	2.3
	C1	3	C1-1	*D. hansenii*	8.1
	C1	4	C1-2	*D. hansenii*	3.5
	C1	5	C1-3	*D. hansenii*	3.5
	C2	6	C2-1	*D. hansenii*	7.0
	C2	1	C2-2	*D. hansenii*	2.3
	D	7	D1	*D. hansenii*	9.3
	E	8	E1	*D. hansenii*	3.5
	E	2	E2	*D. hansenii*	5.8
	F	3	F1	*D. hansenii*	2.3
	K	2	K1	*D. hansenii*	2.3
	O	6	O1	*C. zeylanoides*	4.7

and F1, and *C. zeylanoides* O1 biotypes were detected only at the drying-cellar stage (Table 1). From the remaining biotypes isolated throughout the ripening process, only the D1 biotype of *D. hansenii* was detected in the same proportion at the two stages of the process. A decrease in the number of isolates from K1 of *D. hansenii* was observed during the ripening. On the contrary, more isolates of B1, C2-1 and E2 of *D. hansenii* were detected at the drying-cellar stage than at the post-salting stage. The *D. hansenii* D1 was the predominant biotype at the post-salting stage and *D. hansenii* B1 at the drying-cellar stage (Table 1).

3.3. Differentiation of yeast isolates from dry-cured Iberian ham by their volatile compound production

All of the 86 yeast isolates were tested in triplicate by the production of volatile compounds. Means of volatile compounds were calculated for each yeast biotype differentiated by mtDNA restriction analysis and RAPD-PCR at both ripening stages, post-salting (P) and drying-cellar (DC) (Table 2).

A total of 47 volatile compounds were identified and quantified in the culture medium, some of which were found in trace amounts. They were grouped according to their probable origins as amino acid catabolism (22), lipid oxidation (10), microbial esterification (5) and carbohydrate fermentation products (1). The remaining volatile compounds were grouped as "unknown origin or contaminants" (9) (Table 2).

Branched aldehydes and alcohols derived from amino acid catabolism, such as 3- and 2-methylbutanal, 3- and 2-methylbutanol, 2-methyl-2-pentanol and 2-methyl-1-propanol, were detected in significantly higher amount in some of the inoculated batches than in the control batch (Table 2). However, the branched ethylbenzaldehyde was found at the greatest significant level in the uninoculated batch. These compounds were detected at the highest quantities in the batch inoculated with the *D. hansenii* C2-2 biotype from the drying-cellar stage. 2-Methylbutanoic acid was found only at very low concentrations in some inoculated batches.

Sulphur volatile compounds were identified in all batches, although not all of them were always detected (Table 2). Thus, 3-methylthio-1-propanol, methanethiol and dimethyltrisulphide were not detected in the control batch, showing significant differences with some of the inoculated batches. The *C. zeylanoides* S1-P biotype presented the highest overall amount of sulphur compounds, mainly of dimethyldisulphide which was the most abundant in almost all remaining batches including the control batch. On the contrary, the *D. hansenii* C1-2-DC, C1-3-DC and F1-DC, and *C. zeylanoides* H1-P and H2-P biotypes showed very low production of sulphur compounds (Table 2).

Inside the volatile compounds from lipid oxidation, only the three methylketones identified and hexane were detected in the uninoculated batch (Table 2). The compounds 2- and 3-methylpentane, 2-propanone, 2-butanone, 2-pentanone, butanal and methylbenzene were detected in significantly higher amount in some of the inoculated batches than in the control batch. The *D. hansenii* E1-DC biotype showed the greatest production for most of the hydrocarbons despite the absence of branched 3-methylhexane and methylcyclohexane.

Several esters were found in all batches, excepting the batch inoculated with the *D. hansenii* F1-DC biotype (Table 2). Ethanethioic acid, *S*-methyl ester was the most abundant in the majority of the inoculated batches.

When the volatile compounds generated by the different yeast biotypes detected in both post-salting and drying-cellar stages were compared, generally those isolated from the drying-cellar stage showed higher levels of volatile compounds than those from the post-salting stage (Fig. 3). However, the B1 biotype generated lower amounts of amino acid catabolism, lipid oxidation and microbial esterification products when it was isolated from the drying-cellar stage than from the post-salting stage. The *D. hansenii* C2-2 biotype from the drying-cellar stage showed the highest quantities of volatile compounds originating from catabolism of amino acids and microbial esterification (Fig. 3). This biotype and the *D. hansenii* E1 from the drying-cellar stage showed the greatest levels of compounds derived from lipid oxidation.

4. Discussion

Mitochondrial DNA restriction analysis proved to be a reliable method to differentiate yeasts isolated from dry-cured Iberian ham at species level, since tentative characterization obtained with this method was confirmed by 18S rDNA sequencing. The combination of mtDNA restriction analysis and RAPD-PCR using the primer $(GACA)_4$ showed a higher variability in the tested species than obtained using only the mtDNA restriction analysis. This allowed a better differentiation of yeasts at strain level. Several studies have reported the

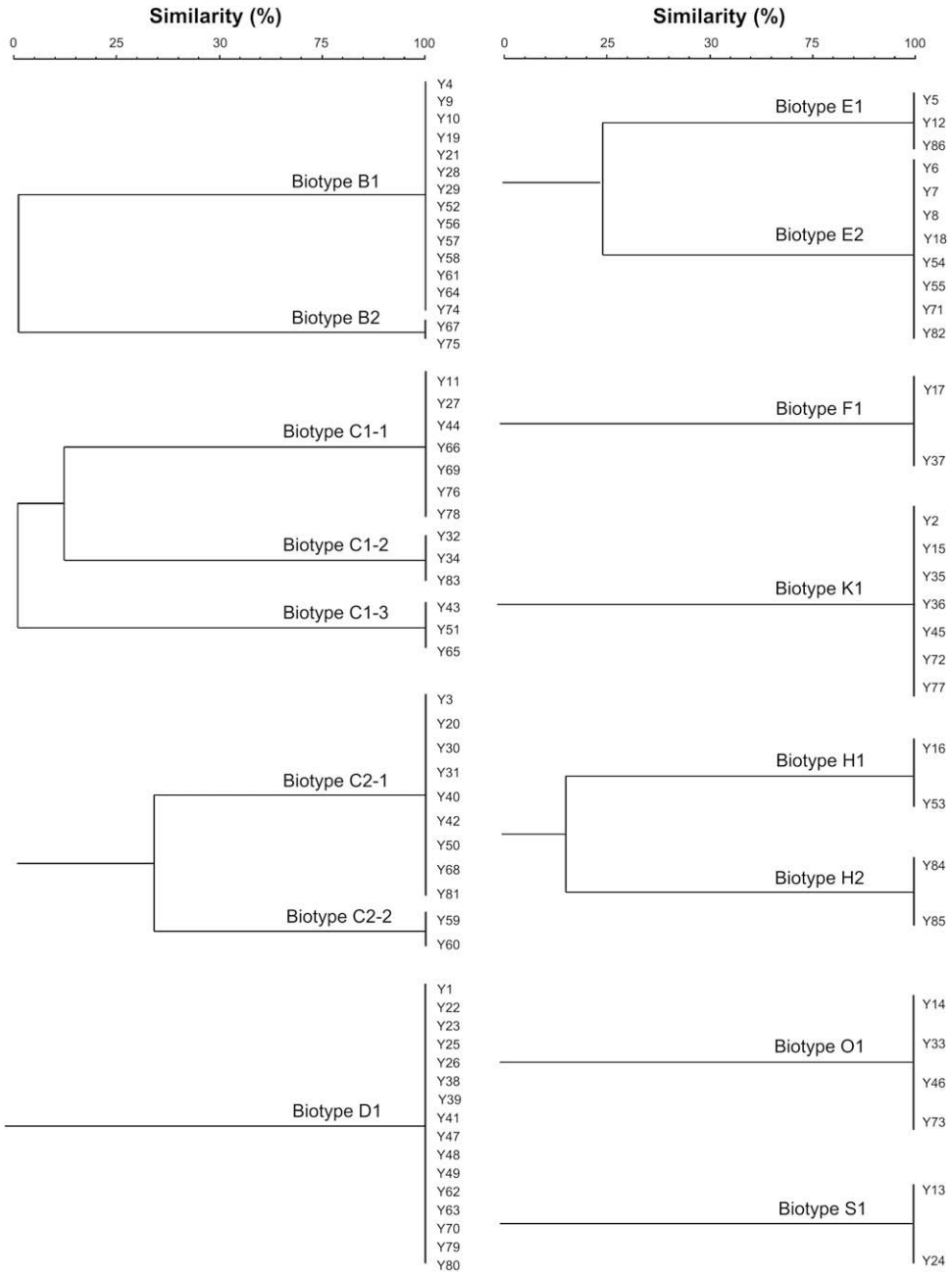

Fig. 2. Dendrogram based on the RAPD-PCR technique using the primer $(GACA)_4$ of yeasts isolated from dry-cured Iberian ham throughout the ripening process and yeast reference strains from the Spanish Type Culture Collection. Distance values between branches in the dendrogram are reported as percentage of similarity (0–100%). Y, yeast strain.

efficiency of mtDNA restriction analysis for typing yeasts (Torriani et al., 1999; Fernández-Espinar et al., 2001; Martorell et al., 2005; Andrade et al., 2006; Nikolaou et al., 2007; Jeyaram et al., 2008). From the results of this work, the combination of this method with RAPD-PCR is useful in the analysis of the diversity of yeast biotypes that usually grow on dry-cured meat products with a long ripening period, such as dry-cured Iberian ham.

Only two species of yeasts were identified in dry-cured Iberian ham in the present study. These species were *D. hansenii* and *C. zeylanoides*, with *D. hansenii* predominating during the whole maturation process. This species is one of the yeasts found most frequently in several types of meat products, including dry-cured ham (Comi and Cantoni, 1983; Núñez et al., 1996; Simoncini et al., 2007). *Debaryomyces maramus*, *Rhodotorula rubra*, *P. carsonii* or *Candida famata*, reported occasionally in dry-cured meat products (Núñez et al., 1996; Simoncini et al., 2007), were not detected in the present work. Thus, there was a low diversity of species in the yeast population growing on dry-cured Iberian ham throughout the ripening. However, a wide variety was found at strain level.

Many biotypes of *D. hansenii* were found during the whole ripening process. On the contrary, most of the biotypes of *C. zeylanoides* were isolated in the post-salting stage and only the biotype O1 was found in the drying-cellar stage. A higher number of yeast biotypes was detected in the drying-cellar stage than in the post-salting stage, probably due to the longer duration of the drying-cellar stage. Some biotypes were detected in only one ripening stage. This happened in the post-salting stage with most of the biotypes of *C. zeylanoides*, while in the drying-cellar stage most

Table 2
Volatile compounds generated in the culture medium by different yeast biotypes, detected by mitochondrial DNA restriction analysis and RAPD-PCR, throughout the ripening process of dry-cured Iberian ham[a].

Compound/origin	Yeast biotypes																				
	Control[b]	B1-P	B1-DC	B2-DC	C1-1-DC	C1-2-DC	C1-3-DC	C2-1-P	C2-1-DC	C2-2-DC	D1-P	D1-DC	E1-DC	E2-P	E2-DC	F1-DC	H1-P	H2-P	K1-P	K1-DC	O1-DC	S1-P
Amino acid catabolism																						
2-Methyl-1-propanol	n.d.	21.34^{ab}	5.09^{a}	12.01^{ab}	18.56^{ab}	2.09^{ab}	0.74^{ab}	13.62^{ab}	25.25^{ab}	45.95^{b}	10.88^{ab}	19.45^{ab}	n.d.	13.21^{ab}	23.83^{ab}	6.20^{ab}	2.83^{ab}	3.25^{ab}	8.37^{ab}	6.40^{ab}	12.12^{ab}	13.63^{ab}
2-Methyl-2-propanol	n.d.	9.70	3.87	2.66	3.95	0.10	0.96	4.19	7.11	19.64	3.50	8.84	n.d.	4.91	8.89	2.81	0.46	0.36	3.72	1.08	6.12	6.24
2-Methyl-1-pentanol	n.d.	n.d.	n.d.	n.d.	0.03	n.d.	n.d.	n.d.	1.19	n.d.	n.d.	0.03	n.d.	0.12	0.54	n.d.	n.d.	n.d.	0.04	n.d.	n.d.	n.d.
2-Methyl-2-pentanol	n.d.	n.d.	0.20^{ab}	0.29^{ab}	0.55^{ab}	n.d.	0.12^{ab}	0.34^{ab}	0.29^{ab}	2.02^{b}	0.14^{ab}	0.64^{ab}	n.d.	0.32^{ab}	0.44^{ab}	n.d.	n.d.	n.d.	0.04^{ab}	0.13^{ab}	0.27^{ab}	n.d.
2-Methylbutanol	n.d.	53.29^{abc}	11.18^{ac}	13.38^{abc}	31.33^{abc}	6.03^{ac}	1.27^{ac}	28.54^{abc}	67.58^{bc}	117.82^{b}	8.14^{ac}	40.70^{abc}	n.d.	27.50^{abc}	66.61^{abc}	0.12^{abc}	n.d.	15.69^{ac}	9.46^{abc}	22.11^{abc}	41.75^{abc}	34.61^{abc}
3-Methylbutanol	n.d.	59.65^{ab}	33.79^{ab}	14.51^{ab}	62.62^{ab}	17.31^{ab}	8.60^{ab}	86.47^{ab}	73.09^{ab}	144.11^{b}	22.55^{ab}	68.29^{ab}	2.36^{ab}	47.52^{ab}	96.20^{b}	54.91^{ab}	41.39^{ab}	20.52^{ab}	33.38^{ab}	19.74^{ab}	54.33^{ab}	65.85^{ab}
3-Methylthio-1-propanol	n.d.	0.05^{a}	0.05^{a}	n.d.	1.65^{ab}	n.d.	n.d.	n.d.	0.09^{a}	4.01^{b}	n.d.	0.34^{a}	n.d.	0.39^{a}	0.90^{a}	n.d.	n.d.	n.d.	n.d.	0.94^{ab}	n.d.	1.42^{ab}
2-Methylpropanal	n.d.	0.35	0.96	2.21	0.84	0.78	n.d.	2.29	1.59	7.05	1.10	3.64	0.44	1.51	4.27	0.13	0.14	n.d.	n.d.	n.d.	1.09	5.44
3-Methylthio-1-propanal	0.08^{a}	n.d.	0.05^{a}	0.18^{ab}	n.d.	n.d.	n.d.	0.12a	0.04^{a}	0.81^{b}	n.d.	0.17^{a}	n.d.	0.08^{a}	0.03^{a}	n.d.	n.d.	n.d.	n.d.	0.18^{ab}	n.d.	n.d.
2-Methylbutanal	0.06^{a}	4.77^{ab}	1.16^{ab}	5.81^{ab}	3.42^{ab}	1.32^{ab}	n.d.	8.59^{ab}	14.04^{ab}	22.03^{b}	3.14^{ab}	9.88^{ab}	n.d.	5.33^{ab}	9.68^{ab}	0.82^{ab}	n.d.	0.22^{ab}	5.70^{ab}	4.84^{ab}	n.d.	1.97^{ab}
3-Methylbutanal	0.28^{a}	7.16^{ab}	3.54^{a}	7.43^{ab}	5.24^{a}	2.80^{a}	0.47^{a}	9.79^{ab}	12.94^{ab}	31.41^{b}	7.15^{a}	12.42^{ab}	0.18^{a}	7.36^{ab}	14.69^{ab}	2.06^{ab}	3.12^{ab}	0.40^{a}	4.95^{a}	5.89^{ab}	7.06^{ab}	8.90^{ab}
2-Methylbutanoic acid	n.d.	0.68	0.03	0.37	0.02	n.d.	n.d.	n.d.	0.14	0.19	n.d.	0.21	n.d.	0.11	n.d.	n.d.	n.d.	n.d.	n.d.	0.12	n.d.	n.d.
Methanethiol	n.d.	3.97^{ab}	1.70^{a}	2.12^{ab}	2.16^{ab}	0.35^{ab}	0.25^{ab}	5.48^{ab}	5.78^{ab}	14.24^{b}	2.76^{ab}	4.78^{ab}	0.15^{ab}	3.14^{ab}	3.17^{ab}	n.d.	0.71^{ab}	0.59	1.06	0.51	3.76	4.41
Dimethyldisulphide	1.21^{a}	13.77^{a}	19.83^{a}	32.11^{ab}	51.19^{ab}	n.d.	0.03^{a}	37.77^{abc}	56.81^{abc}	167.34^{b}	36.80^{a}	59.98^{abc}	0.18^{a}	35.84^{ab}	3.43^{a}	n.d.	n.d.	n.d.	28.53^{a}	13.71^{ab}	24.99^{ab}	183.96^{bc}
Dimethyltrisulphide	n.d.	0.16	0.07	n.d.	0.43	n.d.	n.d.	0.34	0.05	0.66	0.28	0.55	0.05	0.22	0.07	n.d.	n.d.	n.d.	n.d.	n.d.	0.07	n.d.
Carbondisulphide	0.18^{a}	0.10^{a}	n.d.	0.82^{ab}	n.d.	n.d.	0.27^{a}	0.19^{a}	n.d.	n.d.	0.11^{a}	0.02^{a}	3.74^{b}	0.28^{a}	0.98^{ab}	0.33^{ab}	0.03^{a}	n.d.	1.49^{ab}	0.35^{ab}	0.07^{a}	n.d.
Benzeneethanol	n.d.	1.61^{ac}	3.92^{ac}	6.52^{ac}	14.31^{ac}	2.44^{ac}	1.15^{ac}	9.18^{ac}	20.88^{bc}	46.35^{b}	6.25^{ac}	23.08^{bc}	3.07^{ac}	10.57^{ac}	19.60^{abc}	2.84^{ac}	2.65^{ac}	n.d.	8.65^{ac}	9.18^{ac}	20.20^{abc}	21.81^{abc}
Benzeneacetaldehyde	0.80^{a}	0.33^{a}	0.29^{a}	0.77^{ab}	0.55^{a}	0.58^{a}	n.d.	0.58^{a}	0.57^{a}	3.80^{b}	0.54^{a}	0.79^{a}	0.17^{a}	0.64^{a}	0.52^{a}	0.42^{a}	n.d.	n.d.	0.06^{a}	0.64^{ab}	0.33^{a}	0.60^{ab}
Ethylbenzaldehyde	7.78^{a}	n.d.	0.04^{b}	n.d.	0.11^{b}	n.d.	n.d.	0.50^{ab}	n.d.	0.17^{ab}	0.05^{b}	n.d.	n.d.	0.66^{ab}	n.d.	0.86^{ab}	n.d.	n.d.	n.d.	0.33^{ab}	n.d.	n.d.
Benzaldehyde	0.82^{ab}	1.79^{a}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.18^{ab}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2,4-Bis(1,1-dimethylethyl)phenol	n.d.	0.06^{a}	4.18^{a}	6.76^{abc}	6.23^{ab}	0.21^{a}	n.d.	2.78^{ab}	5.63^{ab}	19.09^{bc}	2.09^{a}	6.77^{ab}	0.25^{a}	4.03^{ab}	3.86^{ab}	0.28^{ab}	0.18^{ab}	n.d.	0.96^{a}	6.19^{abc}	18.90^{c}	8.91^{abc}
1,4-Bis(1,1-dimethylethyl)benzene	n.d.	0.34^{a}	2.19^{a}	1.15^{ab}	3.29^{ab}	0.38^{ab}	0.07^{a}	0.7^{ab}	5.96^{ab}	10.73^{ab}	0.86^{a}	3.83^{ab}	0.39^{ab}	2.36^{ab}	2.17^{ab}	0.26^{ab}	0.25^{ab}	0.07^{ab}	0.53^{a}	2.86^{ab}	13.90^{b}	10.29^{ab}
Lipid oxidation																						
2-Methylpentane	n.d.	10.34^{ab}	0.92^{a}	n.d.	1.10^{a}	1.00^{ab}	0.97^{ab}	1.67^{ab}	3.29^{ab}	5.01^{ab}	4.17^{ab}	2.21^{a}	23.57^{b}	3.62^{ab}	0.88^{a}	n.d.	0.28^{ab}	n.d.	n.d.	2.92^{ab}	0.31a	n.d.
3-Methylpentane	n.d.	8.56^{a}	1.04^{a}	n.d.	0.56^{a}	0.68^{a}	1.38^{a}	1.66^{a}	2.62^{a}	7.22^{a}	2.96^{a}	1.81^{a}	39.10^{b}	4.12^{a}	0.33^{a}	n.d.	0.20^{a}	n.d.	0.08^{a}	1.48^{a}	n.d.	n.d.
Hexane	0.83^{a}	35.05^{ab}	11.34^{a}	32.54^{ab}	6.26^{a}	8.39^{ab}	15.00^{ab}	12.35^{ab}	12.44^{ab}	63.19^{ab}	9.85^{a}	26.79^{ab}	67.49^{b}	19.41^{ab}	13.81^{ab}	0.84^{ab}	1.25^{ab}	0.09^{ab}	6.54^{a}	24.03^{ab}	3.47^{a}	13.67^{ab}
3-Methylhexane	n.d.	0.84	0.13	n.d.	0.74	n.d.	n.d.	n.d.	1.17	4.06	0.33	1.81	n.d.	0.67	3.25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Methylcyclohexane	n.d.	1.99	0.38	0.20	1.82	n.d.	n.d.	0.34	3.21	4.63	3.27	3.71	n.d.	1.71	3.49	n.d.	n.d.	n.d.	0.19	1.40	1.69	2.32
2-Propanone	0.99^{a}	16.00^{ab}	9.80^{ab}	10.07^{ab}	11.49^{ab}	2.71^{ab}	2.71^{ab}	8.89^{ab}	11.65^{ab}	35.67^{b}	4.26^{a}	15.70^{ab}	4.51^{ab}	10.18^{ab}	14.97^{ab}	5.26^{ab}	0.61^{ab}	1.56^{ab}	4.13^{a}	8.16^{ab}	10.60^{ab}	9.79^{ab}
2-Butanone	0.28^{a}	1.58^{ab}	6.95^{ab}	3.72^{ab}	18.94^{ab}	0.23^{ab}	0.07^{ab}	7.28^{ab}	29.90^{b}	9.83^{ab}	8.94^{ab}	17.28^{ab}	n.d.	9.97^{ab}	9.40^{ab}	1.95^{ab}	0.03^{ab}	0.48^{ab}	3.64^{ab}	2.01^{ab}	11.60^{ab}	19.81^{ab}
2-Pentanone	1.21^{a}	8.73^{ab}	5.69^{ab}	6.02^{ab}	9.34^{ab}	2.69^{ab}	1.69^{ab}	8.78^{ab}	18.97^{b}	21.89^{ab}	6.90^{ab}	15.38^{ab}	2.05^{ab}	8.99^{ab}	11.82^{ab}	3.26^{ab}	2.18^{ab}	1.32^{ab}	5.35^{ab}	5.36^{ab}	19.13^{ab}	11.40^{ab}
Butanal	n.d.	n.d.	0.14^{abc}	0.77^{a}	n.d.	n.d.	0.24^{ab}	n.d.	0.16a	1.87^{abc}	0.04^{a}	n.d.	n.d.	0.16^{a}	n.d.	0.44^{a}	n.d.	n.d.	n.d.	0.63^{a}	n.d.	2.58^{c}
Methylbenzene	n.d.	n.d.	6.23^{ab}	n.d.	n.d.	n.d.	0.02^{a}	0.11^{a}	n.d.	n.d.	2.87^{a}	0.01^{a}	39.79^{b}	3.34^{a}	0.04^{a}	5.69^{ab}	4.61^{ab}	n.d.	2.29^{a}	n.d.	n.d.	n.d.
Microbial esterification																						
Ethanethioic acid, *S*-methyl ester	n.d.	10.21	1.07	1.36	4.41	0.06	0.04	0.85	8.71	9.67	4.60	3.97	0.42	3.62	12.99	n.d.	0.05	n.d.	0.01	3.33	3.03	3.04
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	0.15^{a}	0.11^{a}	0.13^{a}	1.43^{b}	0.09^{a}	0.25^{a}	0.38a	0.27^{a}	0.14^{a}	n.d.	0.18^{a}	0.33^{a}	0.74^{ab}	0.26^{a}	0.05^{a}	n.d.	0.03^{a}	0.10^{a}	0.07^{a}	0.83^{ab}	0.02^{a}	n.d.
1,2-Benzenedicarboxylic acid, dibutyl ester	0.12^{a}	0.36^{a}	0.16^{a}	0.37^{ab}	0.39^{a}	0.28^{ab}	0.39^{ab}	2.64^{b}	0.11^{a}	0.90^{ab}	0.28^{a}	0.24^{a}	0.48^{ab}	0.42^{a}	0.08^{a}	n.d.	n.d.	0.28^{ab}	0.09^{a}	1.01^{ab}	0.30^{a}	n.d.
1,2-Benzenedicarboxylic acid, bis(2-methoxyethyl) ester	n.d.	0.03^{a}	n.d.	n.d.	0.04^{a}	0.72^{b}	n.d.	0.13^{ab}	0.01^{a}	n.d.	0.02^{a}	0.04^{a}	n.d.	0.06^{a}	0.07^{a}	n.d.	0.06^{ab}	n.d.	n.d.	n.d.	n.d.	0.01^{ab}
Hexanedioic acid, dioctyl ester	n.d.	0.14^{a}	0.12^{a}	0.44^{a}	0.95^{a}	n.d.	n.d.	0.69^{a}	0.28^{a}	8.26^{b}	0.55^{a}	1.19^{a}	n.d.	0.74^{a}	0.78^{a}	n.d.	n.d.	n.d.	0.43^{a}	0.55^{a}	1.67^{a}	1.27^{a}
Carbohydrate fermentation																						
Ethanol	16.30	2.23	3.54	n.d.	5.75	0.10	0.77	1.03	4.44	4.88	0.33	5.48	0.66	4.45	6.17	1.17	n.d.	n.d.	6.62	5.57	4.37	5.13
Unknown origin + contaminants																						
Chloroform	2.56^{a}	16.67^{ab}	7.02^{ab}	11.73^{ab}	8.97^{a}	2.05^{a}	4.21^{a}	12.55^{ab}	14.11^{ab}	20.08^{ab}	7.33^{a}	13.51^{a}	54.65^{b}	12.16^{a}	11.83^{a}	14.79^{ab}	2.47^{ab}	3.63^{ab}	8.33^{a}	8.36^{ab}	10.24^{ab}	12.59^{ab}
1-1′-Biphenyl	n.d.	0.07	0.32	0.58	1.35	0.25	n.d.	0.28	0.76	1.95	0.36	0.93	0.11	0.55	0.60	0.36	n.d.	n.d.	n.d.	0.49	0.98	0.19
4-Methyl-3-penten-2-one	1.00	1.44	0.78	3.10	1.84	0.76	0.32	1.38	0.85	2.29	1.78	1.19	0.90	1.36	1.30	0.82	0.69	n.d.	0.58	0.95	0.23	0.24
Cyclohexanol	n.d.	0.69	0.10	n.d.	1.21	0.04	n.d.	0.46	0.81	1.92	0.17	0.44	n.d.	0.44	0.75	n.d.	n.d.	n.d.	0.17	n.d.	n.d.	1.26

(*continued on next page*)

Table 2 (*continued*)

Compound/origin	Yeast biotypes																			
	Control[b]	B1-P	B1-DC	B2-DC	C1-1-DC	C1-2-DC	C1-3-DC	C2-1-P	C2-1-DC	C2-2-DC	D1-P	D1-DC	E1-DC	E2-P	E2-DC	F1-DC	H1-P	H2-P	K1-P	K1-DC	O1-DC	S1-P
Cyclohexanone	n.d.	0.66	0.16	n.d.	1.36	n.d.	n.d.	0.52	1.41	0.75	0.22	1.79	n.d.	0.64	2.31	n.d.	n.d.	n.d.	n.d.	0.13	n.d.	n.d.
4-Methyl-2-heptanone	1.06	0.22	0.99	1.11	3.09	0.41	0.17	2.06	2.40	6.12	1.21	3.09	0.65	1.80	2.79	0.37	0.97	0.36	0.84	1.66	1.93	1.45
4-Ethyl-1,3-benzenediol	0.33[a]	0.28[a]	0.26[a]	0.70[a]	0.31[a]	1.06a	0.34[a]	0.35[a]	0.30[a]	0.49[a]	0.19[a]	0.31[a]	1.61[ab]	0.45[a]	0.47[a]	0.95[ab]	n.d.	1.08[ab]	0.65[a]	0.18[a]	0.23[a]	n.d.
4-Methoxy-1,3-benzenediamine	n.d.	0.12[a]	0.39[a]	n.d.	0.08[a]	n.d.	0.43[ab]	0.40[ab]	n.d.	n.d.	0.12[a]	n.d.	n.d.	0.13[a]	0.13[a]	0.07[a]	1.88[b]	0.02[a]	0.51[ab]	0.08[a]	n.d.	n.d.
2,6-Bis(1,1-dimethylethyl) -2,5-cyclohexadiene-1,4-dione	0.08[a]	0.49[ab]	5.50[ab]	8.41[abc]	10.31[ab]	0.86[ab]	1.06[ab]	2.76[ab]	14.06[abc]	30.12[bc]	8.55[ab]	12.47[ab]	1.95[ab]	7.62[ab]	9.69[ab]	1.16[ab]	2.55[ab]	1.80[ab]	3.90[ab]	4.03[abc]	35.36[c]	23.09[abc]

Means of volatile compounds were calculated from the strains analyzed for each yeast biotype in both ripening stages, the post-salting (P) and the drying-cellar (DC).

[a] Results are expressed in arbitrary area units ($\times 10^{-6}$) as means of 3 replicates of each strain belonging a biotype. Means in the same row with different lowercase letters indicate significant differences ($P < 0.05$). n.d., not detected.

[b] Control, uninoculated batch.

of the biotypes exclusively detected belonged to *D. hansenii*. This evolution of the yeast biotypes throughout the maturation process of dry-cured Iberian ham may be in relation to changes in the temperature and water activity during ripening. Thus, the temperature of ripening increases from 5 °C at the post-salting stage to values higher than 15 °C at the drying-cellar stage and water activity of the hams decreased from 0.98 at the post-salting stage to values lower than 0.92 at the drying-cellar stage (Ventanas et al., 2001). In addition, at the drying-cellar stage high levels of free amino acids have been reported in the product as a consequence of an intense proteolysis that takes place mainly at this stage (Córdoba et al., 1994). In these conditions, the biotypes of *D. hansenii* show a higher adaptation than those of *C. zeylanoides*, being the last kind of biotypes replaced by those of *D. hansenii* that are more adapted to the ecological conditions of the hams in the drying-cellar stage. These results agree with those previously reported which indicate a higher adaptation of *D. hansenii* than *C. zeylanoides* to maturation conditions of dry-cured meat products (Núñez et al., 1996; Encinas et al., 2000; Cocolin et al., 2006). Consequently, the biotypes of *D. hansenii* seem to be more appropriate for selection as starter cultures.

Regarding the differentiation of yeast biotypes according to their volatile compound production, it can be observed that most of the tested biotypes generated branched aldehydes and alcohols, such as 3- and 2-methylbutanal and 3- and 2-methylbutanol, with the biotype C2-2 of *D. hansenii* showing the highest concentrations. These compounds are derived from the branched amino acids valine, isoleucine and leucine, as a result of Strecker degradation (Ventanas et al., 1992) or microbial metabolism (Durá et al., 2004; Martín et al., 2006) and they have been reported to contribute significantly to the overall flavour of dry-cured hams (Ruiz et al., 1999; Carrapiso et al., 2002; Martín et al., 2006). Thus, these volatile compounds could be used to differentiate yeast biotypes of interest in dry-cured Iberian ham flavour development.

Some of the biotypes detected produced sulphur volatile compounds, especially 3-methylthio-1-propanol, methanethiol and dimethyldisulphide, with the highest production shown by the *C. zeylanoides* S1 biotype. These compounds, derived from sulphur amino acids such as methionine, contribute actively to both the flavours and off-flavours of foodstuffs (Boelens and van Gemert, 1993; López del Castillo-Lozano et al., 2007). Evidence of the yeast contribution to generating sulphur volatile compounds has been previously reported (Spinnler et al., 2001; Arfi et al., 2002; López del Castillo-Lozano et al., 2007).

Most of the yeast biotypes detected produced volatile compounds derived from lipid oxidation, although in lower amounts than those from amino acid catabolism. The compounds 2- and 3-methylpentane, 2-propanone, 2-butanone, 2-pentanone, butanal and methylbenzene, produced in higher amounts by some yeast biotypes, have been reported to add a pleasant flavour to dry-cured meat products (Carrapiso et al., 2002). Thus, these volatile compounds could also be used to differentiate yeast biotypes of interest in dry-cured Iberian ham flavour development.

Esters were produced by the majority of the yeast biotypes assayed, with the highest amount detected in the *D. hansenii* C2-2. Several authors have found that yeasts promote ester generation from the esterification of carboxylic acids and alcohols (Shahidi et al., 1986; Olesen and Stahnke, 2000; Flores et al., 2004). Esters, mainly ethyl esters, have been reported as essential volatile compounds for the typical aroma of dry-cured meat products (Meynier et al., 1999) because of their characteristic fruity notes, their low odour threshold values (Stahnke, 1994) and their contribution to mask rancid odours (Stahnke, 1994; Careri et al., 1993). Their presence, together with 3-methylbutanal, has been associated with a "ripened flavour" (Barbieri et al., 1992; Careri et al.,

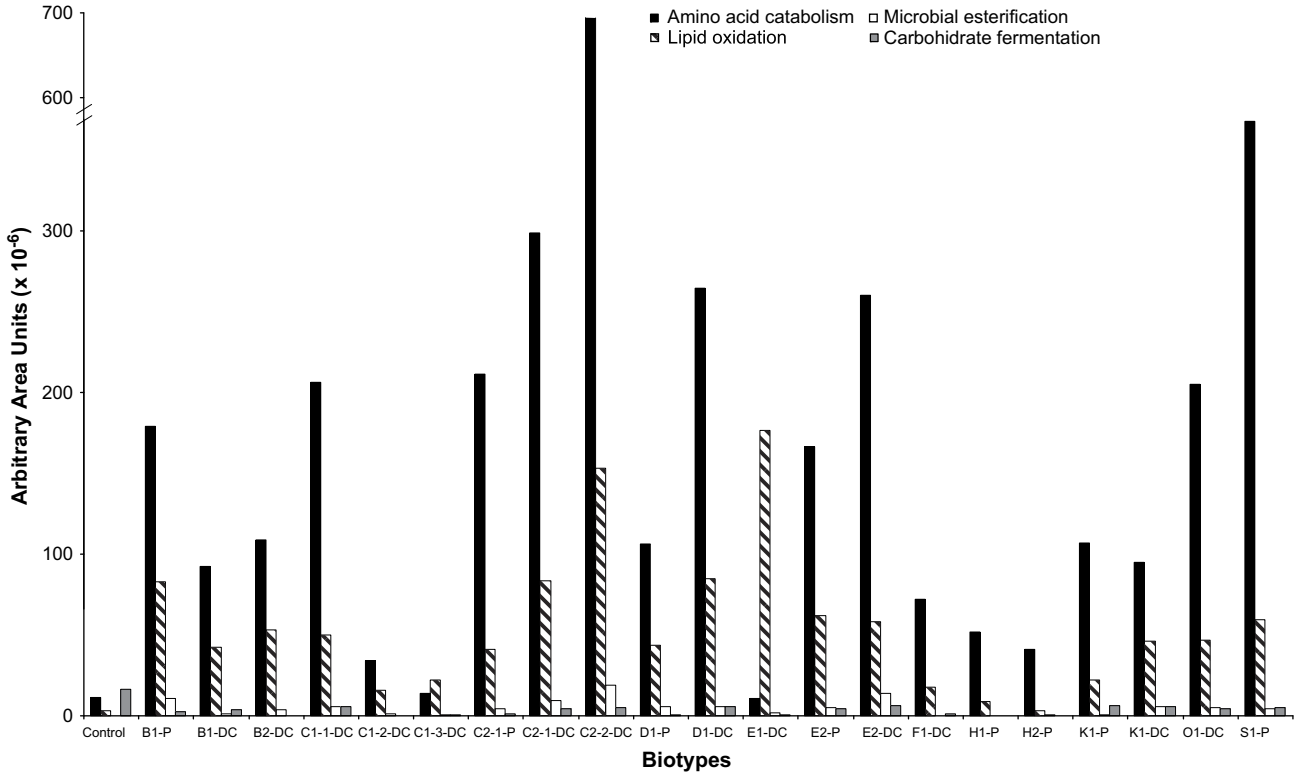

Fig. 3. Accumulated area of volatile compounds according to their origins from uninoculated batch (control) and batches inoculated with the different yeast biotypes detected in the post-salting (P) and drying-cellar (DC) stages of dry-cured Iberian ham.

1993) and could be appropriated to differentiate yeast biotypes of interest in flavour development of dry-cured Iberian ham.

Clear differences between yeast biotypes were detected in the generation of volatile compounds, with the greatest concentrations shown by the biotypes C2-2 of *D. hansenii* and S1 of *C. zeylanoides*. Andrade et al. (2009) found the highest production of volatile compounds in the C2 and E mtDNA restriction biotypes of *D. hansenii* and S of *C. zeylanoides*. Therefore, the combined use of mtDNA restriction analysis and RAPD-PCR distinguishes yeast biotypes with different production of volatile compounds that cannot be found using only mtDNA restriction analysis. Both methods could be very useful as routine techniques for the selection of yeasts of interest in flavour development.

When the volatile compound production of detected yeast biotypes was compared by stage of ripening, in general the biotypes isolated from the drying-cellar stage produced higher amounts than those recovered in the post-salting stage. Therefore, biotypes from the drying-cellar stage produced the highest levels of branched alcohols and aldehydes, hydrocarbons, esters and ketones. The main exception was found in the biotype B1, since strains of this biotype recovered in the post-salting stage yielded higher amounts of volatile compounds than those from the drying-cellar stage. Differences in the production of volatile compounds between yeast strains belonging to the same species have been previously reported (Arrizon et al., 2005; Regodón Mateos et al., 2006). In the present work, the different behaviour between strains of the same biotype could be explained by their particular adaptation to the very distinct ecological conditions of temperature of ripening and water activity of the product found in the drying-cellar stage in comparison with the post-salting stage. The differences in the generation of volatile compounds between strains of the same biotype could not be detected by mtDNA restriction analysis and RAPD-PCR. Thus, additional analysis of the volatile compound production profile is needed to differentiate yeast strains of the same biotype recovered at different stages of ripening. The combination of these three methods may allow selection of yeast biotypes with a high production of volatile compounds involved in flavour development of dry-cured Iberian ham, such as the biotype C2-2 from the drying-cellar stage detected in the present study.

In conclusion, the importance of the combination of RAPD-PCR with mtDNA restriction analysis as a routine method to differentiate yeast biotypes of interest throughout the ripening process of dry-cured Iberian ham was demonstrated in the present work. Clear differences between molecular biotypes were found in the production of volatile compounds, with the highest levels detected in the biotype C2-2 of *D. hansenii*. Analysis of the generation of volatile compounds should be added to mtDNA restriction analysis and RAPD-PCR to differentiate yeast strains of the same biotype. The combination of these three methods could be very useful to select or monitor yeasts as starter cultures in dry-cured meat products.

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References

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. Int. J. Food Microbiol. 107, 48–58.

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 Chem. 113, 457–463.

Arfi, K., Spinnler, H.E., Tache, R., Bonnarme, P., 2002. Production of volatile compounds by cheese-ripening yeasts: requirement for a methanethiol donor for *S*-methyl thioacetate synthesis by *Kluyveromyces lactis*. Appl. Microbiol. Biotechnol. 58, 503–510.

Arrizon, J., Fiore, C., Acosta, G., Romano, P., Gschaedler, A., 2005. Fermentation behaviour and volatile compound production by agave and grape must yeasts in high sugar *Agave tequilana* and grape must fermentations. Antonie van Leeuwenhoek 89, 181–189.

Barbieri, G., Bolzoni, L., Parolari, G., Virgili, R., Careri, M., Mangia, A., 1992. Flavour compounds of dry-cured hams. J. Agric. Food Chem. 40, 2389–2394.

Boelens, M.H., van Gemert, L.J., 1993. Volatile character-impact sulphur compounds and their sensory properties. Perfumer Flavorist 18, 29–39.

Careri, M., Mangia, A., Barbieri, G., Bolzoni, L., Virgili, R., Parolari, G., 1993. Sensory property relationship to chemical data of Italian type dry-cured ham. J. Food Sci. 58, 968–972.

Carrapiso, A.I., Ventanas, J., García, C., 2002. Characterization of the most odor-active compound of Iberian ham headspace. J. Agric. Food Chem. 27, 1996–2000.

Cocolin, L., Urso, R., Rantsiou, K., Cantoni, C., Comi, G., 2006. Dynamics and characterization of yeasts during natural fermentation of Italian sausages. FEMS Yeast Res. 6, 692–701.

Comi, G., Cantoni, C., 1983. Yeasts in dry Parma hams. Industrie Alimentari 22, 102–104.

Córdoba, J.J., Antequera, T., García, C., Ventanas, J., López, C., Asensio, M.A., 1994. Evolution of free amino acids and amines during ripening of Iberian cured ham. J. Agric. Food Chem. 42, 2296–2301.

Durá, M.A., Flores, M., Toldrá, F., 2004. Effect of growth phase and dry-cured sausage processing conditions on *Debaryomyces* spp. generation of volatile compounds from branched-chain amino acids. Food Chem. 86, 391–399.

Encinas, J.P., Lopez-Díaz, T.M., García-Lopez, M.L., Otero, A., Moreno, B., 2000. Yeast populations on Spanish fermented sausages. Meat Sci. 54, 203–208.

Fernández-Espinar, M.T., López, V., Ramón, D., Bartra, E., Querol, A., 2001. Study of the authenticity of commercial wine yeast strains by molecular techniques. Int. J. Food Microbiol. 70, 1–10.

Flores, M., Durá, M.A., Marco, A., Toldrá, F., 2004. Effect of *Debaryomyces* spp. on aroma formation and sensory quality of dry-fermented sausages. Meat Sci. 68, 439–446.

Jessen, B., 1995. Starter cultures for meat fermentation. In: Campbell-Platt, G., Cook, P.E. (Eds.), Fermented Meats. Blackie Academic and Professional, Glasgow, pp. 130–159.

Jeyaram, K., Mohendro Singh, W., Capece, A., Romano, P., 2008. Molecular identification of yeast species associated with 'Hamei' – a traditional starter used for rice wine production in Manipur, India. Int. J. Food Microbiol. 124, 115–125.

López del Castillo-Lozano, M., Delile, A., Spinnler, H.E., Bonnarme, P., Landaud, S., 2007. Comparison of volatile sulphur compound production by cheese-ripening yeasts from methionine and methionine-cysteine mixtures. Appl. Microbiol. Biotech. 75, 1447–1454.

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. Int. J. Food Microbiol. 110, 8–18.

Martorell, P., Fernández-Espinar, M.T., Querol, A., 2005. Molecular monitoring of spoilage yeasts during the production of candied fruit nougats to determine food contamination sources. Int. J. Food Microbiol. 101, 293–302.

Meynier, A., Novelli, E., Chizzolini, R., Zanardi, E., Gandemer, G., 1999. Volatile compounds of commercial Milano salami. Meat Sci. 51, 175–183.

Nikolaou, E., Andrighetto, C., Lombardi, A., Litopoulou-Tzanetaki, E., Tzanetakis, N., 2007. Heterogeneity in genetic and phenotypic characteristics of *Saccharomyces cerevisiae* strains isolated from red and white wine fermentations. Food Control 18, 1458–1465.

Núñez, F., Rodríguez, M.M., Córdoba, J.J., Bermúdez, M.E., Asensio, M.A., 1996. Yeast population during ripening of dry-cured Iberian ham. Int. J. Food Microbiol. 29, 271–280.

Olesen, P.T., Stahnke, L.H., 2000. The influence of *Debaryomyces hansenii* and *Candida utilis* on the aroma formation in garlic spiced fermented sausages and model minces. Meat Sci. 56, 357–368.

Petersen, K.M., Moller, P.L., Jespersen, L., 2001. DNA typing methods for differentiation of *Debaryomyces hansenii* strains and other yeasts related to surface ripened cheeses. Int. J. Food Microbiol. 69, 11–24.

Querol, A., Barrio, E., Ramón, D., 1992. A comparative study of different methods of yeast strain characterization. Syst. Appl. Microbiol. 15, 439–446.

Regodón Mateos, J.A., Pérez Nevado, F., Ramírez Fernández, M., 2006. Influence of *Saccharomyces cerevisiae* yeast strain on the major volatile compounds of wine. Enzyme Microb. Technol. 40, 151–157.

Ruiz, J., Cava, R., Ventanas, J., Jensen, M.T., 1998. Headspace solid phase microextraction for the analysis of volatiles in meat product: dry-cured Iberian ham. J. Agric. Food Chem. 46, 4688–4694.

Ruiz, J., Ventanas, J., Cava, R., Andrés, A., García, C., 1999. Volatile compounds of dry-cured Iberian ham as affected by the length of the curing process. Meat Sci. 52, 19–27.

Shahidi, F., Rubin, L.J., D'Souza, L.A., 1986. Meat flavor volatiles: a review of the composition, techniques of analysis, and sensory evaluation. Crit. Rev. Food Sci. Nutr. 24, 141–243.

Simoncini, N., Rotelli, D., Virgili, R., Quintavalla, S., 2007. Dynamics and characterization of yeasts during ripening of typical Italian dry-cured ham. Food Microbiol. 24, 577–584.

Spinnler, H.E., Berger, C., Lapadatescu, C., Bonnarme, P., 2001. Production of sulphur compounds by several yeasts of technological interest for cheese ripening. Int. Dairy J. 11, 245–252.

Stahnke, L.H., 1994. Aroma components from dried sausages fermented with *Staphylococcus xylosus*. Meat Sci. 38, 39–53.

Torriani, S., Zapparoli, G., Suzzi, G., 1999. Genetic and phenotypic diversity of *Saccharomyces* sensu stricto strains isolated from Amarone wine. Antonie van Leeuwenhoek 75, 207–215.

Vasdinyei, R., Deák, T., 2003. Characterization of yeast isolates originating from Hungarian dairy products using traditional and molecular identification techniques. Int. J. Food Microbiol. 86, 123–130.

Ventanas, J., Córdoba, J.J., Antequera, T., García, C., López-Bote, C., Asensio, M.A., 1992. Hydrolysis and Maillard reactions during ripening of Iberian ham. J. Food Sci. 57, 813–815.

Ventanas, J., Ruiz, J., Córdoba, J.J., 2001. Dry-cured Iberian ham: description of traditional ripening process. In: Dry-cured Iberian Ham Technology. Ediciones Mundi-Prensa, Madrid, pp. 45–72.

Walczak, E., Czaplinska, A., Barszczewski, W., Wilgosz, M., Wojtatowicz, M., Robak, M., 2007. RAPD with microsatellite as a tool for differentiation of *Candida* genus yeasts isolated in brewing. Int. J. Food Microbiol. 24, 305–312.