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ORIGINAL RESEARCH PAPER

MICROBIOLOGICAL EVOLUTION OF *DACIA* SAUSAGE, A DRY CURED ROMANIAN SAUSAGE♦

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Abstract: Selected starter cultures were used to produce the traditional Romanian dry cured sausage, *Dacia*. A control sausage was produced without starter culture (sausage A), one with *L. sakei* CECT 5964 and *S. equorum* SA25 (sausage B) and one with *L. sakei* CECT 5964, *S. equorum* SA25 and *L. acidophilus* CECT 903 (sausage C). Samples from each batch of sausages were taken at 0 (mix before stuffing), and after 2, 4, 7, 14, 21 and 28 days of ripening. Counts of total aerobic mesophilic flora, lactic acid bacteria, salt tolerant flora, and *Enterobacteriaceae* and some physical-chemical parameters (moisture, NaCl, pH and a_w values) were determined. High microbial counts (log CFU) were observed with values at the end of ripening period: for lactic acid bacteria 9.77 (A), 11.47 (B) and 11.19 (C); for total aerobic mesophilic flora 9.89 (A), 11.38 (B) and 11.30 (C); for salt tolerant flora 4.45 (A), 5.31 (B) and 5.27 (C). The starter cultures had a significant inhibitory effect on *Enterobacteriaceae* counts (log CFU), values at the end of ripening period being 1.32 (A), 0.33 (B) and not detected (C). A significant decrease in the pH values is observed until the seventh day of ripening, showing a slight, but progressive increase after the 14th day of ripening. Results show that the production and ripening process in a pilot scale chamber under controlled conditions contributes in obtaining safe and homogeneous products.

Keywords: *dry fermented sausage, lactic acid bacteria, starter cultures*

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INTRODUCTION

Low acid fermented meat products are products highly appreciated by consumers. Normally these products are manufactured in small processing units without addition of starter cultures and the fermentation relies on wild strains [1]. The process favors the growth of the particular microflora, but, is not possible to ensure that the population and variety of microorganisms present in the raw material and in the end product will always be the same and behave the same way. From this point of view the use of selected starter cultures is of great interest.
Selected starter cultures could improve the fermentation process as well as the safety and standardization of fermented sausages. Lactic acid bacteria (LAB) as well as *Micrococcaceae* strains are important microorganisms used as starter cultures in meat fermentation [2]. LAB produce lactic acid quickly if a fermentable sugar is provided, and the growth of spoilage and pathogenic microorganisms is inhibited.
This study followed the microbiological evolution and the changes in some physical-chemical parameters during ripening of *Dacia* sausage, a dry fermented sausage produced with two different starter cultures.

MATERIALS AND METHODS

Sampling

In order to carry out this study, three batches of *Dacia* sausages were produced as it follows: one without starter culture addition (sausage A), one with starter culture consisting of with *L. sakei* CECT 5964 and *S. equorum* SA25 (sausage B) and one with starter culture consisting of with *L. sakei* CECT 5964, *S. equorum* SA25 and *L. acidophilus* CECT 903 (sausage C). From each batch of sausage, samples at 0 days (mix before stuffing), and after 2, 4, 7, 14, 21 and 28 days of ripening were taken. Each sample consisted of two entire units of *Dacia* sausage.

Microbiological analysis

For enumeration of different groups of bacteria, 25 g of sausage sample were aseptically homogenized with 100 mL of 0.1% peptone water also containing 0.85% NaCl and 1% Tween 80 as emulsifier, at 40-45°C for 2 min in Masticator blender (IUL Instruments, Barcelona, Spain). Successive decimal dilutions were prepared. From each sample and on each culture medium, 1 mL of each dilution was inoculated in duplicate on plates and mixed before solidification. Plates of MRS agar and VRBG agar were covered with a layer of the same culture medium before incubation. The microbial groups enumerated in each sample and the culturing and incubation conditions are summarized in Table 1.

Physical and chemical analysis

Moisture and NaCl contents were quantified according to the ISO recommended standards 1442:1997 (ISO, 1997) and 1841-1:1996 (ISO, 1996), respectively.

The pH was measured with a micro pH 2002 pH-meter (CRISON Instruments S.A., Barcelona, Spain) and the water activity (a_w) was measured using a Decagon CX-1 Water Activity System apparatus (Decagon Devices, Pullman, WA, USA). All chemical determinations were made in duplicate in each sample.

Table 1. *Culture media and growing conditions used in the enumeration of each microbial group investigated*

Microbial group	Media	Growing conditions	
		T (°C)	Days
Total aerobic flora	Standard Plate Count agar (Oxoid)	30	2
Lactic acid bacteria	de Man Rogosa Sharpe agar (Oxoid)	30	5
Micrococcaceae	Standard Plate Count agar + 7.5% NaCl	30	2
Enterobacteriaceae	Violet Red Bile Glucose agar (Oxoid)	37	1

RESULTS AND DISCUSSION

Microbial counts

Changes in the number of *Enterobacteriaceae, Micrococcaceae,* LAB and total aerobic bacteria, during the ripening process of *Dacia* sausage are shown in Fig. 1.

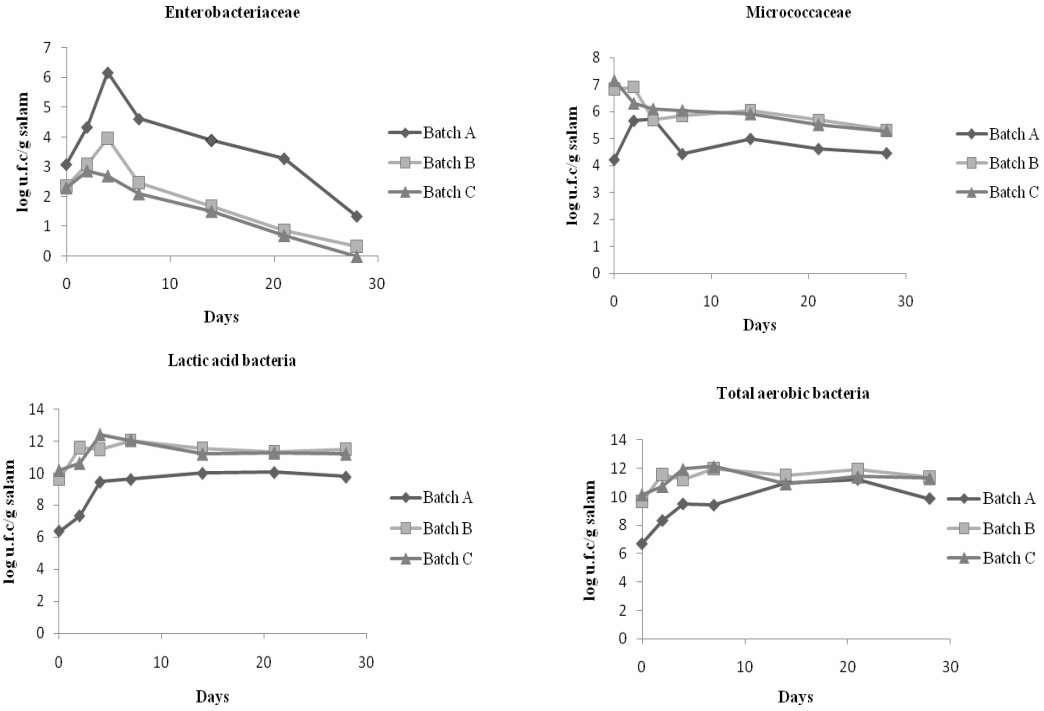

Figure 1. *Microbial growth during the ripening of Dacia fermented sausage in the three batches*

LAB dominated the microflora from the beginning of ripening and their number (10^{11} CFU/g) was similar in batches inoculated with starter culture to total aerobic bacteria as

occurs in other fermented sausages [3] and the same thing happened in those without starter culture but they only reached values of 10^6-10^7CFU/g.
Initial *Enterobacteriaceae* numbers (10^2-10^3 CFU/g) were similar in all batches and in the range usually reported for dry cured sausages [3]. The evolution of these microorganisms during ripening was different between formulations. The batches inoculated had a lower number of *Enterobacteriaceae* after the 14th day of ripening, disappearing at the final of the ripening period.
The decrease in pH may partly explain the reduction and disappearance of these groups of bacteria as other authors have observed [4]. However, not only the pH is responsible for this inhibition because at the same pH the *Enterobacteriaceae* did not disappear until day 28 in non-inoculated batch.
The antimicrobial effect observed in inoculated sausage appears to be due to other compounds besides the low level of pH. We can add the moisture content, water activity and NaCl values.
Micrococacceae belong to the desirable microflora of fermented sausages, because they contribute to the development of the color and flavor [5].
In this study, *Micrococcaceae* counts were about 10^4-10^5 CFU/g in all batches. The reduction of this microbial group during the fermentation is probably due to the application of smoking on the 4th day and rapid pH reduction. These counts were inferior to those of the total aerobic flora, reflecting the competitiveness of these microorganisms in presence of acid-resistant bacteria in phase of active growth [6]. The results were in agreement with Lisazo, Chasco, and Beriain (1999), who considered acidification to be the main cause of *Micrococcaceae* inhibition in dry fermented sausages.
The domination of LAB and *Micrococacceae* in fermented sausages during fermentation and ripening are necessary for a successful production.

Physical-chemical parameters

The values of pH, moisture, water activity and NaCl content are shown in Table 2. The initial pH values of batter were very similar in all batches. At the end of ripening the pH reached values of 5.39 in batch A and C, and 5.19 in the batch B. The slight rise in pH at the end of ripening can be attributed to proteolytic microbiota [7] and to the action of endogenous proteases.
Although the numbers of LAB were high, these results show that higher amounts of sugar are necessary to drop the pH of the sausages below the isoelectric point of myofibrilar proteins (5.1) in normal meat.
The starter cultures showed a slightly significant effect on the moisture content, given that the caliber of the sausages was identical in all cases. Another factor that could influence the dehydration is the relative humidity in the ripening room. This also was the same in all batches. The moisture and water activity were highly influenced by the day of ripening.
The evolution of a_w could be considered as normal for this kind of meat products, being in the first days sufficiently high to support the growth of all groups of bacteria studied.

The content of NaCl, expressed as percentage of dry matter, was practically constant during the process. These values are similar to values described by other authors that vary between 3.64% [8] and 7.26% [9].
The overall evolution of the physical-chemical parameters could be considered as normal for this kind of product [10].

Table 2. *Evolution of pH, moisture, water activity and NaCl content in the three batches*

Batch	**A**		**B**		**C**	
Parameter	**Initial**	**Final product**	**Initial**	**Final product**	**Initial**	**Final product**
pH	6.14	5.39	6.08	5.19	6.09	5.39
Moisture*	61.32	30.20	60.07	33.79	63.61	31.02
a_w	0.961	0.832	0.962	0.827	0.964	0.817
NaCl*	5.60	4.34	4.04	4.73	5.06	4.86

* Expressed as g/100 g of dry matter.

CONCLUSIONS

The lactic acid bacteria were the main microbial group and, as expected in products with a moderate content in salt. The salt tolerant flora presented notably lower counts than those of the lactic acid bacteria. We can conclude that the starter cultures did not affect in a decisive manner the evolution of the psysical-chemical parameters considered in this work. The control of the conditions during the manufacture and ripening, the addition of starter cultures help us to improve the microbiological quality and the safety of this sausage.

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