Design of a Method to Evaluate Yeasts to be Used as Starter Cultures in Dry-Cured Meat Products

Eva M. CASADO, Juan J. CÓRDOBA, María J. ANDRADE and Mar RODRÍGUEZ

Food Hygiene and Safety, Faculty of Veterinary Science, University of Extremadura, Cáceres, Spain

Abstract

Casado E.M., Córdoba J.J., Andrade M.J., Rodríguez M. (2011): **Design of a method to evaluate yeasts to be used as starter cultures in dry-cured meat products**. Czech J. Food Sci., **29**: 463–470.

Some yeasts are involved in flavour development of dry-cured meat products showing a positive impact on the generation of volatile compounds. The aim of this work was to design a method of routine analysis to evaluate the production of volatile compounds by yeasts to be selected as starter cultures. For this purpose, several variations of a minimum culture medium that included free amino acids, oleic acid, and α-ketoglutarate, incubated under similar conditions of water activity and pH as dry-cured meat products, were assayed. In these conditions, the representative yeast strains isolated from a dry-cured meat product were tested. The volatile compounds were analysed using Solid Phase Micro-Extraction and gas chromatography/mass spectrometry. In the designed media, the tested yeasts produced volatile compounds involved in flavour development of dry-cured meat products. In addition, all the strains showed the highest production of these volatile compounds in the complete minimum culture medium witch included α-ketoglutarate and oleic acid.

Keywords: yeasts; volatile compounds; dry-cured meat products; starter cultures; minimum culture medium

Yeast is one of the microbial groups predominant during the ripening period of several food products of intermediate moisture such as dry-cured meat products (Núñez *et al*. 1996; Encinas *et al*. 2000; Osei Abunyewa *et al*. 2000; Cocolin *et al*. 2006; Fernández-López *et al*. 2008). *Debaryomyces hansenii* is the dominant yeast species throughout the maturation of the above products (Fleet 1990; Núñez *et al*. 1996; Encinas *et al*. 2000; Cocolin *et al*. 2006; Aquilanti *et al*. 2007; Andrade *et al*. 2009a, 2010a). Nevertheless, other yeast species such as *Candida zeylanoides, Pichia carsonii*, and *Rhodotorula* spp. can be isolated in ripened products (Núñez *et al*. 1996; Simoncini *et al*. 2007; Andrade *et al*. 2009a, 2010a).

Despite the fact that some authors suggest that proteolytic and lipolytic changes occurring during the maturation of dry-cured meat products are only due to the endogenous enzyme activity (Zhou & Zhao 2007), the contributions of microorganisms growing throughout this period and their enzymes to proteolysis in dry-cured meat products have been demonstrated as well. In this sense, various peptidases and proteases of yeasts have been described suggesting their participation in the ripening of these products (Bolumar *et al*. 2003, 2008). Moreover, strains of *D. hansenii* isolated from dry-cured meat products have shown high proteolytic activity when inoculated on raw pork (Rodríguez *et al.* 1998; Martín *et al*. 2001), dry

Partly supported by of the Spanish Comisión Interministerial de Ciencia y Tecnología, Projects No. AGL2001-0804 and No. AGL2004-3291. Eva M. Casado was the recipient of a Grant of the Regional Government of Junta de Extremadura (Consejería de Educación, Ciencia y Tecnología).

fermented sausages (Durá *et al*. 2004; Patrignani *et al*. 2007), ripened pork loin (Martín *et al*. 2003), and dry-cured ham (Martín *et al*. 2004). On the other hand, *Debaryomyces* spp. have been reported to have a positive impact on the volatile compounds involved in flavour development of dry-cured meat products inoculated with other microorganisms as mixed starter cultures (Flores *et al*. 2004; Martín *et al*. 2006; Andrade *et al*. 2010b). However, it should be noted that the formation of volatile compounds depends on the particular yeast species and the particular strain of the species (Romano 1997; Patel & Shibamoto 2003; Romano *et al*. 2003; Arrizon *et al*. 2005; Regodón *et al*. 2006). To avoid differences between dry-cured meat products from different batches of production due to particular yeast strains growing in these products, yeast strains selected as potent producers of volatile compounds involved in the flavour of dry-cured meat products should be used as starter cultures.

The evaluation of the yeast strains ability to produce volatile compounds can be done in a meat model system (Martín *et al*. 2003) or directly in dry-cured meat products (Durá *et al*. 2004; Martín *et al*. 2006; Patrignani *et al*. 2007; Andrade *et al*. 2010b). However, the former techniques can not be used as routine methods for the evaluation of great numbers of yeast strains, since multiple pieces as sterile samples would be needed. A more feasible technique would be based on a culture medium that emulates the composition of dry-cured meat products through their ripening process. This medium should include free amino acids at their usual concentrations in these products through the ripening process (Córdoba *et al*. 1994a; Martín *et al*. 2001). These compounds have been reported to be used by yeasts in the generation of compounds involved in flavour development (Martín *et al*. 2006). In addition, lipid components such as oleic acid could be used by yeasts in their metabolic pathways of volatile compounds generation related to dry-cured meat products (Ruiz *et al*. 1999; Petrón *et al*. 2004). Thus, the addition of oleic acid should be tested in the design of a culture medium. On the other hand, the addition of α-ketoglutarate in this medium should be assayed in the process of looking for an appropriate method to evaluate the volatile compounds production by yeasts isolated from dry-cured meat products because it favours the conversion of amino acids into flavour compounds (Yvon *et al*. 1998). Finally, the addition of NaCl and lactic acid may allow to obtain the conditions of water activity and pH similar to those of dry-cured meat products (Córdoba *et al*. 1994b; Rodríguez *et al*. 1994).

The aim of this work was to design a method of routine analysis to evaluate the production of volatile compounds by yeasts isolated from dry-cured meat products to be selected as starter cultures. For this purpose, several variations of a minimum culture medium that included free amino acids, oleic acid, and α-ketoglutarate, incubated under conditions of water activity and pH resembling those in dry-cured meat products, were assayed.

MATERIAL AND METHODS

Yeast strains. Twenty-five yeast strains isolated from dry-cured Iberian ham belonging to the main species of yeasts usually found in meat products were used in this study. All the yeasts were purified by repeated cultivation on malt extract agar (MEA) (20 g/l malt extract, 20 g/l glucose, 1 g/l peptone, 20 g/l agar) before the analysis. The yeasts had been grouped by mitochondrial DNA (mtDNA) restriction analysis, using the method described by Andrade *et al*. (2006), in 2 species, *D. hansenii* (21 strains) and *C. zeylanoides* (4 strains).

Inoculation of yeasts in different culture media designed. The 25 yeasts collected from dry-cured Iberian ham were individually inoculated in triplicates on a designed culture medium to determine the volatile compounds production (Na_2HPO_4 buffer 0.1M, pH 6; lactic acid 0.1M; NaCl 5 g/l; oleic acid 4 g/l; ribose 0.5 g/l; creatin 3 g/l; amino acid 4 g/l; and α-ketoglutarate 2.25 g/l). All the samples were incubated at 25°C for 30 days under shaking.

Furthermore, other 2 culture media were tested using the same composition of the former medium but one of them without oleic acid and the other one without α-ketoglutarate, to evaluate the influence of either component on the generation of volatiles by yeasts.

Yeasts were inoculated in all kinds of culture media at 10^6 CFU/ml. Three replicates were incubated without inoculation and used as uninoculated control samples. Microbial counts in the incubated control samples were tested on Plate Count Agar (PCA) and no growth was detected

in any of them. Yeast counts in the inoculated samples were determined on MEA at the end of the incubation period and levels higher than 10^7 CFU/ml were found.

Extraction of volatile compounds. After the incubation period, aliquots of 1.5 ml of culture medium were dispensed into a 5 ml headspace vial (Hewlett-Packard, Palo Alto, USA) and sealed with a PTFE butyl septum (Perkin-Elmer, Foster City, USA) in an aluminium cap. The volatile compounds were collected by Solid Phase Micro-Extraction (SPME) technique (Ruiz *et al*. 1998) with a 10 mm long, 100 µm thick fiber coated with carboxen poly-dimethylsiloxane (Supelco Co., Bellefonte, USA). Prior to the collection of volatiles, the fiber was preconditioned at 220°C for 50 min in the gas chromatograph injection port. The SPME fiber was inserted into the headspace vial through the septum and exposed to the headspace at 42°C for 45 min in a water bath.

Gas Chromatography/Mass Spectrometry (GC/MS) analyses. The chromatographic analyses were performed by using a Hewlett-Packard 5890 S II gas chromatograph connected to a Hewlett-Packard 5971A ion-trap mass spectrometer. A 5% phenyl-95% dimethyl polysiloxane column (50 m × 0.32 mm *i.d.*, 1.05 µm film thickness; Hewlett-Packard) was used for the separation of the volatile compounds. The carrier gas was helium. The injection port was in a splitless mode. The SPME fiber 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, next increased to 150°C at 4°C/min, and then to 250°C at 20°C/minute. To calculate the Kovats index of the compounds, *n*-alkanes (Sigma R-8769) were run under the same conditions. 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 over a range of *m/z* 40–300 for the data collection.

To calculate the Kovats indices for the different compounds, *n*-alkanes (Sigma R-8769) were run under the same conditions. The identification of the volatile compounds of dry fermented sausages was done by comparison of their mass spectra with the data of the Wiley library and the calculated Kovats indices with those reported in the NIST database (NIST 2005) and in the literature (Kondjoyan & Berdagué 1996). Quantification was based on either the total or single ion chromatogram on an arbitrary scale.

Statistical analysis. The data obtained were subjected to one-way analysis of variance. The differences between the means were compared by *LSD* (least-significant difference) test using SPSS software package (vers. 15.0) for Windows (SPSS Inc. Chicago, USA) and were considered to be significant when $P < 0.05$.

RESULTS AND DISCUSSION

A total of 30 volatile compounds were identified in different culture media tested, most of them in the inoculated batches. The volatile compounds included alcohols (8), aldehydes (6), hydrocarbons (4), ketones (4), esters (2), and sulphur compounds (2). The remaining volatile compounds were grouped as "other compounds". Most of the identified compounds have been reported in dry-cured meat products (Flores *et al*. 1998, 2004; Ruiz *et al*. 1998, 1999; Schmidt & Berger 1998; Martín *et al*. 2006; Bianchi *et al*. 2007).

Only 6 volatile compounds were detected in uninoculated control batch, some of them showing (ethanol, ethylbenzaldehyde, 3,4-dimethylbenzaldehyde and chloroform) higher levels than in inoculated batches (Table 1). In the uninoculated control batch, ethanol could derive from the catabolism of lipids or amino acids which were present in the model culture medium. However, with inoculated batches it seems likely that yeasts used ethanol as a carbon and energy source (Flores *et al*. 2000). Ethylbenzaldehyde and 3,4-dimethylbenzaldehyde could also be derived from the catabolism of amino acids. Even though some of these volatile compounds could be considered as environmental contaminants, the 4 compounds have not been reported as essential in the flavour development of dry-cured meat products.

In the inoculated samples, the most abundant compounds were branched aldehydes, alcohols, and hydrocarbons (Figure 1), which were present in significantly higher amounts than in control samples. These compounds have been described as involved in the development of the flavour of dry-cured Iberian ham (Carrapiso *et al*. 2002), which confirms the importance of yeasts in the overall flavour of this product.

The differences found between the inoculated and uninoculated control samples reveal the im-

Table 1. Volatile compounds identified in different culture media individually inoculated with 25 yeast strains isolated from dry-cured ham[A]

	KI	ID	Batches			
			uninoculated complete medium	complete medium	medium without	
					oleic acid	α-ketoglutarate
Hydrocarbons						
Hexane	600	MS + KI	n.d.	0.51	n.d.	n.d.
3,5-Dimethylbenzene	1248	MS + KI	n.d.	0.25	n.d.	0.27
2-Methoxy-2-methylpropane	560	MS	n.d.	n.d.	n.d.	0.08
1,1'-Biphenyl	1367	MS	n.d.	0.92	n.d.	0.23
Aldehydes						
2-Methylpropanal	556	MS + KI	n.d.	0.01	n.d.	0.69
2-Methylbutanal	662	MS + KI	n.d.	1.51	n.d.	n.d.
3-Methylbutanal	654	MS + KI	n.d.	0.49[a]	n.d.	1.80[b]
Ethylbenzaldehyde	1193	MS	7.45	n.d.	n.d.	n.d.
3,4-Dimethylbenzaldehyde	1194	MS + KI	5.37	n.d.	n.d.	n.d.
Benzeneacetaldehyde	1032	MS	n.d.	0.13	n.d.	3.24
Esters						
Propanoic acid, 2-methyl-,1-(1,1-dimethyl-ethyl)-2-methyl-1,3-propanediyl ester	1596	MS + KI	n.d.	0.17	n.d.	n.d.
Ethanethioic acid, methyl ester	692	MS + KI	n.d.	3.88	n.d.	3.68
Alcohols						
Ethanol	< 500	MS	2.01	0.65	n.d.	0.34
2-Pentanol	702	MS + KI	n.d.	1.11	0.35	n.d.
Benzeneethanol	1103	MS	n.d.	8.72	0.52	6.38
2-Methylpropanol	618	MS + KI	n.d.	7.46	1.82	3.63
2-Methyl-2-propanol	521	MS + KI	n.d.	3.67	1.46	n.d.
3-Methylbutanol	728	MS + KI	n.d.	66.49	59.40	39.82
2-Methylbutanol	731	MS + KI	n.d.	55.96	37.59	29.68
2,4-Bis(1,1-dimethylethyl)phenol	1509	MS + KI	n.d.	2.17[a]	n.d.	0.39[b]
Ketones						
2-Propanone	< 500	MS + KI	6.00	8.94	10.74	12.32
2-Butanone	593	MS + KI	3.28	9.49	9.31	12.01
2-Pentanone	687	MS + KI	n.d.	4.32	1.06	2.36
3-Hydroxy-2-butanone	712	MS + KI	n.d.	0.37	0.18	n.d.
Sulphur compounds						
Methanethiol	< 500	MS	n.d.	0.64[a]	n.d.	1.93[b]
Dimethyl disulphide	731	MS + KI	n.d.	3.19	n.d.	n.d.
Other compounds						
Chloroform	609	MS + KI	392.50[a]	107.93[ab]	117.68[ab]	58.83[b]
Limonene	1016	MS	n.d.	0.33	n.d.	n.d.
2,6-Bis(1,1-dimethylethyl)-2,5-cyclo-hexadiene-1,4-dione	1457	MS	n.d.	9.33	5.02	9.71

[A]results are expressed in Arbitrary Area Units ($\times 10^{-6}$) as means of 3 replicates of each strain; means with different letters in the same row are significantly different ($P < 0.05$)

KI – Kovats index; ID – reliability of identification; MS – tentative identification by mass spectrum; KI – mass spectrum and Kovats index agree with those reported in the literature (Kondjoyan & Berdagué 1996) and in the NIST database (http://webbook.nist.gov); n.d. – not detected

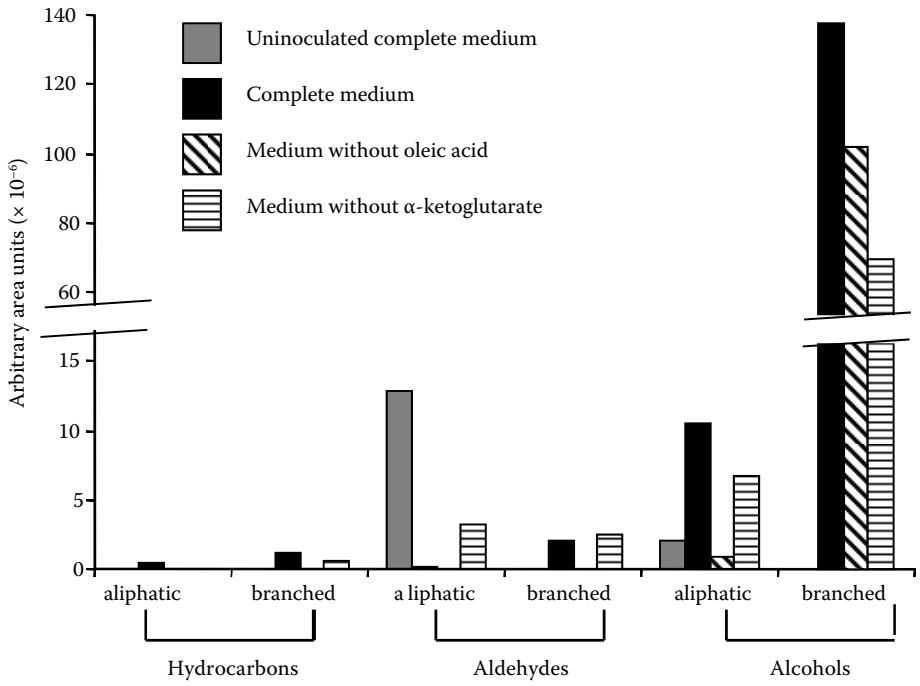

Figure 1. Accumulated area of aliphatic and branched hydrocarbons, aldehydes, and alcohols, in uninoculated batch and three batches inoculated with yeasts (complete, without oleic acid and without α-ketoglutarate media)

portance of yeasts in the generation of volatile compounds as reported by Andrade *et al.* (2009b). In addition, the former results reveal that the tested yeast strains are able to convert the amino acids present in the designed media into those volatile compounds usually reported as involved in flavour development of dry-cured meat products.

When the productions of volatile compounds by the tested culture media were compared, the complete medium, which included α-ketoglutarate and oleic acid, showed the highest number of volatile compounds (26). In the media without α-ketoglutarate and without oleic acid, 19 and 12 volatile compounds were detected, respectively.

The addition of α-ketoglutarate clearly increased the levels of 2-methylbutanal and 3-methylbutanal. In this way, although no significant differences were found, the generation of 2-methylbutanol and 3-methylbutanol, formed by the reduction of 2-methylbutanal and 3-methylbutanal, increased about 2-fold by the addition of α-ketoglutarate (Table 1). The influence of this compound on the increase of some branched alcohols and aldehydes has been observed in fermented sausages (Herranz *et al.* 2003; Tjener *et al.* 2004) as well. Nevertheless, the results might suggest that the addition of α-ketoglutarate is one way of increasing the levels of the flavour-intensive methyl-branched aldehydes and alcohols. That could be of great interest in testing the ability of yeasts in the production of the above compounds to be selected as starter cultures.

The addition of oleic acid to the culture media led to the formation of volatile compounds. The lowest levels of volatile compounds production were found in the culture medium without oleic acid (Table 1). Moreover, it is likely that the presence of oleic acid stimulates microbial growth and the utilisation of compounds by microorganisms producing oxidation derived compounds (Mauricio *et al.* 1997). Although no significant differences in yeast counts in the media inoculated were found (data no shown), it might be possible that the addition of oleic acid increases microbial activity and, consequently, the production of volatile compounds. In this sense, Mauricio *et al.* (1997) showed that oleic acid added to the musts generally increased the levels of the aroma compounds in wine.

Therefore, the complete medium designed in this work is best suited to evaluate the ability of volatile compounds formation involved in flavour development by yeasts isolated from meat products, and to test the yeasts usually growing

in dry-cured meat products to be used as starter cultures. Furthermore, it is a medium which has similar characteristics to those found in the meat products during their ripening. Thus, the complete medium was chosen to evaluate the yeasts isolates obtained from dry-cured ham, specifically *D. hansenii* which is the predominant species in dry-cured meat products during the ripening (Fleet 1990; Núñez *et al*. 1996; Encinas *et al*. 2000; Cocolin *et al*. 2006; Aquilanti *et al*. 2007; Andrade *et al*. 2009a, 2010a). In all the samples inoculated with the different yeasts higher number of volatile compounds was detected in the complete medium than in the remaining culture media tested.

CONCLUSIONS

This study demonstrated that the incubation of yeasts in a medium that includes free amino acids, oleic acid, and α-ketoglutarate followed by further evaluation of volatile compounds production by GC-MS could be used as routine method to evaluate yeasts strains to be used as starter cultures in dry-cured meat products.

References

Andrade M.J., Rodríguez M., Sánchez B., Aranda E., Córdoba J.J. (2006): DNA typing methods for differentiation of yeast related to dry-cured meat products. International Journal of Food Microbiology, **107**: 48–58.

Andrade M.J., Rodríguez M., Casado E.M., Bermúdez E., Córdoba J.J. (2009a): Differentiation of yeasts growing on dry-cured Iberian ham by mitochondrial DNA restriction analysis, RAPD-PCR and their volatile compounds production. Food Microbiology, **26**: 578–586.

Andrade M.J., Córdoba J.J., Sánchez B., Casado E.M., Rodríguez M. (2009b): Evaluation and selection of yeasts isolated from dry-cured Iberian ham by their volatile compound production. Food Chemistry, **113**: 457–463.

Andrade M.J., Rodríguez M., Casado E., Córdoba J.J. (2010a): Efficiency of mitochondrial DNA restriction analysis and RAPD-PCR to characterize yeasts growing on dry-cured Iberian ham at the different geographic areas of ripening. Meat Science, **84**: 377–383.

Andrade M.J., Córdoba J.J., Casado E.M., Córdoba M.G., Rodríguez M. (2010b): Effect of selected strains of *Debaryomyces hansenii* on the volatile compound production of dry fermented sausage "salchichón". Meat Science, **85**: 256–264.

Aquilanti L., Santarelli S., Silvestri G., Osimani A., Petruzzelli A., Clementi F. (2007): The microbial ecology of a typical Italian salami during its natural fermentation. International Journal of Food Microbiology, **120**: 136–145.

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.

Bianchi F., Cantoni C., Careri M., Chiesa L., Musci M., Pinna A. (2007): Characterization of the aromatic profile for the authentication and differentiation of typical Italian dry-sausages. Talanta, **72**: 1552–1563.

Bolumar T., Sanz Y., Aristoy M.-C., Toldrá F. (2003): Purification and properties of an arginyl aminopeptidase from *Debaryomyces hansenii*. International Journal of Food Microbiology, **86**: 141–151.

Bolumar T., Sanz Y., Aristoy M.-C., Toldrá F. (2008): Purification and characterisation of Proteases A and D from *Debaryomyces hansenii*. International Journal of Food Microbiology, **124**: 135–141.

Carrapiso A., Ventanas J., García C. (2002): Characterization of the most odor-active compounds of Iberian ham headspace. Journal of Agricultural and Food Chemistry, **50**: 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 Research, **6**: 692–701.

Córdoba J.J., Antequera T., López-Bote C., García C., Asensio M.A., Ventanas J. (1994a): Evolution of free amino acid and amines during ripening of Iberian cured ham. Journal of Agricultural and Food Chemistry, **42**: 2296–2301.

Córdoba J.J., Antequera T., López-Bote C., García C., Asensio M.A., Ventanas J. (1994b): Hydrolysis and loss of extractability of proteins during ripening of Iberian ham. Meat Science, **37**: 217–227.

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 Chemistry, **86**: 391–399.

Encinas J.P., López-Díaz T.M., García-López M.L., Otero A., Moreno B. (2000): Yeast populations on Spanish fermented sausages. Meat Science, **54**: 203–208.

Fernández-López J., Sendra E., Sayas-Barberá E., Navarro C., Pérez-Álvarez J.A. (2008): Physico-

chemical and microbiological profiles of "salchichón" (Spanish dry-fermented sausage) enriched with orange fiber. Meat Science, **80**: 410–417.

Fleet G.H. (1990): Yeast in dairy products. A review. Journal of Applied Microbiology, **69**: 199–211.

Flores M., Spanier A.M., Toldrá F. (1998): Flavour analysis of dry-cured ham. In: Shahidi F. (ed.): Flavour of Meat, Meat Products and Seafoods. Blackie Academic and Professional, Glasgow, UK: 320–339.

Flores C.L., Rodríguez C., Petit T., Gancedo C. (2000): Carbohydrate and energy-yielding metabolism in non-conventional yeasts. FEMS Microbiology Reviews, **24**: 507–529.

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 Science, **68**: 439–446.

Herranz B., Fernández M., Hierro E., Bruna J.M., Ordoñez J.A., De La Hoz L. (2003): Use of *Lactococcus lactis* subsp. *cremoris* NCDO 763 and α-ketoglutarate to improve the sensory quality of dry fermented sausages. Meat Science, **66**: 151–163.

Kondjoyan, N., Berdagué, J.L. (1996): A compilation of relative retention indices for the analysis of aromatic compounds. Theix, France: Laboratoire Flaveur, Station de Recherches Sur la Viande, INRA.

Martín A., Córdoba J.J., Rodríguez M.M., Núñez F., Asensio M.A. (2001) : Evaluation of microbial proteolysis in meat products by capillary electrophoresis. Journal of Applied Microbiology, **90**: 163–171.

Martín A., Córdoba J.J., Benito M.J., Aranda E., Asensio M.A. (2003): Effect of *Penicillium chysogenum* and *Debaryomyces hansenii* on the volatile compounds during controlled ripening of pork loins. International Journal of Food Microbiology, **84**: 327–338.

Martín A., Córdoba J.J., Núñez F., Benito M.J. Asensio M.A. (2004): Contribution of a selected fungal population to proteolysis on dry-cured ham International Journal of Food Microbiology, **94**: 55–66.

Martín A., Córdoba J.J., Aranda E., Córdoba M.G., Asensio M.A. (2006): Contribution of a selected fungal population to the volatile compounds on dry-cured ham. International Journal of Food Microbiology, **110**: 8–18.

Mauricio J.C., Moreno J., Zea L., Ortega L., Medina M. (1997): The effects of grape must fermentation condition on volatile alcohols and esters formed by *Saccharomyces cerevisiae*. Journal of the Science of Food and Agriculture, **75**: 155–160.

NIST database (2005): Available at http://webbook.nist.gov/

Núñez F., Rodríguez M.M., Córdoba J.J., Bermúdez M.E., Asensio M.A. (1996): Yeast population during ripening of dry-cured Iberian ham. International Journal of Food Microbiology, **29**: 271–280.

Osei Abunyewa A.A., Laing E., Hugo A., Viljoen B.C. (2000): The population change of yeasts in commercial salami. Food Microbiology, **17**: 429–438.

Patel S., Shibamoto T. (2003): Effect of 20 different yeast strains on the production of volatile components in Symphony wine. Journal of Food Composition and Analysis, **16**: 469–476.

Patrignani F., Lucci L., Vallicelli M., Guerzoni M.E., Gardini F., Lanciotti R. (2007): Role of surface-inoculated *Debaryomyces hansenii* and *Yarrowia lipolytica* strains in dried fermented sausage manufacture. Part 1: Evaluation of their effects on microbial evolution, lipolytic and proteolytic patterns. Meat Science, **75**: 676–686.

Petrón M.J., Muriel E., Timón M.L., Martín L., Antequera T. (2004): Fatty acids and triacylglycerols profiles from different types of Iberian dry-cured hams. Meat Science, **68**: 71–77.

Regodón J.A., Pérez F., Ramírez M. (2006): Influence of *Saccharomyces cerevisiae* yeast strain on the major volatile compounds of wine. Enzyme and Microbial Technology, **40**: 151–157.

Rodríguez M., Núñez F., Córdoba J.J., Sanabria C., Bermúdez M.E., Asensio M.A. (1994): Characterization of *Staphylococcus* spp. and *Micrococcus* spp. isolated from Iberian ham throughout the ripening process. International Journal of Food Microbiology, **24**: 329–335.

Rodríguez M.M., Núñez F., Córdoba J.J., Bermúdez M.E., Asensio M.A. (1998): Evaluation of proteolytic activity of microorganisms isolated from dry-cured ham. Journal of Applied Microbiology, **85**: 905–912.

Romano P. (1997): Metabolic characteristics of wine strains during spontaneous and inoculated fermentation. Food Technology and Biotechnology, **35**: 255–260.

Romano P., Fiore C., Paraggio M., Caruso M., Capece A. (2003): Function of yeast species and strains in wine flavour. International Journal of Food Microbiology, **86**: 169–180.

Ruiz J., Cava R., Ventanas J., Jesen M.T. (1998): Headspace solid phase microextraction for the analysis of volatile in meat product: dry-cured Iberian ham. Journal of Agricultural and Food Chemistry, **46**: 4688–4694.

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

Simoncini N., Rotelli D., Virgili R., Quintavalla S. (2007): Dynamics and characterization of yeasts

during ripening of typical Italian dry-cured ham. Food Microbiology, **24**: 577–584.

Schmidt S., Berger R. (1998): Aroma compounds in fermented sausages of different origins. Food Science and Technology, **31**: 559–567.

Tjener K., Stahnke L.H., Andersen L., Martinussen J. (2004): Addition of α-ketoglutarate enhances formation of volatiles by *Staphylococcus carnosus* during sausages fermentation. Meat Science, **67**: 711–719.

Yvon M., Berthelot S., Gripon J.C. (1998): Adding α-ketoglutarate to semi-hard cheese curd highly enhances the conversion of amino acids to aroma compounds. International Dairy Journal, **8**: 889–898.

Zhou G.H., Zhao G.M. (2007): Biochemical changes during processing of traditional Jinhua ham. Meat Science, **77**: 114–120.

Received for publication February 11, 2010
Accepted after corrections November 12, 2010

Corresponding author:

Dr. Mar Rodríguez, University of Extremadura, Faculty of Veterinary Science, Food Hygiene and Safety, Avda. de la Universidad s/n. 10071-Cáceres, Spain
tel.: + 34 927 257 125, e-mail: marrodri@unex.es, http://higiene.unex.es