MICROBIOLOGICAL EVENTS AND EVOLUTION OF HISTIDINE AND TYROSINE DECARBOXYLASE-CONTAINING BACTERIA DURING THE ELABORATION OF "FUET", A SPANISH RIPENED SAUSAGE

A. X. Roig(*), M. M. Hernández, J. J. Rodríguez, E. I. López and M. T. Mora.
Unitat Docent d'Higiene, Inspecció i Control dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona), Spain

Background and objectives: During the elaboration of ripened sausages, biogenic amines can be formed by the action of bacterial decarboxylases. Some of these biogenic amines, such as histamine and tyramine, could be a potential risk for the most sensitive consumers (Bauer *et al.*, 1989). Formation of histamine and tyramine during the elaboration of ripened sausages has been related to the presence of histidine or tyrosine decarboxylase-containing lactic acid bacteria, although presence of histidine and tyrosine decarboxylases has also been described in other bacterial groups, such as enterobacteria, pseudomonads, aerobic spore-forming microorganisms, enterococci and micrococci. The aim of our work has been to study the evolution of these bacterial groups and to relate their evolution with the histidine and tyrosine decarboxylase activity during the elaboration process of "Fuet", one of the most consumed ripened sausages elaborated in Spain, as well as to evaluate the influence of pH and Aw on these parameters.

Materials and Methods: The batter was prepared in a commercial meat plant following their usual technological process, using pork meat trimmings and back fat (90/10), salt, pepper, $NaNO_2$, KNO_3 and sugars (lactose, saccharose and dextrin). The sausages were ripened during 12 days at 15-18ºC and 67-82% relative humidity. By the end of the ripening period "Fuet" is considered ready-to-eat. The sausages were kept under room conditions for five additional weeks (post-ripening storage). Samples were taken immediately on day 0 (after batter preparation), 3rd, 5th (stuffing), 7th, 9th, 13th, 17th (last day of ripening), 24th, 31st, 38th and 53rd day. Total aerobic mesophillic microorganisms (AMM), lactic acid bacteria (LAB), micrococci (MCR), enterococci (ENC), enterobacteria (ENT), spore-forming aerobic microorganisms (SAM) and pseudomonads (PSE) were determined (Roig Sagués et al., 1996). The MPN of histidine and tyrosine decarboxylase-containing bacteria (HDB and TDB respectively) were determined in each sample as described by Roig Sagués et al. (1997a) and Roig Sagués et al. (1997b) respectively, using 5-tube series of Tryptic Soy Broth (Difco) supplemented with 1% L-histidine or L-tyrosine. pH was determined in each sample by inserting a puncture Ingold electrode of 3 mm diameter, connected to a Crison 2001 potentiometer. Aw was measured at 25ºC by a Thermoconstanter automatic analyser (Novasina, Switzerland). Biogenic amines were determined in each sample by the HPLC method described by Veciana-Nogués et al. (1995). Aw and biogenic amines determinations were performed at the Nutrition & Food Science Unit of the Faculty of Pharmacy, University of Barcelona.

Results and discussion: Table 1 shows the evolution of the bacterial groups studied. The main microbiological groups in the batter immediately after preparation (day 0) were micrococci (log 5.27 CFU/g) and pseudomonads (log 5.15 CFU/g). It is during the ripening process (from the 5th day) when the most important changes took place. LAB become the predominant microorganisms after the first four days of the ripening and the highest count was reached the last day of the ripening period (17th day). Micrococci counts increased slightly during the first days of ripening but decreased afterwards to below the initial counts. A similar evolution was observed with the SAM during the last days of the survey. Enterococci counts increased during the first four days of the ripening process, remaining then constant. TDB also increased during the first days of ripening, but afterwards dropped until the last day of ripening (day 17th) increasing again during the post-ripening storage. Only LAB showed a statistical relationship during the survey with TDB (R^2=0.46, $p<0.05$). In previous surveys, enterococci and some LAB were the most powerful tyramine-producing microorganisms isolated from these kind of sausages (Roig Sagués et al., 1997b). Enterobacteria and pseudomonads decreased from the first day of ripening. A close statistical relation was observed between the evolution of HDB and enterobacteria (R^2=0.78, $p<0.01$) and pseudomonads (R^2=0.68, $p<0.01$). Even though enterobacteria and pseudomonads counts dropped during the ripening process until being undetectable, low HDB counts were still detected by the end of this study (over log 1 MPN/g). These results are also in accordance with previous surveys (Roig Sagués et al., 1996). Figure 1 shows pH and Aw evolution. While pH evolution did not show any influence on enterobacteria, pseudomonads and HDB, a significative relation was observed between the Aw and the evolution of these bacterial groups (enterobacteria R^2=0.98, pseudomonads R^2=0.87 and HDB R^2=0.76, $p<0.01$). This fact confirms that Aw is the most important parameter in assuring the microbiological stability of the product and could be a good parameter to control the elaboration process.

Conclusions: Overall, we can conclude that Gram negative bacteria were the responsible of the histidine-decarboxylase activity observed during the production of fuet. However, this activity was not enough to form detectable amounts of histamine during the survey. Enterococci were the main responsible of the tyrosine-decarboxylase activity during the first days of the producing process of "Fuet", although LAB may also contribute to this activity during the last days. This activity was enough to form tyramine along the ripening period, reaching the maximum amount on the 38th day of the survey (129 mg/Kg).

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References:

Bauer, F., Tschabrun, R., & Sick, K. (1989). Histamin in rohwürsten österreichischer herkunft. Wien. Tierärztl. 76, 180-184.

Roig-Sagués, A.X., Hernández-Herrero, M.M., López-Sabater, E.I., Rodríguez-Jerez, J.J. & Mora-Ventura. M.T. (1996). Histidine decarboxylase activity of bacteria isolated from raw and ripened "Salchichón", a Spanish cured sausage. J. Food. Prot. 59, 516-520.

Roig-Sagués, A.X., Hernández-Herrero, M.M., Rodríguez-Jerez, J.J., López-Sabater, E.I. & Mora-Ventura. M.T. (1997a). Histidine decarboxylase activity of *Enterobacter cloacae* (S15/19) during the production of ripened sausages and its influence on the formation of cadaverine. J. Food Prot. 60, 430-432.

Roig-Sagués, A.X., Hernández-Herrero, M.M., Rodríguez-Jerez, J.J., López-Sabater, E.I. & Mora-Ventura. M.T. (1997b). Occurrence of tyramine producing microorganisms in "Salchichón" and tyramine production in sausages inoculated with tyramine producing strain of *Lactobacillus brevis*. J. Food Safety. 17, 13-22.

Veciana-Nogés, M.T.; Hernández-Jover, T.; Mariné-Font, A. & Vidal-Carou, M.C. (1995) Liquid chromatographic method for determination of biogenic amines in fish and fish products. J. AOAC Int. 78, 1045-1050.

TABLE 1. Evolution of microbiological parameters during the elaboration process of "Fuet" (Log((CFU/g)).

Period	Time (days)	Counts								
		AMM	LAB	MCR	SAM	ENT	PSE	ENC	HDB	TDB
Batter	0	5.84	4.91	5.27	4.37	4.07	5.15	4.41	4.14	4.60
aging	3	6.01	4.80	5.18	4.20	3.85	5.35	4.42	3.93	4.46
	5	5.80	4.78	5.04	4.25	3.64	5.00	3.73	4.00	3.97
	7	7.60	7.48	5.22	3.80	3.56	4.87	5.17	3.44	5.20
Ripening	9	8.60	8.53	5.62	4.17	3.52	4.80	5.41	2.94	5.62
	13	8.95	8.65	5.97	4.25	3.38	4.87	5.24	3.74	5.25
	17	8.99	8.89	4.81	4.21	2.42	4.57	5.47	3.38	4.21
	24	8.93	8.75	4.36	4.40	1.79	2.37	5.62	1.59	5.95
Post-	31	8.76	8.69	4.24	4.24	0.93	2.04	4.19	1.60	5.68
ripening storage	38	8.66	8.32	4.67	4.55	-	2.37	5.88	1.28	5.68
	53	8.59	8.30	4.62	4.32	-	-	5.17	2.21	6.00

(-) not detected

Figure 1.
Aw and pH evolution during the elaboration process of "Fuet"