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Ripening technology and microbial biodiversity in the preparation of Nebrodi salame

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Abstract. The production of salame in the Nebrodi area, north-eastern Sicily, is carried out by craftmade technique. An important role in the transformation of the pork meat in salame is done by the technique of preparation and by microorganisms present. Next to a specific technology that provides a technique for preparation / maturation with different characteristics (type of casing, drying / ageing environments) is present a microbial biodiversity that is most evident in a surprising large distribution of species present at a wide range of different biotypes both in reference to lactic acid bacteria and staphylococci. The habitats considered, represented by different samples of salame produced in the Nebrodi area using mature Black Pig meat, show a substantial wholesomeness of meat products where the dominant microflora is represented by specific lactic acid bacteria (*Lactobacillus sake, Lactobacillus curvatus* and *Lactobacillus plantarum*) and stafilococci (*Staphylococcus xylosus* largely dominant*, Staphylococcus equorum* and other minority species). The microflora has shown a complete reliability with good organoleptic and healthiness characteristics of salame, and is available, if necessary, to integrate in a "natural" way the production technology of sausage produced in Nebrodi.

Keywords. Salami – Nebrodi – Ripening – Microorganism – Technology – Black pig.

Technologie de maturation et biodiversité microbienne dans la préparation du salami de Nebrodi

Résumé. *La production de salami dans le Nebrodi, au nord-est de la Sicile, est effectuée artisanalement. La technique de préparation et les microorganismes présents jouent un rôle important dans la transformation de la viande de porc en saucisson. À côté d'une technologie spécifique qui fournit une technique pour la préparation / maturation avec différentes caractéristiques (le type d'enveloppe, les environnements de séchage / maturation) il y a une biodiversité microbienne qui se manifeste surtout dans un nombre surprenant d'espèces présentes dans une large gamme de différents biotypes à la fois en termes de bactéries spécifiques d'acides lactiques et de staphylocoques. Les habitats considérés, représentés par différents échantillons de salami produit dans la zone de Nebrodi utilisant de la viande de porc noir, montrent une salubrité des produits dérivés, et des microflores représentées par des bactéries d'acide lactique (*Lactobacillus sake*, Lactobacillus curvatus *et* Lactobacillus plantarum*) et des staphylocoques (*Staphylococcus xylosus *largement répandue,* Staphylococcus equorum *et d'autres en moindre nombre). Ces ferments ont montré une fiabilité complète avec de bonnes caractéristiques organoleptiques des produits, et sont disponibles pour les intégrer aux technologies de production de saucissons produits dans le Nebrodi.*

Mots-clés. *Salami – Nebrodi – Maturation – Caractéristiques microbiennes – Technologie – Porc noir.*

I – Introduction

In the processing of cured salami an important role is played by both the preparation technique and process parameters in the dough either by microorganisms that play a positive or negative role depending on their nature. The various microorganisms present in the meat can selectively degrade all components of meat lipids, proteins, sugars, etc., transforming them in countless

elementary substances compared to those of beginning, not excluding toxic or malodorous. The control of microorganisms in meats, from raw materials is therefore of vital importance during seasoning.

Salami production in the Nebrodi area (Sicily) is generally carried out according to local traditions of craftmade technique. The preparation of the mixture is done to "a knife tip" using all the cuts of pork. Mixture is added salt, pepper whole and ground and natural essences. Stuffing is carried out in natural pork casing. The seasoning is conducted in natural environment, with an initial phase of drying, can be performed in air-conditioned rooms. Depending on the size of casing used and the environmental temperature and humidity conditions products with different ripening, between 30 and 120 days, are obtained. Genetic characteristics, hygiene and processing techniques maturation influence the final characteristics of the sausages (Diaferia *et al*.,2007, Pirone *et al*., 2007).

An important role on the control of final goods characteristics of the salame is done also by air temperature humidity used during the ripening process (Baldini *et al.,* 2000).

With present work, the thermohygrometric conditions and the microbiological biodiversity of Nebrodi salame have been studied.

II – Material and methods

1. Trend of relative humidity and temperature

They were recorded with the use of portable electronic recorders values of environmental temperature and relative humidity. The values recorded at intervals of 20 minutes were subsequently transferred and stored on personal computers. At intervals of 24 hours, mean, minimum and maximum were then calculated.

2. Isolation and identification of strains

Non-pathogenic strains of staphylococci were isolated and subjected to the following tests:

- biochemical profile by API STAPH (bioMérieux),
- oxidase (bioMérieux),
- coagulase (bioMérieux),
- lisostafine.

3. Technological characterization of isolates

The identified non-pathogenic strains of staphylococci were subjected to the following test technology :

- growth in presence of 5-10% NaCl at temperatures of 10-15-22℃ ,
- proteolytic activity in vitro,
- lipolytic activity,
- biogenic amines production,
- lisostafine resistance,
- lactic acid production.

4. Genetic analysis of strains of staphylococci and lactobacilli

The strains of staphylococci and lactobacilli considered for genetic testing have been reactivated by streaking on appropriate substrates, the colonies developed were removed and diluted in water to be lysed by microwaves .

DNA extracted from each strain was amplified by RAPD-PCR technique, which involves amplification of DNA fragments homologous to a bacterial specific primers and subsequent separation by electrophoresis on agarose gel.

The cycle of RAPD-PCR and primers used for identifying biotypes are shown in Table 1. For the identification of species belonging to the biotypes of lactic acid bacteria identified was run-16s rDNA sequencing, and for species identification of staphylococci belonging to the technique was applied ARDRA.

Table 1. RAPD-PCR and primers used for identifying biotypes

Nome primer	Sequence (5'-3')	Cycle
Rapd2	AgCAgCgTgg	Pre-hold: 94℃ x 5min 94℃ x 1min 29℃ x 1min 72℃ x 2min } 20cycle from 29℃ to 72℃ time 90sec. 94℃ x 30sec 55℃ x 30sec 72℃ x 30sec } 45cycle Final-hold: 72℃ x 5min

Conditions applied for PCR-RAPD.

III – Results and discussion

The identification with biochemical and molecular tests allowed the identification of 15 out of 16 strains as *S. xylosus*; strain (6BNE) was identified as *S. saprophyticus*. All strains grew at 10℃ with 10% NaCl .

All rised at 15 and 21℃ in 2 days even at pH 5.1, while at 10℃ strain s S16NE, S17NE, S18NE 8BNE and B did not grow in pH of 5.1.

At the latter pH value and temperature of 10℃ strains 20NE and 18BNE rised in four days as all those tested at pH 5.4; others showed growth in 7 days.

The ability to grow at low temperature and low pH value is certainly significant from a technological standpoint.

Concerning the qualitative determination of proteolytic strains only 18BNE, S1NE, S18NE show a clear capacity to utilize protein.

Regarding qualitative tests for lipolityc activity only a strain showed metabolic activity toward fat throat while all strains caused tributyrin lipolysis, though with varying degrees of intensity. The quantitative test versus throat fat gave different results: all strains possessed metabolic capacity. The less active strain is 6BNE, the most active one is 8BNE B.

Regarding the production of lactic acid, the values found are obviously different depending on the substrate. In conditions similar to those of a mixture (NIM broth) produced amounts are not relevant (Iaccarino and Pirone, *2008)*.

A minimum of 0.083 g/l for the strain 6BNE and a maximum of 0.341 g/l for the strain 22BNE were found.

In media containing high levels of sugars (APT broth), behaviours are noticeably different than in NIM broth: the production of lactic acid is much higher in APT broth and the results much more variable among them.

Regarding the qualitative test for the production of biogenic amines, all strains are producers under adopted test conditions. The test is only indicative since it provides high concentrations of amino acid precursors.

Quantitative analysis of amino acids concentrations on the product at various stages of maturity, may give more complete and closer results to production conditions. In any case, the test run shows a strain (S18NE) less active because it decarboxylates only tryptophan.

The different production areas showed various thermo-hygrometric characteristics on the seasonality of manufacture (winter and spring), depending by use of natural environments (Table 2).

Table 2. Ranges of temperature and humidity recorded during the tests in different Nebrodi areas

Areas	Month	Temperature (°C)		Relative humidity (%)	
		min	max	min	max
Mirto(ME)	February	7.6	15.6	n.d.	n.d.
	March	7.8	13.5	n.d.	n.d.
	April	12.5	14.6	n.d.	n.d.
Piraino(ME)	February	10.5	17.1	43	86
	March	9.4	15.7	52	82
	April	12.7	14.7	70	75
Troina (EN)	February	10.4	19.6	57	74
	March	10.9	14.1	53	73
	April	12.6	17.6	44	63
Castiglione Siculo (CT)	February	12.1	15.1	45	80
	March	12.5	15.6	41	74
	April	13.3	14.3	55	84
Caronia(ME)	February	14.8	17.6	55	88
	March	15.1	17.5	62	78
	April	14.3	18.0	65	79
Longi(ME)	February	10.4	16.5	41	87
	March	9.9	15.8	45	80
	April	14.3	16.4	56	70
Sinagra(ME)	February	12.1	14.7	80	98
	March	11.6	15.5	53	98
	April	11.5	17.0	47	98

Regarding the genetic analysis, molecular fingerprinting to detect some biotypes of strains used, obtained by the technique of RAPD-PCR using the primer Rapd2, are shown in Figs 1 and 2, respectively for the staphylococci and lactobacilli .

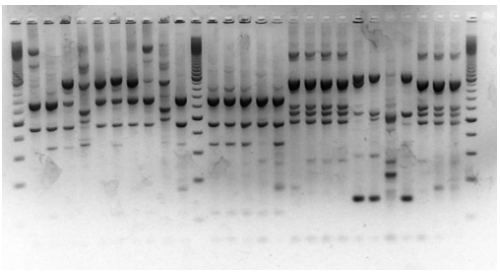

Fig. 1. RAPD profiles of staphylococci isolated from Nebrodi salame.

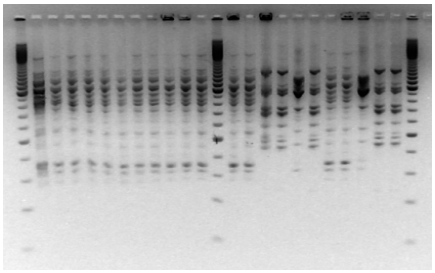

Fig. 2. RAPD profiles of lactobacilli isolated from Nebrodi salame.

5. Identification of species of lactic acid bacteria and staphylococci

A strain for each of identified biotypes of lactic acid bacteria were then subjected to sequence analysis of 16S-rDNA to identify the species 57 strains were identified.

To be able to identify the species of biotypes of stafilococci highlighted with the technique RAPD-PCR, 16S-rDNA of biotypes identified was instead analyzed by the genetic ARDRA technique; 129 strains of staphylococci were identified .

Table 2 and 3 indicate for the strains of lactobacilli and staphylococci, species determined using the ARDRA method, number of identified biotypes, number of strains.

Table 2 . Biotypes and species affiliation of strains of lactobacilli isolated

Biotypes belong to the species	Number of biotypes	Number of strains
Lactobacillus curvatus	3	9
Lactobacillus sakei	7	33
Lactobacillus plantarum	4	13
Waissella	1	2

Table3. Biotypes and species affiliation of strains of staphylococci isolated

Biotypes belong to the species	Number of biotypes	Number of strains
Staphylococcus equorum	4	37
Staphylococcus xylosus	12	59
Staphylococcus epidermidis	1	1
Staphylococcus saprophyticus	4	13
Enterococcus faecium	2	19

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