# *Decontamination technologies of carcasses and fresh meat*

# APPLICATION OF HIGH PRESSURE TO IMPROVE SHELF-LIFE OF THE TYPICAL BLOOD SAUSAGE "MORCILLA DE BURGOS"

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## Background

"Morcilla de Burgos" is a typical cooked blood sausage very popular in Spain. It consists of a mixture of onion, rice, animal fat, blood, different spices and salt stuffed in a natural pork or beef casing. The product is cooked for one hour at 94-95ºC, air cooled to 8-10ºC and finally chilled stored at 4ºC. In general, its composition resembles the English black pudding.

Since the product is subjected to high-temperature cooking during processing, the initial microbiological population of the finished product is very low and the surfaces of the cooked products can be considered sterile. The product is, however, handed after cooking which leads to post-cooking contamination. The yield and type of the microbial contamination of the surface determines the self-life of the product. If the product is packaged under vacuum or modified atmosphere, the dominant spoilage flora will consist of lactic acid bacteria (LAB) (Santos 2001). The typical sensory changes occurring in spoilage of "morcilla de Burgos" are: blowing of the packs, development of drip, slime formation and souring of the product. In some packages blowing has been very pronounced. The self-life of the product packaged under low permeability films has varied from 2 weeks to 6 weeks depending on the initial level of LAB and the presence of particularly active spoilage strains.

The application of high pressure has revealed as a good method to improve the shelf-life of different vacuum-package foods, included meat products as it has been described in Cheftel and Culioli (1997), Lucore at al.(2000) and Sung-Won-Park et al. (2001). Even do some Spanish meat companies use high pressure processing to improve shelf-life of some sliced vacuum packaged meat products.

## Objective

The aim of this study was to evaluate the effect of the high pressure on the microbiota of the blood sausage "Morcilla de Burgos" in order to improve the shelf-life. In this sense, it was also important to evaluate the impact of high pressures to the sensory characteristics of this product.

## Material and Methods

Samples: Two batches of 68 morcillas were stuffed in pork casing and vacuum-packaged in the factory, after cooking and cooling. In each batch, 20 morcillas were used to carry out the sensory analysis and 48 were selected for microbiological analysis.

Treatments: These two batches of morcilla were divided in 3 groups each one, according to the following treatments. Group A was designed as control, group B was treated with 300 MPa and group C with 500 MPa. The treatment time was in both treated groups 10 min and room temperature water was used (15ºC). For the high pressure treatment it was used a pilot plant equipment WAFE-5000/110 developed by NC Hyperbaric (Nicolas Correa Group. Burgos. Spain). This equipment consist of an horizontal vessel of 300 mm diameter and 2000 mm ca of length, with a total capacity of 110 L.

Microbiology analysis: 7 microbiology parameters were measured on day 0 before the high pressure treatment, day 1 the day after treatment, and days 5, 18 and 25. During all this period of time morcillas were kept in refrigeration at 4ºC. The microbiology parameters analysed were: Total viable count (TVC) using pouring-plate method on PCA agar, incubated at 30ºC for 48h. LAB on MRS agar at 30ºC for 48h in anaerobic conditions. Plates were kept in incubating oven in $CO_2$ rich atmosphere (6,0%). *Enterobacteriaceae* on VRBGA agar, double layer pouring-plate method, incubation 37ºC for 24h. *Staphylococcus aureus* on Baird-Parker agar supplemented with egg yolk sodium tellurite emulsion and incubated at 37ºC for 48h. *Pseudomonas sp.* on Pseudomonas agar supplemented with CFC, incubation 30ºC for 48h. *Enterococcus* on Slanetz and Bartley agar, incubation 37ºC for 48h. *Clostridium perfringens* on TSN agar, using the pouring method, and incubated at 37ºC for 48h. Anaerobic conditions were achieved by pouring on solid TSN agar approx. 20mm high agar sealing tap. Two samples of 25g of morcilla were taken to analysis from each treatment. Samples consisted of morcilla slices with casing.

pH along the preservation time was measured with a penetration probe.

Sensory analysis: A discriminatory test was done, comparing the control (without pressure treatment) with morcilla A (300 MPa) or morcilla B (500 MPa) by means of a triangle test. Sensory evaluation was done by a panel consisting of 30 not trained panellists. Panellits were asked to found overall differences between samples. Morcilla was served to the panellist in slices of 1 cm thickness and cooked in microwave till 70ºC in the core. This test was done for each batch.

## Results and Discussion

After the high-pressure treatments the appearance of the morcilla packages were similar to those of the control.

Table 1 shows the results for all microbiological parameters analysed, except for *Clostridium perfringens* that are not shown because no colonies were identified during all the study. These results express the average of the duplicates of the two batches. According to the results it is possible to establish that high pressure is a very effective method to reduce the population of some bacterial groups analysed. This is the case of Gram negative bacteria studied, as *Pseudomonas* and *Enterobacteriaceae.* In the first case, the both treatments eliminate drastically the population of this genus, only a small number of bacteria appear in the 300 MPa treatment in days 18 and 25. The number of *Enterobacteriaceae* colonies decrease 4 logs after high pressure treatments in comparison with the control morcillas, again a slight increase, in the *Enterobacteriaceae* population of those morcillas, has been observed in the last days with the 300 MPa treatment. In general in Gram positive bacteria the reduction of bacterial population has been lower than in Gram negative. In this sense, only 2 logs reduction has been achieved in enterococci and *Staphylococcus*. In Baird-Parker plates two different types of colonies could be distinguished before the treatment and in the plates of the control. Small and black ones referred as *Staphylococcus* spp. and large, flat and brown colonies, which are described as belonging to the genus *Bacillus* (Oxoid and Bioser diagnostics microbiology manuals). After the treatments, the small colonies disappeared and only it was possible to find the large ones, that probably means that the treatments are effective against the *Staphylococcus*

strains, but not against the sporulated bacteria as *Bacillus*. The results show that bacterial growth in PCA and MRS are quite similar, suggesting that these bacterial populations could be the same. However, in the surface of PCA it also grow some big, flat and slightly mucus colonies. These colonies have been described by Santos (2001) as belonging to the genus *Bacillus*. The presence of these kind of bacteria is normal taking into account that a variable number of spices and vegetables as rice and onion form part of the composition of the product. With LAB it seems that high pressure treatments have not been as effective as in the other bacterial groups analysed. In fact the results of Table 1 show that no reduction of LAB population occurred after treatments. However, it can be seen that high pressure treatments affects clearly the growth rate of these bacteria. In this sense, it is necessary 13 days (300 MPa) o more than 16 days (500 MPa) to achieve the same population of LAB in treated morcillas than in control ones. Santos (2001) described strains of *Weissella viridescens* and *Leuconostoc* as the main population of spoilage bacteria in vacuum package morcilla.

Despite, initial water temperature was around 15ºC, it increased during process approximately 4ºC/100 MPa, that means treatment temperature around 33-35ºC. Sung Won Park et al. (2001) working with *W.* viridescens in laboratory media and processed ham showed that the lowest bacteria reduction was achieved in this temperature range. That was probably main factor that affected treatment efficiency.

The pH decrease in all morcillas during the study period from 6.2-6.4 to 4.8-5.0 in control morcillas and in the same rate but slowly in treated ones was observed.

Related to sensory analysis the general appearance of packages and morcillas showed different signal of spoilage as blowing, slime, sour smell and off flavours, pink and green colours, after 8 days in control morcillas and after 15 days in 300 MPa and 18 days in 500 MPa treated morcillas. Different intensity level of those changes was noticed in almost each package. No significant difference was found in discriminatory tests between control and products subjected to pressure treatment, irrespective of the intensity of pressure applied. According with these results, the high pressure treatment does not cause a modification of sensory characteristics of morcilla, or at least the change is not detectable by panellists.

**Conclusions**

High pressure is a very effective method to preserve the traditional Spanish meat product "morcilla de Burgos". It is possible with the conditions used in this study to eliminate or reduce dramatically the population of bacteria belonging to the genus *Pseudomonas*, *Enterobacteriaceae*, enterococci and *Staphylococcus spp*. Although there is not a reduction in spoilage LAB after treatment, this reduce the growth rate of these bacteria *versus* control that implies actually an expanding of the shelf-life. More studies with other treatment conditions will be done in order to improve efficiency of this useful preservative method for traditional meat products.

**Pertinent literature**

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**Table 1** Number of bacteria in control and treated morcilla during storage (log cfu/g)

| Treatments | Day | TVC | LAB | *Enterobacteriaceae* | *Staphylococcus* | *Pseudomonas* | *Enterococcus* |
|---|---|---|---|---|---|---|---|
| Control | 0 | 5,11 | 5,67 | 5,28 | 4,88 | 4,80 | 4,70 |
| | 1 | 5,36 | 5,72 | 5,28 | 4,93 | 4,81 | 5,11 |
| | 5 | 7,08 | 8,00 | 5,76 | 5,04 | 5,59 | 5,43 |
| | 18 | 8,95 | 9,30 | 5,34 | 5,66 | 5,70 | 3,23 |
| | 25 | 9,04 | 9,36 | 4,34 | 2,70 | 6,04 | 3,26 |
| 300 MPa | 0 | 5,08 | 5,68 | 5,28 | 4,88 | 4,84 | 4,74 |
| | 1 | 5,11 | 5,88 | 1,00 | 2,69 | n.d. | 2,81 |
| | 5 | 6,28 | 6,72 | 1,00 | 3,04 | n.d. | 2,40 |
| | 18 | 7,56 | 8,18 | 1,70 | 3,70 | n.d. | 3,11 |
| | 25 | 8,85 | 9,15 | 3,11 | 3,04 | 3,26 | 2,81 |
| 500MPa | 0 | 5,15 | 5,67 | 5,26 | 4,85 | 4,90 | 4,81 |
| | 1 | 5,46 | 5,74 | 1,00 | 2,93 | n.d. | 2,74 |
| | 5 | 6,41 | 6,28 | 1,00 | 2,73 | n.d. | n.d. |
| | 18 | 6,53 | 7,20 | 1,00 | 2,74 | n.d. | 2,36 |
| | 25 | 8,53 | 8,54 | 1,00 | 2,49 | n.d. | 2,81 |