## MICROBIAL POPULATION IN ALTERED DRY-CURED HAM

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## SUMMARY

It has been studied the microbial population of altered Iberian dry-cured hams produced in Olivenza, Higuera la Real and Montanchez, (Badajoz. Spain).

The microflora studied was: mesophyles, psychrotrophic and aerobic bacteria, halotolerant microorganisms, proteolytic and lipolytic bacteria, *Enterobacteriaceae*, <u>Clostridium perfringens</u> and other clostridium species with sulphite activity, faecal streptococci, total coliforms, faecal coliforms, *staphylococci*, lactic bacteria and Salmonellae.

The results obtained suggest that alterations might have a microbial origin. Moreover, the data obtained allow to identify the groups of microorganism responsible for hazard alterations, which is an indispensable step in the control system design (HACCP).

# INTRODUCTION

Extremadura is the most important region of Spain in terms of number of reproductive Iberian sows (70.000), representing 65,5 % of national production.

Iberian pig implement his vital cycle in the ecosystem called "Dehesa", which is mainly constitued of acorn trees and mediterranean forest.

In this habitat, Iberian pig is bred in an extensive management system that make it possible to exercise musculature while animals are looking for acorns in the final feeding period, which will have an enormous influence on the quality of the final products obtained.

Iberian dry cured ham production in Extremadura is about 1.200.000 (A.E.C.E.R.I.B.E.R., 1992). It should be noted that a percentage of that production suffer from some kind of hazard alterations that are responsible for important economic losses in dry-cured ham industry.

Iberian dry-cured ham is a high quality product obtained from Iberian pig carcasses after salting and dry-cured maturing process in the cellars.

Dry-cured ham hazard alterations will depend directly on failures produced during the elaboration process and shortcomings of technological methods applied.

Usually, a reduce number of microorganisms, those successfully adapted to the difficult environmental conditions of ham, are responsible for microbiological alterations (Mossel, 1971).

Salt level and desiccation will limit microorganisms growth since both produce a reduction in water activity (aw).

dry-cured ham obtained from Iberian pigs slaughtered in different areas of Extremadura.

Hazard alterations in ham have been studied by many researchers: putrefaction in american ham was investigated by Mundt and Kitchen in 1951. It was Ingram who studied putrefaction in ham in England (1952). Dempster (1985) studied alterations in cured hams injected with brine solution. Zeller and Renz (1967) identified <u>Proteus rettgeri</u> as the microorganism responsible for hazard alterations in cured ham. In Italy, there have been several authors who have investigated the alterations found more frecuently in italian ham. Cantoni et al., (1969); Giollitti et al. (1971b); Simonetti et al (1983) investigated mephitic alteration and found <u>Proteus vulgaris</u> was the microorganism responsible for this alteration.

In Spain, Arnau et al., (1986) found that Enterobacteriaceae were responsible for spoiled cured ham obtained from White Pig breeds. Lara et al., (1989) identified <u>Lactobacillus curvatus</u> as the microorganisms responsible for alterations in Iberian dry-cured ham.

Several german researchers such as Hechelmann et al., (1980); Hechelmann (1986) consider that *Enterobacteriaceae* and *heterofermentative lactobacillus* are responsible for some of the alterations found in dry-cured ham. Lenges (1986) believe that hazard alterations in ham with bone are due to :

- Deficient refrigeration
- High values of pH.
- High temperature during curing process.

Alterations in dry-cured hams are owed to an heterogeneous microflora that occur during the "resting period". This microflora is a combination of harmless and patogeneous microorganisms which will produce an undesirable smell in subsequent processing phases (Poma, 1987).

#### MATERIALS AND METHODS

Fourteen hams from Iberian pigs slaughtered in different areas of Extremadura (Olivenza, Higuera la Real and Montanchez) were analyzed.

The alterations observed were:

- gas production and swelling
- -colour change in the muscle damaged.
- altered vein due to deficient bleeding
- spoiled nut.

Physical and chemical parameters studied were: ph values, aw and humidity.

The microflora studied was: total mesophyles, total psychrotrophic, halotolerant microorganisms, Enterobacteriaceaes totales, faecal group D enterococci, total coliforms faecal coliforms, clostridium species with sulphite activity, <u>Clostridium perfringens</u>, lactic, bacteria, Micrococaceae, <u>Staphylococcus aureus</u> coagulase positive and Salmonellae.

Surface samples (S), using a square of 25 cm area and 2/3 mm. in thickness as meassurement technique, from *Gracilis* muscle and samples from deep altered musculature (D) were taken under sterile conditions.

Surface and deep samples of 10 gr. each were homogenized with 90 ml. of peptone water in Stomacher. A series of decimal dilutions of the samples were plated into or spread on various specific media and incubated at the optimum temperature for growth and subsequent counting.

For the detection of Salmonellae, 25 gr. samples of each ham were transferred into flasks containing 225 ml. of buffered peptone water.

The methods used for enumeration were:

- mass dilution plate counts
- surface spread plate counts
- most probable number technique (NMP)
- presence and absence in 25 g samples.

Microbiological analyses carried out were:

Total mesophyles on PCA after 3 day incubation at 30°C, total psychrotrophic on PCA after 5 day incubation at 7°C, halotolerant on TSA after 48-72 hours incubation at 30°C., *clostridium* species with sulphite activity on SPS (anaerobic conditions) after 48 hours incubation at 37°C, <u>Clostridium perfringens</u> on TSN (anaerobic conditions) after 24 hours incubation at 44°C, faecal group D *enterococci* on SB after 24-48 hours incubation at 44° C, total *Enterobacteriaceae* on VRBG after 24-48 hours incubation at 37°C, total *coliforms* on VB after 24-48 hours incubation at 37°C using NMP count technique, faecal *coliforms* on VB after 24-48 hours incubation at 44°C, lactic bacteria on ROGOSA (microaerophilic conditions) after 3-5 days incubation at 30°C, *Micrococcaceae* on MSA after 48 hours incubation at 37°C, *staphylococci* on BP after 48 hours incubation at 37°C, *Salmonellae* on SELENITE CYSTINE BROTH and 24 hour incubation at 37°C, then transferred into XLD and AH.

pH values were determined with a Crison 2002 phmeter, aw with electronic Rotroning and humidity by Standard ISO-1442.

## RESULTS AND DISCUSSION

The results of microbiological examination of 14 hams with some kind of alteration are reported in table 1. The counts were analysed after transformation into logarithms Colony Forming Units (lg CFU). **JO** is the abbreviation of hams from Olivenza, **JH** stands for hams from Higuera la Real, and **JM** for hams from Montanchez.

Table 2 shows the results obtained by the same group of researchers in a preliminary microbiological <sup>study</sup> of 9 iberian dry-cured hams with no apparent spoilage processed in the same three slaugterhouses (Higuera, Olivenza, Montanchez).

Comparing table 1 and table 2, some differences between both batches (spoiled and non-spoiled hams) should be noted :

- A higher total amount of microorganism in spoiled hams.

-A higher count of psychrotrophics in spoiled hams.

-High counts of Enterobacteriaceae in some spoiled hams.

-Higher counts of lactic bacteria in spoiled hams.

-Higher counts of Micrococaceae in spoiled hams.

The results demonstrated that in many of the spoiled hams studied, the microflora that might be responsible for those alterations is *lactic bacteria*, since the number of *Lactobacilli* found in spoiled hams (2,30-6,4 lg CFU) is much higher than that found in non-spoiled ones (0-1 lg CFU). This theory is supported by several researchers : Hechelman et al., (1980) sostein that heterofermentative *lactobacillus* cause dry-cured ham spoilage. Lara et al., (1989) isolated and identified <u>Lactobacillus curvatus</u> as responsible for hazard alterations in iberian dry-cured hams. <u>Lactobacillus viridescens</u> presence was first demonstrated in green coloured hams by Evans and Niven Jr. (1951), Niven et al., (1949), Niven Jr. and Evans (1956).

*Micrococaceae* and *staphilococci* counts from deep altered muscle samples,(2,79-6,97 lg CFU) and 3,07-6,13 lg CFU) respectively, are clearly higher in spoiled hams than in non-spoiled ones (2,59 lg CFU). This contamination might be due to an inappropriate temperature (Marin, 1990).

Enterobacteriaceae counts in five of fourteen spoiled hams were much higher than that found in nonaltered hams. Those results are corroborated by Arnau et al., (1986) and Hechelman et al., (1980). Hechelman et al., (1986) mantain that Enterobacteriaceae such as Serratia, Proteus and Enterobacter, are psychrotrophics and thus grow more easily in iberian dry-cured ham during the salting period than other microorganisms since temperature is kept low in that process. This fact would be in accordance with the high counts of psychrotrophic found in our study. Simonetti et al., (1983) and Zeller and Renz (1967) also believe Enterobacteriaceae to be responsible for the spoilage.

From the previus resuls we conclude that, in most cases, different species of microorganisms might be responsible for iberian dry-cured ham hazard alterations. This hypothesis is corroborated by studies made in dry-cured hams obtained from white pig breeds, showing that ham spoilage might be due to a variety of undesirable microorganisms found in altered hams in much higher number than that found in non-altered ones. Marin (1990).

No evidence was obtained of growth of *Clostridium* with sulphite activity nor <u>Clostridium</u> <u>Perfringens</u> nor <u>Salmonellae</u>. The high counts obtained seem to be owed to the fact that spoiled hams present higher humiditiy and aw than non-spoiled ones, as shown in table 3.

# CONCLUSIONS

\*The high counts of Enterobacteriaceae Micrococaceae and lactic bacteria found in altered hams indicate that these microorganisms might be responsible for the hazard alterations.

\*The microflora mentioned above should be considered as a group of risk of hazard alterations in control system designs such as HACCP.

\*The reason for such a high counts might be shortcomings in the technology applied: high humidity ,non appropriate temperature in first processing steps, and high aw values

\*Although some hams from Montanchez had been rejected by consumers, they did not show apparent microbiological alterations.

\*No evidence of growth of harmful microorganisms sush as <u>Clostridium perfringens</u>, Salmonellae, positive coagulase staphylococi or enterotoxic Escherichia coli, was found.

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