

**Research Note**

**Histidine Decarboxylase Activity of *Enterobacter cloacae* S15/19 during the Production of Ripened Sausages and Its Influence on the Formation of Cadaverine**

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**ABSTRACT**

The histidine decarboxylase activity of *Enterobacter cloacae* S15/19 was studied during the production process of *salchichón*, a Spanish ripened sausage. Counts of fecal coliform and histidine decarboxylase bacteria decreased during the production process, showing a good correlation in both inoculated and control samples. In the samples inoculated with *Enterobacter cloacae* S15/19, fecal coliforms were undetectable the last day of the survey, while the population of histidine decarboxylase bacteria was over 2 log MPN/g. Despite the fact that inoculation with *Enterobacter cloacae* S15/19 increased histidine decarboxylase bacteria counts, no differences were observed in the histamine concentration reached, which was undetectable in most of the control and inoculated samples. In contrast, cadaverine concentration increased significantly (*P < 0.01*) in the inoculated samples, suggesting that cadaverine could be used as a hygienic-quality indicator of the raw materials employed in sausage processing.

Key words: Histamine, cadaverine, *Enterobacter cloacae*, ripened sausages

Histamine in ripened sausages has been related to the presence of histidine decarboxylase lactic acid bacteria during the ripening process (3). Some other powerful histamine-forming microorganisms, such as enterobacteria or pseudomonads, have also been reported in raw sausages before the ripening process (7, 11). These microorganisms have also been described as being responsible for histamine formation in fish and fish products (4); however, there are no studies concerning their function in the formation of biogenic amines in dry sausages during the production process.

In previous studies (7) *Enterobacter cloacae* was reported to be a common contaminant of meat destined for sausage production. This study was designed to evaluate the effect of inoculation with a histamine-forming strain, *E. cloacae* S15/19, on histidine decarboxylase activity and on the formation of other biogenic amines during the process of production of cured sausages.

**MATERIALS AND METHODS**

Sausage production and sampling

A short-ripening type of *salchichón*, one of the most popular Spanish cured sausages, was used for this study. The batter was prepared in a commercial meat plant following its usual technological process, using pork meat and fat (90% and 10% respectively), and with the addition per kg of meat and fat of 25 g of salt, 3 g of pepper, 0.9 g of nitrates and nitrites, and 45 g of sugars. After being mixed, the batter was separated into two portions (inoculated and control). One of them was inoculated with a powerful histamine-forming strain, *Enterobacter cloacae* S15/19, isolated from a raw *salchichón* (7). This strain was suspended in Ringer solution (8.5 g of NaCl per liter) to obtain a final concentration close to 10⁶ CFU/g of sausage batter. After inoculation, the batters remained at 5 to 6°C for 5 days and then were stuffed into natural pig gut casings. Sausages were ripened for 12 days at 15 to 18°C and 67 to 82% relative humidity and afterwards kept at room conditions for 5 weeks.

The first sample was taken immediately after batter preparation (on day 0), and afterwards at the 3rd, 5th (stuffing), 7th, 9th, 13th, 17th (last day of ripening), 24th, 31st, 38th, and 53rd day of

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the process. Two sausages were taken each time as a sample and were analyzed together. Two separate trials were carried out for this experiment. Results are given as the mean of the data obtained in both trials.

Microbiological analysis

After disinfection with 70% ethanol and removal of the casing, 10 g of each sample were taken aseptically and homogenized mechanically with 90 ml of 0.1% peptone (Oxoid, Unipath Ltd., Basingstoke, Hampshire, UK) supplemented with 1% Tween 80 (Panreac, Monpllet and Esteban, Barcelona, Spain) (2) in a Stomacher Lab-Blender 400 (Seward Medical, London, UK), and then serially diluted in 0.1% peptone water.

Total aerobic mesophilic microorganisms were enumerated on plate count agar (PCA) (Difco Laboratories, Detroit, MI) at 30°C for 48 h (2): lactic acid bacteria (LAB) on de Man Rogosa Sharpe agar (MRS) (Oxoid) with the pH adjusted to 5.5 and incubated at 30°C for 4 days (9), and fecal coliforms on violet red bile agar (VRBL) (Difco) at 44°C for 24 h (1).

Enumeration of histidine decarboxylase bacteria (HDB) was determined in each sample by the most probable number (MPN) method using 5-tube series of tryptic soy broth (Difco) supplemented with 1% L-histidine (Aldrich Chemical Co., Milwaukee, WIS), with the final pH adjusted to 5.3 and incubation at 30°C for 48 h. Histamine formation was determined in each tube by an enzymatic method (7).

Biogenic amine analysis

Contents of histamine, tyramine, putrescine, and cadaverine were determined by high-performance liquid chromatography (HPLC) (12) at the Nutrition and Food Science Unit of the Faculty of Pharmacy, University of Barcelona. Evaluation of the cadaverine-forming capability by Enterobacter cloacae S15/19 was assessed in NB broth (4) containing 1% lysine (Farmitalia Carlo Erba, Milan) and incubated at 37°C for 18 h by the aforementioned method.

Statistical methods

The statistical methods employed were an analysis of variance (for fecal coliforms, HDB, and putrescine), Friedman (for aerobic mesophilic microorganisms, LAB, histamine, tyramine, and cadaverine) previously verifying normality and variance homogeneity, and a correlation analysis (for all parameters), all performed by using the Statistical Package for Social Sciences (SASS/PC+, SASS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Inoculation of the batter with E. cloacae S15/19 increased fecal coliform counts from 3.7 to 5.2 log CFU/g (Fig. 1). The number of fecal coliforms decreased during the whole process in both inoculated and uninoculated (control) sausage, showing a good correlation with time ($r = -0.95$ in controls and $r = -0.98$ in inoculated samples, $P < 0.001$). Fecal coliform counts on VRBL were observed until day 38 in the control and day 53 in the inoculated samples. Plating on PCA and MRS did not show differences between control and inoculated samples and therefore we can assume that changes in numbers of aerobic mesophils and LAB were not influenced by the inoculation with E. cloacae S15/19. Inoculation also increased HDB counts in the batter from 4.1 log MPN/g in the control to 5.6 log MPN/g in the inoculated samples (Fig. 1). Then the changes decreased until the population’s stabilized, reaching counts over 2 log MPN/g the last day of the survey. A good correlation was observed between population numbers from HDB and VRBL in both control and inoculated samples ($r = 0.90$ and 0.96 respectively, $P < 0.001$).

All these results mean that changes in the numbers of HDB and fecal coliforms in the inoculated samples were clearly influenced by the inoculation with E. cloacae S15/19, increasing both microbiological groups more than 10-fold in the inoculated in relation to the control samples. However, in spite of the fact that fecal coliforms on VRBL and, by extension, the inoculated strain were undetectable the last day of the survey (33rd day), HDB counts were still detectable. Similar results were observed in a previous survey of some retail samples of ripened sausages (7). This finding could be explained by the presence of other histamine-forming microorganisms different than fecal coliforms or by a residual histidine decarboxylase activity, detected in the MPN tubes by the enzymatic method, after the microorganisms responsible have disappeared or were inviable as a consequence of the ripening conditions.

Despite the increase of HDB counts in the inoculated samples in relation to the control samples, histamine concentrations did not reflect it. Histamine was only detected in one.
of the trials, and the content was always below 12 mg/kg of dry matter (data not shown). No differences were observed in the amounts of tyramine and putrescine formed, which showed a highest concentration of 194 mg/kg and 37 mg/kg of dry matter respectively.

Significant differences were observed in the amounts of cadaverine formed in the inoculated and control samples in both trials ($P < 0.01$), being higher in the inoculated ones (Fig. 2). Cadaverine content increased markedly during ripening (between the 5th and the 17th days of the study), with a highest amount of 45 mg/kg (38th day) and 95 mg/kg of dry matter (31st day) in the control and inoculated samples, respectively. In consequence, this result suggests that cadaverine concentration could be used as a hygienic indicator for the fecal contamination of raw materials employed in the elaboration of ripened sausages. This relationship between fecal coliform counts and cadaverine levels had already been reported to occur during the spoilage of raw meat (8).

Although the inoculation with <i>E. cloacae</i> S15/19 was not reflected in an increase of histamine concentration, it clearly influenced cadaverine formation. However, in contrast, <i>E. cloacae</i> S15/19 was able to form high amounts of histamine (6,000 mg/ml) but less cadaverine (170 mg/ml) in NB broth. The decrease of <i>E. cloacae</i> S15/19 counts during the ripening process does not explain the lack of histamine formation in the inoculated samples, since <i>E. cloacae</i> S15/19 was able to decarboxylate lysine, forming cadaverine. This lack could be due to other factors, such as a different influence of the ripening conditions on the formation of each biogenic amine. It also could be explained by bacterial histaminase activity (6) or, less likely, by unavailability of free histidine in the raw materials employed. In our work we did not evaluate the levels of free amino acids in the raw materials, but the amount of free histidine reported in other surveys is quite a lot higher than that of other amino acids, such as lysine (5).

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