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Effect of packaging technology on microbiological and sensory quality of a cooked blood sausage, Morcela de Arroz, from Monchique region of Portugal

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ABSTRACT

Morcela de Arroz (MA), a popular Portuguese blood sausage, with high pH and water activity (a_w), is traditionally commercialized without preservatives and unpacked. This study evaluated the best packaging solution to extend MA shelf life stored at 4 ± 1 °C for 44 days: without packaging (WP), vacuum (VP) and modified atmosphere packaging (MAP) (80% CO₂; 20% N₂). Mesophilic (MTVC), psychrotrophic (PTVC), lactic acid bacteria (LAB), pseudomonads, molds and yeasts, *Enterobacteriaceae*, *Listeria monocytogenes*, *Salmonella* spp., *Bacillus cereus*, *Clostridium perfringens*, sensory properties, pH, moisture and a_w were studied. Moisture and a_w decreased ($p < 0.05$) in WP. pH decreased in WP and MAP during storage. MTVC and PTVC counts increased to values around 7 log CFU/g at 44 days of storage. LAB and *Enterobacteriaceae* counts were higher ($p < 0.05$) in VP. Pseudomonads were inhibited ($p < 0.05$) by MAP after 8 days of storage. Sensory parameters were affected ($p < 0.05$) by packaging and storage time. Globally, MAP performed better.

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1. Introduction

Morcela de Arroz (MA) is a popular cooked rice blood sausage, a ready to eat (RTE) meat product that is traditionally produced in Serra de Monchique (south of Portugal). This product supports the economy of several industries, due to its uniqueness among the traditional Portuguese meat products. MA is produced without addition of chemical preservatives and without the use of any packaging technology. Distribution and storage are performed in a very short period (one week at 0–5 °C). The short shelf life has been an important hurdle to the increase of the production through the diversification of the markets, namely more distant ones.

To our knowledge, there is no information about MA, namely its microbiological, sensory or physical–chemical characteristics, that hinders any attempt to modify the commercialization conditions. Despite the information available about similar blood sausages (Cachaldora, García, Lorenzo, & García-Fontán, 2013; Roseiro, Santos, Almeida, &

Vieira, 1998; Santos, González-Fernández, Jaime, & Rovira, 2003), the specificity of MA makes its shelf life difficult to evaluate by comparison or extrapolation.

Blood sausages are products of difficult preservation due to the rich nutrient composition, high pH (about 6.0) and high a_w reaching values higher than 0.97. Although, the high temperature and cooking time (95–96 °C for around 1 h) during processing eliminates the vegetative forms of microorganisms and only more heat resistant bacteria spores survive the treatment (Silva & Gibbs, 2010), post-cooking contamination on the surface of the product is caused by handling of the product during cooling, refrigeration and post processing operations (Mäkelä & Korkeala, 1987; Oteiza, Chinen, Miliwebsky, & Rivas, 2006; Santos, Diez, González-Fernández, Jaime, & Rovira, 2005).

The use of packaging technology can extend shelf life of meat products by improving safety and stability along shelf life. Vacuum packaging (VP), being cost-effective and easy to apply (McMillin, 2008), has been so far the most widely used packaging technique for different types of meat products (Borch, Kant-Muermans, & Blixt, 1996). However, it presents some disadvantages, such as product deformation and exudate forming. To overcome this problem, modified atmosphere packaging (MAP) has become popular among meat products (Hintlian & Hotchkiss, 1987; Matos, Barreto, & Bernardo, 2005; Pexara, Metaxopoulos, & Drosinos, 2002). CO₂/N₂ atmospheres are accepted as

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particularly adequate for the preservation of meat products, mainly because of the strong growth inhibition of the majority of microorganisms. It is accepted that CO₂ increases the lag phase and generation time of microorganisms, even those that can grow in the absence of oxygen. This inhibitory effect is due to the reduction of pH, inhibition of certain enzymes involved in energy production and damage of the cell membrane. N₂ is used as a filler gas and it has no recognized inhibitory effect. The proportion of CO₂/N₂ in MAP is variable with the product and the desired shelf life (Mullan & McDowell, 2003). Among the several studies made with blood sausages shelf life, Santos et al. (2005) concluded that the combination of 80% CO₂ and 20% N₂ resulted in an improved control of spoilage microbiota and shelf life extension.

The aim of the present study was to evaluate the microbiological, physicochemical and sensory characteristics of Morcela de Arroz packaged in VP, MAP (80% CO₂ and 20% N₂) and without packaging (WP) stored at 4 ± 1 °C in order to investigate the best packaging solution to extend the shelf life period.

2. Materials and methods

2.1. Formulation and processing of the MA

MA was processed in a local industry according to the traditional method. Formulation included Alentejano pig breed meat (loin, shoulder, neck and bacon) (54%); rice (13%), pork blood (6%), water (17%), lard (4%), grinded onion (3%), salt (1%) and spices (ground cumin, ground clove, ground pepper and mint) (2%). Meats were minced in plates with holes of 2 cm diameter and mixed with raw rice, pork blood, water, grinded onion and mint, lard, salt and ground spices (Pereira, Ferreira-Dias, & Matos, 2008). The mixture was stuffed into 60–65 mm diameter natural beef casings. MA was then transferred into a stainless steel cooking vessel and cooked in molho de moura, a broth prepared with water, whole onions, whole garlic, salt and laurel leaves at 91 ± 5 °C for 1.5 h. After cooking (individual weight of each MA around 250 g), products were cooled at room temperature for about 2 h and then transferred into a refrigerated chamber (4 ± 1 °C) until the next day (day 0). The experiment was conducted with three production batches separated in time by 2 months.

2.2. Packaging of the MA

On day 0, 45 samples for each packaging technology studied were individually packed in the producer facility: vacuum packed (VP) in a Belca V-40S packaging machine (Belca, Villabona, Spain) and MAP (80% CO₂/20% N₂) in a Haratek 200A packaging machine (Haratek, Astigarraga, Spain) both in oxygen and water impermeable bags Combitherm XX (dimensions of 230 × 286 mm), 115 µm thickness (Wipak Walsrode GmbH & Co.KG, Walsrode, Germany), with an oxygen transmission rate of 3.0 cm³/m²·24 h·atm at 23 °C and 75% RH, carbon dioxide transmission rate of 13.5 cm³/m²·24 h·atm at 23 °C and 75% RH and a water vapor transmission rate of 1.0 g/m²·24 h at 23 °C and 85% RH. Samples to be studied without package (WP) were transported to the laboratory in sterile sample bags. All samples were transported under refrigeration to the laboratory and stored at 4 ± 1 °C and 80% of RH (Fitoclima s 600 PLH, Aralab, Lisbon, Portugal). Selection of 80% of RH was based on the results of RH measurement in two industries and two retailer's refrigerators. WP samples were taken out from the sterile bags and stored without any package, to simulate the usual practice of commercialization of MA. Nine extra sausages were collected from each batch and transported in the same conditions (under refrigeration in sterile sample bags) to the analysis of the first sampling time, and analyzed immediately on arrival to the laboratory.

2.3. Sampling procedure

One experimental unit, thereafter designated as repetition, was composed by two MA, one to perform microbiological and physicochemical analysis and the other for sensory analysis, once only one MA was not enough for all analysis due to the high consumption of sample by the eighteen member of sensory analysis panel.

The experiment was conducted with 9 repetitions for each experimental condition (packaging × storage time). These 9 repetitions were obtained from 3 producing batches, separated in time by 2 months, collecting 3 samples from each of the 3 batches. Thus, for each storage time, 27 samples were analyzed [3 packaging (WP/VP/MAP) × 3 production batches × 3 repetitions from each batch]. In the first storage time (day 0) only 9 samples were analyzed, once there was no packaging yet (3 production batches × 3 repetitions in each batch). During storage period samples were collected at days 8, 15, 22, 30 and 44 for microbiological, physicochemical and sensory analysis. At first sampling time (day 0) samples not packaged were analyzed immediately after arrival to the laboratory. Extra MA samples packed in each condition were maintained for all the experimental conditions to be used in case of package damage and vacuum or MAP loss. Samples used for sensory analysis panel selection, training and lexicon development are indicated in Sections 2.6.1.1 and 2.6.1.2.

2.4. Physical–chemical analysis

2.4.1. Analysis of gas composition

Oxygen and carbon dioxide concentrations in the head space of the MAP packages were measured with a digital O₂ and CO₂ analyzer (OXYBABY V O₂/CO₂ WITT-Gasetechnik, Witten, Germany). 6 ml gas samples were drawn by the needle of the analyzer through a rubber seal glued on the surface of the pack. Detection limits for O₂ and CO₂ were ± 0.1% and ± 2%, respectively.

2.4.2. pH, moisture and a_w parameters

The potentiometric measurements of pH were carried out by inserting the pin electrode of the pH meter (GLP 21, Crison, Barcelona, Spain) directly into the sample (20.5 ± 0.3 °C). Prior to the moisture and a_w measurements, each sample was ground and homogenized in a domestic food processor. Moisture was determined using a Mettler LP16-M infrared scale (Mettler Instruments AG, Zurich, Switzerland). Rotronic Hygroskop DT was employed to measure the water activity at 25.0 ± 0.01 °C (Rotronic, HygroLab, Bassersdorf, Switzerland). Determinations were made in triplicate.

2.5. Microbiological analysis

Twenty-five-gram slices of MA were taken aseptically and homogenized with 225 ml of sterile Ringer's solution (Merck, Darmstadt, Germany) for 90 s in sterile plastic bag in a lab blender (Masticator, IUL Instruments, Barcelona, Spain). Serial decimal dilutions in sterile Ringer's solution were prepared and 1 ml or 0.1 ml samples of appropriate dilutions were poured or spread onto nutrient agar and selective agar plates. Mesophilic total viable count (MTVC) was determined on Plate Count Agar (PCA, Merck), incubated at 30 °C for 72 h; psychrotrophics (PTVC) were determined on PCA, incubated at 6.5 °C for 10 days; lactic acid bacteria (LAB) on Man Rogosa and Sharpe (Merck) and incubated on anaerobic conditions at 30 °C for 2–3 days; *Enterobacteriaceae* on Violet Red Bile Glucose Agar (Merck), incubated at 37 °C for 24 h; *Pseudomonas* spp. on Pseudomonads Agar Base (Oxoid, Basingstoke, UK) with Cetrimide, Fucidin and Cephalosporin supplement, incubated at 25 °C for 48 h, molds and yeasts on Chloramphenicol Rose Bengal (Merck) and incubated at 25 °C for 5 days; detection of *Salmonella* spp. was performed according to EN 12824 (1997) and detection of *Listeria monocytogenes* according to ISO 11290-1

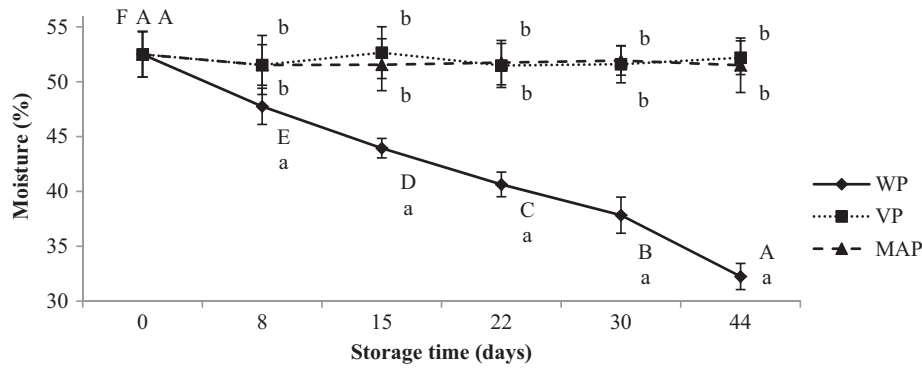


Fig. 1. Effect of packaging technology (without packaging – WP, vacuum packaging – VP and modified atmosphere packaging – MAP, 80% CO₂ and 20% N₂) and of storage time (44 days) at 4 ± 1 °C on moisture content (%). Results are presented as mean ± sd (n = 9 for each packaging/storage time). a–b, means with different letters are different (between packaging technologies at each day of storage) (p < 0.05). A–F, means with different letters are different (same packaging technology along time of storage) (p < 0.05).

(1996); *Bacillus cereus* according to ISO 7932 (2004) and *Clostridium perfringens* as described in ISO 7937 (2004).

The results are presented as log CFU/g. For statistical purposes, when the microorganism count was below the detection limit, it was considered to be zero and when countable colonies were present, but below the countable range, the estimated count was considered for data analysis.

2.6. Sensory analysis

2.6.1. Quantitative descriptive analysis (QDA)

2.6.1.1. Formation of the panel. A panel of 18 assessors was selected from an initial group of 63 recruited individuals (internal recruitment) and subsequently trained how to carry out the necessary analysis. Selection of a shortlist of 38 individuals was based on their capacity to detect and identify different tastes and aromas, ability to memorize sensory attributes, discriminative capacity and accuracy of their vocabulary in describing sensory parameters. Selection of the final 18 assessors was based on their discriminative capacity and repeatability calculated using the data obtained in repeated quantitative descriptive analyses of nine samples of MA in three sessions held at 1-week intervals (ISO 8586-1, 1993). Before performing the evaluation of the samples of the present work, the panel was trained during 10 sessions of 45–60 min. The panelists performed sensory analysis on a total of 27 samples of MA from 12 different origins (2 or 3 samples in each session).

2.6.1.2. Generation of vocabulary. Descriptors for the QDA were generated in five stages according to ISO 11035 (1994): 1 – sessions of free and open generation of vocabulary, where assessors used the descriptors they considered more correct for describing the fresh and aged MA with different storage times, correspondent to different levels of spoilage; 2 – synthesis of vocabulary, determination of frequency of use and elimination of incorrectly used descriptors; 3 – test of an initial list of nineteen descriptors; 4 – data analysis to detect redundant descriptors and selection of the most discriminative descriptors between fresh and not fresh product; 5 – test of the final list. These stages were organized over nine sessions.

For the final list of 16 descriptors, definitions and references were established, proposed by the panel leader, and discussed with the panelists. It was used as references the freshly cooked MA and unpackaged refrigerated MA stored for 7 to 44 days to achieve the desired sensory parameter intensity. The choice of using the product under study rather than other matrixes as a reference was considered to be more useful to generalize sensory concepts, as suggested by Braghieri et al. (2012).

The descriptors retained in the final list to evaluate shelf life of the MA were as follows: Aspect – moisture: moistness surface property relating to the perception of the amount of water; slime: presence of viscous substance in the surface; typical color: characteristic dark brown color; brightness: light reflection due to melting of fat, greasy surface; Aroma – typical odor: characteristic meaty and spicy odor; off-odors: any atypical odor considered not expected in the product; rancid: associated with oxidized fat and oils, putrid: the pungent ammoniac like odor in advanced spoiled meat or fish; sour/fermented:

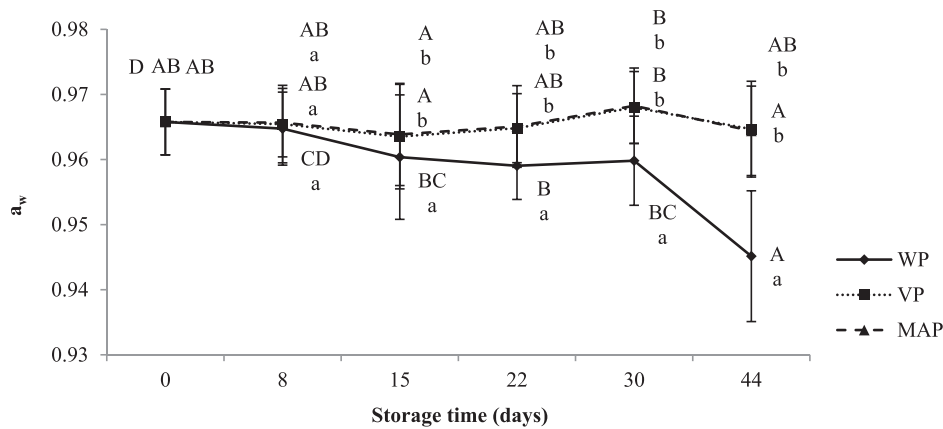


Fig. 2. Effect of packaging technology (without packaging – WP, vacuum packaging – VP and modified atmosphere packaging – MAP, 80% CO₂ and 20% N₂) and of storage time (44 days) at 4 ± 1 °C on water activity (a_w). Results are presented as mean ± sd (n = 9 for each packaging/storage time). a–b, means with different letters are different (between packaging technologies at each day of storage) (p < 0.05). A–D, means with different letters are different (same packaging technology along time of storage) (p < 0.05).

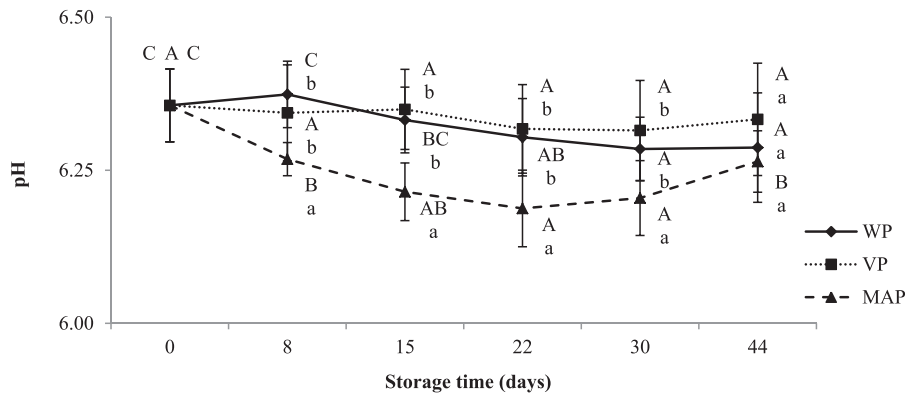


Fig. 3. Effect of packaging technology (without packaging – WP, vacuum packaging – VP and modified atmosphere packaging – MAP, 80% CO₂ and 20% N₂) and of storage time (44 days) at 4 ± 1 °C on pH. Results are presented as mean ± sd (*n* = 9 for each packaging/storage time). a–b, means with different letters are different (between packaging technologies at each day of storage) (*p* < 0.05). A–C, means with different letters are different (same packaging technology along time of storage) (*p* < 0.05).

pungent odor associated to spoiled cooked rice; spoiled wine/vinegary: acetic odor associated to vinegar; moldy/mildew: characteristic odor of molds; Flavor – typical flavor: characteristic meaty, salted and slightly sweet flavor; acid: taste like vinegar or lemon juice; bitter: acrid and harsh taste, as the peel of an orange or coffee dregs; global evaluation of freshness: multifactorial evaluation of freshness, by opposition to spoilage. Additionally, a freshness global evaluation of the entire packed product (external appearance) was introduced. The assessors were asked to score each descriptor in each sample on a scale from 1 (low intensity) to 5 (intense).

2.6.2. Evaluation of the samples

Sessions took place on a sensory analysis laboratory equipped with individual assessment booths and uniform lightening and temperature conditions. To guarantee test repeatability, the samples were cut in 1 cm thick slices and placed in Petri dishes codified with a randomized three digit number and evaluated at room temperature. Spring water and unsalted crackers were provided to clean the mouth between samplings.

2.7. Statistical analysis

The experiment was composed by a total of 144 observations used for statistical analysis [9 samples for day 0 (3 batches × 3 repetitions) + 135 samples (5 storage times × 3 packaging × 3 production batches × 3 repetitions from each batch)].

The analysis of variance was performed with the packaging or storage time as individual effects on the microbiological and sensory variables by one-way ANOVA procedures. When the effect of storage time was analyzed by ANOVA, data from sampling time 0 (day 0) was used for the three packaging conditions. The differences between the mean values were determined using the Tukey test. The relationship between variables was established through Pearson correlation.

The chi-squared test was applied to evaluate the relationship between the presence/absence of *B. cereus* in MA and (1) the packaging technology in each sampling time and (2) the storage period in each package. Statistical significance was set at *p* values less than 0.05. Data analysis was performed using the IBM SPSS Statistics for Windows, Version 19.0 (IBM Corp., 2010).

3. Results

3.1. Physical–chemical analysis

The CO₂ concentration of the MAP samples decreased by 19% during the storage period. The initial moisture content was 52.49 ± 2.08% (Fig. 1) and significantly (*p* < 0.05) decreased in the WP samples during storage time (20% loss). The initial *a_w* was 0.966 ± 0.005 (Fig. 2) and followed a similar trend to that observed for moisture (decreased nearly 0.02 units until 44 days). At day 0, the pH of MA was 6.36 ± 0.06 (Fig. 3). During most part of the storage time, the MAP MA presented a significantly (*p* < 0.05) lower pH comparatively to WP and VP samples, reaching the lowest values at day 22 of the study (pH = 6.19 ± 0.06).

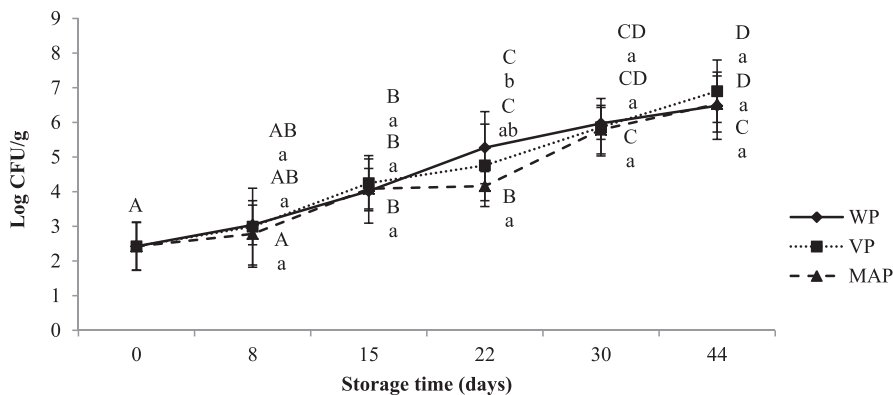


Fig. 4. Effect of packaging technology (without packaging – WP, vacuum packaging – VP and modified atmosphere packaging – MAP, 80% CO₂ and 20% N₂) and of storage time (44 days) at 4 ± 1 °C on mesophilic total viable counts (MTVC) (log CFU/g). Results are presented as mean ± sd (*n* = 9 for each packaging/storage time). a–b, means with different letters are different (between packaging technologies at each day of storage) (*p* < 0.05). A–D, means with different letters are different (same packaging technology along time of storage) (*p* < 0.05).

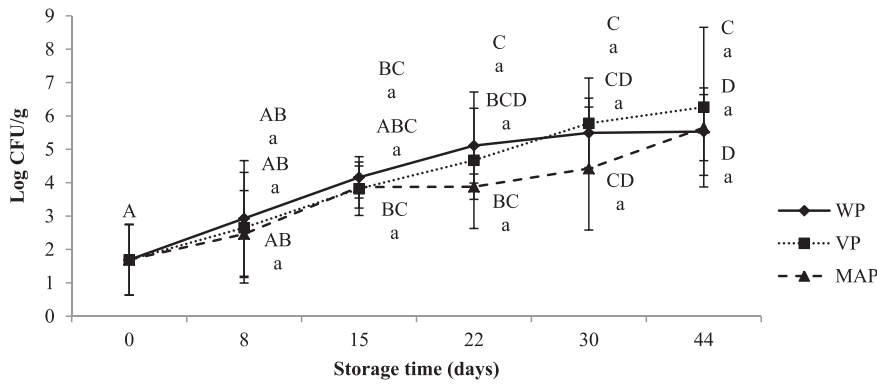


Fig. 5. Effect of packaging technology (without packaging – WP, vacuum packaging – VP and modified atmosphere packaging – MAP, 80% CO₂ and 20% N₂) and of storage time (44 days) at 4 ± 1 °C on psychrotrophic total viable counts (PTVC) (log CFU/g). Results are presented as mean ± sd (n = 9 for each packaging/storage time). A–D, means with different letters are different (same packaging technology along time of storage) (p < 0.05).

3.2. Microbiological analysis

C. perfringens, *L. monocytogenes* and *Salmonella* spp. we're not detected in any of the samples tested during all the experiment in any of the package technologies used. *B. cereus* (results not shown) was not detected in MA freshly prepared (day 0). At 8 days of storage and thereafter it was punctually detected in the three packaging conditions, in a total of 18 samples, without any significant (p > 0.05) association either to packaging or storage time, except at 22 days of storage when all the samples with *B. cereus* were stored WP ($\chi^2 = 9.39, p = 0.09$).

The results of the counts of total viable count at 30 °C (MTVC) and at 6.5 °C (PTVC), LAB, *Enterobacteriaceae* and *Pseudomonas* spp. are presented in Figs. 4, 5, 6, 7 and 8, respectively. During the storage of MA the MTVC and PTVC increased concomitantly from a count of 2.42 ± 0.69 log CFU/g and 1.69 ± 1.06 log CFU/g, respectively, to values around 7 log CFU/g at the end of the storage period (44 days). The increase in the TVC counts was about 1 log CFU/g in each storage interval studied. Generally both TVCs were not affected by the packaging methodology. Only at 22 days of storage a higher (p < 0.05) count of mesophilic was observed in samples stored without package than those packaged in MAP. For the entire storage time of the study the counts of LAB (Fig. 6) were always higher (p < 0.05) in the VP samples than in WP and MAP, except in the day 15 of storage. The counts of LAB increased faster in the VP MA, as inferred by the significant differences (p < 0.05) between the counts at the beginning of the experiment and after 8 days of storage. The higher increase of LAB occurred between 8 and 15 days of storage, nearly 1.5 log CFU/g.

The count of *Enterobacteriaceae* (Fig. 7) was generally lower in MAP samples. At the beginning of the experiment, any *Enterobacteriaceae*

colony was detected. During storage it presented an increase, that was always lower in samples packaged in MAP, with significant differences (p < 0.05) at 8, 22, 30 and 44 days of storage comparatively to VP samples and at days 8, 22 and 30 comparatively to the WP samples. *Pseudomonas* spp. (Fig. 8) were not detected at day 0 of the study, although, in the WP samples their counts significantly increased (p < 0.05) along the storage period, achieving about 1.5 log CFU/g on day 8 of the study and reaching a plateau of 4.5 log CFU/g at 22 days of storage, after which their counts remained similar until the end of the storage period but were always significantly higher (p < 0.05) comparatively to the packaged MA. In the packaged MA, *Pseudomonas* spp. showed a growth rate that was similar both on VP and MAP only until 8 days of storage (p > 0.05). After 8 days of storage on, the MAP MA presented significantly lower (p < 0.05) counts of *Pseudomonas* spp. comparatively to the VP MA. The growth of *Pseudomonads* in MAP was not significantly (p > 0.05) affected by the storage time. The count of molds and yeasts was below the detection limit in all samples until 15 days of storage. At day 22 and thereafter these microorganisms were punctually detected, 2 samples in day 22, 4 in day 30 and 2 in day 44. No association was established between the occurrence of molds and yeasts and the package technology used.

3.3. Sensory analysis

The results of the sensory analysis of WP, VP and MAP MA during 44 days on refrigerated storage (4 ± 1 °C) are presented in Table 1. Sensory parameters were significantly (p < 0.05) affected by the method of packaging and storage time. In general, the sensory quality decreased during storage time. MA stored in MAP presented higher mean scores

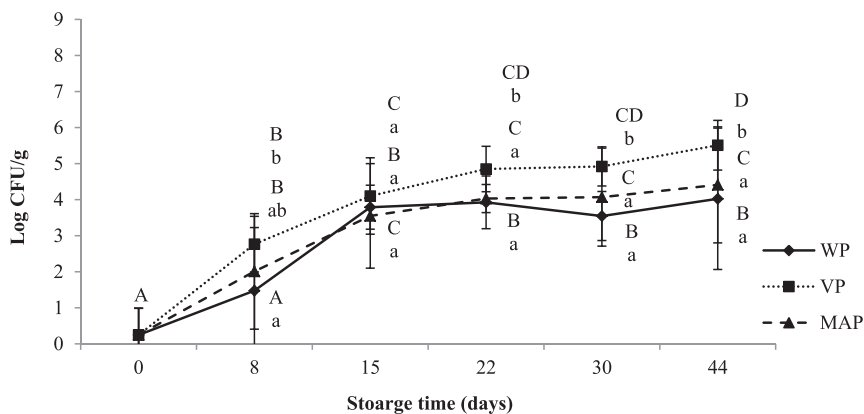


Fig. 6. Effect of packaging technology (without packaging – WP, vacuum packaging – VP and modified atmosphere packaging – MAP, 80% CO₂ and 20% N₂) and of storage time (44 days) at 4 ± 1 °C on lactic acid bacteria (LAB) (log CFU/g). Results are presented as mean ± sd (n = 9 for each packaging/storage time). a–b, means with different letters are different (between packaging technologies at each day of storage) (p < 0.05). A–D, means with different letters are different (same packaging technology along time of storage) (p < 0.05).

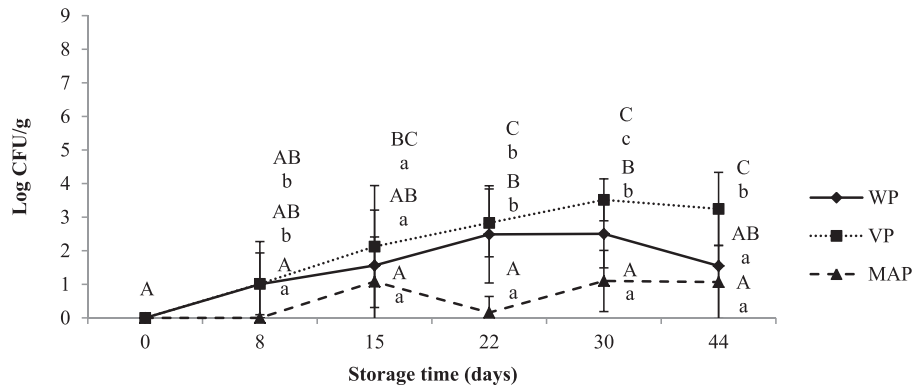


Fig. 7. Effect of packaging technology (without packaging – WP, vacuum packaging – VP and modified atmosphere packaging – MAP, 80% CO₂ and 20% N₂) and of storage time (44 days) at 4 ± 1 °C on *Enterobacteriaceae* (log CFU/g). Results are presented as mean ± sd (*n* = 9 for each packaging/storage time). a–c, means with different letters are different (between packaging technologies at each day of storage) (*p* < 0.05). A–C, means with different letters are different (same packaging technology along time of storage) (*p* < 0.05).

than VP and this higher than WP samples. In the freshly prepared MA, no off-odors, rancid, putrid, sour/fermented, spoiled wine/vinegary, moldy/mildew and putrid odors were detected. The WP presented lower (*p* < 0.05) moisture aspect, typical color and typical odor and flavor at 8 days of storage and thereafter than VP and MAP MA. Presence of off-odors and rancid odor began to be significantly higher (*p* < 0.05) in the WP samples after 8 days of storage. In the WP samples the rancid odor at 44 days of storage was threefold higher than in freshly prepared MA. At 8 days of storage, WP samples began to be considered less (*p* < 0.05) fresh than those packaged, regardless the evaluation made in slices or in packaged MA. The development of the depreciative characteristics: slime, sour/fermented, spoiled wine/vinegary and moldy/mildew odor and acid and bitter tastes were slower than the rancid and off-odors, as they became significantly different in WP samples only after 22 days of storage. From the sensory point of view, VP and MAP had a similar performance for the most of the tested attributes. However, MAP samples were generally more bright (*p* < 0.05 after day 8), and, for longer storage periods (22 days and after), it presented a higher (*p* < 0.05) typical flavor and global freshness (slices or packaged). The evaluation of global freshness in slices or in entire packaged MAP MA during all the experiment reveals that these characteristics were stable during the tested period. The relationship between microbial counts and sensory characteristics was established by Pearson correlations for each packaging technology. Considering only the global freshness (Table 2), it was observed high significant correlations between total viable counts (MTVC and PTVC) and the sensory evaluation of freshness. These correlations were higher in WP samples. When the correlations were established between the specific spoilage microorganisms and the global freshness, it was possible to observe that LAB

were particularly important in the deterioration of VP and MAP, *Enterobacteriaceae* in VP, and *Pseudomonas* spp. in WP samples, as can be observed by the higher correlation coefficient.

4. Discussion

The results for the mean initial pH, *a_w* and moisture content were similar to those found by Santos et al. (2003) in a Spanish blood sausage which confirm that MA is a RTE meat product of difficult preservation. The significant decrease in pH values in the MAP samples comparatively to the VP and WP was probably due to the solubilization of CO₂ in the product, once the conditions were favorable for that, namely the high CO₂ content of the packages and the low temperature used in the experiment (Mullan & McDowell, 2003). Additionally, no correlation was observed between pH evolution and LAB or other microbial group counts for any of the packaged methods, indicating that the pH evolution was independent of the microbial growth.

The traditional method of distribution and commercialization of MA without package (WP) was responsible for a considerable moisture loss. The drying effect during cold storage has several consequences, beyond the direct economic ones due to weight loss. The reduction of *a_w* resulting from the drying of MA was small, when related to the total moisture loss (around 20%). That unexpected relationship might be a consequence of the composition of MA, namely the high level of rice and blood. The binding of water to these components of MA is expected to be weaker than the binding in sausages made only with meat and fat, once carbohydrates have a lower water binding capacity than muscular proteins (Le Maguer, 1987), and the effect of blood on the water holding capacity is variable (Jarmoluk & Pietrasik, 2003). Despite the general

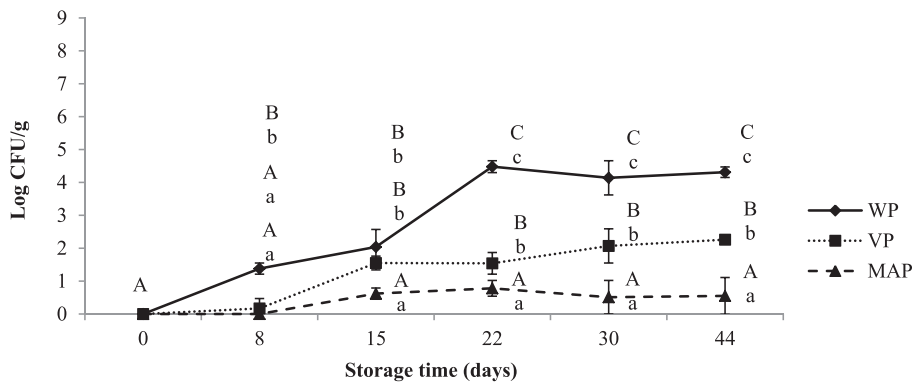


Fig. 8. Effect of packaging technology (without packaging – WP, vacuum packaging – VP and modified atmosphere packaging – MAP, 80% CO₂ and 20% N₂) and of storage time (44 days) at 4 ± 1 °C on *Pseudomonas* spp. (log CFU/g). Results are presented as mean ± sd (*n* = 9 for each packaging/storage time). a–c, means with different letters are different (between packaging technologies at each day of storage) (*p* < 0.05). A–C, means with different letters are different (same packaging technology along time of storage) (*p* < 0.05).

Table 1

Effect of packaging technology (without packaging – WP, vacuum packaging – VP and modified atmosphere packaging – MAP, 80% CO₂ and 20% N₂) and of storage time (44 days) at 4 ± 1 °C on sensory parameters of Morcela de Arroz (MA). Results are presented as mean ± standard deviation (n = 9 for each packaging/storage time).

Sensory parameter	Pack	Storage time at 4 ± 1 °C (days)					
		0	8	15	22	30	44
Moisture	WP	3.98 ± 0.80 ^{1: D}	2.91 ± 0.88 ^{a: B,C}	3.11 ± 1.09 ^{a: C}	2.48 ± 1.22 ^{a: A,B}	2.60 ± 1.39 ^{a: A,B,C}	2.18 ± 1.38 ^{a: A}
	VP	3.98 ± 0.80 ^C	3.51 ± 1.01 ^{b: B}	3.58 ± 0.93 ^{b: B,C}	3.15 ± 0.87 ^{b: A,B}	3.25 ± 0.83 ^{b: A,B}	2.96 ± 0.89 ^{b: A}
	MAP	3.98 ± 0.80 ^B	3.49 ± 0.99 ^{b: A}	3.58 ± 0.91 ^{b: A,B}	3.21 ± 0.94 ^{b: A}	3.43 ± 0.77 ^{b: A}	3.24 ± 0.79 ^{b: A}
Slime	WP	1.04 ± 0.20 ^A	1.13 ± 0.44 ^{a: A}	1.21 ± 0.49 ^{a: A}	1.67 ± 0.91 ^{b: B}	1.74 ± 1.11 ^{b: B}	1.96 ± 1.23 ^{b: B}
	VP	1.04 ± 0.20 ^A	1.30 ± 0.67 ^{a: A,B}	1.36 ± 0.81 ^{a: B}	1.19 ± 0.39 ^{a: A,B}	1.21 ± 0.49 ^{a: A,B}	1.18 ± 0.39 ^{a: A,B}
	MAP	1.04 ± 0.20 ^A	1.23 ± 0.51 ^{a: B}	1.13 ± 0.34 ^{a: A,B}	1.10 ± 0.31 ^{a: A,B}	1.08 ± 0.27 ^{a: A,B}	1.20 ± 0.45 ^{a: A,B}
Typical color	WP	4.58 ± 0.67 ^D	3.55 ± 1.05 ^{a: C}	3.19 ± 0.96 ^{a: C}	1.98 ± 1.02 ^{a: B}	1.87 ± 0.98 ^{a: B}	1.33 ± 0.68 ^{a: B}
	VP	4.58 ± 0.67 ^B	4.02 ± 0.97 ^{b: A}	3.83 ± 0.99 ^{a: A}	3.75 ± 0.76 ^{b: A}	3.60 ± 1.03 ^{b: A}	3.59 ± 0.92 ^{b: A}
	MAP	4.58 ± 0.67 ^B	4.06 ± 1.01 ^{b: A}	4.00 ± 0.90 ^{b: A}	3.71 ± 0.97 ^{b: A}	3.89 ± 0.85 ^{b: A}	3.78 ± 0.97 ^{b: A}
Brightness	WP	3.86 ± 0.81 ^B	2.96 ± 1.06 ^{a: A}	3.04 ± 0.94 ^{a: A}	2.58 ± 1.32 ^{a: A}	2.72 ± 1.34 ^{a: A}	2.53 ± 1.58 ^{a: A}
	VP	3.86 ± 0.81 ^B	3.30 ± 1.17 ^{ab: A}	3.25 ± 1.09 ^{ab: A}	2.98 ± 1.04 ^{ab: A}	2.89 ± 1.03 ^{a: A}	2.98 ± 0.93 ^{a: A}
	MAP	3.86 ± 0.81 ^C	3.47 ± 1.17 ^{b: B,C}	3.42 ± 1.01 ^{b: B,C}	3.13 ± 1.10 ^{b: A,B}	3.11 ± 1.07 ^{a: A,B}	2.92 ± 1.09 ^{a: A}
Typical odor	WP	4.66 ± 0.59 ^D	3.47 ± 1.07 ^{a: C}	3.40 ± 1.05 ^{a: C}	2.13 ± 0.91 ^{a: B}	2.26 ± 1.04 ^{a: B}	1.31 ± 0.62 ^{a: A}
	VP	4.66 ± 0.59 ^D	4.11 ± 0.91 ^{b: C}	3.81 ± 0.95 ^{b: B,C}	3.79 ± 0.80 ^{b: B,C}	3.32 ± 0.94 ^{a: A}	3.59 ± 0.92 ^{b: A,B}
	MAP	4.66 ± 0.59 ^B	4.02 ± 0.89 ^{b: A}	4.08 ± 0.84 ^{b: A}	3.77 ± 0.83 ^{b: A}	4.17 ± 0.73 ^{a: A}	3.82 ± 0.95 ^{b: A}
Off-odors	WP	1.00 ± 0.00 ^A	1.38 ± 0.60 ^{b: A,B}	1.60 ± 0.83 ^{b: B}	2.40 ± 1.28 ^{b: C}	2.47 ± 1.45 ^{b: C}	3.49 ± 1.30 ^{b: D}
	VP	1.00 ± 0.00 ^A	1.21 ± 0.50 ^{ab: A,B}	1.23 ± 0.58 ^{a: A,B}	1.19 ± 0.39 ^{a: A}	1.58 ± 0.84 ^{a: C}	1.49 ± 0.88 ^{a: B,C}
	MAP	1.00 ± 0.00 ^A	1.09 ± 0.35 ^{a: A,B}	1.13 ± 0.40 ^{a: A,B,C}	1.25 ± 0.43 ^{a: B,C}	1.25 ± 0.68 ^{a: B,C}	1.35 ± 0.66 ^{a: C}
Rancid	WP	1.00 ± 0.00 ^A	1.32 ± 0.61 ^{b: A,B}	1.54 ± 0.73 ^{b: B}	2.50 ± 1.36 ^{b: C}	2.64 ± 1.44 ^{b: C}	3.75 ± 1.18 ^{b: D}
	VP	1.00 ± 0.00 ^A	1.04 ± 0.19 ^{a: A}	1.10 ± 0.36 ^{a: A,B}	1.10 ± 0.31 ^{a: A,B}	1.26 ± 0.56 ^{a: B}	1.24 ± 0.62 ^{a: B}
	MAP	1.00 ± 0.00 ^A	1.02 ± 0.14 ^{a: A}	1.04 ± 0.28 ^{a: A}	1.02 ± 0.14 ^{a: A}	1.15 ± 0.50 ^{a: A,B}	1.25 ± 0.63 ^{a: B}
Putrid	WP	1.00 ± 0.00 ^A	1.04 ± 0.19 ^{a: A}	1.00 ± 0.00 ^{a: A}	1.17 ± 0.64 ^{a: A,B}	1.17 ± 0.64 ^{a: A,B}	1.25 ± 0.74 ^{b: B}
	VP	1.00 ± 0.00 ^A	1.00 ± 0.00 ^{a: A}	1.10 ± 0.41 ^{a: A,B}	1.08 ± 0.35 ^{a: A,B}	1.11 ± 0.38 ^{a: A,B}	1.20 ± 0.75 ^{ab: B}
	MAP	1.00 ± 0.00 ^A	1.02 ± 0.14 ^{a: A}	1.02 ± 0.14 ^{a: A}	1.06 ± 0.24 ^{a: A}	1.11 ± 0.47 ^{a: A}	1.14 ± 0.57 ^{a: A}
Sour/fermented	WP	1.00 ± 0.00 ^A	1.11 ± 0.32 ^{a: A,B}	1.13 ± 0.40 ^{a: A,B}	1.46 ± 0.87 ^{b: B}	1.47 ± 0.93 ^{b: B}	1.98 ± 1.21 ^{b: C}
	VP	1.00 ± 0.00 ^A	1.02 ± 0.14 ^{a: A}	1.10 ± 0.36 ^{a: A,B}	1.21 ± 0.50 ^{a: A,B}	1.38 ± 0.71 ^{ab: B}	1.35 ± 0.66 ^{a: B}
	MAP	1.00 ± 0.00 ^A	1.02 ± 0.14 ^{a: A}	1.10 ± 0.36 ^{a: A,B}	1.08 ± 0.28 ^{a: A,B}	1.19 ± 0.59 ^{a: A,B}	1.25 ± 0.69 ^{a: B}
Spoiled wine/vinegary	WP	1.02 ± 0.14 ^A	1.09 ± 0.30 ^{a: A,B}	1.13 ± 0.40 ^{a: A,B}	1.23 ± 0.51 ^{b: A,B}	1.34 ± 0.73 ^{b: B,C}	1.59 ± 0.92 ^{b: C}
	VP	1.02 ± 0.14 ^A	1.08 ± 0.33 ^{a: A,B}	1.08 ± 0.27 ^{a: A,B}	1.08 ± 0.28 ^{ab: A,B}	1.25 ± 0.52 ^{ab: B}	1.22 ± 0.61 ^{a: B}
	MAP	1.02 ± 0.14 ^A	1.02 ± 0.14 ^{a: A}	1.08 ± 0.33 ^{a: A}	1.06 ± 0.32 ^{a: A}	1.13 ± 0.48 ^{a: A}	1.16 ± 0.50 ^{a: A}
Moldy/mildew	WP	1.00 ± 0.00 ^A	1.04 ± 0.19 ^{a: A}	1.04 ± 0.19 ^{a: A}	1.27 ± 0.57 ^{b: A,B}	1.38 ± 0.79 ^{b: B,C}	1.67 ± 0.97 ^{b: C}
	VP	1.00 ± 0.00 ^A	1.02 ± 0.14 ^{a: A}	1.06 ± 0.31 ^{a: A}	1.04 ± 0.20 ^{a: A}	1.11 ± 0.42 ^{a: A}	1.14 ± 0.53 ^{a: A}
	MAP	1.00 ± 0.00 ^A	1.02 ± 0.14 ^{a: A,B}	1.04 ± 0.19 ^{a: A,B}	1.02 ± 0.14 ^{a: A,B}	1.11 ± 0.42 ^{a: A,B}	1.16 ± 0.54 ^{a: B}
Typical flavor	WP	4.62 ± 0.57 ^D	3.29 ± 1.11 ^{a: C}	2.98 ± 0.96 ^{a: C}	2.04 ± 1.07 ^{a: B}	2.12 ± 0.90 ^{a: B}	1.20 ± 0.46 ^{a: A}
	VP	4.62 ± 0.57 ^D	3.96 ± 0.90 ^{b: C}	3.85 ± 0.98 ^{b: B,C}	3.67 ± 0.86 ^{b: A,B,C}	3.32 ± 0.83 ^{b: A,B}	3.42 ± 0.93 ^{b: A,B}
	MAP	4.62 ± 0.57 ^B	3.96 ± 0.92 ^{b: A}	4.12 ± 0.81 ^{b: A}	3.71 ± 0.82 ^{b: A}	4.02 ± 0.84 ^{a: A}	3.70 ± 0.97 ^{b: A}
Acid	WP	1.12 ± 0.39 ^A	1.23 ± 0.51 ^{a: A,B}	1.27 ± 0.63 ^{a: A,B}	1.52 ± 0.84 ^{b: B,C}	1.67 ± 1.14 ^{b: C,D}	2.00 ± 1.26 ^{b: D}
	VP	1.12 ± 0.39 ^A	1.13 ± 0.39 ^{a: A}	1.13 ± 0.40 ^{a: A}	1.23 ± 0.63 ^{a: A}	1.25 ± 0.65 ^{a: A}	1.28 ± 0.57 ^{a: A}
	MAP	1.12 ± 0.39 ^A	1.19 ± 0.48 ^{a: A}	1.13 ± 0.40 ^{a: A}	1.13 ± 0.39 ^{a: A}	1.21 ± 0.60 ^{a: A}	1.26 ± 0.53 ^{a: A}
Bitter	WP	1.06 ± 0.24 ^A	1.12 ± 0.43 ^{a: A,B}	1.25 ± 0.56 ^{a: A,B,C}	1.52 ± 0.84 ^{b: B,C}	1.63 ± 1.09 ^{b: C,D}	2.03 ± 1.40 ^{b: D}
	VP	1.06 ± 0.24 ^A	1.11 ± 0.38 ^{a: A}	1.10 ± 0.36 ^{a: A}	1.15 ± 0.46 ^{a: A}	1.19 ± 0.56 ^{a: A}	1.24 ± 0.52 ^{a: A}
	MAP	1.06 ± 0.24 ^A	1.09 ± 0.35 ^{a: A,B}	1.12 ± 0.38 ^{a: A,B}	1.08 ± 0.35 ^{a: A,B}	1.13 ± 0.44 ^{a: A,B}	1.26 ± 0.63 ^{a: B}
Global freshness	WP	4.68 ± 0.55 ^D	3.11 ± 1.15 ^{a: C}	2.79 ± 0.99 ^{a: C}	1.79 ± 0.82 ^{a: B}	1.72 ± 0.69 ^{a: B}	1.16 ± 0.42 ^{a: A}
	VP	4.68 ± 0.55 ^C	3.94 ± 1.01 ^{b: B}	3.94 ± 0.91 ^{b: B}	3.48 ± 1.01 ^{b: A,B}	3.21 ± 0.95 ^{a: A}	3.37 ± 0.96 ^{b: A}
	MAP	4.68 ± 0.55 ^C	4.02 ± 0.95 ^{b: A,B}	4.11 ± 0.80 ^{b: B}	3.67 ± 0.86 ^{b: A}	3.92 ± 0.78 ^{a: B}	3.73 ± 1.04 ^{b: A,B}
Global freshness of packed ³	WP	4.82 ± 0.48 ²	3.57 ± 1.01 ^{a: D}	3.04 ± 1.07 ^{a: C}	2.17 ± 0.93 ^{a: B}	1.83 ± 0.89 ^{a: B}	1.33 ± 0.55 ^{a: A}
	VP	4.82 ± 0.48	4.11 ± 0.99 ^{b: C}	4.06 ± 0.95 ^{b: C}	3.21 ± 1.13 ^{b: B}	2.87 ± 0.90 ^{b: A,B}	2.57 ± 0.81 ^{b: A}
	MAP	4.82 ± 0.48	4.15 ± 0.84 ^{b: A}	4.25 ± 0.90 ^{b: A}	4.06 ± 0.70 ^{a: A}	3.89 ± 0.82 ^{a: A}	3.92 ± 0.93 ^{a: A}

^{a-c}-means with different lowercase letters in the same column/parameter are significantly different (p < 0.05).

^{A-D}-means with different uppercase letters in the same row are significantly different (p < 0.05).

¹ The first sampling time (day 0) was made before packaging. Mean values were repeated for the three packages only for comparison between storage times.

² For global freshness of packaged product, in the day 0 of the study assessors evaluated only the unpackaged product.

³ Global freshness of the entire packed product (external appearance).

faster deterioration detected by the sensory panel, WP samples did not have a clearly worst microbiological profile, as it might be expected. The reduction of *a_w* due to the drying effect produced a delay in the growth of the microbiota. Despite the small absolute reduction in the *a_w* during the experiment (0.02 units of *a_w*), it occurred at an interval that began to have inhibitory effect on microbiota, particularly on Gram-negative bacteria (Adams & Moss, 2008).

The presence of spore formers like *B. cereus* was probably associated to the contamination of the raw materials and/or to the insufficient heat treatment to inactivate the spores. Nevertheless it does not compromise the safety of MA, once the infective dose for diarrheal or emetic syndrome is clearly higher (Arnesen, Fagerlund, & Granum, 2008) than the counts observed in MA.

The post cooking contamination was probably the main responsible for the MTVC, PTVC and LAB counts observed immediately after

processing. Even for thermotolerant non-spore former Gram-positive bacteria, the heat treatment associated to the cooking of MA was very intense to allow their survival. Once MA was produced at one industry that produces dry-cured meat products, it is acceptable to consider that the contamination of MA was made from the house flora of the industry, composed mainly by Gram positive bacteria, particularly LAB (Mäkelä & Korkeala, 1987). The total viable counts might be also composed by spore formers that survived to the heat treatment (Korkeala, Lindroth, Suihko, Kuhmonen, & Penttilä, 1985). The behavior of total viable counts (MTVC and PTVC) was similar along the experiment, without a clear pattern of differences between MA packaged with different technologies. These results might be justified by the qualitative composition of these total counts. Once the different spoilage microorganisms have particular behaviors in each packaging technology, and it is expected to have a substitution effect when one group is inhibited, the

Table 2
Selected Pearson correlations between microbial counts and sensorial evaluation of global freshness (r, p).

Microbial group	WP	VP	MAP
MTVC	-0.86, <0.001	-0.72, 0.004	-0.72, 0.004
PTVC	-0.83, <0.001	-0.73, 0.003	-0.77, 0.001
LAB	-0.74, 0.002	-0.85, <0.001	-0.88, <0.001
<i>Enterobacteriaceae</i>	-0.49, ns	-0.76, 0.002	-0.33, ns
<i>Pseudomonas</i> spp.	-0.90, <0.001	-0.74, <0.001	-0.33, ns

ns, not significant ($p > 0.05$).

similar MTVC and PTVC were composed by the specific dominant spoilage group in each package. LAB were the dominant spoilage microbiota in VP samples as they usually are in VP meat products (Korkeala & Björkroth, 1997). The microaerophilic needs justifies the ability to grow well in VP, once they can use trace amounts of oxygen dissolved in the VP product (Buchanan, 1986; Korkeala & Björkroth, 1997). MAP and WP samples had similar LAB counts. The reduction of growth in MAP MA can be attributed to the direct effect of carbon dioxide, once it has significant effect on the extension of the lag phase and generation time of certain LAB (Devlieghere, Debevere, & Van Impe, 1998). That was confirmed by the results of Santos, Jaime, et al. (2005), who found an inhibitory effect of MAP (80% CO₂/20% N₂) in the growth of LAB comparatively to VP. However, other authors (Cachaldora et al., 2013; Pexara et al., 2002; Samelis & Georgiadou, 2000) reported that the use of MAP with different amounts of carbon dioxide in cooked meat products did not affect the growth rate of LAB when compared to the use of VP. That apparent controversy might be due to the LAB species present. According to the results obtained in Spanish blood sausages (Diez et al., 2008; Santos, Diez, González-Fernández, Jaime, & Rovira, 2005; Santos, Jaime, et al., 2005) the associated spoilage LAB were heterofermentative mainly members of *Leuconostoc* and *Weissella* genus, that were reported to be more inhibited by high concentration of carbon dioxide. In MA stored WP, the low level of carbon dioxide, combined with the high a_w in the beginning of the storage and low temperature gave a competitive disadvantage to LAB against other psychrotrophic aerobes, namely *Pseudomonas* spp. (Huis in't Veld, 1996).

The behavior of *Enterobacteriaceae* was similar to that of Spanish *Morcilla* using the same MAP of the present work (Santos, Jaime, et al., 2005). In VP and WP samples, *Enterobacteriaceae* were detected from the day 8 of storage and grew until the day 22. After that, the reduction observed in the *Enterobacteriaceae* in WP samples was probably due to the reduction of a_w that was more pronounced in this interval. The continuous growth of *Enterobacteriaceae* until the end of storage period in VP samples was associated to their facultative anaerobic character. The inhibitory effect of carbon dioxide, which is particularly efficient in Gram-negative bacteria (Mullan & McDowell, 2003), justifies the low counts observed in MAP. As expected, the behavior of *Pseudomonas* spp. was highly conditioned by the packaging technology. In WP these aerobic microorganisms grow to higher levels, while the a_w was compatible with their needs. VP retarded considerably the growth of *Pseudomonas* spp. The best inhibitory effect was obtained when MA was packaged in MAP, once this group of spoilage microorganism was present, during all the experiment, in very low counts. In addition to oxygen depletion in the packaging by substitution by nitrogen and carbon dioxide, the direct inhibitory effect of CO₂ was the responsible for the strong inhibition of these Gram-negative aerobic bacteria.

The absence of package as it is usually done by the producers of MA revealed to be the worst choice for the maintenance of the sensory characteristics. These MA supported well the growth of all the spoilage microorganisms studied, resulting in the development of off-odors and sooner than when package is used. Additionally, the absence of package could have contributed to the loss of the characteristic aromas by volatilization and the oxidation of lipids, as inferred by the higher scores for rancid odor. The oxidation of lipids might also have been favored by the high count of *Pseudomonas* spp. in these samples. These bacteria

have an important lipolytic activity (Huis in't Veld, 1996), resulting at one increase of free fatty acids which favors the oxidation phenomena once free fatty acids are more easily oxidized than when esterified in triglycerides (Hamilton, 1994). The introduction of VP represents an improvement in the shelf life. The product maintained good sensorial characteristics during storage, however, mainly due to the growth of LAB and *Enterobacteriaceae* it losses the freshness. The use of MAP revealed to be the best solution to increase shelf life of MA, once almost all the sensory traits were maintained during the 44 days of storage. Though these samples presented high counts of LAB, the principal spoilage microbial group in these MA, the impact of these microorganisms in sensory characteristics was modest, once they have a weak lipolytic and catabolic activity over nitrogen fraction, when compared to *Enterobacteriaceae* and *Pseudomonas* spp. (Huis in't Veld, 1996).

Depending on the initial quality of the product, storage temperature and package construction and design, shelf life of cooked meat products can be extend by two- to four-fold with MAP (Hotchkiss & Langston, 1995). CO₂/N₂ atmospheres are referred as particularly adequate for the preservation of cooked meat products, mainly because of the strong inhibition of the growth of the great majority of microorganisms and of the oxidative phenomena (Hintlian & Hotchkiss, 1987; Matos et al., 2005; Summo, Caponio, Tricarico, Pasqualone, & Gomes, 2010).

5. Conclusions

The present traditional storage and distribution procedure of MA from Monchique region, without packaging, compromises the shelf life of this difficult to preserve ready to eat blood sausage, which was evidenced by sensory and microbiological results, and contributes to considerable economic losses for the producers by compromising the commercialization and the conquest of other markets. Accordingly to the results of the behavior of the microbial groups during the storage period, *Enterobacteriaceae* is associated particularly to the loss of freshness in VP, and *Pseudomonas* spp. in WP samples. MAP using 80% CO₂ and 20% N₂, by its inhibition effect on the growth of LAB, *Enterobacteriaceae* and *Pseudomonas* spp. and on rancidity revealed to be the most efficient on extending the shelf life of Morcela de Arroz.

Conflict of interest

The authors have no conflict of interest to declare.

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