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Short Communication

Characterization of *Staphylococcus* spp. and *Micrococcus* spp. isolated from Iberian ham throughout the ripening process

M. Rodríguez [a,*], F. Núñez [a], J.J. Córdoba [a], C. Sanabria [b], E. Bermúdez [a], M.A. Asensio [a]

[a] *Higiene y Tecnología de los Alimentos, Facultad de Veterinaria, Crta. de Trujillo, 10071 Cáceres, Spain*
[b] *SIA Junta de Extremadura, Badajoz, Spain*

Abstract

The Iberian dry cured ham is an uncooked meat product highly appreciated because of its characteristic flavour. This product is obtained from highly marbled Iberian pig hindlegs after 18–24 months of maturation under natural environmental conditions. The role of Micrococcaceae in the development of the aroma characteristics of this products remains unclear. Identification of Gram-positive, catalase-positive cocci isolated from Mannitol Salt Agar plates showed that *Staphylococcus xylosus* followed by *Staphylococcus equorum* are the predominant organisms, even after 16 months of maturing. A remarkable variety of types of both staphylococci and micrococci are detected at any sampling time. The metabolic activities of these organisms could contribute to the characteristics of the final product.

Keywords: Staphylococci; Micrococci; Dry cured ham

1. Introduction

The Iberian ham is an uncooked dry cured meat product obtained from highly marbled Iberian pig hindlegs. These are salted under refrigeration for about 45–90 days and left to mature, for 14–22 additional months under natural environmental conditions. During the first periods of processing, salting and slow drying are combined with low temperature to reduce the risk of bacterial spoilage. This product has a high commercial value due to its characteristic flavour. The flavour

* Corresponding author. Tel. 34-27-257125. Fax 34-27-257110.

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noticed for Iberian hams suggests intense proteolytic and lipolytic breakdown during maturation (García et al., 1991).

Micrococci are predominant both on the surface and in the depth of the ham, during the complete curing process of Parma (Giolitti et al., 1971a; Baldini et al., 1977; Baldini and Raczynski, 1978; Raczynski et al., 1978), American country style (Kemp et al., 1978, 1982) and Spanish hams (Francisco et al., 1981; Silla et al., 1985; Huerta et al., 1988). The positive role of some *Micrococcus* and *Staphylococcus* spp. in these dry cured meat products, due to their nitrate reductase, catalase, lipolytic and proteolytic activities (Giolitti et al., 1971b; Palumbo and Smith, 1977; Lücke, 1986; Lücke and Hechelmann, 1986) could also contribute to the flavour characteristics of Iberian ham.

The objective of this study was to characterize the changes with time of the populations of micrococci and staphylococci in Iberian ham.

2. Material and methods

Processing of the hams. The study involved eighteen hams from Iberian pigs. After slaughter, the hams were refrigerated at 0°C for 48 h, and then thoroughly rubbed with sea salt containing 1% (w/w) potassium nitrate and trace amounts of nitrites before being stored under salt at 2°C for 8 days. Later, the hams were brushed to remove unabsorbed salt from their surface and kept for 80 days under refrigeration. After that first period, the hams were left to mature, first in a natural drying room with temperatures up to 30°C for five months, and then in a cellar at 16–20°C for 16 months.

Sampling. Samples of 10 g were taken from the surface and the depth of three hams at each of the following stages: fresh, end of salting, end of drying, and 4, 8 and 16 months of aging. Surface samples were taken aseptically from the *Gracilis* muscle. Samples from the interior were taken from the excised *Biceps femoris* muscle, after dipping it in absolute ethyl alcohol and flaming for a few seconds. After homogenizing with 90 ml of peptone water (0.1%), serial dilutions were made in peptone water, and 0.1 ml portions were plated on Mannitol Salt Agar (MSA, Oxoid) which were then incubated for 48 h at 30°C, respectively. In the last two samplings, 50 μg/ml of cycloheximide were added to the MSA agar to prevent the growth of yeasts (Kloos et al., 1974, 1976).

Determination of moisture was made on 5 g samples after drying at 100–102°C in air oven and cooling in a desiccator, according to the method described in ISO Method 1442 (1973). Water activity (a_w) was determined at 20°C by using a thermoconstanter RTD-33 (Novasina, Zürich, Switzerland). Measurement of pH was carried out, immediately after homogenizing 10 g samples with 10 ml distilled deionized water in a OMNI 5000 (OMNI International, Waterbury, CT, USA) for 30 s, using a micropH 2002 (Crison Instruments, Barcelona, Spain).

Identification. Approximately 20% of the colonies recovered on both media were identified. The 457 randomly selected isolates were subjected to the following tests: catalase activity (Harrigan and McCance, 1976); glucose fermentation (Subcommittee on Taxonomy of Staphylococci and Micrococci, 1965, 1971); anaerobic growth in Brewer's Tioglycolate Medium (Evans and Kloos, 1972); acid production from glycerol in the presence of erythromycin and sensitivity to lysostaphin and lysozyme (Schleifer and Kloos, 1975); sensitivity to lysostaphin in tube (Lachica et al., 1971), and furazolidone sensitivity (Von Rheinbaden and Hadlok, 1981).

For species identification the following tests were used: production of pigment and colony diameter on P agar (Naylor and Burgi, 1956) as proposed by Kloos (1982); production of acid from L-arabinose, D-cellobiose, D-mannose, raffinose, sucrose, D-turanose and D-xylose (Devriese et al., 1985); oxidase activity (Faller and Schleifer, 1981); urease activity on urea agar (Christensen, 1946); β-galactosidase activity (International Commission on Microbiological Specifications for Foods, 1978); production of acetoin (Harrigan and McCance, 1976); growth in nutrient agar supplemented with 7.5% (w/w) and 10% (w/w) of NaCl, and growth in nutrient agar at 10°C and 45°C (Baird-Parker, 1979); nitrate reduction in nitrate broth (Difco); motility on Bacto Motility Agar (Difco); coagulase activity by the tube assay and sensitivity to novobiocin (Baird-Parker, 1979); clumping factor using rabbit plasma plus EDTA (Devriese and Hájek, 1980) and detection of DNAse on DNAse Agar (Difco).

For further characterization of the isolates, the following complementary biochemical tests were carried out: growth on P agar at 37°C and growth in Simmons' Citrate Agar (Kloos et al., 1974); phosphatase activity, detection of gelatinase, hydrolysis of casein and production of acid from β-D-fructose, D-galactose, lactose, maltose, D-mannitol, D-melezitose, melibiose, L-rhamnose, D-ribose, salicin, D-trehalose, and xylitol (Devriese et al., 1985).

3. Results and discussion

The changes in moisture, a_w and pH values during the preparation and maturation of the hams are shown in Table 1. The numbers of bacteria obtained

Table 1
Variations of some physicochemical parameters of the *Gracilis* (surface samples) and *Biceps femoris* (interior samples) muscles during the ripening of Iberian ham

	Moisture (%, w/w)		a_w		pH	
	Gracilis	*Biceps femoris*	*Gracilis*	*Biceps femoris*	*Gracilis*	*Biceps femoris*
Fresh	73.23(±0.39)	77.79(±7.86)	0.99(±0.01)	0.99(±0.01)	5.96(±0.06)	5.70(±0.12)
Post-salting	35.88(±1.22)	64.54(±2.47)	0.91(±0.01)	0.97(±0.02)	5.70(±0.06)	5.89(±0.02)
Drying	18.45(±1.21)	58.80(±1.01)	0.82(±0.01)	0.87(±0.01)	6.02(±0.03)	5.86(±0.04)
4 months cellar	18.40(±1.83)	54.72(±2.10)	0.82(±0.02)	0.93(±0.02)	5.74(±0.05)	5.85(±0.03)
8 months cellar	20.50(±1.60)	51.23(±0.71)	0.79(±0.01)	0.90(±0.01)	5.77(±0.15)	5.87(±0.08)
16 months cellar	19.11(±2.98)	50.37(±0.76)	0.76(±0.05)	0.89(±0.01)	5.66(±0.13)	6.12(±0.16)

from the surface were always greater than the numbers from the deep tissues at all the stages of the ripening process. Maximum values were obtained at the end of the salting period, when counts reached 10^8 cfu/g at the surface and 10^4 cfu/g in the deep tissues. Counts decreased continuously until the end of the processing (Fig. 1). From the fourth month of cellar storage, yeast growth on MSA plates without cycloheximide was so high that it was impossible to isolate Micrococcaceae, but cycloheximide effectively inhibited the development of yeasts. The changes in counts of micrococci were similar to the changes described previously for Iberian ham (Francisco et al., 1981).

A total of 457 isolates of Micrococcaceae were obtained, 350 from the surface and 107 from the deep tissues samples. The identifications revealed that *Staphylococcus* spp. constituted the majority (94.7%) of the micrococci isolated from all the samples. Most isolates were *S. xylosus* (69.6%) followed by *S. equorum* (7.7%) and *S. saprophyticus* (7.4%). Occasionally, isolates were identified as *S. cohnii* (1.9%), *S. cohnii urealyticum* (1.3%), *S. arlettae* (0.2%) and *Staphylococcus* spp. (6.6%). No coagulase-positive, DNAse-positive strain was found. The concentration of the various *Staphylococcus* spp. decreased during the ripening process (Table 2).

S. xylosus has also been described as the main species present in other types of dry-cured ham (Carrascosa and Cornejo, 1991; Cornejo and Carrascosa, 1991); however, high rates of other staphylococci, such as *S. sciuri* (Molina et al., 1990) have also been reported.

The *S. xylosus* found in all stages were pigmented, nitrate-reducing and sensitive to lysozyme, did not produce acid from glucose and D-turanose but produced acid from glycerol in the presence of erythromycin and from L-arabinose.

The complementary biochemical tests revealed a great diversity of strains within

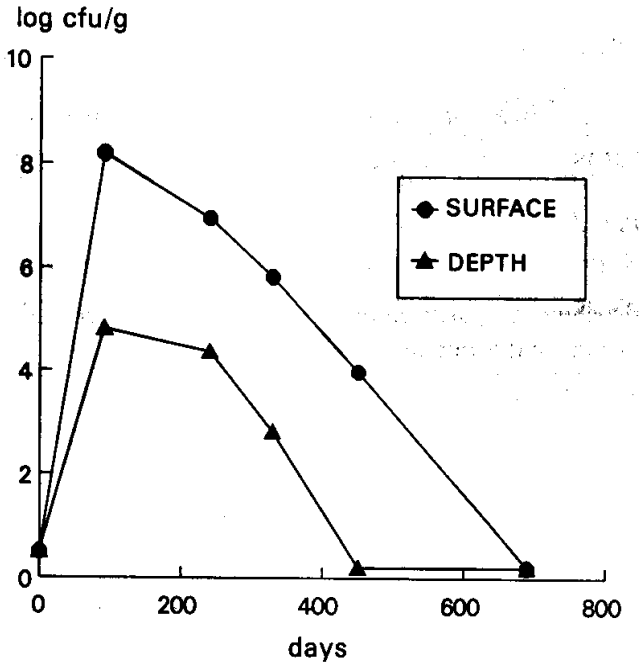

Fig. 1. Changes in Micrococcaceae numbers during the processing of Iberian dry-cured ham.

Table 2
Recovery of the different *Staphylococcus* spp. and *Micrococcus* spp. during the processing of Iberian dry cured ham

	F (log cfu/g)	PS (log cfu/g)	D (log cfu/g)	4 MC (log cfu/g)	8 MC (log cfu/g)	16 MC (log cfu/g)
S. xylosus	n.d.	8.6	7.7	3.3	4.1	n.d.
S. equorum	n.d.	7.3	3.8	3.3	3.4	n.d.
S. saprophyticus	n.d.	7.5	n.d.	4	n.d.	n.d.
S. cohnii	n.d.	7	4.2	n.d.	n.d.	n.d.
Staphylococcus spp.	n.d.	7.5	1.8	6.3	n.d.	n.d.
M. kristinae	n.d.	n.d.	n.d.	3	2.9	n.d.
Micrococcus spp.	n.d.	n.d.	n.d.	2.7	2.9	n.d.

F, fresh; PS, post-salting; D, drying; 4 MC, 4 months of cellar; 8 MC, 8 months of cellar; 16 MC, 16 months of cellar.
n.d., not detected.

the same species, even after 12–16 months of maturation. According to the pattern of these characteristics, 20 different strains *S. xylosus* were obtained, together with 10 strains of *S. equorum* and 11 of *S. saprophyticus*.

The 24 isolates characterized as being members of the genus *Micrococcus* were obtained from the surface of hams after 4 and 8 months of cellar storage. The majority (87.5%) of these isolates were tentatively classified as *M. kristinae*. The remaining members of the genus *Micrococcus* had characteristics common to several species. The low numbers found suggest a negligible role of micrococci for the final characteristics of Iberian ham.

In conclusion, although the number of different species is relatively small, a remarkable variety of types of primarily staphylococci are present in Iberian dry-cured ham. The results indicate that the particular processing of this kind of ham offers an ecosystem suitable for the survival of some micrococci for long periods of time. Therefore, their nitrate reduction and peroxide degradation, as well as the proteolytic and lipolytic activities, could contribute significantly to the characteristics of the final product.

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