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Effect of modified atmosphere and vacuum packaging on some quality characteristics and the shelf-life of "*morcilla*", a typical cooked blood sausage

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ABSTRACT

The effect of modified atmosphere and vacuum packaging on the shelf-life of "*morcilla*", a traditional cooked blood sausage, was investigated. A total of 99 "*morcillas*" were packaged under vacuum and in modified atmosphere using three different gas mixtures: 15:35:50/O_2:N_2:CO_2 (atmosphere 1), 60:40/N_2:CO_2 (atmosphere 2) and 40:60/N_2:CO_2 (atmosphere 3), and stored during 2, 4, 6 and 8 weeks at 4 °C. Shelf life evaluation was based on pH, water activity (a_w), colour (CIE L*, a*, b*, C* and h*), TBARS formation and microbial counts. The results indicated that, in general, storage time affected ($P<0.05$) all parameters whereas no significant differences were observed ($P>0.05$) among packaging conditions. Based on the microbial counts, the shelf-life of "*morcilla*" would be greater than 8 weeks for all packaging conditions. Samples packaged with high CO_2 concentrations (40:60/N_2:CO_2) showed the lowest values of TBARS at the end of storage.

1. Introduction

Blood sausages are traditional meat products in many parts of the world (Díez, Santos, Jaime, & Rovira, 2008). In the north of Spain, "*morcilla*" is the most typical cooked blood sausage. It is made from a mixture 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 90–95 °C and air cooled to 8–10 °C. Generally it is stored under refrigerated conditions (2–4 °C) and consumed after cooking. This product is commercialized as a fresh product with a shelf life of around 8–10 days, limiting its distribution, therefore producers are interested in increasing its shelf life in order to reach more potential markets and satisfy consumer demands (Díez et al., 2008; Santos, Díez, et al., 2005; Santos, González-Fernández, Jaime, & Rovira, 2003; Santos, Jaime, et al., 2005).

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 (Parry, 1993). Modified atmosphere packaging (MAP) and vacuum-packaging (VP), along with refrigeration, have become increasingly popular preservation techniques to extend the shelf life of meat and meat products, which have brought major changes in storage, distribution, and marketing of raw and processed products (Özogul, Polat, & Özogul, 2004). Therefore, modification of the extrinsic parameters of the product ecosystem, like temperature and gaseous atmosphere, is a common practice to extend shelf-life compared with the use of chemical preservatives. The choice of the gas mixture used is influenced by factors like the product sensitivity to oxygen (O_2) and carbon dioxide (CO_2), the colour stabilizing requirements, the initial level of bacterial contamination and the microorganisms capable of growing on the product (Martínez, Djenane, Cilla, Beltrán, & Roncalés, 2005).

A number of studies have been carried out to evaluate the effectiveness of vacuum, gas composition and packaging material on the preservation of dry fermented sausages (Fernández-Fernández, Vázquez-Odériz, & Romero-Rodríguez, 2002; Rubio, Martínez, García-Cachán, Rovira, & Jaime, 2008; Rubio et al., 2007) and cooked meat products (Efthimia, Pexara, Metaxopoulos, & Drosinos, 2002; Møller et al., 2003). Concerning "*morcilla*", few reports are available concerning the packaging effects on microbiological and physicochemical quality of "*morcilla*" during chilled storage (Korkeala & Björkroth, 1997; Santos, Díez, et al., 2005). Thus, this work was focused on the shelf life of "*morcilla*" preserved in vacuum and modified atmosphere packaging during storage at 4 °C. Microbial quality, pH, water activity, colour stability (CIE L*,a*,b*, C* and H* ordinates), and the oxidative stability of lipids was determined at 0, 2, 4, 6, and 8 weeks of storage.

2. Materials and methods

2.1. Sausage formulation and processing

A total of 99 samples were used. The formulation used in the manufacture of the "*morcillas*" included pork lean meat (38.4%), pork dewlap (25.6%), raw onion (12.8%), cooked pork skin (6.4%), water (12.5%), salt (1.3%), supplement (1.3%), sweet paprika (1.0%), spicy

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paprika (0.4%), garlic (0.3%) and blood powder (0.1%). The supplement was the same in all cases and was composed, in unknown proportions, of sugars, skimmed milk powder and sodium nitrite (E_{250}). The different proportions of lean meat, onion, dewlap and skins were minced at low temperature, and then mixed with the remaining ingredients. The meat mixture was stored at 4 °C for 12 h and, after that; it was stuffed into 32–35 mm natural pork casings. Ripening was carried out at 10–12 °C and 75–85% relative humidity during 21 days. The sausages were smoked during approximately the first five days of ripening using smoke from oak wood. The blood sausages were then transferred to a cooking vessel and boiled water at 95–96 °C for around 30 min. After cooking, "*morcillas*" were rapidly cooled at 20 °C during 20 min.

2.2. Packaging of samples

Manufactured "*morcillas*" were divided into four batches of 8 packs and packed under:

Vacuum: the samples were introduced in bags with an oxygen transmission rate of 50 cm^3/m^2/24 h/bar at 23 °C and 75% RH and water vapour transmission rate of 2.6 g/m^2/24 h at 23 °C and 85% RH were subjected to vacuum and sealed in a FRIMAQ V-900 packaging machine (Lorca, Spain).

Gas mixture: "*morcillas*" were packed in trays 300 PS mm thick and sealed with PE film 74 mm thick and with oxygen permeability less than 2 ml/m^2/24 h/bar. The packaging was carried out using a heat sealer Lari3/Pn Cavec T-VG-R-SKIN (Caveco, Milano, Italy). The gas combinations used to package the samples were: 15:35:50/O_2:N_2:CO_2 (atmosphere 1), 60:40/N_2:CO_2 (atmosphere 2) and 40:60/N_2:CO_2 (atmosphere 3). Gas mixtures were supplied by Praxair (Madrid, Spain). All packs were stored at 4 ± 1 °C (simulating retail conditions in a refrigerated chamber). This chamber was illuminated by a standard supermarket fluorescent lamp. Two packages, with three samples each, were taken after 0, 2, 4, 6 and 8 weeks of storage for physico-chemical and microbiological analyses.

2.3. Physico-chemical analysis

2.3.1. pH and a_w parameters

The pH of the samples was measured using a digital pH-meter (Thermo Orion 710A+, Cambridgeshire, UK) equipped with a glass probe for penetration. Water activity (a_w) was determined using a Fast-lab (Gbx, Romans sur Isére Cédex, France) water activity meter, previously calibrated with sodium chloride and potassium sulphate.

2.3.2. Colour evaluation

Objective measurements of colour were performed using a CR-600 colourimeter (Minolta, Osaka, Japan). Each sausage was cut and the colour of the slices was measured three times for each point. A portable colourimeter with the settings: pulsed xenon arc lamp, 0° viewing angle geometry and aperture size 8 mm) was used to measure meat colour in the CIELAB space (Lightness, L*; redness, a*; yellowness, b* (CIE, 1978). Hue (Ho) and chroma (C*) were calculated from the a* and b* values according to the formula:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \text{ and } h^* = acr\ \tan\frac{b^*}{a^*}$$

Before each series of measurements, the instrument was calibrated using a white ceramic tile.

2.3.3. Lipid oxidation

Lipid stability was measured by the 2-thiobarbituric acid (TBA) method proposed by Vyncke (1975). Meat samples of 2 g were taken and mixed with 5% trichloroacetic acid (10 mL) using an Ultra-Turrax (Ika T25 basic, Staufen, Germany) for 2 min. The homogenate was maintained at −10 °C for 10 min and centrifuged at 2054× *g* for 10 min. The supernatant was filtered through a Whatman No. 1 filter paper. The filtrate (5 mL) were taken and mixed with 5 mL TBA solution (0.02 M) and incubated in a water bath at 96 °C for 40 min. The absorbance was measured at 532 nm. Thiobarbituric acid reactive substances (TBARs) values were calculated from a standard curve of malonaldehyde with 1,1-3,3 tetraetoxipropane (TEP) and expressed as mg MDA/kg sample.

2.4. Microbiological analysis

Ten gram 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, also containing 0.85% NaCl and 1% Tween 80 as emulsifier, 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 (TVC) were enumerated in Plate Count Agar (PCA; Oxoid, Unipath Ltd., Basingstoke, UK) incubated at 30 °C for 48 h. Psychrotrophic aerobic bacteria (PVC) were enumerated on Plate Count Agar (PCA; Oxoid, Unipath Ltd., Basingstoke, UK) after incubation at 7 °C for 10 days. Lactic acid bacteria (LAB) were determined on the Man Rogosa Sharpe medium Agar (Oxoid, Unipath Ltd., Basingstoke, UK) (pH 5.6), after incubation at 30 °C for 5 days. *Enterobacteriaceae* was determinate on Violet Red Bile Glucose Agar (Merck, Darmstadt, Germany) after incubation at 37 °C for 24 h. Moulds and yeasts were enumerated using Oxytetracicline Glucose Yeast Extract Agar (OGYE) (Merck, Darmstadt, Germany) with OGYE Selective Supplement (Merck, Darmstadt, Germany), previously incubated at 25 °C for 4–5 days. Pseudomonads were determined on Pseudomonas Selective Agar (Merck, Darmstadt, Germany) with Pseudomonas CFC Selective Supplement (Merck, Darmstadt, Germany), previously incubated at 25 °C for 48 h. Sulfite reducing clostridia were enumerated on the Sulfite Polymyxin Sulfadiazine Agar (Merck, Darmstadt, Germany) with the plates incubated under anaerobic conditions at 44 °C for 24 h.

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

2.5. Statistical analysis

Results were expressed by means ± standard error. Comparison of means was performed by one-way analysis of variance (ANOVA). The least squares means (LSM) were separated using Duncan's test. All statistical test of LSM were performed for a significance level $P<0.05$. Data were analysed using the general linear model of SPSS (SPSS 19.0, Chicago, IL, USA) software package. Correlations between variables ($P<0.05$) were determined by correlation analyses using Pearson's linear correlation coefficient with the above statistical software package.

3. Results and discussion

3.1. Physico-chemical analysis

3.1.1. pH and a_w values

Fig. 1 shows the pH values obtained for the samples during storage. No significant differences ($P>0.05$) in pH values were observed due to the packaging conditions. The mean initial pH of the sausages was 4.72 ± 0.02 and it slightly increased during storage, reaching values from 4.75 to 4.84 after 8 weeks. The trend agrees with the results reported by several authors in other fermented sausages (Fernández, Rozas-Barrero, Romero-Rodríguez, & Vázquez-Odériz,