# Microbiological changes in "Morcilla" preserved in vacuum and modified atmosphere packaging

[1]García-Fontán, M.C.*, [1]García, G., Bermúdez, R., [1]Garrido-Bailón, E., [1]Franco, D. and [1]Lorenzo, J.M.

[1] Meat Technology Centre of Galicia, Rúa Galicia Nº4-Parque Tecnolóxico de Galicia, San Cibrao das Viñas, 32900 Ourense, Spain.
*corresponding author to provide phone: 0034988548276, fax: 0034988548277, e-mail: caminogarcia@ceteca.net

*Abstract*— ***Morcilla*** **is a popular cooked blood sausage. It is made of onion, raw pork of secondary quality, animal fat, blood and different spices according to the local procedures.** ***Morcilla*** **is stuffed in natural pork or beef casings and boiled for about 30 minutes at 90ºC. Traditionally, this product is distributed and sold without packaging. To extend its shelf-life and expand the market, two packaging methods have employed and compared:** ***morcilla*** **stored in vacuum and in modified atmosphere packaging (MAP) using 15% $O_2$+35% $N_2$+50% $CO_2$. Total viable count (TVC), psychrotrophs, lactic acid bacteria (LAB), pseudomonads, enterobacteria, moulds and yeasts, enterococci, sulphite reducing clostridia and** ***Brochothrix termosphacta*** **were analysed during storage (1,2,3,4,5,6,7,8,9 weeks) at 4ºC. Sulfite reducing clostridia and pseudomonas were not detected during storage. On the control sample the population of TVC, psychrotrophs, LAB and enterococci was about 6 log ufc/g, while pseudomonas,** ***Brochothrix*** **and moulds and yeasts count reached 3 log ufc/g. A significant decrease in these groups was observed during storage. The method of packaging only had significant effects on counts TVC ($p<0,05$), psychrotrophs, ($p<0,01$) LAB ($p<0,01$) and enterococci ($p<0,05$) in the first weeks of storage. The rest of the microbiological groups showed a similar behaviour with both packaging.**

*Keywords*— **"*Morcilla*", Microbiological quality, packaging.**

## I. INTRODUCTION

*Morcilla* is a popular cooked blood sausage produced in the north of Spain. It is made of onion, rice (sometimes precooked), animal fat (mainly lard and tallow), blood and different spices according to the local procedure, stuffed in natural or artificial pork or beef casings and boiled for about 1 h at 90-95ºC. Despite its populatity, however, its short shelf life limits its distribution, therefore extending its shelf life is a priority for producers in order to increase the potential market and satisfy consumer demands [1,2,3,4,5].

Modification of the atmosphere within the package by decreasing the oxygen concentration, while increasing the content of carbon dioxide and/or nitrogen, has been shown to significantly prolong the shelf life of perishable food products at chill temperatures [6]. Modified atmosphere packaging (MAP) and vacuum-packaging (VP), along with refrigeration, have become increasingly popular preservation techniques, which have brought major changes in storage, distribution, and marketing of raw and processed products to meet consumer demands [7]. Therefore, modification of the extrinsic parameters of the product ecosystem, like temperature and gaseous atmosphere, is a common practice compared with the use of chemical preservatives. $CO_2/N_2$ atmospheres are particularly suitable for the preservation of cooked and cured meat products, mainly because of the strong inhibition of growth of great majority of microorganisms [8].

A number of studies have been carried out in order to evaluate the effectiveness of vacuum, gas composition and packaging material on the preservation of fresh meat [9,10], dry fermented sausages [11,12,13] cooked meat products [14,15], cooked ham [16], dry-cured ham [17,18,19].

Reports on *Morcilla* are available concerning to the packaging and microbiological quality during chilled storage [3,20]. Thus, this work was focused on studying the microbiological quality of *Morcilla* preserved in vacuum and modified atmosphere packaging during storage at 4ºC.

## II. MATERIALS AND METHODS

### *1. Sampling*

A total of 114 samples were used for this study. The formulation used in the manufacture of the *Morcillas*, included raw onion (9%), pork jowl (26%), lean pork (52%) cooked pork skin (7%), garlic (0.4%), blood (10%), salt and diferent spices. The natural pork casings were preserved with salt and rinsed in clear water prior to use. No nitrite was added to the formulation. The chopped onion and pork jowl, lean pork and cooked pork skin were mixed with the garlic, salt, spices and blood and the sausage emulsion was

stuffed into 35-45 mm casings. Ripening was carried out in storerooms durind 14 days and the pieces were smoked during approximately the first three days of ripening using smoke from oak wood. The blood sausages were then transferred to a cooking vessel and boiled in clear water at 95-96ºC for around 30 minutes. After cooking, *Morcillas* were rapidly cooled at -20ºC during 20 minutes. Samples were divided into two batches. The samples of one of the batches were vacuum packed in a FRIMAQ V-900 packaging machine using bags with an oxygen transmission rate of 50 cm3/m2/24 h/bar at 23ºC and 75% RH and water vapour transmission rate of 2.6 g/m2/24 h at 23ºC and 85% RH. The samples of another batches were then packed in trays of 300 PS mm thick sealed with PE film for gas mixtures of 74 mm thick and with a permeability oxygen less than 2 ml/m2/24h/bar. The packaging was carried out using a heat sealer Lari3/Pn Cavec T-VG-R-SKIN (Cavec, Italy). The composition of the atmosphere used was 15%$O_2$/35%$N_2$/50%$CO_2$ and samples were stored during 0, 1, 2, 3, 4, 5, 6, 7, 8, and 9 weeks at 4º C. Six *Morcillas* were taken directly after cooking for microbiological analysis and pH measurement and during storage two packages, with three samples each one, were randomly selected for pH and microbiological analyses.

### 2. *pH measurement*

pH was measured by blending 25 g of product with 225 mL of distilled water for 2 min. A digital pH-meter Micro pH 2000 (Crison, Barcelona, Spain) was used for the measurement.

### 3. *Microbiological analysis*

Two packages per treatment (vacuum and modified atmosphere packaging) were analysed on each sampling day.

Ten grams of slices of *Morcilla* (including the skin) were aseptically placed into a stomacher bag. It was then homogeneized with 90 mL of sterile 0,1% peptone water in a masticator blender (IUL Instruments, Barcelona, Spain) for 2 min at room temperature. For each sample, appropriate serial decimal dilutions were prepared in Peptone Water solution (0.1%) and duplicate 1 mL or 0.1 mL samples of appropriate dilutions were poured or spread onto total count and selective agar plates.

Total viable counts, were enumerated in Plate Count Agar (PCA; Oxoid, Unipath Ltd., Basingstoke, UK) incubated at 30º C for 48h. Psychrotrophic aerobic bacteria were enumerated on Plate Count Agar (PCA; Oxoid, Unipath Ltd., Basingstoke, UK) after incubation at 7º C for 10 days. Lactic acid bacteria were determined on the Man Rogosa Sharpe medium Agar (Oxoid, Unipath Ltd., Basingstoke, UK) (pH 5,6), after incubation at 30º C for 5 days. Enterobacteriaceae was determinate on Violet Red Bile Glucose Agar (Merck, Darmstadt, Germany) after incubation at 37ºC for 24 h. Moulds and yeasts were enumerated using OGYE Agar Base (Merck, Darmstadt, Germany) with OGYE Selective Supplement (Merck, Darmstadt, Germany), previously ready and incubated at 25ºC for 4-5 days. Pseudomonads were determinated on Pseudomonas Selective Agar (Merck, Darmstadt, Germany) with Pseudomonas CFC Selective Supplement (Merck, Darmstadt, Germany), previously ready and incubated at 25º C for 48h. *Brochothrix termosphacta* was spread on the surface of STAA Agar Base (Oxoid, Unipath Ltd., Basingstoke, UK) with STAA Selective Supplement (Oxoid, Unipath Ltd., Basingstoke, UK), previously ready and incubated at 25º C for 48h. Sulfite reducing clostridia were enumerated on the SPS Agar (Merck, Darmstadt, Germany) with the plates incubated under anaerobic conditions at 44º C for 24h.

After incubation, plates with 30–300 colonies were counted. Microbiological data were transformed into logarithms of the number of colony forming units (cfu/g).

### 4. *Statistical analysis*

Results were expressed by means±standard error. Comparison of means was performed by one-way analysis of variance (ANOVA). Tukey HSD was used to the determine the differences in the mean values ($P<0.05$). Data were analysed using the general linear model of SPSS (SPSS 19.0, Chicago, IL, USA) software package.

## III. RESULTS AND DISCUSSION

The mean initial pH of the *Morcillas* before packaging was 4.72±0.02 and it decreased with storage, regardless of the packaging method, but being more rapid in samples packed under vacuum (Fig. 1). The trend agrees with the results reported by other authors [3], though the initial values were higher (6.31±0.05).

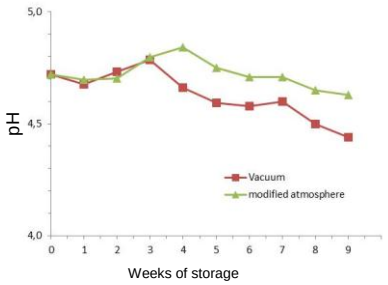


Figure 1. Mean pH values of Morcillas stored under vacuum or under modified atmosphere containing 50% $CO_2$

Sulfite – reducing clostridia were not detected in any sample and pseudomonads only was detected in the control point and in the 3 weeks of storage (Fig.2a). Santos et al. [3] reported that after 27 days, the number of pseudomonads was higher than 8 log cfu/g.

The total mesophilic aerobic count (TVC) and total psychrotrophic aerobic count (PVC) for different *Morcillas* packaging is given in Figure 2b and 2c. The initial value of TVC and PVC (day 0) for samples before packaging were 6.00 and 5.89 log CFU/g respectively, indicative of poor quality of meat and few efficiency of the thermal treatment used. Initial values for TVC and PVC for Morcilla de Burgos [3] were lower. During the first weeks of storage the counts observed were always higher in the *Morcillas* packed in modified atmosphere but from this moment the results were similar for both types of packaging. This is probably is due to the high content in CO2 of the modified atmosphere. Previous research [21] has indeed demonstrated that the concentration of dissolved $CO_2$ in the water phase of a food determines the growth inhibition of microorganisms in a modified atmosphere.

Vacuum-packed *Morcilla* normally begins to spoil after 14-21 days of chill storage [3], mainly due to vacuum loss and the formation of slime and sour odour and taste caused by growth of lactic acid bacteria (BAL) [23,20]. This pattern of spoilage has also reported of other cooked [18,20], and dry meat products [24]. Nevertheless these results do not agree with found in our work. From the week 5 all the counts were kept below 4.5 log cfu/g (Fig. 2d) for vacuum and modified atmosphere packaging indicative of a correct microbiological stabilization of the product.

In general, the storage time affected all microbial microbiota studied in this work, and all decreased with storage. In general, the stability found for the counts from the week 5-6 of storage, could be explained taking into account that packaging in anoxic environments retards microbial growth and delays spoilage due to slow proliferation of bacteria capable of tolerating anaerobic conditions [25]. Pseudomonads and enterobacteria (Fig 2a and 2e) counts were subjected to a significant inhibition and values under the detection limit were found after 4 and 5 weeks of storage, respectively, probably due to the strong competitive effect of lactic acid bacteria on the rest of the endogenous microbiota. Lactic acid bacteria suppress the growth of Gram-negative bacteria by producing organic acids and various antibacterial metabolic products [26,27,28].

Finally to comment that for all the microbial groups (Fig 2a, b, c, d and e) the counts obtained in the week 3 of storage have been much higher to the rest of weeks, probably due to a contamination during post-cooking handling, particularly during the chilling step, just before vacuum or modified atmosphere packaging.

With regard to the type of packaging, the counts obtained for the samples packed in modified atmosphere, were always higher than those samples packed to vacuum. This fact might be due to the anoxic conditions present under vacuum packaging.

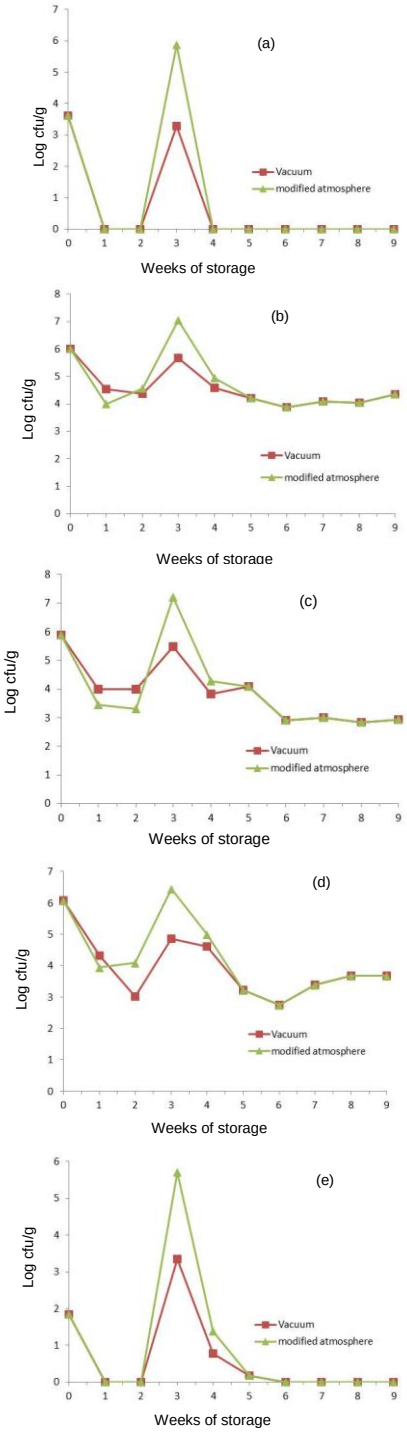


Figure 2 (a) pseudomonads, (b) Total viable counts, (c) psychrotrophs (d) lactic acid bacteria (e) *Enterobacteriaceae*, samples packaged under vacuum and modified atmosphere packaging and storage at 4ºC.

## IV. CONCLUSIONS

The pH decreased with storage, regardless of the packaging method, but being more rapid in samples packed under vacuum.

A significant decrease in TVC, psychrotrophs and lactic acid bacteria, was observed during storage. The method of packaging only had significant effects on counts TVC ($p<0,05$), psychrotrophs, ($p<0,01$) LAB ($p<0,01$) and enterococci ($p<0,05$) in the first weeks of storage. The rest of the microbiological groups showed a similar behaviour with both packaging.

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