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A survey on the microbiological changes during the manufacture of dry-cured lacón, a Spanish traditional meat product

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I. VILAR, M.C. GARCÍA FONTÁN, B. PRIETO, M.E. TORNADIJO AND J. CARBALLO. 2000. This article describes a microbiological study carried out on lacón, a dry-cured meat product made in the north-west of Spain from the fore extremity of pig. Using classical methods, aerobic mesophilic flora, salt-tolerant flora, lactic acid bacteria, Enterobacteriaceae, enterococci, moulds and yeasts were enumerated, some physicochemical parameters (pH, a_w and moisture and NaCl contents) were determined and a representative number of isolates of the salt-tolerant flora (the main microbial group) were identified during the manufacture of five batches. All the microbial groups, with the exception of Enterobacteriaceae and enterococci, reached maximum counts both on the surface and in the interior of the pieces at the end of the post-salting stage and afterwards progressively dropped during the drying–ripening stage. *Staphylococcus xylosus*, *Staph. saprophyticus*, *Staph. simulans*, *Staph. sciuri* and *Micrococcus luteus* were the main species isolated throughout manufacturing. This study will significantly increase knowledge of the microbiology of cured meat products made from entire pieces.

INTRODUCTION

Traditional cured meat products, made from whole meat pieces of pork or beef, are commonly produced and consumed in different countries throughout the world. Dry-cured lacón, a product made in the north-west of Spain (Galicia), which has a wide acceptance by consumers, is among these traditional cured meat products.

Its elaboration is begun by cutting the fore extremity of the pig at the shoulder blade–humerus joint and the stages of the process are very similar to those followed in the production of dry-cured ham.

The information in the scientific literature related to dry-cured lacón is very scarce and refers only to the biochemical characteristics of the end-product (Marra *et al.* 1999). At present, data do not exist on the microbiological and biochemical changes which take place throughout the manufacturing process of this product. These changes are mainly responsible for the quality of the end-product.

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The typical microflora of these meat products is made up of Micrococcaceae, lactic acid bacteria and moulds and yeasts, with the involvement of the Micrococcaceae being very important. Their halotolerant character allows them to be permanently present during the manufacturing of the product, and they play an important part in the formation of the colour, by reducing nitrate to nitrite, and in the proteolytic and lipolytic processes, which could contribute to the development of the characteristic flavour of these products. Their role has been demonstrated in the ripening of fermented sausages (Liepe 1983; Simonetti and Cantoni 1983; Lücke and Hechelmann 1987; Nychas and Arkoudelos 1990; Bersani *et al.* 1991a) and dry-cured hams (Lücke and Hechelmann 1987; Molina *et al.* 1991).

The aim of this work, which forms part of a wider study on both the microbiological and biochemical changes during the manufacture of lacón, was to quantify the microbiological groups present on the surface and in the interior of the pieces during the manufacture of this meat product and to isolate and identify a representative number of strains of the Micrococcaceae family occurring throughout the process.

The existing information in the scientific literature on the microbiology of raw-cured meat products elaborated from whole meat pieces is very limited. This article will contribute to an increase in knowledge in this field.

MATERIALS AND METHODS

Samples

Five batches of lacón were elaborated, as described by Marra *et al.* (1999), by five well-known pigmeat processors. From each batch, samples were taken of fresh pieces, at the end of the salting stage, at the end of the post-salting stage (15 d after removing the pieces from the salt) and at the end of the drying–ripening stage (90 d after removal of the pieces from the salt). Each sample consisted of one whole lacón piece. Samples were transported to the laboratory under refrigeration conditions (below 4 °C) and analysed on arrival.

Microbiological analysis

Surface samples were taken by aseptically removing slices (surface area 100 cm^2 and about 2 mm in thickness) following the method of Fliss *et al.* (1991). Samples of the interior of the pieces were taken with the aid of a sterile metallic rod which was introduced into the musculature at five different points after aseptically removing consecutively three 2-mm thick slices from the surface. Twenty-five grams from the surface and 25 g from the interior of each piece of lacón were homogenized with 100 ml sterile 0·1% peptone water containing 0·85% NaCl and 1% Tween 80 as emulsifier, at 40–45 °C for 2 min in a Stomacher 400 Lab Blender (Seward Medical, London, UK), thus making a 1/5 dilution. Decimal dilutions were prepared by mixing 10 ml of the previous dilution with 90 ml 0·1% sterile peptone water.

In each sample the following microbial groups were enumerated: total mesophilic aerobic flora in standard plate count agar (Oxoid, Basingstoke, UK) after incubation at 30 °C for 48 h; salt-tolerant flora in standard plate count agar (Oxoid) + 7·5% NaCl after incubation at 30 °C for 48 h; lactic acid bacteria in De Man, Rogosa, Sharpe (MRS) agar (Oxoid) acidified at pH 5·6 with acetic acid after incubation at 30 °C for 5 d; moulds and yeasts in oxytetracycline glucose yeast extract agar (Oxoid) after incubation at 22 °C for 5d; enterococci in kanamycin aesculin azide (KAA) agar (Oxoid) incubated for 24 h at 37 °C and Enterobacteriaceae in violet red bile glucose (VRBG) agar (Oxoid) incubated for 24 h at 37 °C.

From each sample and on each culture medium, 1 ml of each dilution was inoculated in duplicate on plates and mixed before solidification. Plates of MRS and VRBG agar were covered with a layer of the same culture medium before incubation. After incubation, plates with 30–300 colonies were counted.

Chemical analysis

Moisture contents were determined following the ISO/R 1442 recommended method. NaCl contents were quantified following the Spanish Official Standard (Carpentier–Volhard method) (Presidencia del Gobierno 1979). The pH was determined in a slurry made by mixing 10 g sample with 10 ml distilled water in a Sorvall Omnimixer homogenizer (Omni International, Waterbury, CT, USA). Measurement was carried out with a pH meter, micro pH 2002 (Crison Instruments, SA, Barcelona, Spain). The water activity (a_w) was measured with a Water Activity System apparatus CX-1 (Decagon Devices, Pullman, WA, USA).

Isolation and identification of strains

Ten colonies were taken at random from Plate Count Agar (PCA) + 7·5% NaCl plates representing each sampling point (five from the surface sample and five from the interior sample) with the aid of a Harrison disc (Harrigan and McCance 1976). The isolates (200 strains, 40 from each batch) were purified by four alternate subcultures in Brain Heart Infusion (BHI) agar and BHI broth (Oxoid).

Gram-positive, catalase-positive cocci grouped in pairs, tetrads or irregular clusters were considered to belong to the Micrococcaceae family and were identified following the methods and criteria described by Baird-Parker (1979), Devriese *et al.* (1985) and Schleifer (1986). The following tests were carried out on each isolate: oxidation and fermentation of glucose and mannitol; assimilation of glycerol in aerobic conditions; growth in furazolidone agar and growth in lysostaphin agar. The aerobic cocci that were furazolidone and lysostaphin resistant and incapable of assimilating the glycerol were considered to be Micrococci; those facultatively anaerobic, incapable of growth in furazolidone and lysostaphin agar and able to assimilate glycerol were considered to be staphylococci. In order to identify the strains at species level the following tests were carried out: colony pigmentation in BHI agar; growth on 7·5, 10 and 15% NaCl agar; characteristics of growth in Baird–Parker agar; esculin hydrolysis; growth at 10 °C; acetoin production; nitrate reduction; presence of oxidase, alkaline phosphatase, urease, arginine dihydrolase, ornithine decarboxilase, β-galactosidase, arginine arylamidase, pyrrolydonil arylamydase, β-glucuronidase; novobiocin resistance; acid production from N-acetyl glucosamine, L-arabinose, D-cellobiose, D-fructose, glucose, glycerol, lactose, maltose, D-

mannitol, D-mannose, raffinose, D-ribose, sucrose, D-trehalose and D-turanose.

Statistical analysis

Correlations between log counts of the microbial groups and biochemical parameters were determined with the aid of the computer program SPSS/PC$^+$ version 3.1 (SPSS, Chicago, Illinois, USA).

RESULTS AND DISCUSSION

Changes in chemical parameters and microbial counts throughout manufacture

Table 1 shows the evolution of the chemical and physical parameters studied during lacón manufacture.

Throughout the manufacturing process of lacón batches a pronounced drop in moisture content was observed, which is initially attributed to the effect of the salting process (the diffusion of salt to the interior of the pieces is accompanied by the emergence of tissue fluids to the exterior) and later to the dehydration which is produced due to the progressive increase in temperature and to the effect of low relative humidity values in the post-salting and drying–ripening rooms.

The pH remained fairly stable throughout the whole process.

A fall in a_w values could be seen both in the surface and interior samples from the beginning of the process, especially noticeable during the post-salting and drying–ripening stages. The fall in a_w was more pronounced in the surface samples, which was to be expected since the fall in the moisture content was higher and the NaCl percentages were also higher than in the interior samples.

The NaCl content, expressed as a percentage of total solids, noticeably increased at the end of the salting stage and continued to increase in the interior portion during the post-salting and drying–ripening stages. The lower NaCl values in the surface samples may seem strange since the salt spread from the most external part to the interior of the pieces. This observation could be due to the fact that the pieces are washed on the outside after the salting stage and this washing operation must reduce the NaCl on the surface.

The final average NaCl contents observed were in the range of values previously described in dry-cured lacón by Marra *et al.* (1999).

Table 2 shows the changes in the counts of the microbial groups enumerated during the manufacturing process.

In almost all the microbial groups studied, high counts, both on the surface and in the interior of the fresh pieces, were observed; this indicates the low standard of hygiene in the handling of the pieces in the quartering rooms and in the manufacturing plants. Counts of total mesophilic flora in the interior of the raw pieces were very high (10^3 cfu g^{-1}). However, similar counts to those in this study were observed by Mercier *et al.* (1989) in the interior of the raw pieces used in the manufacture of bündnerfleisch, by Huerta *et al.* (1988) in the interior of the raw pieces of ham and by Taylor (1976) in the pieces used in the manufacture of bilton. These high counts could be due to the penetration of extrinsic flora from the outside and to its rapid multiplication in the interior because of favourable conditions.

Table 1 Changes in some biochemical parameters during the manufacture of dry-cured lacón (average values ± S.D. of the five batches)

		Sampling points			
		A	B	C	D
Moisture (g 100 g^{-1})	Surface	72·1 ± 4·4	60·4 ± 2·7	43·5 ± 6·3	25·2 ± 5·8
	Interior	71·2 ± 4·4	59·8 ± 4·8	46·9 ± 6·8	38·6 ± 8·4
NaCl (g 100 g^{-1}	Surface	1·9 ± 0·2	30·7 ± 3·3	15·4 ± 1·5	17·7 ± 2·7
total solids)	Interior	1·4 ± 0·2	13·8 ± 4·0	17·1 ± 2·9	20·5 ± 3·3
NaCl (g 100 g^{-1} moisture)	Surface	0·7 ± 0·1	20·1 ± 1·3	20·3 ± 4·4	58·0 ± 22·4
	Interior	0·6 ± 0·1	9·0 ± 1·7	19·5 ± 4·0	35·7 ± 15·7
pH	Surface	5·98 ± 0·24	5·98 ± 0·29	6·26 ± 0·25	5·95 ± 0·18
	Interior	6·02 ± 0·19	6·07 ± 0·30	6·13 ± 0·21	6·16 ± 0·14
a_w	Surface	0·992 ± 0·001	0·851 ± 0·008	0·795 ± 0·041	0·705 ± 0·019
	Interior	0·992 ± 0·001	0·938 ± 0·007	0·855 ± 0·037	0·767 ± 0·052

A, Raw pieces; B, after salting; C, after post-salting stage; D, after drying–ripening stage.

Table 2 Changes in $\log_{10}$ cfu g^{-1} of main microbial groups during the manufacture of dry-cured lacón (average values ± S.D. of the five batches)

Microbial group		Sampling points			
		A	B	C	D
Total mesophilic aerobic flora	Surface	6·23 ± 1·67	5·33 ± 0·65	9·10 ± 0·21	4·79 ± 1·55
	Interior	3·03 ± 1·96	2·01 ± 0·62	6·11 ± 0·49	1·99 ± 1·47
Salt-tolerant flora	Surface	4·85 ± 0·79	4·99 ± 1·00	9·17 ± 0·21	4·91 ± 1·52
	Interior	1·71 ± 1·07	1·79 ± 0·78	6·15 ± 0·48	1·69 ± 1·66
Lactic acid bacteria	Surface	3·39 ± 0·90	3·17 ± 0·38	5·85 ± 0·70	3·45 ± 0·98
	Interior	1·56 ± 1·48	0·53 ± 0·85	3·30 ± 0·60	1·25 ± 1·23
Moulds and yeasts	Surface	4·55 ± 1·25	4·25 ± 0·72	7·12 ± 0·28	4·22 ± 1·69
	Interior	1·23 ± 1·13	1·18 ± 1·10	4·33 ± 0·52	1·17 ± 1·17
Enterococci	Surface	1·78 ± 1·19	1·04 ± 0·51	0·89 ± 0·80	0·16 ± 0·22
	Interior	0·08 ± 0·18	–	–	–
Enterobacteriaceae	Surface	3·32 ± 1·63	–	–	–
	Interior	0·79 ± 1·08	–	–	–

A, Raw pieces; B, after salting; C, after post-salting stage; D, after drying–ripening stage.
–, Absence in 0·2 g.

The counts of the total mesophilic aerobic flora and halotolerant flora on the surface were of the order of 1 log unit higher than those found by other authors (Huerta *et al.* 1988; Silla *et al.* 1989) in fresh pieces of ham. The counts of these two microbial groups in the interior of the pieces were nevertheless similar for the total mesophilic aerobic flora and of the order of 1 log unit lower for the halotolerant flora than those observed by Huerta *et al.* (1988) in fresh pieces of ham. The counts of lactic acid flora both on the surface and in the interior basically agree with those found by Huerta *et al.* (1988) and Silla *et al.* (1989) in fresh pieces of ham. The counts of Enterobacteriaceae were similar to those found by Silla *et al.* (1989) and slightly lower than those determined by Huerta *et al.* (1988); the counts of enterococci were slightly lower than those determined by Huerta *et al.* (1988) in fresh pieces of ham.

After salting, the total aerobic mesophilic flora fell 1 log unit, on average, both on the surface and in the interior of the pieces. The Enterobacteriaceae disappeared completely after salting, both on the surface and in the interior; however, they were also absent in the interior of the fresh pieces in three of the five batches studied. This salting effect is logical if it is taken into account that the microorganisms of the Enterobacteriaceae family are among those most sensitive to salt; they normally only multiply at a_w values higher than 0·93, although there are some exceptions such as halotolerant strains of *Enterobacter* which can easily grow at a_w values of 0·90. The rest of the microbial groups did not undergo any significant variations after salting either on the surface or in the interior of the pieces, which do not seem to be affected, at least quantitatively, by this operation. Our observations seem to verify that salt, by itself, has an inhibiting effect only on the Gram-negative flora.

The enterococci (counts in KAA agar) were not detected in the interior of the lacón pieces except in one of the fresh pieces; on the surface of the pieces the average counts were much lower at the end of the post-salting stage and enterococci had practically disappeared at the end of the drying–ripening stage.

Except for the Enterobacteriaceae and enterococci, the rest of the microbial groups reached maximum counts, both on the surface and in the interior of the pieces, at the end of the post-salting stage. The increase in the counts of the total aerobic mesophilic flora, halotolerant flora and fungal flora during the post-salting stage has also been shown by other authors during the manufacture of other meat products, such as ham (Huerta *et al.* 1988; Silla *et al.* 1989; Carrascosa *et al.* 1992) and Spanish beef cecina (García *et al.* 1995).

From the end of the post-salting stage a fall in the counts of all the microbial groups was observed, possibly due to the inhibitory effect of the low a_w values on the flora (see Bersani *et al.* 1991b). The fall in water activity seems to be decisive in the fall in the counts during the drying–ripening stage, a consequence of the loss of moisture in the pieces caused by the higher temperatures (12 °C) and lower relative humidities (70%) in the rooms where

this elaboration stage takes place. The average moisture percentage of the pieces fell at the end of the drying–ripening stage in comparison with the values observed at the end of the post-salting stage. In consequence, the salt concentration underwent a considerable increase, particularly on the surface of the pieces, which limits the microbial growth. The a_w underwent a progressive decrease to final average values of 0·705 on the surface of the pieces and 0·767 in the interior, although the values of this parameter which are capable of having an inhibitory effect on many microbial populations (values close to 0·85) are reached after salting in the surface of the pieces and at the end of the post-salting stage in the interior.

Owing to the high NaCl concentrations which were reached both on the surface and in the interior of the pieces immediately after salting, from this sampling point on, the total mesophilic aerobic flora counts practically coincided with those of the salt-tolerant flora which is a consequence of the selective action of the high salt contents (high correlation coefficient values were observed between the total mesophilic counts and the salt-tolerant flora counts, both on the surface ($r = 0{\cdot}92$; $P < 0{\cdot}001$) and in the interior ($r = 0{\cdot}90$; $P < 0{\cdot}001$)) of the pieces. This effect was previously observed in other meat products which underwent a salting process followed by a drying–ripening process (Giolitti *et al.* 1971; Graham and Blumer 1971; Francisco *et al.* 1981; Carrascosa *et al.* 1988; Huerta *et al.* 1988; Silla *et al.* 1989; Rodríguez *et al.* 1994; García *et al.* 1995). In fact, the salt-tolerant bacteria belonging to the Micrococcaceae family (and mainly the coagulase-negative staphylococci) are the micro-organisms which are usually isolated in the greater proportion in raw-cured meat products such as ham (Huerta *et al.* 1988; Silla *et al.* 1989; Carrascosa *et al.* 1992) or Spanish beef cecina (García *et al.* 1995). These micro-organisms can remain until the final stages of the manufacturing process since, apart from their salt-tolerant character, they can resist low a_w and high osmotic pressure conditions (Carrascosa and Cornejo 1991).

Identification of Micrococcaceae strains isolated throughout manufacture

Of the 200 strains isolated from the PCA + 7·5% NaCl culture medium, 154 strains (77% of the isolates) were considered to be Micrococcaceae (85 strains from the surface and 69 from the interior samples), which indicates that, in this study, this culture medium showed a fairly high selectivity for the isolation of Micrococcaceae.

In this study the number of isolated strains belonging to the *Staphylococcus* genus was much higher than those belonging to the *Micrococcus* genus. Of the Micrococcaceae strains isolated from the surface of the pieces 84% belonged to the *Staphylococcus* genus while only 16% belonged to the *Micrococcus* genus. A similar result was obtained in the interior of the pieces where 74% of the strains were of the *Staphylococcus* genus and 26% of the *Micrococcus* genus.

The predominance of isolates of the *Staphylococcus* genus in comparison with those of the *Micrococcus* genus appears as a common phenomenon in the majority of studies on the characterization of the microbial flora in raw-cured meat products (Graham and Blumer 1971; Francisco *et al.* 1981; Von Rheinbaben and Seipp 1986; Molina *et al.* 1989; Kotzekidou 1992; Rodríguez *et al.* 1994; García *et al.* 1995) and fermented sausages (Comi *et al.* 1992). Moreover, the isolation of staphylococci generally continues throughout the manufacturing process, while that of micrococci takes place only in some particular phases of the manufacture.

Of the 85 Micrococcaceae strains isolated from the surface of the pieces, 19 were identified as *Staphylococcus xylosus*, 16 as *Staph. saprophyticus*, 12 as *Staph. simulans*, 10 as *Staph. sciuri*, seven as *Staph. capitis*, six as *Staph. equorum*, one as *Staph. epidermidis*, 13 as *Micrococcus luteus* and one as *M. lylae*.

Of the 69 Micrococcaceae strains isolated from the interior of the pieces, 14 were identified as *Staph. saprophyticus*, 10 as *Staph. xylosus*, nine as *Staph. simulans*, seven as *Staph. capitis*, five as *Staph. equorum*, five as *Staph. sciuri*, one as *Staph. warneri*, 17 as *M. luteus* and one as *M. varians*.

The absence of the *Staph. aureus* species is a guarantee of the hygienic quality of the product.

Tables 3 and 4 show the distribution at the sampling points of the strains isolated from the surface and the interior of the pieces, respectively.

The most constant staphylococcal species throughout the whole ripening process were the novobiocin-resistant staphylococci (*Staph. xylosus*, *Staph. saprophyticus and Staph. sciuri*), both in the samples from the interior and in those from the surface of the lacón pieces. *Staphylococcus equorum* only reached relatively high percentages in the surface samples at the end of the post-salting stage.

Staphylococcus xylosus has been denoted as the most abundant species in other raw-cured meat products made from whole pieces, such as ham and Italian bresaole (Von Rheinbaben and Seipp 1986; Molina *et al.* 1989; Bersani *et al.* 1991b; Carrascosa and Cornejo 1991; Cornejo and Carrascosa 1991; Rodríguez *et al.* 1994). This species is, in fact, one of the most resistant to unfavourable environmental conditions (McMeekin *et al.* 1987; Chandler and McMeekin 1989), the predominant Micrococcaceae species during the curing process of ham and has been proposed for the elaboration of a starter culture destined to ensure a good development of the manufacturing process of ham, as well as of other meat products which undergo a curing pro-

Table 3 Distribution at the sampling points of the Micrococcaceae species isolated from the surface of the pieces during the manufacture of dry-cured lacón (five batches)

	Sampling points							
	A		B		C		D	
Species	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%
Staph. xylosus	8	32	4	16	4	16	3	12
Staph. saprophyticus	5	20	6	24	1	4	4	16
Staph. simulans	–		6	24	6	24	–	
Staph. sciuri	3	12	4	16	–		3	12
Staph. capitis	5	20	–		–		2	8
Staph. equorum	1	4	1	4	4	16	–	
Staph. epidermidis	–		–		1	4	–	
M. luteus	–		2	8	5	20	6	24
M. lylae	–		–		1	4	–	

A, Raw pieces; B, after salting; C, after post-salting stage; D, after drying–ripening stage.

Table 4 Distribution at the sampling points of the Micrococcaceae species isolated from the interior of the pieces during the manufacture of dry-cured lacón (five batches)

	Sampling points							
	A		B		C		D	
Species	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%
Staph. saprophyticus	6	24	2	8	–		6	24
Staph. xylosus	3	12	2	8	5	20	–	
Staph. simulans	–		5	20	4	16	–	
Staph. capitis	1	4	–		1	4	5	20
Staph. sciuri	5	20	–		–		–	
Staph. equorum	–		2	8	2	8	1	4
Staph. warneri	–		1	4	–		–	
M. luteus	–		7	28	8	32	2	8
M. varians	–		–		–		1	4

A, Raw pieces; B, after salting; C, after post-salting stage; D, after drying–ripening stage.

cess (Hammes *et al*. 1985; Lücke and Hechelmann 1987). *Staphylococcus xylosus* has also been frequently isolated from fermented sausages, as shown by several studies (see Comi *et al*. 1992). This species is also one of the microbial species with a greater genetic variability, which could lead to the existence of multiple biotypes with different phenotypical characteristics (Kloos 1980); this is why strains of the same species can have differences in their biochemical characteristics. This phenomenon could be due to the adaptation capacity of a species to different environmental conditions (Rodríguez Jovita 1997).

Staphylococcus sciuri is, together with *Staph. xylosus*, one of the Micrococcaceae species which is isolated in the highest proportion at different stages of the manufacturing process of ham (Von Rheinbaben and Seipp 1986; Molina *et al*. 1989).

Staphylococcus saprophyticus and *Staph. equorum* were also isolated by different authors in other raw-cured meat products (Cantoni and Pizzo 1980; Delarras and Laban 1981; Simonetti and Cantoni 1983; Comi *et al.* 1986; Seager *et al.* 1986; Cornejo and Carrascosa 1991; Kotzekidou 1992; Rodríguez *et al.* 1994; García *et al.* 1995; Rodríguez Jovita 1997). *Staphylococcus equorum* was the most abundant staphylococcal species isolated from Spanish beef cecina (García *et al.* 1995). Moreover, *Staph. equorum* is, together with *Staph. xylosus,* one of the most abundant species in fresh meat samples (Schleifer *et al.* 1984; Kloos 1990; Prieto *et al.* 1995).

With regard to the novobiocin-sensitive staphylococcal species, *Staph. simulans* was only isolated after the salting and post-salting stages. *Staphylococcus capitis* was isolated from fresh pieces and after the drying–ripening stage on the surface of the pieces; in the samples from the interior it was only significantly isolated at the end of the drying–ripening stage. Other novobiocin-sensitive staphylococcal species isolated were *Staph. epidermidis*, in the surface samples, and *Staph. warneri,* in the interior samples, both in a low proportion.

The novobiocin-sensitive staphylococcal species isolated by us from lacón coincided with those isolated by other authors in other meat products (Delarras 1980; Langlois *et al.* 1989; Kloos and Schleifer 1992). The proportions in which these authors isolated the species were also low and similar to ours.

The staphylococcal species resistant to novobiocin usually enter the product with the salt but they can also come from contamination of other types as they are widespread in nature and are species which are considered normal on the skin of many farm animals; their presence in the samples from the interior of the cured meat pieces is generally associated with their penetration with the salt, being capable of being added with it or being present as surface contaminants of the pieces. The species sensitive to novobiocin mainly come from contamination from human and animal skin (Schleifer 1986; Comi *et al.* 1992); this microbial group has mainly been isolated from human skin (Kloos and Musselwhite 1975; Kloos 1990) and its presence in meat products is related to the handling of the pieces before salting.

From the staphylococcal species isolated from lacón, and from their proportions, it can be deduced that salt seems to be the main source of contamination of lacón. Nevertheless, some of the staphylococcal species resistant to novobiocin (*Staph. saprophyticus* and *Staph. sciuri*) can come from the pig skin as they can appear as contaminants in animal carcasses a few hours after slaughter. Kotzekidou (1992) isolated *Staph. saprophyticus* from Greek basturna in a proportion of 32% in comparison with the total staphylococcal isolates and attributed its presence to contamination deriving from pig skin; the presence of this species is important since it is a micro-organism which can occasionally be pathogenic (Samelis *et al.* 1998).

Within the *Micrococcus* genus, the predominant species, which also represented almost all of the micrococci isolated both on the surface and in the interior of the pieces, was *M. luteus* which reached important percentages of the total isolates from the salting stage until the end of the manufacturing process. *Micrococcus luteus* is not commonly isolated in raw-cured meat products. This species is found to be predominant in human skin (Kloos *et al.* 1974) and its presence in lacón could be related to the handling of the pieces. Despite the fact that *M. varians* is the predominant species of animal origin (Schleifer *et al.* 1984), we have only isolated one strain of this species, which came from the interior of a lacón piece at the last sampling point. Our data agree with those of other authors (Van der Riet 1982; Kotzekidou 1992; García *et al.* 1995) who have isolated this species in very scarce proportions from other meat products. Nevertheless, this species is included in the starter cultures usable in raw meat products (Lücke and Hechelmann 1987). Finally, *M. lylae* was only isolated from the surface of one piece at the end of the post-salting stage; its origin also seems to be related to the handling of the pieces as it is a species which is normally present on human skin (Kloos *et al.* 1974).

In this study it was verified that the novobiocin-sensitive staphylococcal species and the micrococci were replaced by the novobiocin-resistant staphylococcal species as the manufacturing process progressed, which agrees with the results of similar studies (Samelis *et al.* 1998).

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