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The contribution of *Penicillium aurantiogriseum* to the volatile composition and sensory quality of dry fermented sausages

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Abstract

An atoxygenic, proteolytic and lipolytic strain of *Penicillium aurantiogriseum* was tested for its ability to accelerate the production of volatile compounds and to improve the sensory properties of dry fermented sausages. The following batches of sausages were prepared: control; superficially inoculated with a spore suspension; added with an intracellular cell free extract; and superficially inoculated and added with the intracellular cell free extract. Higher levels of lipid oxidation products were found in the aroma extracts of sausages without a mould cover. In contrast, branched aldehydes and alcohols presented higher concentrations in superficially inoculated and extract added sausages, while esters only showed higher concentration in the first ones. The sensory analysis showed that sausages prepared with both treatments received the highest scores in all the properties evaluated, which demonstrated both the potential of this mould as producer of volatile compounds and the effectiveness of combining both treatments.

Keywords: Dry fermented sausages; Moulds; *Penicillium aurantiogriseum*; Volatile compounds; Cell free extract; Texture; Sensory analysis

1. Introduction

Moulds have been used in the production of fermented meats for many centuries. Initially, sausages were passively inoculated by moulds associated with the environment (rooms, caves, equipment, etc.), which grew on the surface of the sausage forming a layer. In such products mould species settled randomly, the dominant ones being those which were better adapted to the ripening conditions (temperature, a_w on the surface, competitive phenomena, etc.). However, the colonisation of the sausage surface by an inappropriate mould can lead to faulty products and increase the risk of mycotoxin production (Cook, 1995). For this reason, the inoculation of sausages with selected mould strains is advantageous and, nowadays, is a common practice in the fermented meat product industry. The mould starter cultures play an important role in the taste and aroma development of these products (Cook, 1995; Lücke, 1998).

Many compounds are involved in sausage aroma and they derive from several sources: carbohydrate fermentation, lipid breakdown and proteolysis, as well as seasoning and smoking. Proteolytic and lipolytic activities have been detected in mould strains isolated from dry fermented sausages (Toledo, Selgas, Casas, Ordóñez, & García, 1997; Trigueros, García, Casas, Ordóñez, & Selgas, 1995) and dry hams (Huerta, Sanchís, Hernández, & Hernández, 1987), giving rise to amino acids and fatty acids. The further transformation of these substances into volatile compounds is essential for the development of the characteristic flavor of fermented sausages and it seems that certain moulds are involved in these reactions. It is known that some mould strains have the ability to degrade amino acids into branched aldehydes and alcohols (Greenberg & Ledford, 1979;

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Karahadian, Josephson & Lindsay, 1985), and compounds such as 2- and 3-methyl-1-butanol, 2- and 3-methylbutanal and 2-methylpropanal are important components for dry sausage aroma (Montel, Masson, & Talon, 1998). Furthermore, it has been shown that the action of certain mould enzymes on free fatty acids leads to the formation of various aroma compounds, including methylketones and, by reduction, the corresponding secondary alcohols (Creuly, Larroche, & Gros, 1992; Karahadian et al., 1985; Kinsella & Hwang, 1976).

The purpose of this work was to examine the formation of flavor compounds in sausages inoculated with a *Penicillium aurantiogriseum* strain, both superficially inoculated and/or added with its intracellular cell free extract (ICFE), and to evaluate the effect on the sensory properties. This species was chosen because it is endowed with extracellular proteolytic and lipolytic (Trigueros et al., 1995), which can potentiate the release of volatile compounds precursors (Bruna, Fernández, Herranz, Ordóñez, & Hoz, 2001) and in vitro amino oxidase activities (Bruna, Fernández, Hierro, Hoz, & Ordóñez, 2000).

2. Material and methods

2.1. Preparation of the fermented sausages

Four separate batches of fermented sausages were manufactured: batch C (control) consisted of the initial ingredients, seasonings and curing salts; batch S was similar to batch C but was superficially inoculated with a spore suspension of *Penicillium aurantiogriseum* immediately after stuffing; batch E contained an ICFE of *P. aurantiogriseum* (101 mg protein/kg) and batch E + S, which was similar to batch E but it was also superficially inoculated with the spore suspension immediately after stuffing. To adjust the moisture content of the different batches, the corresponding amount of phosphate buffer was added to batches C and S. Potassium sorbate (25%) was sprayed on the surface of batches C and E to avoid the growing of moulds. The mixtures were stuffed into collagen sausage casings (40 mm in diameter) and left to ripen in an Ibercex ripening cabinet, model G-28 (A.S.L., San Fernando de Henares, Spain).

The preparation of the spore suspension, the ICFE, the formulation of the sausages and the ripening conditions have been described in a previous paper (Bruna et al., 2001). Sausage manufacture was done three different times. The four batches mentioned before were manufactured with different ingredients but same formulation and technology at different periods of the year.

2.2. Analysis of volatile compounds

Volatile compounds were analyzed by GC–MS as described by Elmore, Mottram, Enser, and Wood (2000). Twenty-five grams of ground sausage were introduced into a 250 ml conical flask and equilibrated for 30 min at 30°C. Volatile compounds were extracted at 30°C by a nitrogen flow of 40 ml/min for 1 h and adsorbed on a steel trap (105×3 mm i.d.) containing 85 mg of Tenax TA (Scientific Glass Engineering Ltd., Milton Keynes, UK). A standard (131 ng of 1,2-dichlorobenzene in 1 μl of hexane) was added to the trap at the end of the collection and excess solvent and any water retained on the trap were removed by purging the trap with nitrogen at 40 ml/min for 5 min. Three separate headspace collections were carried out for each sausage batch.

GC–MS analyses were performed on a Hewlett-Packard 5972 mass spectrometer fitted with a HP5890 Series II gas chromatograph and a G1034 Chemstation. A CHIS injection port (Scientific Glass Engineering Ltd.) was used to thermally desorb the volatiles compounds from the Tenax trap onto the front of a CP-Sil 8 CB low bleed/MS fused silica capillary column (60 m×0.25 mm i.d., 0.25 μm film thickness, Chrompack, Middelburg, The Netherlands). During the desorption period of 5 min, volatile compounds were cryofocused by immersing 15 cm of column adjacent to the injector in powdered solid CO_2 while the oven was held at 40°C. The solid CO_2 was then removed and chromatography achieved by holding at 40°C for 2 min followed by a programmed rise to 280°C at 4°C/min. A series of *n*-alkanes (C_6-C_{22}) was analyzed, under the same conditions, to obtain linear retention index (LRI) values for the aroma components.

The mass spectrometer was operated in electron impact mode with an electron energy of 70 eV and an emission current of 50 μA. Compounds were identified by comparing their mass spectra and LRI values with those of authentic standards. Approximate quantities of the volatiles were estimated by comparing their peak areas with those of the 1,2-dichlorobenzene internal standard, obtained from the total ion chromatograms, using a response factor of 1.

Each of the following analysis were done by triplicate, therefore each value presented in this work is the mean of nine data.

2.3. Texture analysis

A TA.XT 2i/25 texture analyser (Stable Micro Systems, Surrey, UK) equipped with a cylindrical probe P/25 was used to determine hardness, cohesiveness, adhesiveness, gumminess, chewiness and springiness, and a reversible probe to determine the maximum cutting force and the cutting work (Bourne, 1978). This procedure involved cutting samples approximately 1.5 cm high and 2.5 cm wide which were compressed twice to 50% of their thickness. The following parameters were defined: hardness (H) = maximum strength required to

achieve compression; area of the first compression ($A1$) = total energy required for the first compression; area of the second compression ($A2$) = total energy required for the second compression; adhesiveness = area under the abscissa after the first compression; springiness (S) = height the sample recovers between the first and second compression; cohesiveness (C) = $A2/A1$; gumminess (G) = $H \times C$; chewiness (Ch) = $S \times G$; maximum cutting strength = maximum height on the cutting graph; and total cutting work = area under the cutting curve.

Each batch was analysed five times, therefore each value presented in this work is the mean of 15 data.

2.4. Sensory analysis

This was carried out at the end of ripening by a panel of 20 tasters in an ISO normalised testing room. These were all members of Departamento de Higiene y Tecnología de los Alimentos and had been previously trained in the sensorial assessment of meat products. A triangle test was carried out (ISO, 1981) by asking the tasters to choose the sample they thought it was different and the reason for choosing it.

The colour, texture, odour and taste were also assessed using a non-structured hedonic scale in which samples were given scores of 1 (very poor) to 10 (excellent). The global quality was calculated from the expression: Overall quality = (Colour × 0.1) + (Texture × 0.25) + (Odour × 0.15) + (Flavour × 0.5). This expression was calculated taking into account the opinion of the 18 tasters who, in a study on commercial fermented sausages, had been asked to assess the relative importance of the different sensory characteristics (Bruna, Fernández, Hierro, Hoz, & Ordóñez, 1999).

2.5. Statistical analysis

ANOVA was used to search for significant differences between mean values of the different results. Comparison between batches was performed by the Student Newman–Keul's test using SigmaStat 1.1 (Jandel Corporation, San Rafael, USA). Significance level was established at $P < 0.05$.

Furthermore, data were analysed using principal component analysis (PCA), compromising a correlation matrix of average scores with 4 rows (batches) by 4 columns (attributes). Overall quality was not included as this attribute results from the addition of colour, odour, texture and flavour. The results of the PCA were represented as biplots (Risvik, McEwan, & Rödbotten, 1996) where both the batches [principal components scores (PC-scores)] and attributes (PC-loadings) are represented on the same plot. On the biplot a scaling factor of 0.25 was applied to PC-loadings of the sensorial attributes. SAS system 8.0 was used for the statistical analysis.

3. Results and discussion

3.1. Volatile compounds

About 82 volatile compounds were identified and quantified across the four different samples. They included 25 hydrocarbons, 19 aldehydes, 12 alcohols, seven terpenes, six acids, five esters, five ketones, two furans and one sulfur compound. Table 1 shows the mean quantities after 22 days of ripening. Compounds are grouped according to their chemical class. Hydrocarbons have not been included as they have relatively high odour threshold values (Drumm & Spanier, 1991) and are, therefore very unlikely to contribute to sausage flavor.

Most of the compounds were found in all four batches of sausage. However, there were major quantitative differences in the volatile profiles of the different batches. In general, the samples grouped into those which were not inoculated with spore (C and E) and those which were inoculated (S and E + S).

3.2. Volatile compounds from lipid oxidation

The compounds formed via lipid oxidation, such as aliphatic aldehydes (2-butenal, pentanal, hexanal, 2-hexenal, heptanal, nonanal, decanal), certain ketones (2-butanone, 2-pentanone and 2-heptanone) and alcohols (1-penten-3-ol), showed large differences when the batches inoculated on the surface (S and E + S) and the non-inoculated ones (C and E) were compared. All the above mentioned compounds reached lower concentrations (1.6–3.7 fold) in mould inoculated sausages than their respective controls (batch S vs. C and batch E + S vs. E). Some compounds (2-butanone, 2-pentanone and 2-butenal) were not even detected in mould covered sausages. This is of great relevance since lipid oxidation products play an important role on the sapid and aromatic characteristics of dry fermented sausages (Berdagué, Monteil, Montel, & Talon, 1993).

Aldehydes are probably the most interesting of the lipid-derived volatiles as they have low odor threshold values (Shahidi, Rubin, & D'Souza, 1986). Saturated aldehydes potentiate the odor, whereas 2-enals and 2,4-dienals provide sweet, fruity and fatty attributes (Hamilton, 1989). Saturated aliphatic aldehydes from C_5 up to C_{10} were detected in all batches. However, as mentioned above, the levels of these compounds were markedly higher in the sausages whose surface was not inoculated with the mould. A similar pattern was observed with the alkenals 2-hexenal and 2-heptenal. These results are in agreement with previously reported data from analysis of TBARS values on these products (Bruna et al., 2001). This confirms the antioxidative effect exhibited by moulds by protecting against light, consuming oxygen and degrading peroxides (Cook, 1995; Lücke, 1998). This may be considered as a positive effect on flavor,

Table 1
Volatile compounds (ng/100 g) found in headspace extracts of dry fermented sausages after 22 days of ripening[a]

Compound	Mean concentration in headspace (ng/100 g) Batch[b]				LRI[c]
	C	E	S	E+S	
Alcohols					
Ethanol	n.d.	19a	71b	85b	503
1-Propanol	n.d.	n.d.	177a	91a	521
2-Methyl-1-propanol	21a	44a	649b	140c	609
1-Butanol	n.d.	n.d.	26a	27a	653
2-Butanol	45a	23a	2211b	2179b	676
2-Methyl-1-butanol	43a	142b	179b	239c	744
3-Methyl-1-butanol	44a	393b	870c	1064d	740
2,3-Butanediol	458a	381a	92b	184b	769
1-Pentanol	131a	229b	440c	129a	772
1-Penten-3-ol	625a	258b	60c	65c	672
1-Hexanol	19a	32a	289b	122c	867
1-Octen-3-ol	39a	56a	272b	315b	986
Aldehydes					
Butanal	n.d.	137a	101a	n.d.	593
2-Methylpropanal	16a	112b	365c	653d	609
2-Methylbutanal	151a	280b	530c	672d	674
3-Methylbutanal	422a	1420b	4027c	5407d	666
2-Butenal	56a	34a	n.d.	n.d.	657
2-Methyl-2-butenal (*E*)	25a	10a	n.d.	n.d.	739
Pentanal	686a	749a	286b	265b	705
Hexanal	3691a	3718a	1357b	1407b	807
2-Hexenal	32a	21a,b	14b	11b	862
Heptanal	110a	138a	59b	74b	906
2-Heptenal	60a	61a	29a	23a	963
Octanal	59a	68a	37a	41a	1006
Nonanal	190a	162a	67b	38b	1108
2-Nonenal	7a	6a	16a	18a	1165
2,4-Nonadienal	n.d.	n.d.	3a	3a	1222
Decanal	26a	32a	7b	9b	1209
2-Decenal	14a	13a	16a	11a	1267
Benzaldehyde	46a	58a	51a	17b	972
Benzeneacetaldehyde	9a	26a	92b	24a	106
Ketones					
3-Hydroxy-2-butanone (*acetoin*)	419a	518b	93c	109c	749
2-Butanone	320a	736b	n.d.	n.d.	604
2,3-Butanedione (*diacetyl*)	178a	415b	125a	117a	612
2-Pentanone	1217a	532b	n.d.	n.d.	692
2-Heptanone	56a	57a	21b	17b	893
Esters					
Ethyl acetate	42a	60a	398b	346b	612
Methyl propanoate	11a	13a	65b	60b	627
Ethyl butanoate	n.d.	n.d.	63a	89a	805
Methyl hexanoate	4a	6a	11a	13a	934
Ethyl hexanoate	n.d.	n.d.	43a	48a	1003
Acids					
Acetic acid	2203a	3887b	107c	79c	702
Propanoic acid	10a	11a	36b	44b	745
2-Methylpropanoic acid	9a	12a	25b	33b	579
2-Methylbutanoic acid	5a	12b	6a	15b	838
3-Methylbutanoic acid	n.d.	31a	6b	40a	891
Butanoic acid	n.d.	n.d.	31a	40a	820

(*continued on next page*)

Table 1 (*continued*)

Compound	Mean concentration in headspace (ng/100 g) Batch[b]				LRI[c]
	C	E	S	E+S	
Terpenes					
α-Pinene	2391a	2270a	2529a	3541b	937
β-Pinene	2697a	2670a	2829a	2559a	985
β-Myrcene	466a	517a	593a	532a	989
δ-3-Carene[d]	3922a	4398a	3893a	3965a	1016
Limonene	1686a	1730a	1742a	1952a	1035
Linalool	27a	30a	43a	32a	1101
α-Terpineol	6a	5a	12a	3a	1203
Sulfur compounds					
Dimethyl disulfide	37a	77b	17a	11a	752
Furans					
2-Methylfuran	633a	865b	216c	122c	633
2-Ethylfuran	40a	58b	36a	14a	703

[a] Means in the same row with different letters are significantly different ($P<0.05$). n.d., not detected.

[b] Batch C (control); batch E (control batch added with an intracellular cell free extract of *Penicillium aurantiogriseum*, 101 mg protein/kg); batch S (control batch superficially inoculated with a spore suspension of *P. aurantiogriseum*); batch E+S (batch E superficially inoculated with the spore suspension of *P. aurantiogriseum*).

[c] Linear retention index on a CP-Sil 8 CB low bleed/MS column.

[d] Tentatively identified by comparison with mass spectrum and LRI in Adams (1995).

since rancidity is usually referred as a negative attribute in meat products (Konopka, Guth, & Grosh, 1995; Shu-Mei, Gray, Booren, Crackel, & Gill, 1995). Similar results were found by Leistner, Ayres, and Lillard (1965) in cured hams covered with moulds and by Bruna, Fernández, Hierro, Ordóñez, and Hoz (2000) in dry sausages ripened with a *Mucor racemosus* strain.

Three methylketones were identified and quantified, namely 2-butanone, 2-pentanone and 2-heptanone. Methylketones have odour threshold values higher than those of their isomeric aldehydes (Seik, Albin, Sather, & Lindsay, 1971); therefore, at similar concentrations, they are relatively unimportant in the flavour of meat products, but could add spicy, fruity and fatty notes to the product (Grosch, 1982; Molimard & Spinnler, 1996). They may be formed from fatty acids by chemical (autooxidation) or enzymatic (β-oxidation) reactions during the free fatty acid metabolism by moulds. In the latter case, the pathway involves decarboxylation of a ketoacid to the corresponding methylketone. Therefore, the main final products are odd-carbon methylketones and, by reduction, the corresponding secondary alcohols. Since 2-pentanone and 2-heptanone reached higher concentrations in non-inoculated batches (C and E), and no odd-carbon secondary alcohols were detected in inoculated sausages (S and E+S), it can be concluded that methylketones were mainly derived from the chemical oxidation of free fatty acids. The higher levels of these compounds in non-inoculated sausages confirm the antioxidative effect of the mould cover (Bruna, Fernández, Hierro, Ordóñez, & Hoz, 2000; Bruna et al., 2001; Núñez, 1995).

Two furans, 2-methylfuran and 2-ethylfuran, were detected in the headspace volatiles from all experimental sausages, batches C and E containing the highest levels. Since both compounds are products of lipid oxidation (Mottram, 1991) similar considerations as those proposed for the aldehydes and methylketones can be made.

3.3. Volatile compounds from amino acid breakdown

Some differences in the volatile compounds derived from amino acids were found between batches, mainly with the three branched aldehydes (2-methylpropanal, 2-methylbutanal, 3-methylbutanal) and their corresponding alcohols (2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol).

These branched aldehydes have been suggested to make a significant contribution to the overall flavor of dry fermented sausages (Stahnke, 1995) and dry hams (Careri, Mangia, Barbieri, Bolzoni, Virgili, & Parolari, 1993; Ruiz, Ventanas, Cava, Andrés, & García, 1999) where they have been associated with a "ripened aroma" (Ruiz et al., 1999). 2-Methylpropanal, 2- and 3-methylbutanal can be derived from amino acids (valine, isoleucine and leucine, respectively) via Strecker degradation (Barbieri, Bolzoni, Parolari, & Virgili, 1992; García, Berdagué, Antequera, López-Bote, Córdoba, & Ventanas, 1991; Hofmann, Münch, & Schieberle, 2000; Ventanas, Córdoba, Antequera, García, López-Bote, &

Asensio, 1992) or by microbial metabolism (Degorce-Dumas, More, Goursaud, & Leveau, 1984; Hinrichsen & Pedersen, 1995). According to previous studies in dry cured ham (García et al., 1991; Ventanas et al., 1992), the latter pathway is the most probable in fermented sausages since the Strecker degradation normally requires heat or very long ripening periods and is favoured by relatively low a_w (about 0.85). The increase in the concentration of these compounds may be attributed to the L-amino oxidase activity of the fungal strain and also to the increase in the concentration of certain amino acids due to the proteolytic activity developed on the surface (Blom, Hagen, Pedersen, Holck, Axelsson, & Naes, 1996; Bruna et al., 2001; Hagen, Berdagué, Holck, Naes, & Blom, 1996). The addition of the ICFE produced a significant increase in these compounds.

The 2- and 3-methylbutanol and 2-methylpropanol are formed by reduction of the corresponding aldehydes (Ha & Lindsay, 1990; Stahnke, 1994). The transformation of amino acids into aldehydes, alcohols and carboxylic acids is a very common pathway in fungal metabolism (Molimard & Spinnler, 1996). The presence of these compounds has been described in many mould ripened foods (Börjesson, Stöllman, & Schnürer, 1990, 1993; Jacobsen & Hinrichsen, 1996; Jelén & Wasowicz, 1998; Larsen & Frisvad, 1995; Molimard & Spinnler, 1996) and could contribute to the desirable flavor attributes of some cured meat products, like Iberian ham (Ruiz et al., 1999).

The batches which showed the highest levels of these compounds were E + S and S, followed by batches E and C. In batches E + S and S total branched chain aldehydes and alcohols levels were, respectively, 12- and 9-fold higher than those recorded in batch C, while the content of batch E was 3-fold higher than that of the control batch. These results suggest a possible activity of *P. aurantiogriseum* (both the ICFE and the superficial inoculation) on the amino acids precursors of these compounds, especially when the mould was superficially inoculated. The drop in the content of isoleucine and valine (Bruna et al., 2001) due to the addition of the ICFE supports in part this fact. However, the levels of leucine were not found to decrease. Börjesson et al. (1990) also detected 3-methylbutanal and 3-methy-1-butanol among the volatiles produced in different agars inoculated with spore suspensions of *P. aurantiogriseum*. As mentioned above, amino acids can be transformed to α-ketoacids by oxidative deamination involving oxidoreductases and then to aldehydes by decarboxylases. Oxidoreductases have been found in some moulds species such as *Geotrichum candidum*, which has a deaminative activity on glutamic and aspartic acids (Greenberg & Ledford, 1979) and also on tryptophan, leucine, methionine and phenylalanine (Gueho & Buissière, 1975). Bruna, Fernández, Hierro, Ordóñez, and Hoz (2000) also detected higher levels of these branched aldehydes and alcohols in sausages added with extracts of *M. racemosus*.

3.4. Volatile compounds from microbial activity

Several esters were detected in the sausages. They arise from the esterification of carboxylic acids and alcohols. The enzymes involved in this reaction are present in many species of yeasts, moulds and bacteria (Gatfield, 1988; Jelén, & Wasowicz, 1998), and esters have been reported as important volatiles in fermented sausages (Mateo, & Zumalacárregui, 1996; Meynier, Novelli, Chizzolini, Zanardi, & Gandemer, 1999; Stahnke, 1995). On the other hand lower concentrations are found in dry cured ham (Buscailhon, Berdagué, & Monin, 1993; Ruiz et al., 1999; Timón, Ventanas, Martín, Tejeda, & García, 1998), where microbial counts are very low (Lücke, 1986; Molina & Toldrá, 1993). Free fatty acids are generated as a result of the action of lipases, either endogenous or microbial, while alcohols are derived from different sources such as sugars fermentation and amino acids and/or lipids catabolism (Molimard & Spinnler, 1996). It has been reported previously (Bruna et al., 2001) that the superficial inoculation of the sausages with *P. aurantiogriseum* produced a significant release of some organic acids, like acetic, propionic, butyric and, probably, hexanoic due to the superficial lipolytic activity or to the catabolism of certain amino acids (Stahnke, 1995). The increase in concentration of all ethyl esters in superficial inoculated batches (S and E + S) can be attributed to the higher growth of *Micrococcaceae* (Montel, Reitz, Talon, Berdagué, & Rousset-Akrim, 1996; Stahnke, 1995) as a result of the higher pH found in these batches (Bruna et al., 2001). Esters are of particular interest because of their characteristic fruity notes and their low odour threshold values. Their presence, together with 2- and 3-methylbutanal, has been associated with a "ripened flavor" (Barbieri, Bolzoni, Parolari, & Virgili, 1992; Careri et al., 1993).

Ethyl acetate was found to be the most abundant ester in all batches, but its concentration was much higher (7–10 fold) in superficially inoculated sausages (batches S and E + S) than in the non inoculated ones (batches C and E), which coincides with the corresponding acetic acid contents determined by HPLC (Bruna et al., 2001). Although acetic acid has also been determined by GC–MS, these results can not be considered as accurate because this acid coelutes with other volatile compounds and its area is calculated by difference from the others. Other authors (Croizet, Denoyer, Tran, & Berdagué, 1992; Stahnke, 1994; Mateo & Zumalacárregui, 1996) also found this ester to be the most prominent in dry fermented sausages. Yoshioka and Hashimoto (1981) described the formation of this ester by the esterification of ethanol with acetyl coenzyme

A. Similarly, the highest levels of other esters were detected in superficially inoculated sausages, while sausages without inoculation showed very low amounts of esters, some of them could not even been detected. These results are in agreement with other studies which show the ability of *Penicillium* and *Mucor* strains to produce esters (Adamek, Bergström, Börjesson, & Stöllman, 1992; Bruna, Fernández, Hierro, Ordóñez, & Hoz, 2000; Larsen & Frisvad, 1995).

The production of 8-carbon alcohols and ketones (octanol, 3-octanol, 1-octen-3-ol, 2-octen-1-ol, 3-octanone, 1-octen-3-one) is very common in fungal metabolism (Jelén & Wasowicz, 1998; Larsen & Frisvad, 1995). 1-Octen-3-ol was detected in all batches, especially in those superficially inoculated (S and E + S). This alcohol, which has a mushroom-like odour and a low threshold value (0.01 ppm), plays a major role in the flavor of some mould-ripened cheeses (Molimard & Spinnler, 1996). It has also been described in dry fermented sausages (Berdagué et al., 1993; Edwards, Ordóñez, Dainty, Hierro, & Hoz, 1999; Meynier et al., 1999). The highest levels of 1-octen-3-ol were found in batches superficially inoculated with *P. aurantiogriseum* which can be attributed to the mould metabolism (Jelén & Wasowicz, 1998; Larsen & Frisvad, 1995). Tressl, Bahri, and Engel (1982) have proposed linoleic acid as the natural precursor of 1-octen-3-ol in mushrooms. A lipoxygenase and a hydroperoxide lyase are the main enzymes involved in the biosynthesis of this alcohol (Wurzenberger & Grosh, 1984). The production of 1-octen-3-ol by *Penicillium* spp. have been reported by other authors in culture media (Börjesson et al., 1993), cereals (Börjesson et al., 1990), cheese (Larsen & Knochel, 1997) and cured meat (Martín, 1999).

Another two alcohols, ethanol and 1-propanol, were also present in higher concentrations in superficially inoculated batches. These two alcohols, together with 2-methyl-1-propanol, 3-methyl-1-butanol and 1-octen-3-ol, are the main metabolites produced by *P. aurantiogriseum* (Börjesson et al., 1990; Jelén & Wasowicz, 1998).

3.5. Volatile compounds from spices

The volatile terpenes accounted for 45% of the total volatiles in all batches. Most of these were monoterpenes hydrocarbons and only two terpene alcohol were detected. Although some fungi can generate terpenes, the similar concentrations found in all batches seems to indicate that all of them come from the spices added. The results obtained are in agreement with other authors (Berger, Macku, German, & Shibamoto, 1990; Meynier et al., 1999; Stahnke & Zeuthen, 1992) who found that terpene levels were as much as 50% of the volatiles identified in dry fermented sausages.

3.6. Texture analysis

Table 2 shows the texture profile of experimental sausages after 22 days of ripening. The values recorded in the texture analysis were similar to those described by other authors (Ansorena, Gimeno, Astiasarán, & Bello, 1998; Gimeno, Astiasarán, & Bello, 1999a, b; Tabilo, Flores, Fiszman, & Toldrá, 1999). As expected, most parameters were not modified by the addition of the ICFE, since the extract did not show any proteolytic and/or lipolytic activity.

On the other hand, the superficial inoculation with *P. aurantiogriseum* caused a significant decrease ($P < 0.05$) in hardness and related-parameters (gumminess and chewiness). This effect can be attributed to the higher pH observed in superficially inoculated batches (Bruna et al., 2001) as this parameter is directly related to water holding capacity (Hamm, 1986). However, moisture and a_w were not significantly different between batches (Bruna et al., 2001), probably due to the low humidity of the ripening chamber. Therefore, the decrease in

Table 2
Texture analysis (mean ± standard deviation) of dry fermented sausages after 22 days of ripening[a]

Parameter	Batch[b]			
	C	E	S	E + S
Hardness (N)	178.6 ± 12.5a	166.6 ± 15.7a	128.6 ± 24.9b	124.0 ± 18.6b
Adhesiviness (N)	−0.86 ± 0.39a	−1.15 ± 0.46a	−0.74 ± 0.37a	−0.48 ± 0.28a
Springiness (m)	0.0038 ± 0.001a	0.0036 ± 0.0008a	0.0040 ± 0.0007a	0.0041 ± 0.001a
Cohesiviness	0.44 ± 0.01a	0.42 ± 0.05a	0.41 ± 0.06a	0.38 ± 0.08a
Gumminess (N)	80.5 ± 4.3a	69.6 ± 4.5b	52.2 ± 8.6c	46.4 ± 10.7c
Chewiness (J)	0.35 ± 0.01a	0.25 ± 0.05b	0.21 ± 0.06b,c	0.15 ± 0.05c
Cutting force (N)	107.8 ± 20.1a	80.4 ± 19.8a,b	92.7 ± 26.1a	65.1 ± 22.3b
Cutting work (J)	0.00086 ± 0.0003a	0.00061 ± 0.0001a	0.00068 ± 0.0003a	0.00046 ± 0.0001a

[a] Means in the same row with different letters are significantly different ($P < 0.05$).

[b] Batch C (control); batch E (control batch added with an intracellular cell free extract of *Penicillium aurantiogriseum*, 101 mg protein/kg); batch S (control batch superficially inoculated with a spore suspension of *P. aurantiogriseum*); batch E + S (batch E superficially inoculated with the spore suspension of *P. aurantiogriseum*).

hardness, gumminess and chewiness may be associated to the proteolytic and lipolytic activity of the live mould (Monin et al., 1997; Tabilo et al., 1999). The decrease in hardness may be considered as positive attribute in some sausage varieties such as "salchichón" (Melendo, Beltrán, Jaime, Sancho, & Roncalés, 1996) while in other types where meat and fat are very finely minced, like "chorizo de Pamplona", is considered as a negative attribute (Gimeno et al., 1999b).

3.7. Sensory analysis

Triangle test (data not shown) gave statistically significant differences ($P<0.05$) among all batches. Maximum differences ($P<0.001$) were found when comparing mould covered sausages with those non inoculated. These differences were mainly derived from odour and flavour. The minimum number of right answers required to achieve significant differences ($P<0.05$) were obtained for batches added with the ICFE. These differences were mainly based in odour.

Table 3 shows the results of the descriptive sensory analysis. Neither texture nor colour showed significant differences between batches. The addition of the ICFE increased odour and flavour scores (18 and 25%, respectively) and consequently, overall quality (16%). This fact could be explained by the accumulation of volatile compounds derived from the amino acid breakdown, mainly 2-methylpropanal, 2- and 3-methylbutanal and their respective alcohols.

The superficial inoculation of *P. aurantiogriseum* increased both odour (61%) and flavor scores (30%), which can be attributed to the increase of the volatile compounds derived from the amino acid catabolism, the accumulation of esters derived from the mould metabolism and the lower oxidation rate observed in these sausages, as a consequence of the antioxidative activity of *P. aurantiogriseum* (Bruna et al., 2001).

The combination of both treatments (Batch E+S) produced the greatest increases in odour (89%) and flavor scores (70%) when compared with control batch.

A PCA on the conventional profiling data revealed that a total of 88% of the information was explained by the first principal component and 10% by the second. A graphical plot of this is shown in Fig. 1. PC1 is related to the four attributes and PC2 is mainly representing

Table 3
Sensory analysis (mean±standard deviation) of dry fermented sausages after 22 days of ripening[a]

Characteristic	Batch[b]			
	C	E	S	E+S
Odour	4.9±0.7a	5.8±0.7b	7.9±0.8c	9.3±1.0d
Colour	7.8±1.5a	7.7±1.3a	8.0±1.6a	8.0±0.8a
Texture	6.9±1.1a	7.3±0.6a	7.7±0.9a	7.8±0.8a
Flavour	5.5±1.1a	6.9±1.0b	7.2±1.2b	9.4±0.9c
Overall quality[c]	5.9±1.2a	6.9±0.9b	7.5±1.1b	8.8±0.9c

[a] Means in the same row with different letters are significantly different ($P<0.05$).

[b] Batch C (control); batch E (control batch added with an intracellular cell free extract of *Penicillium aurantiogriseum*, 101 mg protein/kg); batch S (control batch superficially inoculated with a spore suspension of *P. aurantiogriseum*); batch E+S (batch E superficially inoculated with the spore suspension of *P. aurantiogriseum*).

[c] Overall quality = (colour×0.1) + (texture×0.25) + (odour×0.15) + (flavour×0.5).

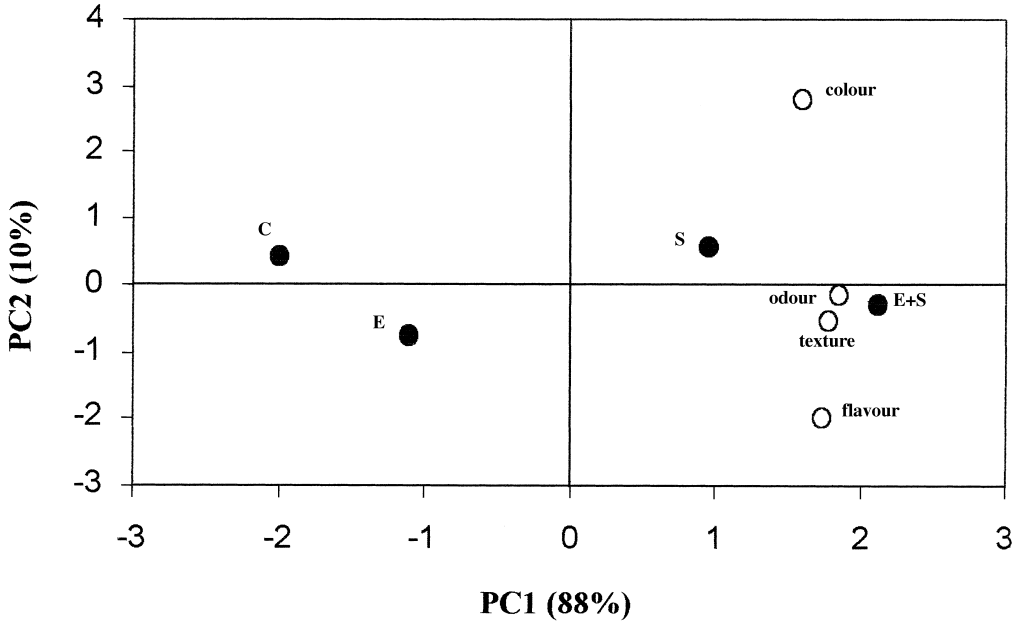

Fig. 1. Description of the main tendencies of variation in sensory quality: PCA for PC1 and PC2 for sensory attributes analysed on experimental sausages.

colour and flavour but these two variables are correlated to PC2 in the opposite way. PC1 is the most important variable to explain the differences between batches because it collects 88% of the total variability.

PC1 shows a clear difference between superficially inoculated (S and E + S) and non inoculated batches (C and E) as the first ones obtained higher scores for all the attributes analysed. PC1 can also explain the differences between batches added with the ICFE (E and E + S) and non added batches (C and S) as the first ones also obtained higher scores at PC1, although not as big as the observed between superficially inoculated and non inoculated batches.

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