MICROBIOLOGICAL CHANGES DURING THE RIPENING OF "CHORIZO DE CEBOLLA", A TRADITIONAL FERMENTED SAUSAGE MADE IN THE NW OF SPAIN.

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INTRODUCTION

"Chorizo de cebolla" is a fermented sausage which is traditionally made using artisanal procedures throughout Galicia (NW of Spain). At present its consumption is increasing and its elaboration at industrial level is reaching a very high proportions.

It is elaborated from pork of secondary importance (dewlap, back of neck, bits of bacon, diaphragm) to which lung and cooked skin are occasionally added. The meat is minced and salt, garlic, sweet and spicy paprika and marjoram are added, leaving the resulting mass to settle for 12 to 24 hours. In the industrial manufacturing a mixture of sugars, preservatives, colourants and antioxidants are usually added to the mass. The onion (cebolla), whether raw or cooked, is chopped finely. Sometimes pumpkin (*Cucurbita pepo*) is added, in this case it is pealed, chopped, and strained for approximately a day. After having been left standing, the onion and pumpkin are added in varying proportions to the mass. All the ingredients are kneaded and generally placed in thick pig intestines. After this process, the sausages undergo a drying-ripening which can last from 10 days to 2 months. In the first few days of this drying-ripening period, a slight heating, almost always accompanied by smoking, is usually applied. The final product is always consumed after boiling.

The studies carried out up to now on this type of sausage only refer to the biochemical characterization of the final product (Prieto et al., 1995).

Given that the microbial fermentation seems to be the most important process in the elaboration of the raw-cured sausages, it is very important to know the levels the different microbial groups of technological interest reach throughout the ripening of this sausage and the evolution which they follow. It would also be of interest to know the levels and evolution of some microbial groups of hygienic and sanitary interest. Further on, the microbial species present will be identified and their role in the ripening process will be determined. The results of these studies will be of great usefulness in obtaining a product of high and uniform quality at industrial level, through the use of starter cultures and the suitability of the ingredients used in the formulation of the mass.

MATERIALS AND METHODS

Samples

In order to carry out this study, 4 batches of "chorizo de cebolla", 2 homemade and 2 industrially made, were elaborated following the procedure described in the introduction. Samples from mass prior to introduction into the intestines (0 day) and from sausages at 2, 7, 14, 21, 28 and 42 days of ripening were taken from each batch. Each sample was made up of one whole sausage.

Microbiological analysis

The homogeneizates and dilutions were made following the recommendations of the ICMSF (1978). 25 g of each sample (after discarding aseptically the intestine) were homogeneized with 100 ml of sterile peptone saline water at 40-45 ºC for 2 min in a Stomacher 400 Lab Blender (Seward Medical, London), thus making a 1/5 dilution. Successive decimal dilutions were prepared by mixing 10 ml of the previous dilution with 90 ml of 0.1 % sterile peptone water.

Aerobic mesophilic flora were enumerated in Standard Plate Count Agar (Oxoid) (ICMSF, 1978), after incubation at 30 ºC for 48 hours. Salt tolerant flora (presumptive *Micrococcaceae*) in Standard Plate Count Agar (Oxoid) + 7.5 % NaCl after incubation at 30 ºC for 48 h. Lactic acid bacteria (presumptive Lactobacilli) in M.R.S. Agar (Oxoid) previous acidification up to pH 4.6 using acetic acid, after incubation at 30 ºC for 5 days. Moulds and yeasts in Oxytetracycline Glucose Yeast-Extract Agar (Oxoid) (Mossel et al., 1970) after incubation at 25 ºC for 4 days. Enterococci in Kanamycin Aesculin Azide Agar (Oxoid) (Mossel et al., 1978) after incubation at 37 ºC for 24 h. Staphylococci in Baird-Parker Agar (Oxoid) (Baird-Parker, 1962) after incubation at 37 ºC for 24 h. Finally, *Enterobacteriaceae* were enumerated in Violet Red Bile Glucose Agar (Oxoid) (Mossel et al., 1962) after incubation at 37 ºC for 24 h. Plates of M.R.S. agar and V.R.B.G. agar were covered with a layer of the same culture medium before incubation. After incubation, plates with 30 to 300 colonies were counted.

RESULTS AND DISCUSSION

Tables 1 and 2 show the evolution of the studied microbial groups during the ripening of the batches of "Chorizo de cebolla" made by artisanal (homemade) and industrial methods, respectively.

In general the counts of the different microbial groups underwent an increase in the first few days of ripening, reaching maximum counts between days 7 and 14, and afterwards falling slightly until the end of the ripening process. The staphylococci maintained fairly constant levels throughout ripening. The *Enterobacteriaceae*, after undergoing an increase of about one log. unit in the first two days of ripening, gradually fell until totally disappearing on day 42 of ripening in the industrially elaborated batches.

The evolution of the different microbial groups and the counts shown by each one of them are, although with some differences, similar to those observed in other fermented sausages such as Lebanon (Smith and Palumbo, 1973), Pepperoni (Palumbo et al., 1976), Salchichón (Serrano Moreno, 1979), Chorizo (Mendoza et al., 1983), and Chorizo de León (Seco Álvarez, 1985).

The lactic acid bacteria (presumptive Lactobacilli) underwent a very fast proliferation in the first few days becoming the microbial group with the highest counts after days 2-7 of ripening. This seems to demonstrate that "Chorizo de cebolla", as would be expected from a sausage with their characteristics, undergo a true lactic fermentation. The proliferation of the lactic acid bacteria, with the consequent production of lactic acid and fall in pH values, seems to be one of the determining factors of the important decrease, even disappearance, of the Gram-negative bacteria (*Enterobacteriaceae*).

With regard to the differences between the batches elaborated artesanally (homemade) and those made industrially, the multiplication of lactic acid bacteria during the first few days of ripening seems to be quicker in the industrially produced batches. These batches show lower values of *Enterobacteriaceae* and a more marked fall in this microbial group throughout ripening. The industrially produced batches show slightly higher enterococci counts and mould and yeast counts slightly lower than the artisanally produced batches.

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TABLE 1. Changes in different microbial groups (log. c.f.u./g) during the ripening process of "Chorizo de cebolla" made by artisanal methods. (Data are the average ± standard deviation values of two batches).

	Ripening time (days)						
Microbial group	0	2	7	14	21	28	42
Aerobic mesophilic flora	7.28±0.77	7.66±0.32	8.96±0.12	9.04±0.33	8.59±0.04	8.49±0.05	7.99±0.31
Salt tolerant flora	5.94±0.03	6.19±0.07	6.85±0.31	6.69±0.25	6.32±0.03	6.06±0.25	5.93±0.56
Lactic acid bacteria	4.68±0.09	6.92±0.26	8.98±0.05	9.02±0.33	8.65±0.08	8.42±0.04	8.16±0.33
Moulds and Yeasts	5.40±0.46	5.72±0.43	6.48±0.23	6.68±0.82	6.59±0.37	6.32±0.82	5.81±1.07
Enterococci	3.89±0.35	4.61±0.03	5.45±0.47	4.89±0.19	5.51±0.33	4.99±0.43	4.70±0.31
Staphylococci	3.94±0.48	4.08±0.75	3.94±0.71	3.30±0.67	3.58±0.95	3.78±0.22	3.72±0.21
Enterobacteriaceae	5.69±0.12	6.83±0.60	5.71±0.31	4.17±0.54	1.71±1.31	2.66±0.10	0.95±0.55

TABLE 2. Changes in different microbial groups (log. c.f.u./g) during the ripening process of "Chorizo de cebolla" made by industrial methods. (Data are the average ± standard deviation values of two batches).

	Ripening time (days)						
Microbial group	0	2	7	14	21	28	42
Aerobic mesophilic flora	6.83±0.43	8.80±0.15	8.90±0.08	8.85±0.04	8.73±0.06	8.40±0.17	8.31±0.01
Salt tolerant flora	5.98±0.66	7.18±1.05	7.15±1.42	6.88±1.38	6.81±1.35	5.89±0.59	5.63±0.53
Lactic acid bacteria	5.62±1.46	8.80±0.11	8.92±0.08	8.85±0.07	8.84±0.05	8.61±0.13	8.63±0.01
Moulds and Yeasts	4.45±0.22	4.16±0.35	4.72±0.44	5.02±0.07	3.98±1.74	4.40±0.54	2.93±1.33
Enterococci	4.61±2.36	6.25±1.99	7.03±0.13	6.76±1.42	7.22±0.71	6.17±0.71	6.48±1.22
Staphylococci	3.37±1.02	3.71±0.51	4.87±0.39	4.79±0.33	4.73±0.32	4.25±0.37	4.35±0.29
Enterobacteriaceae	3.74±0.36	4.80±1.27	4.12±1.16	2.54±0.03	1.93±0.46	0.86±0.86	**

**Absence in 0.2 g.