Use of Bacterial Cultures in the Ripening of Fermented Sausages

GRACIELA M. VIGNOLO, AIDA P. de RUIZ HOLGADO, and GUILLERMO OLIVER*  
Centro de Referenda para Lactohacilos (CERELA), Chacabuco 145, 4000 Tucumán, Argentina;  
Facultad de Bioquimica, Quimica y Farmacia, Universidad Nacional de Tucumán.  
(Received for publication September 29, 1988)

ABSTRACT

Pure bacterial cultures were used in the production of Argentina salami, the most popular cured meat product in the country. Three selected groups of two strains each of Lactobacillus plantarum and Micrococcus varians were used as a starter cultures, with two different sugar concentrations. Apart from the use of these starter cultures, the salami was manufactured under the same conditions normally used in industrial production. When 0.6% sucrose was used, the levels of L. plantarum and M. varians were 10^8 and 10^7 CFU/g, respectively, on the second day of ripening. Similar levels of lactic acid bacteria were found in non-inoculated sausages at 2 d post-preparation. Under these conditions, coliforms decreased significantly. The final pH of the inoculated and uninoculated sausage were 5.0 and 5.2, respectively, after 7 d of ripening. When 0.9% glucose plus 0.6% sucrose was added, the level of lactic acid bacteria was 10^9 CFU/g on the second day, a value that remained constant in the inoculated sausages to the end of the ripening period. Staphylococci showed a marked decrease in population, while coliforms disappeared on the second or third day. The final pH of 4.40 or 4.55 was reached within 4 d. The product obtained under these conditions had a firm texture, a good slicing surface and pleasant flavor and aroma. The use of starter cultures in cured dry Argentine-style sausage shortened the ripening period from 14 to 7 d.

The inoculation of meat pastes with mixtures of lactobacilli and micrococci and/or nonpathogenic staphylococci, to improve keeping quality and safety, and enhance consumer acceptability of the product, has become common practice to the manufacture of cured dry sausages (12,13). The lactic acid produced during the fermentation process, from sugars added to the sausage mixture, lowers the pH, imparting a tangy flavor to the product, and denatures the meat protein. This denaturation, which also results in water release, is largely responsible for the texture associated with fermented sausages. Moreover, the low pH attained inhibits the development of contaminating microflora (7). The nonpathogenic micrococci and/or staphylococci that are added or present reduce nitrates to nitrites, which, together with the myoglobin in the meat, give the product its characteristic color, and also prevent the development of off-flavors.

The inoculation of selected strains has as its main objective the prevention of the risks inherent in the action of spontaneous flora. That is why there is so much research on the isolation from meat products of technologically appropriate strains, which are commercialized later on (1,4,5,6,8).

A very important function of starter cultures in dry sausage fermentation is the inhibition of growth of undesirable microorganisms. Thus, lactic acid bacteria contribute to microbial food safety in these products because, if not all, food poisoning bacteria are inhibited by the lactic acid produced. A number of investigators have studied growth and survival of Staphylococcus aureus during the processing of a variety of fermented sausages. Daly et al. (3) studied the effect of different starter cultures that consisted of a Lactobacillus-Micrococcus combination (11) and suggested that micrococcus species were responsible for the inhibition of enterotoxin production. Christiansen et al. (2), demonstrated that the pH drop resulting from the fermentation of glucose was the most important factor controlling botulism toxin in fermented sausages. In the same way, Masters et al. (10) observed that Salmonella newport and S. typhimurium were eliminated completely by the use of an L. plantarum starter culture in summer sausage. As demonstrated above, the use of starter cultures in the manufacture of fermented meat products is desirable for a number of reasons. Lactic acid bacteria produce lactic acid quickly and consistently if a fermentable sugar is provided; the growth of spoilage and pathogenic microorganisms is inhibited; batch-to-batch variation in the product is decreased because starter cultures are steady in their acid production; finally, processing times are reduced because it is not necessary to select and grow the desired microbial species by using natural inoculation techniques such as "back-slopping" (adding meat reserved from a previous successful fermentation to the sausage mix).

It is the aim of the present work to determine whether inoculated strains used as starter cultures can reproduce and exert a favorable effect on a typical dry fermented sausage,
and also to select the best combination of strains for the fermentation.

MATERIALS AND METHODS

Cultures

The following strains were used: Lactobacillus plantarum GV 419 + Micrococcus varians GV 820 (Mixture A); Lactobacillus plantarum GV 415 + Micrococcus varians GV 31 (Mixture B); and Lactobacillus plantarum GV 417 + Micrococcus varians GV 718 (Mixture C). These strains were isolated from dry sausage and characterized as previously reported by Vignolo et al. (14).

Preparation of sausages

The formulation and inoculation of the sausage mixture was conducted in a meat products factory under the manufacturing conditions normally applied there. The following ingredients were employed: 30% beef, 50% pork, 12% pork fat, 3.2% NaCl, 0.6% sucrose, 100 ppm NaN₃, 0.4% pepper, 0.2% garlic and wine. The meat components, curing salts, and other ingredients were thoroughly mixed and the sausage mix was then divided into 1 kg portions. Each batch was inoculated with 8 ml of an 18 - 24 h culture of L. plantarum and M. varians used as starter cultures, yielding an initial population of 5 x 10⁷ - 5 x 10⁸ CFU/g for lactobacilli and 10⁶ - 10⁷ CFU/g for micrococci. The inoculated paste was stuffed into natural casings and incubated at 24°C.

The sugars used were 0.6% sucrose and 0.6% sucrose plus 0.9% glucose.

Sampling procedure

Samples from each batch were collected at 0 (after stuffing), 1, 2, 3, 4, 5, 6, 7, and 8 d. A 10 g sample of the sausage was aseptically ground in a VIRTIS homogenizer with 90 ml of 0.1% sterile peptone + 4% NaCl. Serial dilutions were made and the plating was done in duplicates.

Media and growth conditions

Total aerobic count on PCA, 2 d at 30°C; lactic acid bacteria on acidified Rogosa SL agar (RSL), 5 d at 30°C; micrococci on phenol red mannitol salt agar (MSA), 3 d at 30°C; molds and yeasts on potato dextrose agar (PDA), 5 d at 25°C; staphylococci on Baird-Parker with tellurite-egg yolk emulsion, 1 d at 37°C; and total coliforms in brilliant green bile (2%), 1 d at 37°C. Cell counts in each medium were made in duplicate in plates containing 30 to 300 colonies.

pH measurement

A 3 g sample of sausage was blended with 9 ml of distilled water for 10 min. The pH of the slurry was determined with an ORION Research pH-meter.

RESULTS AND DISCUSSION

The growth and development of lactic acid bacteria, micrococci, staphylococci, coliforms, molds, and yeasts were studied during the ripening of salamis with 0.6% sucrose content.

Determinations were done simultaneously in salami inoculated with starter cultures and in a uninoculated control. Results are shown in Fig. 1, 2, 3 and 4.

The initial level of lactic acid bacteria in the non-inoculated salami was 1 x 10⁴ CFU/g up to the fourth day of ripening after which the cell count increased up to 2 x 10⁸ CFU/g, a figure that remained constant until the end of the 8 d of ripening.

In the case of inoculated salami there was good growth of the starter culture. The initial number of lactic acid bacteria ranged between 8 x 10⁵ and 7 x 10⁶ CFU/g. In the case of salami inoculated with mixtures B and C (Fig. 2 and 3) a greater bacterial development was observed during the first 2 d (1 x 10⁷ and 8 x 10⁶ CFU/g, respectively), followed by a decrease (1 x 10⁶ CFU/g mean value) on the third and fourth days, the values remaining constant from then on till the end of the ripening process.
Salami inoculated with mixture A (Fig. 1) showed an initial level of $4 \times 10^8$ CFU/g lactic acid bacteria. This level did not vary up to the eighth day.

The micrococci of starter cultures A and B grew rapidly during the first 24 h of ripening, with levels up to $1 \times 10^9$ CFU/g, from then on there was a remarkable decrease ($3 \times 10^8$ and $1 \times 10^7$ CFU/g, respectively) on the third day of ripening. The decrease of micrococci was even more noticeable in mixture C.

In the case of uninoculated control salami (Fig. 4), the initial amount of micrococci remained constant during the entire ripening process.

The development of microorganism considered contaminants was also studied. There was no significant difference in the development of staphylococci, either in inoculated or in uninoculated salami. Coliforms, on the other hand, showed a significant decrease in number after the first 24 h in salami inoculated with starter cultures. Uninoculated controls (Fig. 4) showed no variation in the number of coliforms up to the third day, at which time the number of coliforms in inoculated salami ranged from 10 to 100 CFU/g.

No significant differences were observed between the mold and yeast count of inoculated and control salamis. The decrease in the number of lactic acid bacteria observed when 0.6% of sucrose was used (Fig. 1, 2, and 3) might have been caused by a depletion of the carbohydrate source. On the basis of this assumption, an experiment was carried out, adding a large amount of sugar (0.6% sucrose + 0.9% glucose) to the meat mix. Results are shown in Fig. 5, 6, and 7.

Under the above conditions, the maximum values for lactic acid bacteria (between $1 \times 10^7$ and $4 \times 10^8$ CFU/g) were reached on the second day of ripening, remaining fairly constant throughout the following days with a slight increase on the eighth day of ripening.
The micrococci added to the mixtures were slightly inhibited by the lactic acid produced in the presence of 1.5% total sugars, but still reached a cell count of $5 \times 10^6$ CFU/g on the fourth day (9).

The acidity of the meat paste was expected to stimulate the development of molds and yeasts. However, this was not the case, and the behavior of this microbial group was similar to that observed in the experiment with 0.6% sucrose; there was even a tendency to a decrease in their cell count toward the end of the ripening period.

The levels of staphylococci found were maximum at 24 h (mixture A and C), and 48 h (mixture B) of the ripening period; then a significant decrease occurred, especially in the case of starter mixture A, which showed a level of $8 \times 10^4$ CFU/g on the eighth day.

The most important results were found in the case of coliforms, which disappeared from salamis after 2 d of ripening with starter mixtures A and C, and on the third day following inoculation with mixture B.

The reason for such inhibition might be the large amount of acid produced by lactic acid bacteria when the sugar concentration was raised. In order to confirm this hypothesis, the pH values during the ripening period were measured.

When salami contained 0.6% sucrose, final pH was 5.0 and 5.2 in the case of inoculated and uninoculated samples, respectively, though the final value was reached more quickly in the former group (Fig. 8). The addition of 0.9% glucose + 0.6% sucrose caused a decrease in the pH in the salami paste inoculated with the three mixtures assayed, so that on the eighth day of ripening pH values ranged between 4.40 and 4.45 (Fig. 9). The lowest value was reached with mixture C, perhaps because of the high acidifying ability of the strain *L. plantarum* GV 417 included in the starter culture.

These results agree with those published by Liepe (8) who, in assays on an industrial scale, obtained a pH value
of 4.70 after 5 d of ripening using a mixture of \textit{Lactobacillus pentosus} and \textit{Staphylococcus carnosus}.

Fig. 10 shows the mean development of NaCl concentration and moisture. No significant differences were observed in the values obtained for inoculated and un inoculated salami. After 7 d of ripening, the NaCl content was 5.6% and water percentage was 45%.

On the basis of the above results, it may be concluded that the starter cultures tested performed better in the presence of higher sugar levels in the past leading to increased acid production and causing a more significant inhibition of staphylococci and coliforms.

Moreover, the low pH values obtained at the end of the process assured the greater stability of the product, as well as a firmer texture and a neat slicing surface. The flavor obtained in the experiment with 1.5% total sugars was mild and slightly acid.

The addition of the nitrate-reducing strains was responsible for color formation and color retention.

Salami produced with starter cultures required from 7 to 8 d to reach the same organoleptic characteristics as those produced with the traditional techniques, requiring 14 to 15 d for the ripening process to be completed.

We believe that, from an industrial standpoint, these results provided a strong justification for the use of selected strains in the manufacture of Argentine-style cured dry sausage.

ACKNOWLEDGMENTS

This work was partially supported with grants from Secretaría de Ciencia y Técnica (Argentina) and PID 3-09100/85.

The authors wish also to thank Dr. Ana María Strasser de Saad for helpful discussion and Marta Núñez de Kairuz for technical assistance.

REFERENCES