SPECIES OF *MICROCOCCACEAE* DURING THE MANUFACTURING AND RIPENING OF DRY-CURED LACÓN.

I. Vilar, C. García-Fontán, A.I. Marra, M.E. Tornadijo, and J. Carballo*.

Área de Tecnología de los Alimentos. Facultad de Ciencias de Orense, Universidad de Vigo. Campus de Las Lagunas, 32004 Orense, Spain.
* Corresponding author. E-mail: Carbatec@uvigo.es.

INTRODUCTION. The cured lacón is a meat product characteristic of Galicia (a region placed in the nort west of Spain). Its elaboraction is begun by cutting the fore extremity of the pig at the shoulder plade-humerus joint (the hoof may be eliminated) and the stages of the process are very similar to those followed in the elaboration of dry-cured ham.

The pieces, once selected and classified by weight, are dry salted with coarse salt, forming mounds on the floor, alternating between pieces and salt. The period of time the pieces spend in the mound is more or less 1 day per Kg of weight and the temperature of the salting room is around 3 °C. After the salting stage, the pieces are taken from the mound, brushed (sometimes washed) and are transferred to a post-salting room where they stay for more or less a fortnight at a temperature of 5 °C and a relative humidity of around 85 %. Once the post-salting stage has finished, the pieces undergo a drying-ripening process for which they are transferred to a room at 12 °C and 70 % of relative himidity. They remain in this room for around a month and half (the length of the drying-ripening process is very variable depending on the needs of the market). The final product, depending on the degree of ripening, can be eaten raw or cooked.

In a previous study (Vilar et al., 1997) it was shown that the salt tolerant flora (*Micrococcaceae*) is the largest microbial group throughout the elaboration and ripening of dry-cured lacón.

The importance of the microorganisms of the *Micrococcaceae* family in the cured meat products has been investigated by various authors (Cantoni et al., 1970; Frey, 1983; Hammes, 1986; Lücke, 1986; Molina et al., 1991) placing emphasis on the role of these microorganisms in the development of the flavour of these products. Even in the case of ham and dry sausages, specific starter cultures composed of microorganisms from this family have been proposed and evaluated (Lücke and Hechelmann, 1987; Selgas et al., 1988; Carrascosa and Cornejo, 1991; Cornejo and Carrascosa, 1991).

The aim of this work is to identify the species which belong to the *Micrococcaceae* family present throughout the elaboration and ripening of lacón and then to determine their role in the ripening process of this meat product.

MATERIALS AND METHODS. *Samples* - Five batches of lacón were elaborated by five well-known pigmeat industries. Samples were taken from each batch at the end of the salting stage (A), at the end of the post-salting stage (a fortnight after removing the pieces from the salt) (B), and at the end of the drying-ripening stage (two months after removing the pieces from the salt) (C). Each sample was made up of one whole lacón.

Microbiological analysis - Twenty-five g of the surface and 25 g of the inner in each piece of lacón were homogeneized with 100 ml of sterile saline peptone water at 40 to 45 °C for 2 min. in a Stomacher 400 Lab Blender (Seward Medical, London), thus making the 1/5 dilutions. Decimal dilutions were prepared by mixing 10 ml of the previous dilution with 90 ml of 0.1 % sterile peptone water. One ml of each dilution were plated in duplicate in Standard Plate Count Agar + 7.5 % NaCl. Plates were incubated at 30 °C for 48 hours. After incubation, plates with 30 to 300 colonies were counted.

Isolation and identification of strains - At the end of the incubation period, from the count plates, 10 random colonies were taken from each piece (5 from the surface sample and 5 from the inner sample) with the aid of a Harrison disc (Harrigan and McCance, 1976). The 140 strains isolated (75 from the surface and 65 from the inner of the pieces) were purified by means of 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 (*Micrococcaceae*) were identified by comparing their biochemical and culture characteristics with the patterns described by Schleifer et al. (1984) and Schleifer (1986). The strains were assigned to the *Staphylococcus* genus or to the *Micrococcus* genus on the basis of anaerobic fermentacion of glucose and aerobic assimilation of glycerol. According to the methods described by Baird-Parker (1979) and Devriese et al. (1985), each isolate was tested for oxidation and fermentation of glucose; oxidation and fermentation of D-mannitol; growth at 10 °C; growth in presence of 10 and 15 % NaCl; presence of urease, arginie dihydrolase, ornithine decarboxylase, alkaline phosphatase, pyrrolidonyl arylamidase, β-galactosidase, arginine arylamidase, oxidase, β-glucuronidase; acetoin production (Voges-Proskauer reaction); nitrate to nitrite reduction; esculin hydrolysis; novobiocin resistance; acid production aerobically from N-acetyl glucosamine, L-arabinose, D-cellobiose, D-fructose, glycerol, lactose, maltose, D-mannose, raffinose, D-ribose, sucrose, D-trehalose, D-turanose.

RESULTS AND DISCUSSION. Of the 75 strains isolated from the surface of the 15 pieces of lacón studied during the manufacturing and ripening process, 57 were considered to be *Micrococcaceae* (44 from the genus *Staphylococcus* and 13 from the genus *Micrococcus*) and were identified as *Staphylococcus simulans* (11 isolates), *S. xylosus* (10), *S. saprophyticus* (9), *S. sciuri* (6), *S. equorum* (5), *S. capitis* (2), *S. epidermidis* (1), *Micrococcus luteus* (12), and *M. lylae* (1). The distribution of these isolates over the sampling points is shown in Table1.

Of the 65 strains isolated from the inner of the 15 pieces studied, 54 were considered to be *Micrococcaceae* (36 from the *Staphylococcus* genus and 18 from the *Micrococcus* genus) and were identified as *Staphylococcus simulans* (9 isolates), *S. saprophyticus* (8), *S. xylosus* (6), *S. capitis* (6), *S. equorum* (5), *S. sciuri* (1), *S. warneri* (1), *Micrococcus luteus* (17), and *M. varians* (1). Table 2 shows the distribution of these isolates over the sampling points.

Of a total of 140 strains isolated from the plates of Standard Plate Count Agar + 7.5 % NaCl, 111 strains (80 %) were considered to be *Micrococcaceae* which shows that this medium has a fairly high selectivity for the *Micrococcaeae* isolation from lacón.

We have be able to confirm, both on the surface and in the inner of the pieces, a predominance of the staphylococci on the micrococci, a phenomenon previously observed by other authors in different cured meat products (Molina et al., 1989; Kotzekidou, 1992; García et al., 1995).

Staphylococcus simulans, S. saprophyticus, S. equorum and *Micrococcus luteus* were isolated in similar proportions on the surface and in the inner of the pieces, while *S. xylosus* and *S. sciuri* were present more on the surface of the pieces, and *S. capitis* was more

abundant inside the pieces.

S. simulans was the predominant species of staphylococci in lacón. However, it is necessary to point out that this species was not found either on the surface or inside the pieces at the end of the drying-ripening period (see Tables 1 and 2). *S. simulans* was isolated in considerable proportions in other cured meat products such as Spanish beef cecina (García et al., 1995) and Greek basturma (Kotzekidou, 1992). *S. xylosus* and *S. saprophyticus* were isolated in very important percentages both on the surface and inside the pieces of lacón. *S. xylosus* was the most abundant species of staphylococci from dry cured hams (Cornejo and Carrascosa, 1991), bresaola (Bersani et al., 1991), and Bündnerfleisch (Mercier, 1989). *S. saprophyticus* was also isolated in important proportions in Spanish beef cecina (García et al., 1995) and Greek basturma (Kotzekidou, 1992). Another important species in lacón was *S. equorum*; this species, which represented the predominant staphylococci in Spanish beef cecina (García et al., 1995), has been described in fresh meat (Schleifer et al., 1984; Kloos, 1990), however its presence in cured meat-products is very scarce.

With regard to the species of Micrococci, *M. luteus* was by far the most quantitatively important species both on the surface and inside the pieces. It is not normal to find high proportions of micrococci inside the pieces due to their aerobic character. However, some species of micrococci can act as facultative anaerobic. The primary habitat of *M. luteus* is on the skin of mammals (Schleifer, 1986) which is why it is not strange to find their predominant presence both on the surface and inside the pieces of lacón.

The responsability of the isolated staphylococci and micrococci strains in the ripening process of lacón will be determined in the future by comparing their proteolytic and lipolytic activities with the proteolytic and lipolytic phenomena which take place during the ripening of this cured meat-product.

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Table 1.- Changes in the species of *Micrococcaceae* in the surface of the pieces during the manufacturing and ripening of dry cured lacón. Five batches.

	Sampling points		
Species	A	B	C
S. simulans	5	6	-
S .xylosus	3	4	3
S. saprophyticus	4	1	4
S. sciuri	3	-	3
S. equorum	1	4	-
S. capitis	-	-	2
S. epidermidis	-	1	-
M. luteus	2	5	5
M. lylae	-	1	-

Table 2.- Changes in the species of *Micrococcaceae* in the inner of the pieces during the manufacturing and ripening of dry cured lacón. Five batches.

	Sampling points		
Species	A	B	C
S. simulans	5	4	-
S. saprophyticus	2	-	6
S. xylosus	2	4	-
S. capitis	-	1	5
S. equorum	2	2	1
S. sciuri	-	1	-
S. warneri	1	-	-
M. luteus	7	8	2
M. varians	-	-	1