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Generation of non-protein nitrogen and volatile compounds by *Penicillium chrysogenum* Pg222 activity on pork myofibrillar proteins

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Abstract

A non-toxigenic strain of proteolytic *Penicillium chrysogenum* Pg222 isolated from dry-cured ham was tested for its ability to generate non-protein nitrogen (NPN) and volatile compounds from muscle myofibrillar proteins. The activity of mold led to higher accumulation of non-protein and amino acidic nitrogens than control samples. Volatile compound analysis revealed the presence of branched compounds, such as 3-methylbutanal and 3-methylbutanol only in samples inoculated with the mold. Similarly, compounds such as ethanol, propanol and 2-methoxy ethanol were detected only in inoculated samples at all sampling time. 3- and 2-methylpentane, benzoic and acetic acids, 2-butanone and 2-ethylhexanol, pyridine and 3-carene were detected occasionally, but only in the Pg222 batch. The proteolytic activity of *P. chrysogenum* Pg222 lead to accumulation of soluble NPN compounds, in addition to the generation of volatile compounds of great interest for dry-cured meat products. Therefore, this mold could be appropriate to be used as a non-toxigenic starter culture during the ripening of dry-cured meat products to stimulate proteolysis and flavour development.

Keywords: *Penicillium chrysogenum*; Volatile compounds; Non-protein nitrogen; Dry-cured meat products

1. Introduction

Proteolysis is considered to be one of the major processes involved in aroma development in dry-cured meat products (Ordóñez et al., 1999; Martín et al., 2001). During ripening, protein breakdown yields small peptides and free amino acids (Córdoba et al., 1994a, b; Martín et al., 1998b), which are involved in taste and flavour development (Flores et al., 1997; Ruiz et al., 1999; Ordóñez et al., 1999).

The proteolysis in dry-cured meat products has been attributed mainly to endogenous enzymes (Toldrá, 1998), but these enzymes may be inhibited by salt and curing agents during the ripening process (Sárraga et al., 1989; Rico et al., 1991; Toldrá et al., 1993). On the other hand, micro-organisms isolated from dry-cured meat products, mainly molds, have shown high proteolytic activity during ripening time (Rodríguez et al., 1998; Bruna et al., 2001), and could be used to accelerate proteolysis. However, control of the fungal population growing on these products is essential, because most of the molds isolated from dry-cured meat products are toxigenic (Núñez et al., 1996, 2000; Sosa et al., 2002). Therefore, selected non-toxigenic strains could be used as starter cultures to make sure of the positive contribution of molds in the ripening of dry-cured meat products.

A non-toxigenic strain of *Penicillium chrysogenum* isolated from dry-cured ham (Núñez et al., 1996) has shown high hydrolytic activity for meat proteins (Rodríguez et al., 1998; Martín et al., 2001; Benito et al., 2003). However, before this strain is proposed as a starter culture, it should be tested for its ability to

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contribute to taste and flavour development. Several proteolytic micro-organisms used as starter cultures in dry-cured meat products are able to hydrolyse meat proteins, but do not contribute to the accumulation of free amino acids and the generation of volatile compounds (Ordoñez et al., 1999). The further transformation of free amino acids, released as a consequence of proteolytic activity, into volatile compounds is essential for the development of the characteristic flavour of dry-cured meat products. It is known that some mold strains are able to degrade amino acids into branched aldehydes and alcohols (Karahadian et al., 1985; Bruna et al., 2001). The ability of proteolytic *P. chrysogenum* to generate volatile compounds from meat proteins should be assayed. Since myofibrillar proteins are the most hydrolysed proteins in dry-cured meat products (Córdoba et al., 1994a), a direct test on those proteins might be very valuable to know the contribution of the proteolytic activity of *P. chrysogenum* Pg222 to accumulation of compounds related to taste (soluble non-protein nitrogen (NPN) compounds) and flavour development (volatile compounds).

The aim of this work was to know the contribution of proteolytic activity of *P. chrysogenum* Pg222 to the generation of NPN and volatile compounds, to determine its potential to be used as a starter culture for dry-cured meat products.

2. Materials and methods

2.1. Mold strain

The strain of *P. chrysogenum* Pg222 has been isolated from dry-cured ham and it has not shown toxicological effects in some biological assays, including brine shrimp test and cytotoxicity against VERO cells (Núñez et al., 1996). This strain has shown proteolytic activity when growing on meat slices (Rodríguez et al., 1998).

2.2. Extraction of myofibrillar muscle proteins

Pork loins were removed from carcasses immediately after slaughter. The exterior surface of the muscles was sterilized by searing as described by Dainty and Hibbard (1980). The burnt tissues were removed down to a depth of ca. 5 mm, using sterile instruments in a laminar flow cabinet Bio Flow II (Telstar, Spain). Sterile tissues were then cut into small pieces, and ca. 20 g samples were placed in Stomacher bags. The extraction of proteins was carried out following the method described by Rodríguez et al. (1998). Sarcoplasmic proteins were removed from muscle after homogenization and washing three times with 200 ml of sterile 0.03 M, pH 7.4 potassium phosphate buffer. Myofibrillar proteins were extracted with 200 ml of sterile 0.55 M IK, 0.05 M sodium phosphate buffer pH 7.4 containing 200 mg/l of chloramphenicol (Sigma, St. Lois, Missouri) to prevent bacterial growth.

2.3. Culture conditions

Culture medium was obtained by mixing the above myofibrillar proteins extract with sterile nutrient broth and NaCl to reach a final concentration of 1.6 mg of protein per ml, 0.1% (wt/vol) of nutrient broth and 5% (wt/vol) NaCl. This medium was designed to favour proteolysis by mold adding low amount of other nutrients, and to reach a salt concentration similar to dry-cured meat products.

P. chrysogenum Pg222 was inoculated with 0.1 ml of a suspension containing ca. 10^7 spores/ml in 15 ml of medium, and incubated at 25 °C for 4 days. Non-inoculated medium was also incubated as a control. The sterility of the control batch was confirmed by determining the absence of the growth of micro-organisms on plate count agar (Oxoid, Unipath, Basingstoke, UK). All samples were taken in triplicate every 4 h after an initial incubation of 24 h.

2.4. Analysis of total protein

The concentration of protein was determined following the Bradford method (Bradford, 1976) using 100 μl of culture media.

2.5. Analysis of non-protein nitrogen

NPN was determined by the method of Johnson (1941) using 4 ml of culture media after protein precipitation with 0.6 M perchloric acid, as described by De Ketelaere et al. (1974).

2.6. Analysis of amino acid nitrogen

Amino acid nitrogen (AN) was determined from the 0.6 N perchloric protein precipitation fraction after peptide precipitation with sulfosalicilic acid 10%, according to Martín et al. (1998a).

2.7. Extraction of volatile compounds

Samples of 1 ml of culture media were put into a 10 ml headspace vial (Hewlett-Packard, Palo Alto, California, USA) and sealed with a PTFE butyl septum (Perkin-Elmer, Foster City, California, USA) in an aluminium cap. Volatile compounds were extracted by Solid Phase Micro-Extraction technique (SPME) (Ruiz et al., 1998) with a 10 mm long, 100 μm thick fibre coated with carboxen-poly dimethylsiloxane (Supelco Co., Bellefonte, Pennsylvania, USA). Prior to collection of

volatiles, the fibre was preconditioned at 250 °C for 50 min at the GC injection port. The SPME fibre was inserted into the headspace vial through the septum and exposed to headspace for 30 min at 50 °C in a water bath. During this time samples were homogenized by shaking.

2.8. Gas chromatography/mass spectrometry (GC/MS) analyses of volatile compounds

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) and helium as carrier gas were used for the separation of volatile compounds. The carrier gas was helium. The injection port was in a splitless mode. The SPME fibre was kept in the injection port at 250 °C during the whole chromatographic run. The temperature program was isothermal for 5 min at 35 °C, then increased to 150 °C at 4 °C/min, and to 250 °C at 20 °C/min. 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 data collection. The NIST/EPA/NIH mass spectral library and Kovats indexes were used to identify the volatile compounds.

2.9. Statistical analysis

Statistical analysis of the data was carried out using one-way analysis of variance, and the mean was separated by Tukey's honest significant difference test using SPSS for Windows, 10.0 (SPSS Inc., Chicago, Illinois, USA).

3. Results

3.1. Total protein

The concentration of total protein remained unchangeable in control samples throughout the incubation time. However, the amount of total protein decreased in samples inoculated with Pg222 (Table 1), reaching a statistically significant difference from the control after 84 h of incubation.

3.2. Non-protein nitrogen

Throughout the incubation time there was no changes in NPN concentration in control samples (Table 1). The concentration of NPN in inoculated samples increased showing a maximum at 72 h (Table 1), and then decreased, reaching similar values to those in the control samples at 92 h of incubation.

3.3. Amino acidic nitrogen

The concentration of AN remained unchangeable in control samples throughout the incubation time. However, in inoculated samples the amino acidic nitrogen showed a steady increase, reaching statistically significant differences to the control batch after 72 h of incubation time. At 92 h of incubation, the concentration of AN in the inoculated samples showed a decrease as compared with the earlier sampling time (Table 1).

3.4. Volatile compounds

In the GC/MS analyses, a total of 16 volatile compounds were identified and quantified (Table 2). 2-propanone was detected in both control and Pg222 batches, but always in higher amounts ($P<0.05$), in the inoculated than in the control samples (Table 3). 3-methylbutanol, ethanol, propanol and 2-methoxy ethanol were detected only in inoculated samples and

Table 1
Effect of *P. chrysogenum* Pg222 on total proteins (TP), non-protein nitrogen (NPN) and amino acid nitrogen (AN) at different incubation times[a]

Hours	TP (mg/ml)		NPN (μg/ml)		AN (μg/ml)	
	Control	Pg222	Control	Pg222	Control	Pg222
24	1.6±0.14	1.5±0.16[1b]	65±12.5	62±20.6[1]	49±2.3	36±3.9[1]
36	1.6±0.12	1.5±0.01[1]	66±14.8	61±25.3[2]	38±2.0	33±2.5[1]
48	1.6±0.03	1.4±0.02[1]	70±6.7	68±34.9[2]	41±1.9	43±0.7[1]
60	1.6±0.09	1.3±0.12[1]	80±11.5	196±5.8a[3,4]	40±2.9	49±2.2[1,2]
72	1.5±0.04	1.3±0.17[1]	84±8.33	386±11.1a[4]	35±4.5	56±0.1a[2]
84	1.5±0.07	0.9±0.31a[1,2]	69±9.5	138±38.5[2,3]	45±1.3	87±6.4a[3]
92	1.5±0.09	0.6±0.07a[2]	71±5.9	56±14.0[2]	42±6.9	54±1.7a[2]

[a]Data are given as mean±standard deviation.
[b]For a given incubation time (row), values followed by a letter are significantly different ($P<0.05$) from its control. For a given determination (column), values with different numbers as superscript are significantly different ($P<0.05$).

Table 2
Volatile compounds from control and *P. chrysogenum* Pg222 treated samples using SPME CG-MS

Compound	Peak number[a]	Reliability of identification[b]
Alkanes		
2-methylpentane	3	A
3-methylpentane	4	A
Aldehydes		
3-methylbutanal	8	A
Alcohols		
Ethanol	1	B
Propanol	5	A
Butanol	10	A
3-methylbutanol	12	A
2-methoxy ethanol	11	A
2-ethylhexanol	14	A
Acids		
Acetic acid	9	B
Benzoic acid	17	A
Ketones		
2-propanone	2	A
2-butanone	6	A
Others compounds		
2,4 bis(1,1-dimethylethyl) phenol	16	A
Pyridine	13	A
3-carene	15	A

[a]Peak number in chromatogram of samples.
[b]The reliability of the identification or structural proposal is indicated by the following symbols: A, mass spectrum and retention time identical to those of an authentic sample; B, mass spectrum consistent with spectra found in NIST. EPA. NDH library.

in all sampling time. 3-methylbutanal, 3- and 2-methylpentane, benzoic and acetic acids, 2-butanone and 2-ethylhexanol, pyridine and 3-carene were detected occasionally, but only in Pg222 batch. Most of the above compounds were found in the inoculated batch the last sampling time (Table 3).

4. Discussion

The proteolytic effect of *P. chrysogenum* Pg222 was evidenced by lower amount of total protein in the inoculated than in the control samples after 84 h incubation. Furthermore, the hydrolytic effect of Pg222 was noted by higher accumulation of NPN and AN in inoculated than control samples. The evolutions of NPN, and AN in inoculated samples were very similar. A gradual increase of AN concentration is observed to reach statistical differences at 72 h of incubation, when NPN concentration is maximal. AN continued increasing until 84 h, probably due to the release of amino acids from peptides. The decreasing protein concentration together with the degradation of peptides and consumption of AN by mold metabolism, led to a drop in NPN, and AN amounts after 72 and 84 h of incubation, respectively.

Just about 15% of NPN corresponded to AN at 72 h of incubation, and only at 92 h almost the total NPN matched to AN. The limited amount of NPN and AN found is probably due to the restricted quantity of proteins present in the culture medium. It is expected that in dry-cured meat products, where the proteins substrate are not limited, *P. chrysogenum* Pg222 may generate larger amounts of peptides and AN from myofibrillar proteins. When some mold strains have been used as starter cultures in dry-fermented sausages noticeable increases of free amino acid contend were reported. Thus, surface inoculation with *Mucor racemosus* or *P. aurantiogriseum* increased the free amino acid content by 44–46% (Bruna et al., 2000, 2001). Furthermore, different molds isolated from dry-cured ham, including *P. commune* and *P. chrysogenum*, particularly the strain Pg222 here tested, increased some free amino acids when grown on pork slices or loins ripened under controlled conditions (Rodríguez et al., 1998; Martín et al., 2001). Mold contribution to NPN and AN formation may result essential in dry-curing of big meat pieces, where curing agents (Flores et al., 1997) and free amino acids released (Flores et al., 1998) produce a strong inhibitory effect on some muscle aminopeptidases. On the other hand, the AN released is taken by the mold to be involved in different metabolic pathways yielding a large number of new products such as methyl-aldehydes, alcohols and ketones of great interest to flavour development of these products.

Volatile compound analysis revealed formation of 3-methyl-butanol in Pg222 treated samples as early as 28 h of incubation. This branched alcohol could derive from Erhlich's pathway of the leucine metabolism or from aldehyde 3-methyl-butanal degradation, which is formed by the Strecker pathway for leucine degradation (Molimard and Spinnler, 1996). 3-methyl-butanal was detected at 60 h, reaching the highest values at 72 h, when AN concentration showed the maximum value. At the last sampling time this compound was not detected, probably due to its transformation to 3-methylbutanol by the route previously indicated. The former branched compounds have been associated with flavour of dry-cured meat products (Careri et al., 1993; Hinrichsen and Pedersen, 1995; Montel et al., 1998; Stahnke, 1995; Ruiz et al., 1999). In addition, these compounds have been reported with low threshold values (Stahnke, 1994). Thus, the generation of these compounds may have a high impact on dry-cured ham flavour.

Ethanol, propanol and butanol, only detected in the inoculated batch, have been reported to be formed by transesterification (or reduction) of the activated acyl-CoA derivatives from amino acid catabolism,

Table 3
Volatile compounds (arbitrary area units)/10^6 from control and inoculated samples with *P. chrysogenum* Pg222 at the different incubation times assayed

Compound	28h		36h		48h		60h		72h		84h		92h	
	Control	Pg222	Control	Pg222	Control	Pg222	Control	Pg222	Control	Pg222	Control	Pg222	Control	Pg222
Alkanes														
2-methylpentane	nd	nd	nd	nd	nd	nd	nd	nd	nd	22.82^{++}	nd	nd	nd	nd
3-methylpentane	nd	nd	nd	nd	nd	nd	nd	nd	nd	32.82^{++}	nd	nd	nd	nd
Aldehydes														
3-methylbutanal	nd	nd	nd	nd	nd	nd	nd	42.66^{++}	nd	300.14^{++}	nd	129.2^{++}	nd	nd
Alcohols														
Ethanol	nd	703.85^{++}	nd	1650.44^{++}	nd	2096.64^{++}	nd	1164.11^{++}	nd	1356.02^{++}	nd	891.06^{++}	nd	780.34^{++}
Propanol	nd	64	nd	nd	nd	75	nd	141.45^{++}	nd	123.01^{++}	nd	68.23^{++}	nd	20.67^{++}
Butanol	nd	nd	nd	nd	nd	22.85^{++}	nd	nd	nd	4.2^{++}	nd	301.9^{++}	nd	190.32^{++}
3-methylbutanol	nd	39.82^{++}	nd	64.19^{++}	nd	51.42^{++}	nd	32.21^{++}	nd	28.13^{++}	nd	57.83^{++}	nd	174.82^{++}
2-methoxy ethanol	nd	15.73^{++}	nd	24.68^{++}	nd	51.42^{++}	nd	10.51^{++}	nd	42.92^{++}	nd	3.11^{++}	nd	141.63^{++}
2-ethylhexanol	nd	nd	nd	nd	nd	74.98^{++}	nd	53.12^{++}	nd	10.23^{++}	nd	nd	nd	nd
Acids														
Acetic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	363.68^{++}
Benzoic acid	nd	nd	nd	nd	nd	nd	nd	5.23^{++}	nd	nd	nd	nd	nd	nd
Ketones														
2-propanone	197.56	1135.66^{++}	108.63	2115.93^{++}	160.86	1251.51^{++}	59.23	281.14^{++}	93.53	241.4^{++}	66.21	525.6^{++}	49.20	947.71^{++}
2-butanone	nd	nd	nd	nd	nd	nd	nd	nd	nd	58.84^{++}	nd	315.11^{++}	nd	13.08^{++}
Other compounds														
2,4 bis(1,1-dimethylethyl) phenol	nd	nd	nd	37.33^{++}	nd	44.55^{++}	nd	nd	nd	nd	nd	nd	nd	42.25^{++}
Pyridine	nd	61.01^{++}	nd	47.5^{++}	nd	nd	nd	nd	nd	129.55^{++}	nd	nd	nd	nd
3-carene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	27.09^{++}

Values with $++$ as superscript are significantly different ($P<0.05$) respect to its control.
nd, not detected.

followed by additional reduction reactions (Thierry and Maillard, 2002; Charlotte et al., 2002). Although these alcohols have higher threshold values than methyl branched compounds (Montel et al., 1998), their presence may also affect and modulate the final flavour of dry-cured ham. In addition, ethanol is the precursor of several esters (Molimard and Spinnler, 1996), which have very low threshold values.

The methyl ketones 2-propanone and 2-butanone also detected in higher amount in inoculated samples, have been reported as essential in ripened products due to its aromatic notes to fruity, flower and musty and its low threshold (Molimard and Spinnler, 1996). Several molds strains have been reported to produce the above methyl ketones in different laboratory media (Jacobsen and Hinrichsen, 1998; Sunesen et al., 2004).

P. chrysogenum Pg222 seems to influence the generation of acetic and benzoic acids, since there was detected in the inoculated samples. It has been reported that some mold strains are able to produce a significant release of some organic acids due to the catabolism of certain amino acids (Stahnke, 1995).

From the results it can be concluded that the proteolytic activity of *P. chrysogenum* Pg222 lead to accumulation of soluble NPN compounds, in addition to the generation of volatile compounds of great interest for dry-cured meat products, even with the restricted quantity of proteins in the culture medium and the limitation in incubation time (less than 4 days). It is expected that in dry-cured ham, where the protein substrate is not limited, and time of ripening is much longer, *P. chrysogenum* Pg222 may generate large amounts of soluble NPN and volatile compounds. Although these compounds may be formed mainly on the surface, they could migrate by water to the deep of the ham. Therefore, this mold could be appropriate to be used as a non-toxigenic starter culture during the ripening of dry-cured ham to stimulate proteolysis and flavour development. *P. chrysogenum* Pg222 may be inoculated on the surface of the hams at the beginning of drying stage.

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References

Benito, M.J., Córdoba, J.J., Alonso, M., Asensio, M.A., Núñez, F., 2003. Hydrolytic activity of *Penicillium chrysogenum* Pg222 on pork myofibrillar proteins. Int. J. Food Microbiol. 89, 155–161.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72, 248–254.

Bruna, J.M., Fernández, M., Hierro, E.M., Ordóñez, J.A., de la Hoz, L., 2000. Improvement of the sensory properties of dry-fermented sausages by the superficial inoculation and/or the addition of intracellular extracts of *Mucor racemosus*. J. Food Sci. 65, 731–738.

Bruna, J.M., Hierro, E.M., de la Hoz, L., Mottram, D.S., Fernández, M., Ordóñez, J.A., 2001. The contribution of *Penicillium aurantiogriseum* to the volatile composition and sensory quality of dry fermented sausages. Meat Sci. 59, 97–107.

Charlotte, E., Schöller, G., Gürtler, H., Pedersen, R., Molin, S., Wilkins, K., 2002. Volatile metabolites from Actinomycetes. J. Agric. Food Chem. 50, 2615–2621.

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

Córdoba, J.J., Antequera, T., Ventanas, J., López, C., García, C., Asensio, M.A., 1994a. Hydrolysis and loss of extractability of proteins during ripening of Iberian ham. Meat Sci. 37, 217–227.

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

Dainty, R.H., Hibbard, C.M., 1980. Aerobic metabolism of *Brochothrix thermosphacta* growing on meat surface and in laboratory media. J. Appl. Bacteriol. 48, 387–396.

De Ketelaere, A., Demeyer, D., Vandekerckhove, P., Vervaeke, I., 1974. Stoichiometry of carbohydrate fermentation during dry sausage ripening. J. Food Sci. 39, 297–300.

Flores, M., Aristoy, M.C., Spanier, A.M., Toldrá, F., 1997. Non-volatile components effects on quality of "serrano" dry-cured ham as related to processing time. J. Food Sci. 62, 1235–1239.

Flores, M., Aristoy, M.C., Toldrá, F., 1998. Feedback inhibition of porcine muscle alanyl and arginyl aminopeptidases in cured meat products. J. Agric. Food Chem. 46, 4982–4986.

Hinrichsen, L.L., Pedersen, S.B., 1995. Relationship among flavor, volatile compounds, chemical changes and microflora in Italian-type dry-cured ham during processing. J. Agric. Food Chem. 43, 2932–2940.

Jacobsen, T., Hinrichsen, L., 1998. Bioformation of flavour by *Penicillium candidum*, *Penicillium nalgiovense* and *Geotrichum candidum* on glucose, peptone, maize oil and meat extract. Food Chem. 60, 409–416.

Johnson, M.J., 1941. Isolation and properties of a pure yeast polypeptidase. Proceedings of the Third International Congress of Microbiology, New York, 348pp.

Karahadian, C., Josephson, D.B., Lindsay, R.C., 1985. Contribution of *Penicillium* sp. to the flavours of Brie and Cammembert cheese. J. Dairy Sci. 68, 1865–1877.

Martín, L., Córdoba, J.J., Antequera, T., Timón, M.L., Ventanas, J., 1998a. Effects of salt and temperature on proteolysis during ripening of Iberiam ham. Meat Sci. 49, 145–153.

Martín, L., Antequera, T., Ruiz, J., Cava, R., Tejeda, J.F., Córdoba, J.J., 1998b. Influence of processing conditions of Iberian ham on proteolysis during ripening. Food Sci. Technol. Int. 4, 17–22.

Martín, A., Córdoba, J.J., Rodríguez, M.M., Núñez, F., Asensio, M.A., 2001. Evaluation of microbial proteolysis in meat products by capillary electrophoresis. J. Appl. Microbiol. 90, 163–171.

Molimard, R., Spinnler, H.E., 1996. Compounds involved in flavor of surface mold-ripened cheeses: origins and properties. J. Dairy Sci. 79, 169–184.

Montel, M.C., Masson, F., Talon, R., 1998. Bacterial role in flavour development. Meat Sci. 49, S111–S123.

Núñez, F., Rodríguez, M.M., Bermúdez, M.E., Córdoba, J.J., Asensio, M.A., 1996. Composition and toxigenic potential of the mould population on dry-cured Iberian ham. Int. J. Food Microbiol. 32, 185–197.

Núñez, F., Díaz, M.C., Rodríguez, M., Aranda, E., Martín, A., Asensio, M.A., 2000. Effects of substrate, water activity, and temperature on growth and verrucosidin production by *Penicillium polonicum* isolated from dry-cured ham. J. Food Prot. 63, 231–236.

Ordóñez, J.A., Hierro, E.M., Bruna, J.M., de la Hoz, L., 1999. Changes in the components of dry-fermented sausages during ripening. CRC Crit. Rev. Food. Sci. Nutr. 39, 329–367.

Rico, E., Toldrá, F., Flores, J., 1991. Effect of dry-curing process parameters on pork muscle cathepsin B, H and L activity. Z. Lebensm. Unters. Forsch. 193, 541–544.

Rodríguez, M.M., Núñez, F., Córdoba, J.J., Bermúdez, M.E., Asensio, M.A., 1998. Evaluation of proteolytic activity of micro-organisms isolated from dry-cured ham. J. Appl. Microbiol. 85, 905–912.

Ruiz, J., Ventanas, J., Cava, R., Timón, M.L., García, C., 1998. Sensory characteristics of Iberian ham: influence of processing time and slice location. Food Res. Int. 31, 53–58.

Ruiz, J., García, C., Díaz, M.C., Cava, R., Tejeda, J.F., Ventanas, J., 1999. Dry-cured Iberian ham non-volatile components as affected by the length of the curing process. Food Res. Int. 32, 643–651.

Sárraga, C., Gil, M., Arnau, J., Monfort, J.M., Cussó, R., 1989. Effect of curing salt and phosphate on activity of porcine muscle proteases. Meat Sci. 25, 241–249.

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

Stahnke, L.H., 1995. Dried sausages fermented with *Staphylococcus xylosus* at different temperatures and different ingredient levels. Part II. Volatile components. Meat Sci. 41, 193–209.

Sunesen, L.O., Trihaas, J., Stahnke, L.H., 2004. Volatiles in a sausage surface model-influence of *Penicillium nalgiovense*, *Pediococcus pentosaceus*, ascorbate, nitrate and temperature. Meat Sci. 66, 447–456.

Sosa, M.J., Córdoba, J.J., Díaz, C., Rodríguez, M., Bermúdez, E., Asensio, M.A., Núñez, F., 2002. Production of cyclopiazonic acid by *Penicillium commune* isolated from dry-cured ham on a meat extract-based substrate. J. Food Prot. 65, 988–992.

Thierry, A., Maillard, M.B., 2002. Production of cheese flavour compounds derived from amino acid catabolism by *Propionibacterium freudenreichii*. Lait 82, 17–32.

Toldrá, F., 1998. Proteolysis and lipolysis in flavour development of dry-cured meat products. Meat Sci. 49, S101–S110.

Toldrá, F., Cerveró, C., Rico, E., Part, C., 1993. Porcine aminopeptidase activity as affected by curing agents. J. Food Sci. 58, 724–726.