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NITRATE-FREE NATURAL ALTERNATIVES TO NITRITES IN SPANISH DRY-FERMENTED SAUSAGE: EFFECTS ON MICROBIOLOGICAL STABILITY AND QUALITY

Martínez B.*, Vieira C., Rubio B.

Estación Tecnológica de la Carne. Instituto Tecnológico Agrario de Castilla León (ITACyL), Spain.

*Corresponding author email: mardomma@itacyl.es

I. INTRODUCTION

The classification of nitrite-cured meat as a Group 1 carcinogen by the International Agency for Research on Cancer [1] has intensified research into natural nitrate sources, such as vegetable extracts, to formulate clean-label meat products. Despite being perceived as a safer option, these alternatives still pose a risk of N-nitrosamine formation due to residual nitrite content in the final product [2]. Thus, addressing this issue requires innovative strategies that eliminate the need for nitrification salts; however, eliminating nitrification salts without a suitable replacement could compromise not only the sensory and technological properties of the product but also its microbiological safety. Given these concerns, the development of a truly nitrate- and nitrite-free alternative is essential to ensure both food safety and product quality. This study aimed to evaluate the effectiveness of nitrate-free natural ingredients as substitutes for nitrites in the production of Spanish dry-fermented sausage ("salchichón") assessing their impact on physicochemical properties, microbiological stability, and sensory characteristics.

II. MATERIALS AND METHODS

Two batches of fermented sausages were manufactured, one of them *Natural batch,* with a blend of different plant-derived ingredients (rosemary, acerola, beetroot and radish) and a starter culture (ELCE Br, Danisco) and the other *Chemical batch,* with nitrates and nitrites added (150 and 100 ppm, respectively). All the sausages were manufactured the same day, using the same technology and according to a traditional formulation for Iberian "salchichón". The different sausage mixtures were stuffed into casings (40mm ø) and were placed in a drying chamber (Hermekit, Cenfrío, Spain) at 3-5ºC and 82-87% RH for 7 days and 8-10ºC and 75-80% RH until the end of the ripening process (43 days).

Five samples of each batch were taken after 0, 7 and 43 days of processing to carry out the following analyses: pH, water activity (Decagon CX-2 AQUALAB hygrometer), nitrate and nitrite content (HPLC-UV), total viable aerobic counts (3M Petrifilm Aerobic Count Plate -3M, Madrid, Spain- at 30ºC for 72 h), lactic acid bacteria counts (MRS Agar -Scharlau, Spain-at 30ºC for 72 h), *Micrococcaceae* counts (MSA Agar -Scharlau, Spain- at 37ºC for 48 h), molds counts (DG 18 -Scharlau, Spain- at 25ºC for 5 days) and yeast counts (3M Petrifilm Yeasts and molds Count Plate -3M, Madrid, Spain-at at 25ºC for 5 days). *Enterobacteriaceae* counts (3M Petrifilm Enterobacteriaceae Count Plate -3M, Madrid, Spain- at 37ºC for 24h) were analyzed after 0 and 43 days. Finally, in the cured product (43 days), triangle test and hedonic tests (measurement satisfaction degree and preference test) were performed by a sensory panel consisting of 19 semi-trained members. In satisfaction degree test, panelists evaluated appearance, odour and in-mouth evaluation into a five-point hedonic scale anchored ''dislike extremely'' and ''like extremely'. In the preference tests, the panelists were asked to indicate which of the two types of "salchichón" they preferred.

Data sets were statistically analyzed using one-way analysis of variance (ANOVA) to identify significant differences within each batch during the manufacturing process and between batches at each sampling point. The means were separated by Tukey-honest significant difference test at 5% level. Data analyses were conducted using Statgraphics Centurión XVIII-X64.

III. RESULTS AND DISCUSSION

At time 0, the pH of the "natural" sausages was approximately 0.2 units lower ($P<0.05$) than that of the "chemical" sausages (6.2). This pH difference persisted throughout the processing stages, resulting in final pH values of 5.0 for the natural sausages and 5.2 for the chemical sausages. Regarding water activity, no differences between batches were observed at any sampling time. Initial water activity values were 0.97, decreased to 0.96 after 7 days and to 0.88 at the end of the ripening.

The results of microbial counts are shown in Table 1. Although significant differences were observed for most of the parameters analyzed on day 0, these differences between batches disappeared after processing. *Micrococcaceae* and yeast counts in natural "salchichón" were initially highest ($P<0.05$), but decreased after ripening. At the end of

the processing, both the natural batch and the chemical batch showed an increase in total viable aerobic and lactic acid bacteria counts, and a decrease in yeast and *Enterobacteriaceae* counts. In the final product, *Enterobacteriaceae* counts were below the detection limit, likely attributable to the competitive effect exerted by lactic acid bacteria. In our study, consistent with findings reported for other products such as salami, the replacement of nitrates and nitrites enabled the production of microbiologically safe products, with minimal impact on key physicochemical parameters such as water activity and pH [3].

Table 1 – Mean value, standard deviation and results of the statistical analysis (One-Way ANOVA) obtained from the microbiological quality assessment of the two types of sausages produced.

Microbial groups (log ucf/g)	*Batch*	Sampling points		
		0 day	7 days	43 days
Total viable aerobic	*Natural*	$_{a}$ 5.5 ± 0.14 ^{A}	$_{b}$ 5.9 ± 0.22 ^{A}	$_{c}$ 8.5 ± 0.20
	Chemical	$_{a}$ 6.8 ± 0.09 ^{B}	$_{b}$ 7.0 ± 0.04 ^{B}	$_{c}$ 8.8 ± 0.07
Lactic acid bacteria	*Natural*	$_{a}$ 4.3 ± 0.09 ^{A}	$_{b}$ 5.9 ± 0.21 ^{A}	$_{c}$ 8.7 ± 0.53
	Chemical	$_{a}$ 6.1 ± 0.51 ^{B}	$_{a}$ 6.6 ± 0.10 B	$_{b}$ 9.0 ± 0.02
Micrococcaceae	*Natural*	4.8 ± 0.10 ^{A}	5.3 ± 0.47 ^{A}	5.5 ± 0.54
	Chemical	$_{b}$ 6.7 ± 0.08 ^{B}	$_{b}$ 6.6 ± 0.03 ^{B}	$_{a}$ 6.0 ± 0.19
Yeasts	*Natural*	$_{b}$ 2.8 ± 0.13 ^{A}	$_{b}$ 2.3 ± 0.87	$_{a}$ ND
	Chemical	3.1 ± 0.02 ^{B}	3.0 ± 0.18	1.7 ± 1.73
Molds	*Natural*	3.9 ± 0.02 ^{A}	4.9 ± 0.90	5.1 ± 0.90
	Chemical	4.5 ± 0.11 ^{B}	5.4 ± 0.32	3.5 ± 2.64
Enterobacteriaceae	*Natural*	$_{b}$ 2.9 ± 0.39	//	$_{a}$ ND
	Chemical	$_{b}$ 3.5 ± 0.18	//	$_{a}$ 1.6 ± 0.31

^{AB} Different superscripts in the same column mean significant differences between batches (P<0.05).
$_{abc}$ Different subscripts in the same row mean significant differences between sampling points (P<0.05).

Finally, the triangle test comparing “natural” with “chemical" sausages batches were well distinguished (P< 0.001). However, in the sensory evaluations conducted to assess consumer satisfaction regarding the appearance, odour and in-mouth evaluation of the two types of sausages, statistically significant differences (P < 0.05) were observed only in appearance. On other hand, although the appearance of "chemical" sausages was rated more favorably, no differences were found in preference test. Other studies [4] have emphasized that the omission of added nitrates and nitrites in meat products has a limited effect on sensory quality.

IV. CONCLUSION

The evaluated nitrate-free natural ingredients do not compromise the “salchichón” manufacturing process, its safety, or alter its sensory attributes in an unfavorable way.

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