ENTEROCOCCUS SPP. AND LEUCONOSTOC SPP. IN ITALIAN STYLE SALAMI PRODUCED IN THE UNITED STATES

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Background - Enterococci are gram-positive bacteria that fit within the general definition of lactic acid bacteria (Franz et al., 1999). Modern classification techniques resulted in the transfer of some members of the genus Streptococcus, notably some of the Lancefield's group D streptococci (Deibel, 1964), to the new genus Enterococcus (Schleifer and Kilpper-Bälz, 1984). Enterococci have been implicated in outbreaks of food borne illness, and they have been ascribed a detrimental role in food. However they have been shown rarely to be involved in food poisoning outbreaks (Giraffa et al., 1995) and only *Enterococcus faecalis* has been demonstrated to cause changes in meat products, such as softening of German style sausages or deterioration of salami (Cantoni et al., 1987; Papa et al., 1990; Cattaneo et al., 1994). In fact enterococci are frequently detected at high levels in several kinds of full-ripened salami with no alteration of the sensory properties (Dellapina et al., 1994). Since enterococci are enteric organisms commonly found in meat and milk products they have also been considered as indicators of fecal contamination (Cooper and Ramadan, 1955; Whittenbury, 1965). However, once introduced into a food-processing plant, they can become established, and the subsequent contamination of a food product may be of environmental origin rather than fecal origin. Moreover, several researchers postulate that enterococci may contribute to the development of the typical sensory properties of certain fermented meat products, such as salami (Holley et al., 1988). In addition, the ability of enterococci to contribute to cheese ripening is well known (Trovatelli et al., 1987; Gatti et al., 1993) and they are used in the starter cultures for certain type of cheeses (Ledda et al., 1994). The use of enterococci in starter cultures for fermented sausages has also been proposed (Berwal and Dinchev, 1993). Enterococci have a possible contribution to the ripening of cheese due to their lipolytic (Carrasco de Mendoza et al. 1992; Chandler et al., 1979; Mucchetti et al., 1982), proteolytic (Denti et al., 1994; Shugart and Beck, 1964; 1966; Villani and Coppola, 1994) and caseinolytic (Mucchetti et al., 1982; Shugart and Beck, 1964; Somkuti and Babel, 1969; Wallace and Harmon, 1970) activities. Moreover it has been stressed their contribution to flavor producing due to the attitude of produce acetoin, diacetyl and acetaldehyde (Schmidt and Lenoir, 1972). *Enterococcus* strains have been isolated from numerous, diverse nondairy environments including fermented sausages (Aymerich et al., 1996). In fact enterococci are frequently detected at high levels in several kind of full-ripened salami with no alteration of the sensorial properties (Dellapina et al., 1994). Enterococci fit within the general definition of lactic acid bacteria and their association with the human environment and their beneficial interaction, both in food and in the human intestinal tract, combined with the long tradition of lactic fermented foods in many cultures, have led to the general conclusion that this group may be 'generally recognizes as safe' ('GRAS') (FDA, Department of Health and Human Services, 1997). The aim of this work was to investigate the presence of enterococci and *Leuconostoc* spp. in Italian style salami produced in the US and to compare their presence to sensory evaluation.

Objectives - In an attempt to explore the relationship between enterococci composition of Italian style salami produced in the US and consumer acceptability, the objective of this work is to analyze several types of salami for the presence of *Enterococcus* spp. and *Leuconostoc* spp. and to correlate bacteriological analysis to the sensory evaluation..

Methods – i) *Description of salami*. A series of fully ripened salami belonging to 14 different types were purchased from local wholesale shops. Their characteristics are described in Table 1. *ii) Counts, isolation and identification of strains*. After homogenization and dilution the following microbial groups were evaluated: *Enterococcus* spp. on Kanamycin aesculin azide (KAA, Oxoid, Inc., NY, USA) agar incubated at 42°C in air for 18-24 hrs; *Leuconostoc* spp. on Bile aesculin (BA, Oxoid) agar incubated at 37°C in air for 18-24 hrs. All round white or gray colonies grown on KAA agar, about 2 mm diameter, surrounded by black haloes were considered to be group D streptococci; all colonies grown on BA agar, irrespective of diameter, surrounded or not by black haloes were considered to be *Leuconostoc* spp. Both these 'presumptive' counts were confirmed by further analyzing 10 colonies per plate. Colonies of presumed enterococci or *Leuconostoc* spp. were then subjected traditional tests and API 20 STREP (BioMérieux, Inc., Missouri, USA). Each colony was subcultured into brain heart infusion broth (BHI, Oxoid) at 37°C for 24h and then tested for the following characteristics: cell morphology after Gram staining, production of catalase, growth in 6.5% NaCl BHI agar at 37°C, growth in the presence of 4% bile salts at 37°C, hemolysis type on tryptone soya (TS, Oxoid) agar to which 5% of ram blood had been added, at 37°C. Complementary biochemical tests for species identification, were performed on colonies grown on blood agar using API 20 STREP (BioMérieux). Computer program APILAB plus V 3.3.3 (BioMérieux) was used for the results. iii) S*ensory evaluation*. A panel of 6 assessors were presented with 5 samples per session, in 3 sessions, in a balanced order of presentation. Samples were rated according to 4 attributes (plus the acceptability with a scale from unacceptable to very good, see Figure 1) on a 9 cm undifferentiated scale with the anchor points: absent and extremely intense.

Results and discussion – Data from microbiological analysis are shown in Table 2. Seven types of salami had a predominant *Enterococcus* spp. microflora, among the observed microbial groups, in three types both microbial groups were balanced, whilst in three types *Leuconostoc* spp. appeared to be the predominant microflora. The results of sensory evaluation are presented in Figure 1. There were no significant interactions between the two factors (predominant microflora represented by *Enterococcus* spp. or *Leuconostoc* spp.) included in the model. Salami where the predominant microflora was represented by *Enterococcus* spp. were rated as less colored and less bitter, while those with a larger presence of *Leuconostoc* spp. were rated as slightly more salty and bitter. Differences in the sensory evaluation suggest that further study must be carried out to characterize the cultures to be used as a starter with respect to their ability of developing bitter peptides and/or other flavor compounds.

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Table 1. Description of the salami analyzed.

#	Description	Notes
1	Pepperoni	whole
2	Italian dry *salame*	whole
3	Pepperoni	sliced, packaged (keep refrigerated)
4	Pepperoni	whole
5	Hard salami	whole, with added citric acid
6	Italian salami	whole, with added citric acid
7	Hard salami	whole, with added citric acid
8	Genoa salami	whole, with added citric acid
9	Italian dry *salame* (hot)	sliced, packaged (keep refrigerated)
10	Italian dry *salame* (light)	sliced, packaged (keep refrigerated)
11	*Salame toscano*	sliced, packaged (keep refrigerated)
12	Italian dry *salame*	whole
13	Garlic Italian dry *salame*	sliced, packaged (keep refrigerated)
14	Italian dry *salame*	sliced, packaged (keep refrigerated)

Table 2. *Enterococcus* spp. and *Leuconostoc* spp. in full-ripened salami - Log CFU/g.

Types →		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Enterococcus spp.	Mean	3,01	3,09	4,45	5,69	5,05	5,73	4,00	2,83	5,53	n.d.	6,04	3,84	2,48	2,00
	s.d.	0,06	0,10	0,28	0,12	0,24	0,30	0,00	0,16	0,24	-	0,16	0,17	0,00	0,00
Leuconostoc spp.	Mean	n.d.	4,50	n.d.	n.d.	n.d.	5,73	4,24	3,60	n.d.	3,95	n.d.	n.d.	4,73	4,30
	s.d.	-	0,14	-	-	-	0,30	0,14	0,21	-	0,11	-	-	0,18	0,24

n.d.: not detected in 15 grams.

Figure 1. Sensory attributes of three groups of salami (E>L: *Enterococcus* spp. Log CFU/g higher than *Leuconostoc* spp.; E=L Log CFU/g similar; L>E : *Leuconostoc* spp. Log CFU/g higher than *Enterococcus* spp).