MICROBIAL EVOLUTION IN VACUUM-PACKAGED DRY-CURED HAM

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SUMMARY

The evolution of the microbial population in vacuum-packaged Iberian dry-cured ham has been studied. The raw material was dry-cured ham processed during 12 months by craft methods. Samples of unitary portions and slices were vacuum-packaged and stored at a temperature of 7°C\1.
Microbial analyses carried out on this material were: total amounts of mesophyles and psychrotrophics, yeasts, moulds, lactobacilli, Brochothrix termosphacta, Micrococcaceae, coliforms, Escherichia coli, group D streptococci, sulphite-reducing clostridia spores, positive-coagulase staphylococci and Salmonellae. The microbiological analyses were carried out before the vacuum-packaging process and after 2, 5 and 8 months of cold storage.
The results obtained show that microbial population change during storage and it is also affected by sample presentation (portions and slices) . However, the results make evident that the craft method studied assured the hygienic and sanitary quality of the Iberian dry-cured ham. At the same time, the vacuum-packaging process seems to guarantee the quality of this product for, at least, 8 months. So, this conservation method could be adequate for commercial distribution.
Possible microbiological risk groups of slicing and vacuum-packaging of Iberian dry-cured ham are pointed out; so as critical control points of this process in order to establish them on HACCP system for Iberian dry-cured ham.

Introduction-

The packaging of alimentary products, among other aspects, protects them from microbiological changes and from the deterioration caused by agents like oxygen, humidity and light; it prevents the transmission of strange odors and the loss of individual smell, and it may still increase their quality during the cure (STEWART and AMERINE, 1973).
From the microbiological development point of view, vacuum-packaging creates a reducing environment which doesn't allow the development of aerobic microorganisms, presenting, however, favorable conditions to the development of an anaerobic fauna, where we can stand out the Clostridium botulinum. Thus, it is necessary to prevent possible risks.
In the perspective of using packaging during the technologic process of the dry-cured ham, KEMP et al. (1983, 1986) studied the use of this type of packaging from a certain phase of the process in order to reduce the time of cure and the loss of weight.
The selling of vacuum-packed dry-cured ham is getting more and more common, rendering the product more accessible and increasing, at the same time, its shelf life.
This work studies the evolution of the microbiological flora from samples of Iberian pig vacuum-packed dry-cured ham; this kind of pig is a rustic breed which production is limited to the southwest of the Iberian peninsula. It is the first time that such study is done about the Iberian pig dry-cured ham.
The production of this kind of dry-cured ham has an increasing role in the evolution of the economy in disfavored areas lacking resources.

Material and methods

There were used five Iberian dry-cured hams submmitted to a cure of twelve months after salting. The material in study was craft made. There was a salting period of 25 days, in a natural environment, with a temperature between 10 and 14°C and a relative humidity between 65 and 85%. The post-salting period lasted 21 days under environmental conditions similar to those of the salting period. The drying process lasted 120 days with temperatures between 12°C in the begining, and 26°C in the end. The relative humidity ranged between 75% and 55%. Maturation was done in a cold cellar.

After taking the bones out of the hams, using cross cuts regarding the longitudinal axis in each one of them there were collected 7 samples with an unitary weight of about 250g: 4 unitary portions and 3 groups constituted of homogeneous slices. One portion of each dry-cured ham was analysed the very day it was cuted; the other samples were vacuum-packed and kept in a temperature of 7°C\1. After 2, 5 and 8 months of conservation two samples of each dry-cured ham were analysed: an unitary portion and a whole of slices.

The used package was a multicover film, composed by polyethylene, ethylvinylalcool and polyamide. According to the producer, the film is permeable to the oxygen (35cm3/m2/24h/bar) to the carbonic gas (150 cm3/m2/24h/bar) and to the water vapour (20g/m2/24h).

The microbiological determinations consisted in the counting of the following microorganisms: total mesophyles (culture medium referred in Table 1, incubation at 30°C for 48 hours), psychrotrophics (culture medium referred in Table 1, incubation at 6,5°C during a period of 10 days), moulds and yeasts (culture medium refered in Table 1, added with 0,5% of Kanamicin, incubation at 25°C for 5 days), Brochothrix thermosphacta (Gardner medium (COLLINS and LYNE, 1989), incubation at 20°C for 5 days), lactobacilli (Man, Rogosa and Sharp -Difco-, incubation at 30° C for 74 hours), Micrococaccaeae (Manitol Salt Agar -Difco-, incubation at 30°C for 72 hours), colifoms (Brilliant Green Bile 2% -Difco-, incubation at 30°C for 48 hours) and Escherichia coli (Brilliant Green Bile 2% -Difco- and Peptone 1% -Difco-, incubation at 44,5°C for 48 hours), group D streptococci (Kanamicin Aesculin Azide Agar -Oxoid-, incubation at 37°C for 48 hours), sulphite-reducing clostridia spores (Sulfit Polimixin Sulfadiazine Agar -Difco-, incubation at 44,5°C for 72 hours), positive-coagulase staphylococci (Baird-Parker Medium -Oxoid-, incubation at 37°C for 24 hours) and the search for Salmonellae. This seach required the following: previous enrichment of Buffered Peptone Water -Oxoid-, incubation at 37°C for 18 hours, enrichment of Tetrathionate Broth Base -Difco- and Selenite Broth -Difco-, incubation at 42°C and 37°C, respectively, for 24+24 hours. Isolation in Brilliant Green Agar -Oxoid- and Desoxycholate Citrate Agar -Oxoid-, incubation at 37°C for 24+24 hours. Suspect colonies inoculated in Triple Sugar Iron Agar -Difco-, incubated at 37°C for 24 hours.

The counting of total mesophyle and psychrotrophic microorganisms, moulds and yeasts, Brochothrix thermosphacta and lactobacilli was carried out for each of the samples under study. The remaining determinations, in each period of analysis, were made after a compound sample representing the surface and depth of all the samples conserved in unitary portions and in slices.

Several variance analyses were carried out taking into account the Sample presentation (with 2 levels: portions and slices) and Time (with 4 levels: 0, 2, 5 and 8 months) factors.

Results and discussion

Overall, the countings of mesophyle and psychrotrophic microorganisms, and yeasts showed an increase between periods of 0 and 2 months, a decrease between periods of 2 and 5 months and another increase up to the period of 8 months (Figures 1, 2 and 3). These results may be due to the remaining oxygen and the increase in water activity (a_w) after packaging, followed by a decrease of aw and oxygen intake. The microbiological development between the periods of 5 and 8 months may be due to a possible loss of vacuum. The variations of the a_w values are confirmed by ELIAS (1993), with a similar material.

A microscopic examination of the preparations of colonies, after coloration, showed that the psychrotrophic microflora was almost exclusively composed by yeasts.

In works carried out with Parma dry-cured ham, GIOLITTI et al. (1971) obtained values for the mesophyle flora inferior to 10^2 c.f.u. g^{-1} inside the muscular masses and between 10^2 and 10^4 c.f.u. g^{-1} on the surface fat. CORNEJO et al. (1988) in works done with white breed cured ham, in the begining of the drying period obtained countings on the surface of $77x10^6$ c.f.u. g^{-1} for mesophyle microorganisms and of $90x10^4$ c.f.u. g^{-1} for psychrotrophics. On the surface of the Parma dry-cured ham with a cure period of 13 months CANTONI et al. (1971) obtained yeast countings of $30x10^4$ c.f.u. g^{-1}. For the surface of Serrano dry-cured ham GIMÉNEZ (1992) obtained yeast countings of 10^5 u.f.c. g^{-1}.

The mould countings obtained relatively low results (Figure 4), with a maximum of $19x10^2$ c.f.u. g^{-1} in the period of 0 months.
The counting of lactobacilli (Figure 5) showed decreasing values during the conservation time and the results of the period of 8 months were inferior to the detection limit of the used method. These results may be explained by the advanced level of maturation of the studied material. CANTONI et al. (1971), with samples taken from the surface of the Parma dry-cured ham with a cure period of 13 months obtained values of lactobacilli inferior to 50 c.f.u. g^{-1}. An identical result was obtained by GIOLITTI et al. (1971) when they analysed representative depth samples of the same type of dry-cured ham with a cure period of 12 months. RACZYNSKI et al. (1978) studied the surface flora of two groups of Parma dry-cured ham, one with 148 days and another with 170 days and obtained countings of $200x10^4$ and $6x10^4$ c.f.u. g^{-1}, respectively. DELLAGLIO et al. (1984) studied the lactic acid flora of the vacuum-packed San Daniele dry-cured ham and verified that the dominant species were Lactobacillus curvatus and L. casei. According to several authors (SCHLLINGER and LÜCKE, 1988; DEBEVERE, 1989) spoilage of vacuum-packed meat products is specially done by lactic acid bacteria.
The evolution of the countings of Micrococcaceae (Figures 6 and 7) showed superior results in the countings carried out on the surface, probably because they were aerobic microorganisms (LÜCKE, 1986). In the Parma dry-cured ham with a cure period of 13 months CANTONI et al. (1971) found, on the surface, contents of micrococci of $12,5x10^4$ c.f.u. g^{-1} and GIOLITTI et al. (1971) in deeper parts of the same type of dry-cured ham with a cure period of 12 months obtained countings inferior to 500 c.f.u. g^{-1}. POMA (1987), for the San Daniele and Parma dry-cured hams refers countings of micrococci of 10^6 c.f.u. g^{-1} at the end of the salting period and between 10^2 and 10^4 c.f.u. g^{-1} at the end of the post-salting period. BALDINI et al. (1977) and RACZYNSKY et al. (1978), refer concentrations of Micrococcaceae in the beginning of the drying period of 10^7 c.f.u. g^{-1}.
According to the opinion of several authors (BALDINI et al., 1977; RACZYNSKY et al., 1978; ARNAU, 1987; POMA, 1987; CARRASCOSA et al., 1989; GIMÉNEZ, 1992) the Micrococcaceae family composes the major flora of the dry-cured ham.
The countings of Brochothrix thermosphacta produced inferior results at the detection limit of the used method in each analysed sample. The fact that the B. thermosphacta, although an optional aerobic, develops better in anaerobiosis (SNEATH and JONES, 1986; TAYLOR et al., 1990) and the maturation level of the dry-cured ham used in this work, which is related to relatively low values of pH (between 5,64 and 6,16) and of a_w (between 0,822 and 0,894) (ELIAS, 1993), may explain the obtained results.
The variance analysis, regarding the Sample presentation factor, only showed significant differences ($p\backslash 0,05$) in the countings of psychrotrophic microorganims and yeasts (Tables 3 and 4) corresponding, in both cases, the higher results to the slice samples.
As far as the Time factor is concerned, only the countings of mesophyle microorganisms and yeasts (Tables 2 and 4) show significant differences ($p\backslash 0,05$) among the results obtained in the different times of analysis.
The higienic and sanitary control was carried out through the countings of the coliforms and of Escherichia coli, of group D streptococci, of sulphite-reducing clostridia spores and of psitive-coagulase staphylococci as well as through the search for Salmonellae. The obtained results are exposed in Tables 9 to 12.
The counting of E. coli produced inferior results to the limit of detection of the used method and the search for Salmonnellae was always negative.
These results suggest adequate higienic conditions as well as good manufacturing processes .

Conclusions

The obtained results don't allow us to affirm that there is an influence between the way the samples to be conserved (portions or slices) are prepared and the microbiological development, however the countings of psychrotrophic microorganisms and yeasts were significantly ($p\backslash 0,05$) superior in the samples kept in an assemblage of slices.
Comparing the results obtained in the several analyses carried out on different days there were significant differences ($p\backslash 0,05$) for the countings of mesophyle microorganisms and yeasts. However, the results obtained at the period of time of 8 months and at the period of time of 0 months were never significantly different.
The results of the higienic and sanitary control suggest that it is possible, under the conditions adopted for this work, to conserve dry-cured ham during a period of time of 8 months, at least, without affecting negatively the salubriousness of the product.

The anatomic structure of the Iberian dry cured ham is interrupted through the chopping before the vacuum-packaging. This practice removes the most efficient critical control point concerning the microbiological risk (CCP1). During the conservation of the vacuum-packed Iberian dry-cured ham, refrigeration must be considered the CCP1 of the product.
The Staphylococcus aureus and the Clostridium botunilum, although not detected, must be considered groups of epidemiologic risk. The yeasts shoul be considered as a group of alterative risk.

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