RELATIONSHIP BETWEEN MICROBIAL POPULATION AND PHYSICO-CHEMICAL PARAMETERS IN BONE TAINTED DRY-CURED HAMS

Yolanda Miranda, Marta Ordoñez, Isabel Jaime, Jordi Rovira.
Department of Biotechnology and Food Science. University of Burgos. Plaza Misael Bañuelos s/n. 09001 Burgos. Spain.

Background

Spoilage of dry cured ham during its manufacture process is a serious problem that can make impossible its human comsuption. Spoilage can be related to different causes and it can be seen in various presentations, one of the most important is the bone tainted hams which lies in a deep putrefaction of ham that causes bad smell, presence of gas and a modification of tissue texture. The presence of gas induces a swollen appearance of the ham. Putrefaction is probably linked to microbial action, and it seems that different members of the family *Enterobacteriaceae* play a main role in this process (Arnau et al., 1987; Marin et al., 1992; Sanabria et al., 1997). By the moment, some of the bacterial species isolated from swollen hams are *Proteus vulgaris*, *Serratia liquefaciens* and *Leclercia adecarboxylata* (Sanabria et al., 1997; Marin et al., 1992).

Objectives: The purpose of this study was to evaluate the physico-chemical parameters of swollen hams and the bacterial microbiota involved in this alteration process, comparing them with normal ham.

Methods

Samples: Four swollen hams (SH) and an unspoiled control (CH) were used. The hams were classified as spoiled by experts and all of them showed an external swollen. Hams analysed were in the fifth month of the ageing period, and they were chosen from a batch that follows a slow dry-cured process (12 months). Samples were taken from the area of the ham which includes the muscles: *biceps femoris*, *semitendinosus*, *semimembranosus* and *gracilis*. This studied area was divided in three different zones (A-C) for facilitating the study of relation that could exit between physicho-chemical parameters and the microbiota of the ham. The areas included the zone close to fat (A) that comprises part of *biceps femoris*, a medium zone (B) including part of *biceps femoris* and part of *semitendinosus* and *semimembranosus* muscles, and the zone linked to the other surface of the ham (C) that includes as well a part of *semimembranosus* and *gracilis* muscles.

Microbiological methodology: Samples were taken under aseptic conditions from the inner part of each described zone. Twenty-five grams of each sample were homogenised in 225 ml of Ringers´ solution. Homogenates were diluted consecutively 1:10 in this solution and colony counts were achieved in different media as described, depending on the bacterial group studied: Total viable count (TVC) were isolated on PCA agar at 30 °C, 3 days; total psycrotrophic bacteria on PCA agar but incubation was at 7 C, 5 days; total anaerobes onto Schaedler agar (anaerobic conditions) at 37°C, 48 h; *Enterobacteriaceae* onto VRBG agar at 37°C, 48h. Identification of *Enterobacteriaceae* was carried out from Hektoen plates, previously inoculated. Lactic acid bacteria (LAB) were isolated onto MRS agar (microaerobic conditions) at 37°C, 48h.; *Clostridium perfringens* on TSN at 37°C, 48h; *Micrococaceae* onto MSA agar at 37°C, 48h.

Identification methods: Colonies of *Enterobacteriaceae* isolated from the VRBG agar or Hektoen agar plates were grown in nutritive agar. According to the criteria in Bergeys Manual, the following tests were carried out: Gram stain, oxidase and catalase production. The identification was carried out following the commercial method API 20E (BióMerieux).

Physico-chemical analysis: In each of the described zones, were determined: pH, moisture calculated measuring weight loss at 103±2°C at constant weight, chlorides by Method of Charpentier-Volhard and water activity (Aw) with AquaLAB CX-2.

Results and discussion

The physico-chemical characteristics of the studied hams are described in Table 1. Comparison between normal/unspoiled and swollen hams shows the presence of some distinctive physico-chemical characteristics. Chloride concentration was clearly lower in swollen hams, and the pH was higher than normal hams (6.3 *vs* 5.8). Regarding to the moisture and the Aw, the data show that there are remarkable differences among the established zones, being the moisture and the Aw higher in the zones A and B than in zone C. This fact also stands when the comparison is done between swollen hams and control ham.

Microbiological data are shown in table 2 and 3. Bacterial counts are increased in spoiled hams compared with the control ham in agreement with the findings reported by Sanabria et al. (1994), and they are higher (10^3-10^8 ufc/g) than the counts found in bone tainted/swollen hams by other authors (10^2-10^3 ufc/g) (Marin et al., 1992). This result could be associated to the period of ageing of the hams. These authors analysed hams from nine to twelve months and in this study earlier ageing hams were used.

In the spoiled hams the colonisation of zones A and B was higher than in zone C. This factor would be in accordance with the increase in moisture and Aw in those zones.

High *Enterobacteriaceae* counts were isolated in all spoiled hams (Sanabria et al., 1994; Marin et al., 1992). The bacterial species identified that belong to this family were *Proteus mirabilis*, *Hafnia alvei*, *Serratia liquefaciens*, other species of *Serratia* and *Erwinia* spp. These species have a number of characteristics, such as their proteolytic ability and their psicrotolerant behaviour, that allow their adaptation at this micro-environment, their rapid multiplication and the production of different compounds in the hams that contribute to the deterioration of the product.

An interesting fact was the presence of different species in each ham studied. The isolation of different species arises some interesting queries related with the spoiling capacity of each microbial species. The most spoiled and swollen ham (SH 1) showed a great reduction in chloride

concentration and the species isolated from this ham was *Proteus mirabilis*, not previously described. *Hafnia alvei* was isolated from other ham (SH 4). In the second and third spoiled hams, mixed cultures were obtained including *Serratia liquefaciens* and *Erwinia* spp., *Serratia liquefaciens*, *Serratia plymuthica*, and *Erwinia* spp., respectively. Carrascosa *et al.* described *Proteus vulgaris* as an important pathogen involved in ham spoilage (Sanabria et al., 1997). Our findings suggested that other species of *Proteus*, as *Proteus mirabilis* can be related to this form of food spoilage.

Clostridia and lactic acid bacteria were not isolated from the samples of the studied hams.

Conclusions

- The bone tainted hams analysed in earlier stages of the dry-cured process present higher counts of microorganisms than hams analysed in later stages.
- In the spoiled hams the colonization in the muscles of external (close to fat) and the medium zones is higher than in muscles of internal zone. This fact would be in accordance with the increase in moisture and Aw in these zones.
- The high counts of *Enterobacteriaceae* in swollen hams induce to consider that this bacterial group could be the causing agent of this alteration.
- The most spoiled and swollen ham showed the least chloride concentration and the species isolated from this ham was *Proteus mirabilis*, not previously described.

In view of the results obtained, the spoiling ability of *Proteus mirabilis* is going to be studied experimentally in brief.

References

Arnau J., Hugas M., Monfort J.M.(1987). Defectos en el jamòn curado. En "Jamón Curado: Aspectos Técnicos".IRTA, Barcelona.

Marín M.E., de la Rosa M.C., Carrascosa A.V. and Cornejo I. (1992). Microbiological and physico-chemical aspects of spoiled dry-curec Spanish hams. Fleischwirtsh. 72, 1553-1557.

Sanabria C., Fallola A., Sabio E., Vidal-Aragón M.C., Carrascosa A., Ferrera J.L. (1994). Microbial population in altered dry-cured ham. 40th ICoMST. The Hague, Netherlands.

Sanabria C. , Fallola A., Carrascosa A.V. (1997): Inducción de alteración microbiana en jamón ibérico. Eurocarne 59,49-56.

Table 1. Physicho-chemical characteristics of the normal and swollen hams studied.

	SH 1			SH 2			SH 3			SH 4			CH	
Variables	A	B	C	A	B	C	A	B	C	A	B	C	BF	SM
Aw	0.969	0.963	0.962	0.947	0.944	0.926	0.965	0.964	0.954	0.955	0.957	0.945	0.943	0,935
Moisture %	66.3	61.5	56.3	63.1	62.8	55.3	66.0	65.6	59.9	61.2	65.2	59.8	62.6	58.5
Chlorides %	5.4	4.7	5.1	11,2	10.6	9.2	6.7	7.6	6.6	6.3	7.8	5.1	11,9	12,2
pH		6.3			6.1			6.2			6.2		5.8	

Chlorides % in dry matter (DM) SM: *semimembranosus* BF: *biceps femoris*

Table 2. Microbiological population in swollen hams and control one: colony counts.

	SH 1			SH 2			SH 3			SH 4	CH
Microorg. (c.f.u/g)	A	B	C	A	B	C	A	B	C		
TVC	$2.4*10^7$	$1.1*10^8$	$8.5*10^5$	$1.1*10^4$	$8*10^6$	$5.0*10^1$	$2.1*10^5$	$1,8*10^4$	$1.8*10^3$	$2.5*10^6$	$9.0*10^1$
Psychrotrophics	$1.8*10^2$	$1.2*10^2$	$6,3*10^3$	$1.0*10^4$	$1.6*10^7$	—	$9.0*10^4$	$1.6*10^4$	$1.9*10^3$	$2.6*10^6$	—
Anaerobe	$3.5*10^7$	$1.0*10^8$	$5.5*10^4$	$4.6*10^3$	$9.7*10^6$	—	$4.1*10^4$	$9.3*10^3$	$1.6*10^3$	$2.9*10^4$	$1.0*10^2$
Enterobacteriaceae	$1.2*10^7$	$4.6*10^7$	$3.9*10^5$	$1.9*10^3$	$1.4*10^3$	—	$3.1*10^3$	$9.5*10^2$	—	$7.0*10^3$	—
Lactic acid bacteria	—	—	—	—	—	—	—	—	—	—	—
Cl. perfringens	—	—	—	—	—	—	—	—	—	—	—
Gram positive cocci	—	—	—	—	$4.0*10^1$	—	—	$2.9*10^2$	$2.3*10^2$	$4.6*10^6$	$7.0*10^3$

Table 3. Distribution of different *Enterobacteriaceae* in the studied hams.

	Identified Species
SH 1	*Proteus mirabilis*
SH 2	*Serratia liquefaciens*
	Erwinia spp.
SH 3	*Serratia liquefaciens*
	Erwinia spp.
	Serratia plymuthica
SH 4	*Hafnia alvei*