APPLICATION OF LACTATE AND DIACETATE TO IMPROVE THE SHELF-LIFE OF THE BLOOD SAUSAGE "MORCILLA DE BURGOS"

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Background

Morcilla de Burgos is a typical blood sausage from Spain. It is made of a mixture of onion, rice, animal fat (mainly lard), blood and spices. All these ingredients are mixed and stuffed in natural casings and then cooked in water at 94 ºC during 45-60 min, after this cooking step they are air cooled at room temperature. *Morcilla* is sold without packaging in local markets and vacuum packaged in retailing shops. Preservation of *morcilla* is quite difficult, because it is a product with a high water activity (0.984), moisture (around 60%) and pH (up to 6.1) together with a rich nutrient composition (Santos et al., 2003). Vacuum packaged *morcilla* usually lasts around 21 to 30 days depending on the initial contamination and the storage conditions. In general, the spoilage of *Morcilla de Burgos* is due to the growth of heterofermentative lactic acid bacteria that produce changes in the coloration of the casing from black to pink or greenish, blowing of packages and forming ropy slime.
Sodium lactate has been used for several years, in the meat industry because of capability to increase flavour, shelf life and increase the microbiological safety of these products. The antimicrobial effects of lactate are due to their water activity lowering ability and the inhibitory effect of the lactate ion (Koos, 1992; Houstma, 1993). In that sense, they have been successfully applied in the extension of the shelf life of cooked ham by reducing the counts of psychrotrophs, faecal streptococci and enterobacteria and reducing as well the formation of ropy slime (Rondinini et al., 1996). Apart from this role in the extension of the shelf life of meat products, lactate plays and important role inhibiting the growth of foodborne pathogens as *E. coli* O157:H7, *B. cereus*, *L. monocytogenes*, *Clostridium botulinum*, *Staphylococcus aureus*, *Y. enterocolitica* and so on (Bolton, 1998; Shelef, 1994; Miller and Acuff, 1994). One of the problems derivate of using sodium lactate is the salty taste, when it is used in high concentration (> 3%). This problem may be overcome using other lactate derivates as potassium lactate, and adjusting the salt content in the product (Wilmink, 2000). Recent studies have shown the synergic effect of using together lactate derivates and diacetate, against several foodborne pathogens (Mbandi and Shelef, 2001; Glass et al., 2002).

Objectives

The aim of this work was to investigate the effect of several lactate derivates, and the combination of lactate and diacetate, on the extension of the shelf life and microbial quality of the blood sausage *morcilla de Burgos*. The improvement of the shelf life of this product is very important in order to expand the selling market, because *morcilla de Burgos* has a very popular market in Spain as a "tapa". It is also very important to prove that the use of these substances as preservatives do not decrease the sensory properties of the final product.

Materials and methods

Samples: Two trails of four batches of 25 K of *morcilla* with the same composition were elaborated in a typical *morcilla* factory. In batch A we added 3% potassium lactate (Purasal P Hipure. Purac.); in batch B 3% mixture of sodium lactate and potassium lactate (Purasal LITE. Purac.); batch C was considered as control and no lactate derivates was added; and finally batch D was elaborated with 2,5% of a mixture of potassium lactate and diacetate (Purasal P Optiform. Purac.). After the mixtures were made, they were stuffed in beef natural casings and open air cooked at 94ºC during almost 1 hour. After cooking step they were air cooled before being individually vacuum packaged. In that moment, they were brought to the lab in ice boxes to keep them at cool temperature. Packages were kept in a dark place at 4ºC for 32 days, and two packages of each batch were analysed at 0, 5, 10, 15, 20, 27 and 32 days.

Microbial analysis: A slice of 25 g of *morcilla* were sterile weighted, diluted in 225 ml of Ringer solution (Oxoid. Basingstoke, UK), and homogenised before preparing 1/10 serial dilutions. According to the results obtained in a previous work (Santos, 2001), six different microbiological parameters were chosen to evaluate the effectiveness of the treatments:
Total Viable Count plated on PCA agar plates (Oxoid) and incubated at 30ºC during 48 hours.
Enterobacteria were tested in VRBGA agar (Oxoid) incubated at 30ºC during 48 hours.
Pseudomonads were plated on Pseudomonads agar (Oxoid) supplemented with CFC (Cetrimide, Fucidine, Cefaloridine, Oxoid) at 30ºC during 48 hours.
Lactic acid bacteria (LAB) were grown in MRS agar (Biokar Diagnostics, Beauvais, France) and incubated at 30ºC for 48 hours in anaerobic conditions.
Pyschrotrophs were deep plated on PCA agar and plates were incubated at 7ºC during 10 days.
Clostridium perfringens were tested inoculating 1ml of sample dilution in a tube with 20 ml of TSN agar (Tryptone Sulfite Neomycin, Biokar Diagnostics) and incubated at 45ºC during 24-48 hours, in anaerobic conditions. Black colonies were considered as positive.
After taking the sample for microbial analysis, pH was measured in four different points of the *morcilla*, with a penetrating probe.

Sensory analysis: to evaluate if the treatments proposed could introduce some sensory changes in the products, triangle and ranking test were carried out twice for each trial, using a consumer panel (n=30). *Morcilla* were cut in 1.5 cm slices width and microwave heated till 70ºC. Triangle test were only made to evaluate differences between treatments C (control) and D (lactate-K + diacetate), because we thought we obtained the best results with treatment D due to the synergic effect between both preservative substances, according with the literature. The results of this test were analysed in the correspondent tables. Ranking test was made with all four batches of *morcilla* in the same panel session after the triangle test, to evaluate the preference between all four batches. The results were analysed with the aid of Kramer's tables and Friedman test.

Results and discussion

The evolution of pH and the microbiological results during the storage time are shown in table 1. It is possible to observe that pH drops more quickly in batch C than in the others and in 27 days of storage the pH is below 5.00, while in the other batches it keeps above this value. This fact was easily observed; when samples from batch C were opened due to the penetrating sour smell they produce.
On day 0, the same day that *morcilla* were made and packaged, only some colonies grew on PCA agar (TVC). These colonies were irregular in shape and some of them were mucous and they were associated to members of the sporulating genera *Bacillus*, because there are some vegetable ingredients in the composition of *morcilla de Burgos* than can harbour these bacteria, and because after the cooking step it seems that only sporulating bacteria can survive. In the remaining parameters no growth were detected for this sampling day. These results are in accordance with the results obtained by Santos (2001) and Borek et al. (2002) in our lab with same product.
Remarkable rapid growth of bacteria in the product was observed in the first five days, especially in TVC, LAB and Psychrothrophs. This growth could be the quick development of LAB that had favourable conditions to grow. In fact, it is possible to observe that counts of TVC, LAB and Psychrotrophs are quite similar from day 5 to the end of the storage time. Santos described in previous works (2001) the same situation in this product, as well as the static evolution of enterobacteria and pseudomonads during all the storage time. The presence of these microbial populations that appears 24 hours after packaging is due to the post-cooking contamination in the cooling and packaging steps (Santos, 2001; Borek et al., 2002).
LAB is the spoilage dominating population, especially members of the genera *Leuconstoc* and *Weisella* (Santos, 2001). It is in this microbial group where the effects of the different treatments were more significant. In batch C *morcilla*, the spoilage came about around the days 20-25 of the storage time, while in the other treatments spoilage took place later, especially in *morcilla* of batch B; in that case there was a difference of almost two logs in TVC at the end of the storage time. Less effective seems to be the combination of potassium lactate and diacetate. A similar evolution can be seen for psychrotrophs. No growth in TSN agar was detected.

Triangle test done on the second and 7 day of storage showed only a significant difference ($p<0.05$) in one of the four test performed between *morcilla* of batch C and D. Regarding to the ranking test no significant differences were detected on the test days with both statistical methods (Kramer and Friedman).

Conclusions

According to the results found in this study, it seems that the application of a combination of potasium lactate and sodium lactate may have a possitive effect, in the extension of the shelf life of *morcilla de Burgos* this effect could be around 12 days, from 20 to 32 days. Besides this combination does not seem to affect the sensory properties of the product. Something similar happens with treatment A (potassium lactate), however the combination of potassium lactate and diacetate seems to have no effect in improving the shelf life of *morcilla de Burgos*. New studies shoud be done to prove the effectivines of lactate derivates on foodborne pathogens in this product.

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Tables

Table1. Evolution of the main microbial populations in vacuum packaged *morcilla de Burgos* treated with lactate derivates and stored at 4ºC. (log cfu/g).

	Days						
	0	**5**	**10**	**15**	**20**	**27**	**32**
pH							
A	6.39	6.36	5.99	5.92	5.63	5.27	5.08
B	6.35	6.41	6.26	6.10	6.28	5.48	5.23
C	6.33	6.20	5.62	5.60	5.27	4.80	4.82
D	6.12	6.14	5.81	5.75	5.55	5.22	5.01
Total Viable Count							
A	4.30	7.50	8.10	8.15	8.30	9.60	8.50
B	4.16	6.80	7.60	8.00	7.50	9.60	8.70
C	4.30	7.90	8.40	8.40	8.50	8.90	9.60
D	4.25	7.00	8.40	8.20	8.25	8.30	9.70
Enterobacteria							
A	-	2.90	4.00	3.80	3.70	2.20	2.00
B	-	2.70	3.00	4.40	4.80	4.20	3.20
C	-	3.40	4.00	4.60	5.10	5.20	3.80
D	-	3.00	3.20	3.40	3.80	4.00	3.00
Pseudomonads							
A	-	2.00	3.70	4.00	4.30	3.40	3.30
B	-	2.40	4.20	4.90	5.00	3.20	3.20
C	-	4.00	4.30	4.40	4.30	3.50	3.20
D	-	-	-	-	-	3.00	3.00
Lactic Acid Bacteria							
A	-	5.00	6.30	6.65	7.70	6.65	6.54
B	-	4.30	5.70	6.54	6.60	6.74	6.16
C	-	6.20	6.60	7.30	7.20	7.54	8.41
D	-	4.30	6.30	7.30	7.95	7.80	7.66
Psychrotrophs							
A	-	5.00	6.30	6.50	6.40	6.20	6.50
B	-	5.15	6.87	6.20	6.50	6.80	7.00
C	-	6.00	8.00	7.82	8.20	8.00	8.00
D	-	6.40	6.30	7.26	7.00	7.00	7.70

A: 3% of lactate-K
B: 3% of lactate-Na + lactate-K
C: Control
D: 2.5% of lactate-K + diactetate
-: no growth detected