PE8.17 Differentiation of yeasts of technological interest in Iberian dry-cured ham 135.00

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***Abstract*—The efficiency of mitochondrial DNA restriction analysis (mtDNA RFLP) and random amplification of polymorphic DNA (RAPD-PCR) to differentiate yeasts growing on Iberian dry-cured ham throughout the ripening with different volatile compounds generation was analyzed. For this aim, 338 yeasts isolated from Iberian dry-cured ham were used. The combination of mtDNA RFLP and RAPD-PCR allowed differentiating yeasts at strain level. Clear differences between yeast biotypes were detected in the volatile compounds generation, showing the biotype C2-2 of *Debaryomyces hansenii*, the predominant yeast species identified, the highest levels of volatiles. The combination of mtDNA RFLP and RAPD-PCR was an efficient method to differentiate yeast biotypes with different ability to produce volatile compounds, and might be useful for the selection of yeasts with desirable characteristics as starter cultures in dry-cured ham.**

***Index Terms*— Dry-cured ham, Molecular techniques, Volatile compounds, Yeasts**

I. INTRODUCTION

Yeasts are one of the predominant microbial groups throughout most of the ripening process of Iberian dry-cured ham. *Debaryomyces hansenii* has been reported to be the commonest yeast species in this product [1]. Different biotypes of yeasts have been found in Iberian dry-cured ham with different ability to produce volatile compounds involved in flavour development of Iberian dry-cured ham [2]. Therefore, it should be of great interest to differentiate the main yeast biotypes growing on Iberian dry-cured ham in relation to the generation of volatile compounds. For this purpose different molecular techniques will be evaluated.

The aim of this work was to analyze the effectiveness of mitochondrial DNA restriction analysis (mtDNA RFLP) and random amplification of polymorphic DNA (RAPD-PCR) to differentiate yeast biotypes growing on the hams with different production of volatile compounds.

II. MATERIALS AND METHODS

II.1. Molecular differentiation of yeasts isolated from Iberian dry-cured ham.

A total of 338 yeast strains isolated from Iberian hams at different industries of the four Spanish Protected Designations of Origin of this product were differentiated by their molecular profiles. They were obtained by the combined use of mtDNA RFLP with the restriction enzyme *Hae*III and RAPD-PCR with primer $(GACA)_4$ [3]. Species identification of representative isolates of each molecular pattern obtained was performed by 18S ribosomal DNA (rDNA) sequencing.

II.2. Differentiation of yeasts isolated from Iberian dry-cured ham by their volatile compound production

A culture medium that emulates the composition of Iberian dry-cured ham during the maturation process was used for differentiating yeasts isolated from this product by their volatile compound production [2]. The medium was inoculated with representative isolates of each molecular pattern and then samples of both inoculated and uninoculated medium were incubated for 30 days at 25 ºC with shaking. Uninoculated culture medium was used as control. After the incubation, the extraction of volatile compounds was performed by solid phase micro-extraction technique (SPME). Volatile compounds analysis was performed using a Hewlett-Packard 5890 S II gas chromatograph coupled with a Hewlett-Packard 5971A ion-trap mass spectrometer. The individual volatile compounds were identified through their mass spectra by comparison with NIST/EPA/NIH library and their Kovats indices. To calculate the indices of the different compounds, n-alkanes (Sigma R-8769) were run under the same conditions as the samples.

III. RESULTS AND DISCUSSION

The combination of mtDNA RFLP and RAPD-PCR yielded 16 different molecular patterns or biotypes (Table 1). The combination of both methods allowed the differentiation of yeasts at strain level. When sequencing of the 18S rDNA was used the H1, H2, O1 and S1 biotypes were identified as *Candida zeylanoides* and the remaining as *D. hansenii* (Table 1). There was a low diversity of species in the yeast population growing on Iberian dry-cured ham throughout

the ripening, being *D. hansenii* the predominant species during the whole maturation process. However, a wide variety of biotypes was detected at strain level.

A total of 47 volatile compounds were produced by different biotypes of yeasts differentiated by mtDNA restriction analysis and RAPD-PCR. These compounds were classified according to their most likely origin 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). The lowest levels of volatile compounds were detected in the control batch (Figure 1). Clear differences between yeast biotypes in the production of volatile compounds were detected (Figure 1). Most of the tested biotypes produced branched aldehydes and alcohols derived from amino acid catabolism, such as 3- and 2-methylbutanal and 3- and 2-methylbutanol, with the biotype C2-2 of *D. hansenii* showing the highest levels in most cases (Figure 2). These compounds have been reported to contribute considerably to the overall flavour of meat products [4,5]. The biotype E2 of *D. hansenii* presented the greatest amounts of esters (Figure 1) and the biotype S1 of *C. zeylanoides* the highest overall level of sulphur compounds (Figure 2). The ability of yeasts to produce volatile sulphur compounds has been previously reported [6,7]. High amounts of the former compounds could contribute to an undesirable flavour [8]. Besides volatile compounds derived from lipid oxidation, such as 2- and 3-methylpentane, 2-propanone, 2-butanone, 2-pentanone, butanal and methylbenzene were detected in significantly higher amount in some of the inoculated samples than in the control samples.

On account of the biotype C2-2 produced the highest levels of volatile compounds related to the flavour of Iberian dry-cured ham, this biotype of *D. hansenii* could be considered for use as starter culture in this meat product. Therefore, the combined use of mtDNA RFLP and RAPD-PCR permitted distinguishing yeast biotypes with different generation of volatile compounds.

IV. CONCLUSION

The combination of mtDNA RFLP and RAPD-PCR was an efficient method to differentiate yeast biotypes with different ability to produce volatile compounds, and might be useful for the selection of yeasts with desirable characteristics as starter cultures in dry-cured ham.

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Table 1. Identification of yeast biotypes isolated from Iberian dry-cured ham by 18S ribosomal DNA (rDNA) sequencing. Yeast biotypes were obtained by mitochondrial DNA restriction analysis and RAPD-PCR with primer $(GACA)_4$.

Yeast biotypes	**Identification by 18S rDNA sequencing**
B1	*Debaryomyces hansenii*
B2	*D. hansenii*
C1-1	*D. hansenii*
C1-2	*D. hansenii*
C1-3	*D. hansenii*
C2-1	*D. hansenii*
C2-2	*D. hansenii*
D1	*D. hansenii*
E1	*D. hansenii*
E2	*D. hansenii*
F1	*D. hansenii*
H1	*Candida zeylanoides*
H2	*C. zeylanoides*
K1	*D. hansenii*
O1	*C. zeylanoides*
S1	*C. zeylanoides*