Food Microbiology, 1996, **13**, 375–381

ORIGINAL ARTICLE

Characterization of Enterobacteriaceae strains isolated during industrial processing of dry-cured hams

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Growth trends for the Family Enterobacteriaceae were studied during the industrial fast and slow dry-curing of hams. In both dry-curing processes, levels of the micro-organisms in that Family were low, as was the water activity. In all, a combined total of 280 strains of micro-organisms belonging to the Enterobacteriaceae were isolated during the two dry-curing processes. The number of species identified decreased as curing progressed. At the end of the fast-curing process, Leclercia adecarboxylata *was the only species present, whereas at the end of the slow-curing process, it was the most prevalent species, accompanied by* Klebsiella pneumoniae *subsp.* pneumoniae *and* Enterobacter agglomerans*. The overall hygiene quality of the hams was good because of the technological characteristics of the dry-cured ham processes.*

Introduction

Spanish dry-cured ham is a traditional meat product, that is termed 'Serrano' ham if made from various breeds of white hogs and 'Ibérico' ham if made from Iberian (or black) hogs. Over the period 1978–1987 the annual production was 20–22 million hams (Paz and Hernández 1989). Similar types of ham are produced in Italy (Baldini 1985) and the United States (Karrer et al. 1987).

The Micrococcaceae is the prevalent microbiological group in Serrano hams (Cornejo et al. 1992), with *Staphylococcus xylosus* being the predominant species (Carrascosa and Cornejo 1991). There are also low levels of pathogenic micro-organisms (Marín et al. 1994). However, no information is available of micro-organisms belonging to the Family Enterobacteriaceae, which are considered to be hygienic indicators.

The aim of the present study was, therefore, to consider the evolution of the *Enterobacteriaceae* during industrial dry-curing of Serrano ham in order to improve the knowledge on the succession of the species during processing, and to assess the hygienic level of processes and products.

Materials and Methods

Samples were taken during two industrial dry-curing processes of Spanish Serrano ham manufacture, fast curing and slow curing, each carried out at a separate meat processing plant.

Fast curing

Fast curing was performed on hams with

Received: 2 March 1996

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0740-0020/96/050375+07 $25.00/0

bones, skin and subcutaneous fat present, average weight 8 kg. The fresh hams were dry-salted by piling up the hams with a combination of sea salt, nitrates and nitrites (98 : 0·5 : 1·5), for 9–11 days at 5°C. After salting, the surface of the hams was brush cleaned under running tap water. The salted hams were hung during 25–30 days at 5°C for post-salting, and dried 25–30 days at 15°C and 15–20 days at 20°C. Finally, hams were aged during 15–20 days at 30°C.

Slow curing

Slow curing was performed on hams with bone and fat entire but with the skin partially removed, average weight 11 kg. The fresh hams were dry-salted by piling up the hams with a combination of sea salt, nitrates and nitrites (98 : 1·5 : 0·5), for 8–10 days at an ambient temperature of 5°C. After salting, the surface of the hams was brushed clean under running tap water. The salted hams were hung in cold stores at 5°C for 40–45 days for post-salting, and then dried during 25–30 days at 14°C, 20–25 days at 25°C, 25–30 days at 32°C and 10–15 days at 35°C. Finally hams were aged during 60 days at 18–20°C. During both curing processes the relative humidity decreased from close to 100% to around 60%.

An initial batch of 20 hams was numbered at each factory to take samples during processing. Three hams were used at each sampling time. Samples were taken from the surface of the hams both fresh (F), post-washing (PW), and after post-salting (PS). Inner samples were taken from the inner part of the ham muscles on completion of the post-salting stage (PS), on finalization of drying (FD), and on finalization of the process (FP). Surface samples were taken aseptically using a sterile knife; sample slices were <3 mm thick. The inner samples were taken using a sterile trier. All samples were collected under a laminar-airflow hood. After sampling, 10 g of sample was weighed out and diluted by homogenizing under aseptic conditions in a Masticator blender (IUL) in 90 ml of 0·1% peptone water (Difco). Successive dilutions were performed using the same diluent.

Microbiological assays

Appropriate dilutions were pour-plated on tryptone soya agar (BBL) to obtain aerobic mesophile counts (at 30°C for 3 days), and on VRBG agar (Oxoid), in two layer plates, to obtain Enterobacteriaceae counts (at 37°C for 48 h), that were also determined using the most probable number (MPN) method following UNE standard no. 34-557-83 for meat products (Anon. 1983).

Identification methods

Colonies were isolated from the VRBG agar (Oxoid) in the count plates or after streaking from MPN broth on plate count agar (Difco) for identification. In both cases isolation was carried out randomly and comprised over 50% of the colonies present. Strains were identified according to the identification criteria in Bergey's Manual (Brenner 1984). The following tests were employed: Gram stain, motility, aerobic and anaerobic growth, oxidase and catalase production, oxidation/fermentation of glucose, IMVIC, growth in CNK broth, fermentation of D-xylose, growth on McConkey agar, glucose/lactose use, hydrogen sulphide and urease production, lysine decarboxylase and galactosidase production (Cowan and Steel 1993). These biochemical tests were complemented by the API 20E strip system (Biomerieux).

Physicochemical analysis

Variations in pH were recorded according to the method of Bacus (1984) and water activity (a_w) was estimated according to the method of Palmia (1982).

Results

The value of the coefficient of variation (CV= s.e./$\bar{x}$×100) was less than 30% for all microbiological and physicochemical parameters.

Microbial counts during the fast-curing and slow-curing processes are presented in Table 1. The concentration of enterobacteria on the surface underwent a decline during salting of at least 2 log cycles, probably as a

consequence of the low temperature, progressive dehydration, increased NaCl concentration, and washing. This decrease was not detected for aerobic mesophiles, which increased again post-salting. The inner counts (Table 1) were lower and, in the case of the Enterobacteriaceae, decreased to less than 10 cfu g^{-1} at the end of curing. Nevertheless, this group of bacteria slightly increased from post-salting to finalization of drying in fast dry curing (Table 1). Final counts of aerobic mesophiles were at 10^4 cfu g^{-1}, at the end of both fast curing and slow curing.

Table 2 shows the changes taking place in the physicochemical parameters during fast and slow curing. The pH values were slightly higher than 6, and the a_w values in the finished product had decreased to levels lower than 0·92. The decrease in a_w during the drying phase was lower in the fast process (Table 2).

The results of the taxonomic study of the members of the Enterobacteriaceae are shown in Tables 3 and 4, for fast and slow curing processes, respectively. Using the preliminary tests of Cowan and Steel (1993), only 280 of the 317 strains isolated belonged to the Enterobacteriaceae. The most abundant species was *Leclercia adecarboxylata*, which yielded a total of 57 strains during fast curing (Table 3) and a total of 48 strains during slow curing (Table 4). *Hafnia alvei* was the predominant species in fresh hams during slow curing (Table 4) followed by *Escherichia coli*.

In the fast curing process isolation of *L. adecarboxylata* began post-washing and post-salting (Table 3). In addition, *L. adecarboxylata* was the only enterobacteria detected at the end of processing. The trend during the slow-curing process was similar (Table 4), except for the fact that *L. adecarboxylata* was prevalent from the drying stage. In addition, *Klebsiella pneumoniae* subsp. *pneumoniae* and *Enterobacter agglomerans* were also present at the end of the slow-curing process.

Discussion

During the curing processes studied, the drop in the Enterobacteriaceae counts was con-

Table 1. Microbiological counts during the fast (A) and slow (B) dry-curing processes (cfu or MPN g^{-1})

		Surface counts			Inner counts		
		F*	PW	PS	PS	FD	FP
Aerobic plate count	A	$1·74\times10^5$	$1·07\times10^5$	$7·59\times10^7$	$4·07\times10^4$	$1·58\times10^5$	$3·31\times10^4$
	B	$2·14\times10^6$	$5·01\times10^4$	$7·94\times10^8$	$5·89\times10^5$	$9·33\times10^4$	$2·63\times10^4$
Enterobacteriaceae							
Total count	A	$2·51\times10^3$	$5·90\times10$	<10	$3·80\times10$	$1·20\times10^2$	<10
	B	$1·62\times10^4$	<10	<10	$5·60\times10$	<10	<10
MPN	A	$>2·40\times10^3$	$1·47\times10$	5·47	$8·72\times10$	$1·17\times10$	3·6
	B	$>2·40\times10^3$	6·13	3	<3	$1·42\times10$	4·3

F, fresh ham; PW, post-washing; PS, post-salting; FD, finalization of drying; FP, finalization of the curing process.

Table 2. pH and water activity (a_w) during the fast (A) and slow (B) dry-curing process

		Surface			Inner		
		F	PW	PS	PS	FD	FP
pH	A	6·41	6·01	6·42	6·24	6·30	6·30
	B	6·10	5·90	6·80	6·20	5·96	6·13
a_w	A	—	0·8456	0·9198	0·9441	0·9374	0·9189
	B	—	0·8042	0·9201	0·9294	0·8964	0·8869

F, fresh ham; PW, post-washing; PS, post-salting; FD, finalization of drying; FP, finalization of the curing process.

comitant with the progressive reduction in a_w. Both the Enterobacteriaceae counts and a_w were lower during slow curing, because the technical conditions responsible for the decline, namely, the combination of salting and desiccation, were able to act on the substrate for longer periods during slow curing. A_w at the surface increased post-salting because washing removed the supersaturation of salt from the surface. The increase (one log cycle) recorded for the total Enterobacteriaceae inner counts (Table 1) during fast-curing process might be attributed to the increase in temperature and the slight decrease in a_w (Table 2) taking place from the post-salting to the finalization of drying. A_w values lower than 0·93 have been reported to inhibit enterobacteria in Italian hams (Campanini and Casolari 1983).

Similar enterobacterial counts post-salting have been reported in Italian hams (Baldini et al. 1977, Rackzynski et al. 1978). The increase in aerobic mesophiles on the surface has also been recorded in American hams (Kemp et al. 1978, 1980) and in Spanish hams (Carrascosa et al. 1992). These findings suggest that the nature of the substrate and the technical characteristics of processing exert similar effects on the microbial population present, even though the processes carried out in the different countries are not identical.

The decrease in the number of enterobacteria, the low levels at the end of curing, and

Table 3. Distribution and incidence of enterobacterial species isolated during the fast dry-curing process

Stage	No. of strains	Species identified	I.P.S. (%)
Fresh ham	22	*Escherichia coli*	51·2
	4	*Klebsiella pneumoniae* (subsp. *ozaenae*)	9·3
	3	*Proteus mirabilis*	7·0
	3	*Citrobacter freundii*	7·0
	2	*Yersinia ruckeri*	4·7
	2	*Enterobacter agglomerans*	4·7
	1	*Serratia fonticola*	2·3
	1	*Proteus vulgaris*	2·3
	1	*Serratia plymuthica*	2·3
	1	*Klebsiella oxytoca*	2·3
	1	*E. coli* (inactive)	2·3
	1	*Serratia liquefaciens*	2·3
	1	*Hafnia alvei*	2·3
Post-washing	11	*Leclercia adecarboxylata*	39·3
	5	*H. alvei*	17·8
	4	*C. freundii*	14·3
	4	*S. liquefaciens*	14·3
	2	*Rhanella aquatilis*	7·1
	1	*Enterobacter intermedium*	3·6
	1	*Serratia marcescens*	3·6
Post-salting	25	*L. adecarboxylata*	75·7
	4	*E. intermedium*	12·1
	2	*S. liquefaciens*	6·1
	2	*E. agglomerans*	6·1
Finalization of drying	16	*L. adecarboxylata*	100
Finalization of the curing process	5	*L. adecarboxylata*	100
Total	125		

I.P.S. (%), Incidence per sample of the species identified.

the pH variation, were quite similar to those recorded in Italian (Baldini et al. 1977) and American (Langlois et al. 1979) hams.

E. coli was the most prevalent species in fresh hams during the fast-curing process (Table 3). This species has never been implicated in outbreaks of food poisoning caused by consumption of Spanish Serrano ham (Marín et al. 1991), although it has been involved in outbreaks caused by consumption of other foodstuffs (Notermans et al. 1994). Hence the presence of this organism is indicative of low levels of hygiene, but not necessarily related with faecal contamination.

The number of species present decreased post-washing and during the subsequent stages as processing advanced. The species that persisted until the end of processing were present post-washing but were not present pre-salting. This fact suggests that they have adapted to processing conditions, and therefore, they are present at the processing facility, or that they have a concomitant resistance to salt, which could be species or strain dependent.

According to Bersani et al. (1984) and Hechelmann et al. (1974, 1980), the microorganisms involved in alterations taking place in dry-cured hams during cold storage

Table 4. Distribution and incidence of enterobacterial species isolated during the slow dry-curing process

Stage	No. of strains	Species identified	I.P.S. (%)
Fresh ham	15	*Hafnia alvei*	50·0
	6	*Escherichia coli*	20·0
	3	*Enterobacter intermedium*	10·0
	2	*Citrobacter freundii*	6·7
	2	*Yersinia ruckeri*	6·7
	1	*Klebsiella oxytoca*	3·3
	1	*Edwarsiella ictaluri*	3·3
Post-washing	6	*Leclercia adecarboxylata*	37·5
	6	*Serratia marcescens*	37·5
	1	*C. freundii*	6·2
	1	*Serratia liquefaciens*	6·3
	1	*Klebsiella pneumoniae* (subsp. *pneumoniae*)	6·3
	1	*Klebsiella pneumoniae* (subsp. *ozaenae*)	6·3
Post-salting	4	*S. marcescens*	36·4
	2	*C. freundii*	18·2
	2	*Erwinia* sp.	18·2
	1	*Hafnia alvei*	9·0
	1	*Edwarsiella* sp.	9·0
	1	*Serratia* sp.	9·0
Finalization of drying	26	*Enterobacter cloacae*	34·2
	26	*L. adecarboxylata*	34·2
	11	*C. freundii*	14·5
	8	*Enterobacter* sp.	10·5
	5	*Pantoea agglomerans*	6·6
Finalization of curing	16	*L. adecarboxylata*	72·7
	5	*K. pneumoniae* (subsp. *pneumoniae*)	22·7
	1	*Enterobacter agglomerans*	4·5
Total	155		

I.P.S. (%), Incidence per sample of the species identified.

are cold-tolerant enterobacteria belonging to the genera *Serratia, Proteus, Enterobacter, Citrobacter, Klebsiella, Hafnia,* and *Yersinia,* with *Serratia liquefaciens* being the species most often isolated. Stiles (1981) reported that *Serratia* and *Enterobacter* were the genera most commonly present on working surfaces in the meat processing industry. The species found in this study belonged mainly to the genera listed above, hence, it would seem reasonable to assume that the enterobacteria present in the initial stages of processing are common contaminants to all meat products from warm-blooded animals.

As already stated above, *L. adecarboxylata* was the species most frequently isolated and identified in both slow and fast processes. *L. adecarboxylata,* formerly *Escherichia adecarboxylata* (Leclerc 1962), is one of the pigmented Enterobacteriaceae (Tamura et al. 1986). This species can be found in freshwater, in the environment, and rarely in faeces. It is regarded as a species responsible for the alterations in foodstuffs (Leclerc, 1962).

To our knowledge, the Enterobacteriaceae levels and species found in the processes studied are harmless to man, and therefore, are of minor interest from a hygienic point of view. Faulty curing could explain the presence of banal saprophytic enterobacterial species present in fresh hams, which may sometimes cause alterations in the cured hams, as has also been described in the case of other cured meat products (Hechelmann et al. 1980, Campanini and Casolari, 1983, Lenges 1986).

Acknowledgements

The authors wish to thank Ms María J. Martín and Ms María V. Santamaría for their technical assistance. This study was performed in the framework of CICYT project ALI-92-0145 under the auspices of the Spanish Ministry of Education and Science.

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