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The effect of nitrite and starter culture on microbiological quality of "chorizo"—a Spanish dry cured sausage

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Abstract

The effect of nitrite and starter culture on the survival of Enterobacteriaceae, Micrococcaceae, Lactic acid bacteria and other microorganisms was evaluated during ripening of "chorizo", a Spanish dry sausage. Sodium nitrite 50 and 150 ppm and Lactobacillus sake CL35 added to the "chorizo" have a significant inhibitory effect on Enterobacteriaceae counts but did not on Micrococcaceae. The use of *Lact. sake* could be an adequate safety factor in this product.

Keywords: Non-fermented sausage; "Chorizo"; Antibacterial activity; *Lact. Sake*; Curing salts

1. Introduction

"Chorizo" is the most popular Spanish dry fermented sausage and more than 20 varieties of it have been described (Spanish Ministry of Agriculture, Fisheries and Food, 1983). In the province of León (northwest Spain) a particular variety is produced, made with pork meat and fat, salt, garlic, Spanish paprika and oregano, but no curing agents, sugar or starter cultures are added to it. It is heavily smoked and ripening and drying are carried out under natural climatic conditions during the coldest months of the year.

The preparation technique of this type of "chorizo" is still basically a family art with the use of rudimentary utensils and natural casings. The sausages are hand kneaded and stuffed without any aseptic measures taken. Generally, it appears that the hygiene level is very poor throughout the traditional preparation of this food. There are several posible sources of contamination of "chorizo" with enteric pathogen microorganisms. There are also some factors which favour the growth of Enterobacteriaceae during sausage production including a high initial pH value, a high initial water activity, a low concentration of fermentable carbohydrates, low numbers of lactobacilli in the fresh sausage mixture, the absence of nitrate or nitrite (very low levels as salt contaminants) and, sometimes, not low enough ripening temperatures.

Generally, Enterobacteriaceae are rarely found in long-ripening-period meat products due to their high sensitivity to acidity and desiccation (Lücke, 1986). Nevertheless, it is possible that if the contamination is high, the conditions of inappropriate storage and early consumption, may lead to outbreaks of food-borne infections and intoxications as several authors have pointed out (Lücke, 1985; Schillinger & Lücke, 1989).

Several reports have been published on the inhibitory effect of nitrite on food pathogens (Albalas & Roberts 1977; Túrantas & Ünlütürk 1993; Wirth 1989). However, studies on this effect in dry sausages are scarce, and in many cases, their conclusions are conflicting (Collins-Thompson, Krusky, Usborne, & Hauschild, 1984; Leitsner, 1986).

Micrococcaceae and Lactic acid bacteria (mainly lactobacilli) present in meat during the manufacturing of "chorizo" play an important role in the physico-chemical development and final characteristics of this (Silla, 1989) and other similar products (Hammes, 1986; Lücke, 1986). The role of lactobacilli is not limited to their action on ripening. Strains of several species of this genus isolated from fermented meat products have been found to inhibit the growth of many organisms

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Table 1
Microbiological media and incubation conditions

Microbial groups	Media	Growing conditions	
		T^a (°C)	Days
Total aerobic flora	PC agar (Oxoid)	30	2
Lactic acid bacteria	MRS agar (Oxoid)	30	2
Micrococcaceae	MS agar (Oxoid)	30	2
Enterobacteriaceae	VRBG agar (Oxoid)	30	1
Sulphite reducer *Clostridium*	SPS agar (Merck)	37	2
Staphylococcus aureus	Baird-Parker agar (Merck)	37	2

associated with food spoilage (including pathogenic strains). In a previous study, the authors identified from "chorizo" a strain of *Lactobacillus, Lact.sake CL35*, which is able to inhibit Gram-positive and Gram-negative bacteria, with the exception of some members of the genus *Micrococcus* (Fernández & Díez, 1992). Thus, it may be possible to use this microorganism to improve the microbiological and organoleptic qualities of "chorizo".

The aim of this research was to study the effect of the use of *Lact. sake* starter culture and different concentrations of nitrite on the growth of Enterobacteriaceae, Micrococcaceae, Lactic acid bacteria and other groups of microorganisms during the ripening of "chorizo" made by traditional procedures.

2. Materials and methods

2.1. Sausage manufacture and sampling

The sausages used in this study were prepared following traditional procedures (Lois, Gutierrez, Zumalcárregui, & Lopez, 1987) and the fermentation and drying steps were carried out in rooms subjected to natural climatic conditions. Samples were smoked daily for 6–8 h during the first 10 days of ripening.

The formulation consisted of 78% (w/w) lean pork, 18% (w/w) pork back fat, 2.5% (w/w) Spanish paprika, 1.5% (w/w) NaCL, 1% (w/w) garlic and 0.01% (w/w) of oregano. Meat components and the remaining ingredients were throughly and manually mixed. The mixture was aged at 5–7 °C for 24 h. Prior to stuffing, four batches of around 5 kg each were prepared: one without starter or nitrite (Batch 1), others (Batches 2 and 3) were added with 50 and 150 ppm of nitrite respectively and the last (Batch 4) inoculated with 10 ml kg^{-1} of a suitable dilution of BHI broth culture of *Lact. sake* CL 35 in order to obtain an initial population of 10^3–10^4 cfu g^{-1}.

Individual samples of "chorizo" (aproximately 400 g each, 35–40 mm diameter) were taken from each batch at day 0 (immediately after mixing and filling all ingredients) and at days : 2, 4, 6, 8, 12, 17, 24, 32, 37, 52 and 66 and immediately processed in the laboratory.

2.2. Microbiological analysis

For enumeration of different groups of bacteria 25 g of "chorizo" samples were aseptically homogenized with 100 ml of 0.1% peptone water containing 1% of Tween 80 for 2 min in a Colworth Stomacher 400. Serial decimal dilutions were made in sterile 0.1% peptone water and plated onto growth media in duplicate. Culturing and incubation conditions are shown in Table 1.

2.3. Physical and chemical analysis

Water activity measurements were carried out using the moisture equilibrium method with saturated salt solutions, according to Serrano (1979). pH was determined in slurries made from 10-g samples in 10 ml distilled water blended in a Sorvall Onnimixer. Measurements were made with a Crison pH meter model MicropH 2001. Sodium nitrite was determined by the Spanish Official Recommended method (Presidencia del Gobieno, 1979).

3. Results and discussion

Changes in the numbers of total aerobic bacteria, lactic acid bacteria Enterobacteriaceae and Micrococcaceae during the ripening process of "chorizo" are shown in Fig. 1.

Lactic acid bacteria dominated the microflora from the beginning of ripening and their number (10^8–10^9 cfu g^{-1}; Fig. 1B) was similar in batches 1, 2 and 3 to total aerobic bacteria (Fig. 1A) as occurs in other fermented sausages (Obradovic, Cavoski, Kenecki, Perunovic, Radovanovic, & Popovic, 1983; Samelis, Maurogenakis, & Metaxopoulos, 1994; Sanz, Flores, Toldrá, & Feria, 1997a). No significant differences in this group of bacteria were detected in nitrite made sausages (batch 2 and 3) with regard to the control sample (batch 1).

Counts of total aerobic bacteria were significantly lower in inoculated sausage (batch 4) than in the other formulations during the whole ripening process. This may be due to the fermentation process. The fermentation promotes the growth of lactic acid bacteria

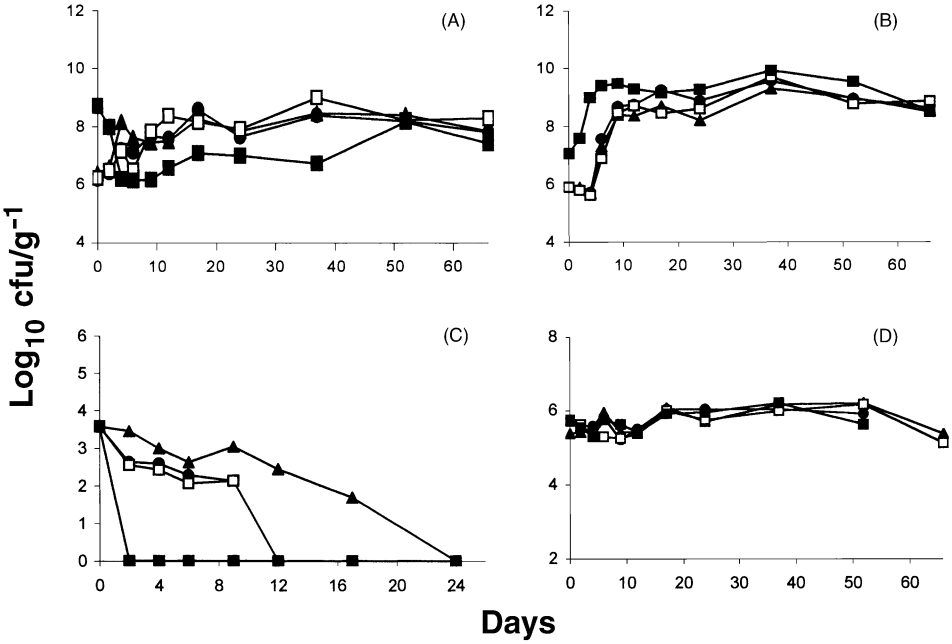

Fig. 1. Microbial counts [(A) Total aerobic bacteria (B) Lactic acid bacteria (C) *Enterobacteriaceae* and (D) *Micrococcacea* during the ripening process of chorizo. Batches: batch 1 (▲) without starter and nitrites; batch 2 (●) with 50 ppm. of sodium nitrite; batch 3 (□) with 150 ppm. of sodium nitrite and batch 4 (■) with starter and without nitrites.

(Demeyer, Verplaetse, & Gistelinck, 1986) and causes the reduction of total aerobic bacteria and the disappearance of Enterobacteriaceae within a few days (Lizaso, Chasco, & Beriain, 1999). Our results agree with these findings.

Initial *Enterobacteriaceae* numbers (10^3–10^4 ufc g^{-1}) were similar in all batches and in the range usually reported for this type of traditional products (Dominguez, Gutierrez, López, Seco, & Zumalacárregui, 1989; Sanz, Flores et al., 1997a). However, the evolution of these microorganisms during ripening was clearly different between formulations. Fig. 1(C) shows that there were no longer any viable cells of Enterobacteriaceae after 48 h of inoculation of starter culture in batch 4 while in the other three samples a significative number of viable cells were still present after 8–10 days (in the control sausage they did not disappear until after 24 days). The large and rapid decrease in pH (Fig. 2) that occurred in inoculated sausage (probably as a result of the high acidifying capability of the starter used for inoculation) may partly explain the reduction and disappearance of these groups of bacteria as other authors have observed (Raccah, 1992). However, not only the pH is responsible for this inhibition because at the same pH or lower the Enterobacterioaceae did not dissapear until day 24 in non-inoculated batches and the decrease in viable counts of total aerobic bacteria were much less pronounced than in the inoculated one.

The antimicrobial effect observed in inoculated sausage appears to be due to other compounds besides the low level of pH. It could possibly be due to antibiotic

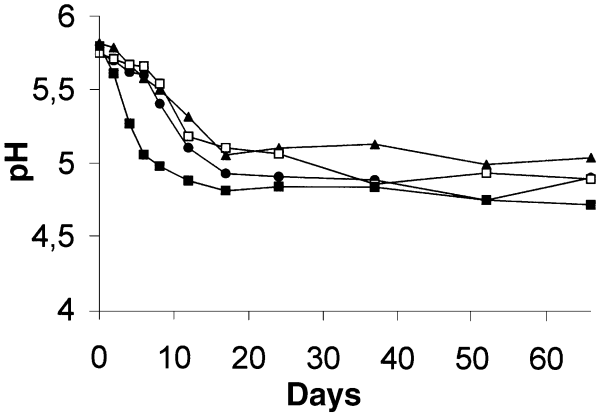

Fig. 2. Evolution of pH during ripening of "chorizo". Batch 1 (▲) without starter and nitrites; batch 2 (●) with 50 ppm. of sodium nitrite; batch 3 (□) with 150 ppm. of sodium nitrite and batch 4 (■) with starter and without nitrites.

like sustances excreted by *Lact. sake* used as starter. The antimicrobial effect of *Lact. sake CL35* "in vitro" was reported by the authors in a previous paper (Fernández & Diez, 1992).

In this study, Micrococcaceae counts were about 10^5–10^6 ufc g^{-1} in all batches (Fig. 1D) and surprisingly, their growth was not significantly affected either by the presence of *Lact. sake* (the resistance of Micrococcaceae to the antimicrobial activity of *Lact. sake* CL35 was shown by the authors in the above mentioned work), by that of nitrites, or by acidification. This latter does not agree with other authors (Lizaso et al., 1999; Samelis et al., 1994) who consider the acidification as the main

cause of Micrococcaceae inhibition in dry fermented sausages. These results are of great importance and very positive technologically speaking since it is known that Micrococcaceae improves sensory properties of dry sausages because of their intense lypolitic and proteolytic activity (Lücke, 1986; Nychas & Arkoudelos 1990; Sutic & Joksinovic, 1973).

The evolution of microflora in nitrite made sausages (batches 2 and 3) was similar to the control batch except for Enterobacteriaceae. Levels of this group were lower than those of free-nitrite sausages (Fig. 1C). The inhibitory effect of nitrite is correlated with its initial concentrations and to the reduction in pH. This has also been observed in fermented sausages by other authors (Lücke, 1986; Sanz, Vila, Toldrá, Nieto, & Flores, 1997).

Absence of sulphite reducing *Clostridium* and *Staphylococcus aureus* was observed from the beginning and during the ripening process.

The evolution of a_w (similar in all batches) could be considered as normal for this kind of meat product reaching values between 0.760–0.788 in the final product.The a_w in the first 25 days was sufficiently high (>0.890) to support the growth of all groups of bacteria studied.

In conclusion, the microbiological stability of "chorizo" after processing depends on the combination of several hurdles (action of nitrite, water activity reduction, acidification...) but the use of starter culture (*Lact. sake CL35*) in the manufacture of this meat product ensures its safety even if consumed immediately after stuffing. Further studies are necessary to confirm the influence of starter culture and nitrites on the sensory properties of this product.

Acknowledgements

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