Microbial evolution during the curing of Spanish serrano hams. The influence of some preservatives on the microbial flora.

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INTRODUCTION

On the Spanish market coexist different kinds of hams: Spanish serrano hams is the most worthy for consumers because of its quality owing to the pig race, the age, the exercise and the sex.
The crossing of Iberian pigs and Duroc Jersey has lately proliferated having some advantadges and disadvantdges.
In pigs, the amount of intramuscle fat is a race feature and increases with the animal age. Iberian pigs have a great amount of fat veining.
With equal diet, the pure Iberian pig fat is more unsaturated than crossed Iberian pigs, whereas the fat melting point is lower. The higher the percentage of Duroc crossing the higher the melting point.
From a feeding point there are three kinds of Iberian pigs: those fed exclusively on acorn, those on comercial feeding-stuff and those called "recebo" meaning pigs fed on a mixture of those above mentioned.
The best Spanish serrano hams are those from pigs exclusively fed on acorn.
We call acorn the fruit of different trees: Quercus ilex, Quercus suber, Quercus lusitanica.
The manufacturing process of Spanish serrano ham is longer than normal ham because of its great amount of intramuscle fat slowing dehydration and salt equalization.
The juiciness of Spanish serrano ham is due to the stimulating effect of fat and salt upon the salival flow. As the curing process occurs in a non-controlled curing room, ham suffers a high temperature during summer, which produces fat melting. This fat prevents the ham from being affected by atmospheric humidity changes.
The animals must reach enough weight to get a strong depree of fat muscle infiltration and good size of hams. It is important to notice that the amount of veining increases with age.
In Iberian pigs the boar taint problem does not exist, because all animals are castrated in order to get carcasses with greater fat percentage.
In Spain, boric acid was traditionally used as a surface ham preservative. After the restriction of its use by government law many different preservatives appeared on the market.
In this study the evolution of different microbial parameters in several lots of hams was followed seeking the effectiveness of preservatives compared to control hams.

MATERIAL AND METHODS

Two hundred hams from Iberian pigs were selected by parameters of pH $\leq$ 6,2, Ta $\leq$ 4ºC), they were fed on acorn and commercial feeding-stuffs. Hams were subdivised into 5 lots (A, B, C, D, E) a commercial preservative was assigned to each lot and one acted as control (lot A). Preservative B was composed of boric acid. C was benzoic acid, sorbic acid, citric acid, adipic acid, ethyl alcohol, pepper and rice flour as excipient. D was propylic ester of p-hidroxibenzoic acid. E was potassium nitrate, lactic acid, sodium chloride and water as excipient.
Preservatives were added after salting and before salt-equalization. B, C and D were added through a gentle rubbing, and E was injected into 4 different areas of the ham.
Fiteen hams were analized: three from each lot. Internal and superficial samples were taken throughout the process: selection, post-salting, salt equalization, mid-curing and the end of curing.
Superficial sampling was carried out by the swab method in 2 ml. of dilution liquid scanning the sampling area marked by an inox. circle of 26 cm ø. The surface microbiological parameters studied were: aerobic total count and moulds and yeasts.
Internal sampling was carried out by an sterile punch collecting about 20 grs of sample. It was homogenised through an stomacher 400 with peptone 0,1% and Nacl 4% in a proportion of 1:9. Serial decimal dil.lutions were made.
The internal microbiological parameters studied were: aerobic total count, halotolerant flora, Micrococcaceae, lactic acid bacteria, lipolytic microorganisms, Vibrio costicola and Brochothrix thermosphacta.
The culture medium used were: Agar Plate Count (OXOID) for aerobic total count, Tripticasa Soya Agar (OXOID) plus 4% NaCl for halotolerant flora, Mannitol Salt Agar (MERCK) for Micrococcaceae. All microorganisms grown on MSA. have been considered as Micrococcaceae. MRS Agar (OXOID) for lactic acid bacteria in double layer agar and incubated in anaerobiosi Tributirine Agar (OXOID) for lipolytic microorganisms. Gardner Agar (1966) for Brochothix thermosphacta. Classified according to Cantoni (1983). Crystal violet Kannamicina Agar (Gardner 1973) for Vibrio costicola. Classified according to Gardner (1980) sabourand 2% dextrose Agar (MERCK) for yeasts and moulds.
manufacturing process: After quartering, hams were refrigerated to get an internal temperature of 3 ºC approx., salted in piles during 12-15 days at 4 ºC and 85-90% relative humidity. Non-refined salt was used with nitrates as impurity.
After salting, hams stood in a refrigerated chamber for salt-equalization at 4 ºC and 85-90% HR for approx. 3 months, in a controlled curing room for a month and 10-12 months in a non-controlled curing room.
Spanish serrano hams need a long curing time, since the loss of water is difficult owing to the fatness/lean ratio.

RESULTS AND DISCUSSION

1. Surface flora evolution throughout curing

During curing the ham surface changes from fresh and tender meat with high humidity to a product with low aw, a certain degree of salt concentration and good growth of fungi.
In the salt equalization period the surface counts of m.o. rose considerably despite salt concentration because during salting there was a selection of halotolerant flora so aerobic total count and yeasts and moulds selected must be able to grow in a salted medium from 4% to 8% NaCl
In this stage the hams still a fresh product with high surface humidity favouring the growth of bacteria and fungi despite the temperature (4 ºC). Yeasts grow better than fungi. Mucor mucedo is a contaminant species commonly formed in the SE period, it gives a cotton-like aspect to the meat.
The aerobic total count and yeast and mould count stayed the same during the first days in the curing room, nonetheless there was a decrease of aw which encourapes the development of moulds.
The aerobic total count decreased with curing.
The fungi found in hams grow better in xerofiles atmospheres so the decrease of aw favours its growth or maintains it.

2. Internal flora evolution throughout curing

Aerobic total count and halotolerant flora followed a parallel evolution throughout curing (figure B2), having the same counts so, halotolerant microorganisms of ham were viable in a salt-free medium but they achieved a narrow colony diameter.
During salt equalization halotolerant counts decreased because of the atmospheric conditions in the room: law temperature and high relative humidity.
Spanish serrano hams are cured in non-controlled curing rooms. Usually they leave the salt-equalization room in spring, so curing is done at the temperatures and humidities of spring, summer and fall.
While curing aw lesseus, limiting the growth of halotolerant flora and keeping its counts with slight variatiores.
Micrococcaceae : Micrococcus and Staphylococcus coagulasa negatives were the major flora in hams. They kept the same evolution as aerobic total count and the halotolerant flora. There were not any clear conclusions about the role of Micrococcaceae in curing, however there are evidences of a lipolytic action from Micrococcaceae in curing.
Lactic acid bacteria counts diminished during salt-equalization because of its sensitivity to law temperatures.
In the curing room, the concentration rose due to high temperatures favouring its growth. At the end of curing they completely disappeared.
Brochothrix thermosphacta is a microorganism commonly found in vacuum-packaged meat. It was isolated in high counts at thebeginning of the process. The viability of B. thermosphacta lessened as the process progressed. By the end of curing no isolation of this microorganism was achieved. Spanish serrano hams have a major fatness/lean ratio preventing a quick decrease of water activity inside the ham which favours the viability of B. thermosphacta till mid-curing. This is typical of Spanish serrano hams. In Spanish white pig hams Brochothrix thermosphacta disappears immediately after salting.

3. Influence of different preservatives on the surface flora of Spanish serrano hams

As seen in figures A1 and A2 the surface flora was not affected by the different preservatives Each lot of hams with preservatives followed the same evolution of aerobic total count and yeasts and fungi as the control hams.
Hams with boric acid as preservative developped a purple-violet fungi belonging to the genera Aspergillus. This fungi is very appreciated by the "connoisseurs". Itis traditionally thought that purple-violet fungi contributes to thedevelopment of ham organoleptic characteristics because of its lipolytic action on the surface fat.

4. Influence of different preservatives on the internal flora of Spanish serrano hams.

Internal aerobic total count, halotolerant flora and Micrococcaceae concentration (Figures F1, F2,F3)in control and preservatives hams were similar throughout the curing process.
There were no big differences between different preservatives although hams with boric acid had the lowest counts.
According to lactic acid bacteria counts (Figure F4) there were no clear differences between control and preservative hams. However hams injected with preservative E had a higher count in mi-curing than the other hams although this disappeared at the end of curing. In preservative C hams there was no isolation of lactic acid bacteria after the SE period.
Brochothrix thermosphacta was slightly affected by different preservatives (Figure F5).Its counts remained positivein control and preservative C hams until mid-curing. In preservative D and E hams these bacteria lost viability during SE.
Preservative B hams with boric acid did not present this microorganism throughout the process.
Vibrio costicola was not isolated in any case.

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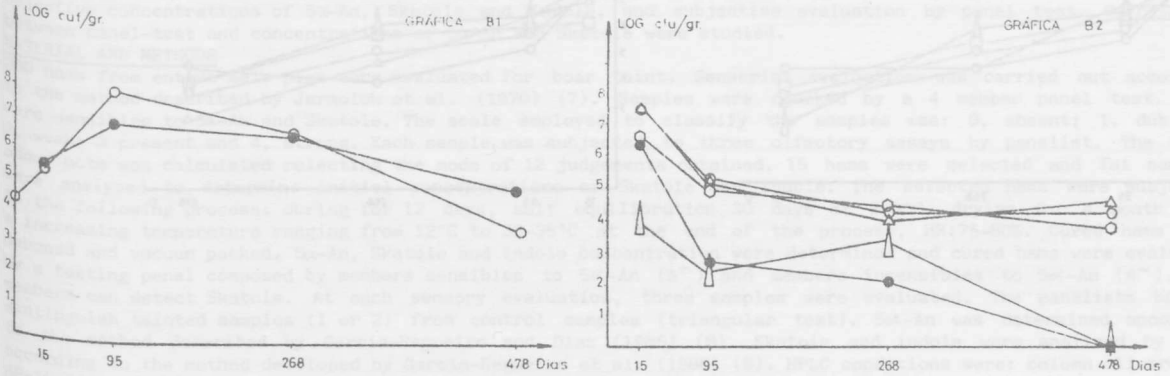
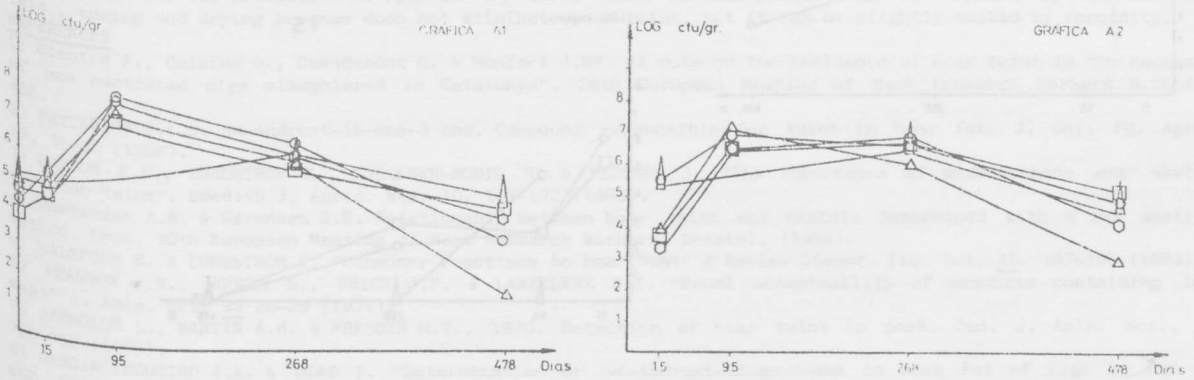
FIGURE B1 - Surface microbial evolution of control hams.
(○ aerobic total count, ● yeasts and moulds).
FIGURE B2 - Internal microbial evolution of control hams.
(○ aerobic total count, △ halotolerant flora, ⬡ Micrococcaceae, ▯ lactic acid bacteria, □ lipolytic microorganisms, /
● Brochothrix thermosphacta).



FIGURES A1 - A2 surface microbial evolution of different lots of hams. With several preservatives (○lot A, △lot B, □lot C, /
⬡lot D, ▯lot E).

A1 - aerobic total count. A2 - yeasts and moulds.



FIGURES F1 - F5. Internal microbial evolution of different lots of hams with several preservatives. (○ lot A, △ lot B, □ lot C, ⬡ lot D, ⏢ lot E).
F1 aerobic total count, F2 yeasts and moulds, F3 Micrococcaceae, F4 acidlactic bacteria, F5 Brochothrix thermosphacta).