USE OF MODIFIED ATMOSPHERE PACKAGING TO EXTEND THE SHELF-LIFE OF "MORCILLA"

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Background: traditional meat products from Spain are more known every day. In relation to these meat products, "morcilla" is a popular product in Spain, especially "morcilla" from Burgos. This product resembles to the English black pudding and it is made of rice (sometimes pre-cooked), onion, fat, blood from slaughtered pigs and cattle, and different spices, stuffed in natural pork or beef casings and boiled about 90 min at 90-95°C. In general this product is distributed unpacked in a limit market and with a short shelf-life (10-12 days) due to their high moisture and the absence of a lactic fermentation. Nowadays, producers of "morcilla" from Burgos (about 40) are very interested in extending the shelf-life of this product in order to reduce product returns and increase access to distant markets. Normally cooked meat products are chilled stored, usually in vacuum-pack or in modified atmosphere pack (MAP) or stored in aerobic atmosphere (Borch et al., 1996). MAP (CO_2 and N_2) was used in this experiment to extend the shelf-life of this product.

Objectives: the aim of this work was to determine the shelf-life of the different groups of "morcilla" previously established, according to their microbiological characteristics, stored at 4°C using two different concentrations of CO_2 in the packs ($50\%CO_2/50\%N_2$ and $30\%CO_2/70\% N_2$).

Methods: according to the total viable count (TVC) in the casing and in the internal zone all products were distributed in the following four groups: **HH**: external TVC>5 log CFU/g and internal TVC> 3 log CFU/g; **HL**: external TVC>5 log CFU/g and internal TVC< 3 log CFU/g; **LH**: external TVC<5 log CFU/g and internal TVC> 3 log CFU/g; **LL**: external TVC<5 log CFU/g and internal TVC< 3 log CFU/g. For each group two atmospheres were tested: $50\%CO_2/50\%N_2$ (50/50) and $30\%CO_2/70\% N_2$ (30/70). Film used for the experiment was polyamide/polyethylene (40/100) with low permeability. The ratio of gas volume/meat volume was approximately 1:2. Samples were stored in cold rooms at 4°C until sampling. Two packs were opened at 0, 7, 14, 21, 28, 35, 42 days for physicochemical, microbiological and sensory analysis.
Physicochemical tests: pH, Aw, drip loss, moisture and percentage of CO_2 and O_2 using a portable Combi Check 9800-1(PBI Dansensor, Denmark), were analysed weekly.
Microbiological tests: total viable count (TVC) and psychrotrophic in PCA incubated at 30°C 48h, and at 7°C 8 days, respectively, lactic acid bacteria (LAB) in MRS incubated in anaerobic conditions at 30°C 48h, heterofermentative LAB in APT broth incubated at 30°C 48h and Enterobacteriaceae in VRBGA incubated at 37°C 24-48h were determined in the internal zone and in the skin of the sausage.
Sensory analysis: the product were evaluated weekly by an experienced, trained 3-5 member sensory panel using a 5-point scale for the assessment of overall appearance, odour and taste where 5=excellent and 1=unacceptable. Also intensity of exudate and off-odour was assessed with a 5-point scale (1=absence, 5= high intensity). The time in days before the panel considered the overall appearance to be at the limit of acceptability (score=3) was defined as shelf-life of the product. For this purpose when a score of 3 was passed, interpolation between scores on successive weeks was used (Silla and Simonsen, 1985).

Results and discussions: the initial pH of the product was significantly different ($P<0.05$) for each product and it was observed a significant decrease ($P<0.05$) during the storage time in each product with the two atmospheres. The initial pH decreased from 6.84±0.38 until 6.06±0.38 after 42 days of storage. Concerning to drip loss, moisture and Aw, there were hardly changes during the storage period.

No growth of Enterobacteriaceae was detected for any group during the storage period as would be expected for a cooked product. Initially growth of psychrotrophic and LAB was not detected in the internal zone. On the contrary, Bacillus strains appeared in the MRS plates at first sampling days. This fact suggests that these bacteria, which are frequently introduced to processed meats by spices (Linch and Potter, 1988), were present as resistant forms during the cooking process and they could survive the heat treatment.

For group HH, TVC in the internal zone were similar (near to 5 log CFU/g) during the storage for both atmospheres although psychrotrophic and LAB (especially heterofermentative LAB) increased with time until reaching values near to TVC. The increase in psychrotrophic LAB could be a result of the selective inhibition of normal spoilage bacteria that is produced by MAP as many authors have reported for different meat products (Holley et al., 1994; Jeremiah et al., 1995; Nissen et al., 1996). The external TVC, psychrotrophic and LAB (heterofermentatives) counts strongly increased until values near to 8-9 log CFU/g finding the highest bacterial numbers for atmosphere 50/50. Although in the initial step after packaging, the level of carbon dioxide decreased for all groups due to the solubilization of CO_2 in the product, there was a remarkable increase of this gas for group HH during the storage period. The most obvious changes in sensory attributes during cold storage of this product were the presence of visible colonies on the product surface and the development of sour and acid odour and taste. According to these facts it can be conclude that LAB, especially heterofermentative LAB, are the responsible of pH drop and contribute to the blowing of packs, exudate development and souring of the product. Group HH had the lower shelf-life with both atmospheres, but regarding to odour and taste scores higher values were found with the atmosphere 30/70 (Table 1).

The general behaviour for group HL according to external microbiological evolution was similar to group HH with slightly lower counts for the atmosphere 30/70. In the internal zone for atmosphere 50/50, TVC reached 5 log CFU/g as group HH although initial TVC was <3 log CFU/g, while for atmosphere 30/70 counts of TVC, psychrotrophic and LAB remained low and presence of

heterofermentative LAB were not detected. Although microbiologically atmosphere 30/70 should be better, the lowest shelf-life was achieved with this gas mixture for this group. On the contrary, group LH external counts were lower with atmosphere 50/50 and similar bacterial numbers were found for the microbiological parameters in the internal zone for both atmospheres, but the sensory evaluation gave better scores to the "morcillas" packed under 30% CO_2 and the shelf-life was nine days longer.

Finally in group LL, external TVC increased until 7-8 log CFU/g and all the external counts were rather higher with 30% of CO_2, but in the internal zone TVC did not experimented a large increase with atmosphere 30/70 while with the another gas composition TVC reached values close to 5 log CFU/g. No heterofermentative LAB was detected during the storage with atmosphere 30/70. The higher shelf-life and the best sensory parameters were achieved with atmosphere 50/50.

Conclusions: group highly contaminated HH had the lowest shelf-life under both atmospheres, which indicates that lower initial levels of microorganisms present influence on the success of MAP.
Atmosphere 50/50 seems to be more efficient for "morcillas" belonging to group LL, but in relation to the groups HL and LH not clear correlation between microbial counts and sensory parameters has been found to determine the shelf-life. This makes neccessary investigate an alternative atmosphere composition, and also it could be useful to find some physicochemical parameters correlated to sensory evaluation, which provide a good measurement of spoilage.

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Table 1. Effects of CO_2 level and storage on sensory parameters for the different groups

Group	MAP	shelf-life	Days of storage 7	14	21	28	35	42
Overall appearance								
HH	50/50	17	4.2^{xa}	3.7^{a}	2.0^{b}	1.4^{bc}	1.0^{c}	1.0^{c}
	30/70	17	3.4^{ya}	3.3^{a}	2.3^{b}	1.3^{c}	1.0^{c}	1.0^{c}
HL	50/50	31	4.2^{ab}	4.5^{xa}	3.8^{xabc}	3.5^{xbc}	1.3^{d}	1.7^{d}
	30/70	17	3.5^{a}	3.0^{yab}	2.8^{yabc}	2.0^{ycd}	2.2^{bc}	1.0^{d}
LH	50/50	21	4.0^{a}	3.7^{a}	3.0^{xb}	2.3^{xc}	1.7^{d}	2.0^{xcd}
	30/70	30	4.0^{a}	3.3^{b}	4.0^{ya}	3.5^{yab}	1.3^{c}	1.1^{yc}
LL	50/50	31	4.6^{a}	4.5^{xa}	4.0^{xa}	4.0^{xa}	1.3^{b}	1.0^{b}
	30/70	18	4.1^{a}	3.3^{yb}	2.7^{yb}	1.7^{yc}	1.3^{c}	1.0^{c}
Odour								
HH	50/50		4.0^{a}	2.8^{b}	1.1^{xc}	1.0^{xc}	1.0^{xc}	1.0^{xc}
	30/70		3.6^{a}	3.3^{a}	3.0^{ya}	2.3^{yb}	2.5^{yb}	2.6^{yb}
HL	50/50		4.0^{xa}	4.3^{xa}	3.5^{xab}	2.8^{bc}	2.3^{cd}	1.5^{d}
	30/70		3.0^{ya}	2.5^{yab}	2.5^{yab}	2.0^{b}	2.8^{ab}	2.0^{b}
LH	50/50		4.0^{a}	3.5^{ab}	3.1^{bc}	2.6^{xcd}	1.0^{xe}	2.0^{d}
	30/70		3.6^{a}	3.3^{a}	3.7^{a}	3.3^{ya}	3.0^{ya}	1.3^{b}
LL	50/50		4.4^{a}	4.8^{a}	3.7^{xb}	3.3^{xb}	1.7^{xc}	1.0^{xc}
	30/70		4.4^{a}	4.3^{a}	2.8^{yb}	2.5^{yb}	2.7^{yb}	3.0^{yb}

Group	MAP	Days of storage 7	14	21	28	35	42
Off-odours							
HH	50/50	1.0^{a}	2.0^{b}	4.8^{xc}	4.6^{xc}	4.7^{xc}	4.0^{xc}
	30/70	1.0^{a}	2.7^{cd}	1.3^{yab}	3.0^{yd}	2.0^{yabcd}	2.1^{ybcd}
HL	50/50	1.3^{a}	1.0^{a}	1.1^{xa}	1.8^{xa}	2.7^{xb}	3.3^{b}
	30/70	1.3^{a}	1.8^{ab}	4.3^{yc}	3.7^{yc}	4.0^{yc}	2.5^{b}
LH	50/50	1.0^{a}	1.3^{xab}	1.3^{ab}	2.5^{xc}	2.5^{c}	2.0^{xbc}
	30/70	1.3^{a}	2.2^{yb}	1.0^{a}	1.0^{ya}	1.5^{ab}	3.8^{yc}
LL	50/50	1.0^{a}	1.0^{a}	1.6^{b}	1.9^{bx}	3.3^{xc}	5.0^{xd}
	30/70	1.0^{a}	1.0^{a}	2.2^{b}	2.7^{yb}	1.0^{ya}	1.0^{ya}
Taste							
HH	50/50	4.1^{a}	3.0^{b}	1.5^{xc}	NT	NT	NT
	30/70	4.1^{a}	3.7^{ab}	3.3^{yb}	3.0^{b}	1.5^{c}	2.0^{c}
HL	50/50	3.8^{a}	4.3^{xb}	4.0^{xa}	2.3^{xc}	2.5^{c}	NT
	30/70	4.0^{ya}	3.3^{yab}	3.2^{yb}	3.0^{yb}	NT	NT
LH	50/50	4.0^{xa}	3.7^{ab}	3.5^{ab}	3.0^{b}	NT	NT
	30/70	3.3^{ya}	3.7^{a}	3.7^{a}	3.0^{a}	3.5^{a}	NT
LL	50/50	4.3^{a}	4.8^{b}	2.1^{xc}	3.0^{xd}	1.3^{e}	NT
	30/70	4.3^{a}	4.3^{a}	3.0^{yb}	2.0^{yc}	NT	NT

^{a,b,c,d,e} Means in the same row with different superscripts differ ($P<0,05$) because of the storage time effect.
^{x,y} Means in the same column within each group with different supercripts differ ($P<0,05$) because of the level of CO_2 effect.
NT: not tested