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Production of *Napoli* salami from some swine autochthonous genetic types. IV. Characteristics of the mycoflora†

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SUMMARY - Fourth six salami, distinct by genetic type, sex and plants of seasoning were examined to show eventual relationships among these factors and the food quality characteristics, rate of fungal colonization and species of moulds grown on them. Yeast were not considered for this study. Rate of colonization was much higher salami seasoned at Angri (SA) than in those seasoned at Morcone (BN). On the contrary, neither the genetic type nor the sex of swine influenced the rate of fungal colonization of the salami. The species of fungi most frequently isolated in salami seasoned both at Angri and Morcone, were *P. aurantiogriseum* and *P. nalgiovense*. The occurrence of *P. brevicompactum*, *P. griseofulvum*, *P. implicatum* and *P. italicum* was low. Only rarely fungi of other genera (*Fusarium*, *Trichoderma*, *Cladosporium*, *Mucor*, *Aureobasidium*) were isolated. A rather high probability that food quality characteristics of the salami depend on high level of fungal colonization was found. Tests on artificial inoculation of salami by selected isolates of *P. aurantiogriseum* and *P. nalgiovense* and on production of mycotoxins either *in vitro* or *in vivo* are in progress.

Key words: Pig, autochthonous genetic type, salami, mycoflora characteristics.

RESUME - *"La production de saucisson type* Napoli *avec des porcins de races autochtones. IV. Caractéristiques de la mycoflore". On a étudié 46 types différents de saucisson suivant le type génétique, le sexe de l'animal et le lieu de séchage, afin de signaler les relations éventuelles entre ces facteurs et les caractéristiques organoleptiques, le degré de colonisation de champignons et le type de champignon présent sur la surface des saucissons. Les levures n'ont pas été prises en considération. On a détecté de façon constante un degré de contamination hautement supérieur dans les saucissons séchés dans l'établissement de Angri (SA) par rapport aux saucissons séchés à Morcone (BN). Au contraire, ni le type génétique, ni le sexe des animaux n'ont influencé le niveau de contamination par champignon dans les saucissons étudiés. Les espèces de champignons que l'on retrouve plus fréquemment, aussi bien à Angri qu'à Morcone, sont les suivantes :* Penicillium aurantiogriseum *et* P. nalgiovense*, tandis que la présence d'autres tels que* Penicillium *spp.* (P. brevicompactum, P. griseofulvum, P. implicatum *et* P. italicum) *est légèrement inférieure. On retrouve rarement des champignons appartenant à d'autres genres :* (Fusarium, Trichoderma, Cladosporium, Mucor, Aureobasidium)*. D'après les résultats obtenus on peut établir une relation constante entre le degré de colonisation et les qualités organoleptiques du produit après 45 jours de séchage. On est en train de réaliser des essais préliminaires d'inoculation artificielle de champignons isolés de* P. aurantiogriseum *et* P. nalgiovense *sur des saucissons pour vérifier la possibilité d'une action de bioamélioration sur ces champignons.*

Mots-clés : *Porc, types génétiques autochtones, saucisson, caractéristiques de la mycoflore.*

Introduction

Salami produced both industrially and handmade are quickly colonized by a composite of fungal flora during seasoning.

Regarding the taxonomy of the involved fungi and the role they play on the ripening and quality of the salami, several works by foreign (Leistner and Ayres, 1968; Hadlok *et al.*, 1975; Andersen, 1995;

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Filtenborg *et al*., 1996;) and Italian authors (Carbone, 1910; Dragoni and Cantoni, 1979; Grazia *et al*., 1986a; Grazia, 1989) have indicated the strong predominance of *Penicillium* species and their active involvement in the regulation of humidity, de-acidification, the improvement of the characteristics of taste and smell, as well as other aspects that positively intervene on the food quality characteristics of the product, can be globally indicated as bioamelioration.

On the other hand, it is ascertained that in nature about 70-80 per cent of *Penicillium* species are capable of producing mycotoxins, many of which are effectively detected in the cultural media of the isolates belonging to many variants species that colonize salami, as well as in the salami itself (Grazia *et al*., 1986a; Mutti *et al*., 1988: Filtenborg *et al*., 1996).

On the basis of the evidence reported, there have been initiatives to select fungal strains that, once artificially inoculated on salami, are able to interfere with colonization of undesirable fungi by a highly rapid growth rate and, chiefly, to improve the food quality characteristics of the product (Grazia *et al*., 1986b). Today, such starter strains are rather commonly used in industry.

Since studies on mycology of cured meats conducted in our country regard environments, technologies and products pertinent to Northern Italy, the purpose of the present work is to make a similar comparison for Southern Italy. Furthermore, to better characterize the research, was taken in consideration the salami typical of Naples, and, as additional factors, the genetic type and sex of the swine utilized.

Materials and methods

Salami were prepared according to Cappuccio *et al.* (1998) from pigs of the Calabrese, Cinta Senese and Siciliano genetic types. Fourth six salami, different for plants of seasoning (Angri, near Salerno, and Morcone, near Benevento), sex and genetic type, were tested. For each of them both the rate of fungal colonization and the species of fungi isolated were determined.

Two slices, whose area corresponding nearly to about 40% of the square of the whole area of the salami, geometrically considered as a cylinder, were cut from different points of each salami on 45th day of seasoning.

From these slices the core was cut off to obtain an annulus 1 cm of tick. These portions were cut in pieces, and homogenized in an homogenator with a proper amount of sterile distilled water. The homogenate was filtered through four layers of cheesecloth and dilutions of 10^{-3} to 10^{-6} in sterile distilled water were made. One ml of each dilution was put in four Petri dishes and a proper amount of melted PDA (potato extract-dextrose-agar) at 45°C temperature containing antibiotics (chloramphenicol 50 g/l and chlortetracycline hydrocloride 50 g/l) was added to each dish by gentle shaking. After incubation at room temperature for 5-8 days, fungal colonies were counted and the rate of fungal colonization of each salami (No. conidia/cm^2 of surface) was calculated. Yeasts were not included in this work.

Identification of *Penicillium* species was made on the basis of the macro- and microscopic characteristics exhibited by the isolates grown on CYA and MEA at 25°C for at least 7 days, according to Pitt (1979, 1988) and Pitt and Hocking (1985), but also to Samson *et al*. (1995) and Stolk *et al*. (1990).

To compare the food quality characteristics of the salami with the relative rate of fungal colonization, the results of a panel tests carried out at ConSDABI (Zullo *et al.,* 1998) were utilized. Differences among means were valued by Least Significant Difference test (LSD) at $P<0.05$.

Results and discussion

The rate of fungal colonization was very variable, from a minimum of 0.3×10^6 conidia/cm^2 for a salami from a Calabrese male swine to a maximum of 212×10^6 conidia/cm^2 for a salami from a Calabrese female swine (Table 1).

Table 1. Rate of fungal colonization (millions of conidia/cm^2) for each salami within the considered factors

Plants	Genetic type					
	Calabrese		Cinta senese		Siciliano	
	Male	Female	Male	Female	Male	Female
Morcone	24	12	5	10	18	19
	0.3	-	9	-	4	1
	0.3	-	13	-	16	3
	1	-	-	-	32	4
	-	-	-	-	4	4
	-	-	-	-	11	-
Angri	69	14	9	22	38	93
	148	35	15	-	18	61
	25	212	4	-	70	84
	5	-	-	-	24	62
	81	-	-	-	33	34
	-	-	-	-	20	14
	-	-	-	-	14	16

In general, the rate of fungal colonization of the products examined was greatly influenced by plants: the mean rate of fungal colonization of the salami seasoned at Angri (47◊10^6 conidia/cm^2) resulted near 5-fold greater than that of salami seasoned at Morcone (9.5◊10^6 conidia/cm^2). This was statistically confirmed by significance of the difference. On the contrary, differences among genetic types, as well as among sexes, were not statistically significant. Differences obtained from interaction between plants and genetic type, were significant for salami of Calabrese and Siciliano genetic types coming from Angri and Morcone, but not for Cinta Senese coming from the two plants of seasoning. A total absence of significance was found by analysis of the interaction between genetic type and sex and between plant and sex (Table 2).

Table 2. Means of rate fungal colonization (millions of conidia/cm^2) within considered factors and significance of the comparisons

Genetic type		Sex		Plants	
Calabrese	12.9^a	Male	11.6^a	Morcone	5.2^a
Cinta senese	9.2^a	Female	18.0^a	Angri	29.1^b
Siciliano	16.2^a				

Plants	Genetic type		
	Calabrese	Cinta senese	Siciliano
Morcone	2.0^a	8.4^a	6.7^a
Angri	41.2^b	10.4^a	32.4^b

a,b: Different letters within each factor indicate significant level of $P<0.05$

To a high rate of colonization corresponds, on the other hand, a small variety of moulds, either at genus or species level. More common species belong to *Penicillium* Link and resulted to be *P. aurantiogriseum* and, at to a minor extent, *P. nalgiovense* in salami from swine of all three genetic types. *P. aurantiogriseum* is one of the more common moulds in nature, very frequent on cereals and

different kinds of food (Pitt, 1988). As ripened meat colonizer and, particularly, as salami colonizer it has repeatedly reported (Haslock *et al.*, 1975; Pitt and Hocking, 1985) often with the synonym of *P. verrusosum* var. *cydopium* (Westling) Samson, Stolk et Hadlock (Grazia *et al.*, 1986a; Grazia, 1989; Leistner and Eckardt, 1979; Filtenborg *et al.*, 1996). *P. nalgiovense* seems to be prevalent among the mycoflora of the salami after few weeks of ripening (Filtenborg *et al.*, 1996), is frequent (7th position) in the *Penicillium* list from meat products provided by Leistner and Eckardt (1979), while has been isolated only rarely from salami produced both industrially and handmade (Grazia *et al.*, 1986a). It has been reported also from fresh meats (Dalla Valle *et al.*, 1987). A moderate presence was shown by *P. brevicompactum* and *P. griseofulvum*, this latter one only in salami of Cinta Senese genetic type; a slight presence was shown by *P. implicatum* and *P. italicum* only in salami of the Siciliano genetic type. Species belonging to other genera were found only rarely (not higher then 0.3%) with the exception of *Trichoderma koningii* that represented 2.7% of the fungal isolates from salami prepared with meat of the Calabrese genetic type (Table 3).

Table 3. Frequency (%) of each fungal species over the total of fungi isolated from salami of the different genetic types

Specie	Genetic type			
	Calabrese	Cinta senese	Siciliano	Total
Aureobasidium pullulans	0.1	0.0	0.1	0.08
Cladosporium cladosporioides	*	0.0	*	0.0
Fusarium oxysporum	*	0.0	0.3	0.2
Mucor recemosus	*	0.0	*	0.0
Penicillium aurantiogriseum	75.2	73.2	82.9	79.3
" *brevicompactum*	6.9	0.0	5.6	5.0
" *griseofulvum*	4.1	13.1	1.8	4.4
" *implicatum*	0.0	0.0	0.1	0.04
" *italicum*	0.0	0.0	0.1	0.04
" *nalgiovense*	10.8	13.6	9.1	10.4
Trichoderma koningii	2.7	0.0	0.0	0.8

*Isolated presence

The possible influence of fungi on food quality characteristics of salami is shown by a regression line in Fig. 1. Although to a high global opinion of the panel test often corresponds a low rate of fungal colonization, the coefficient of regression shows a rather high probability that the food quality characteristics of the salami depends on high level of colonization by fungi.

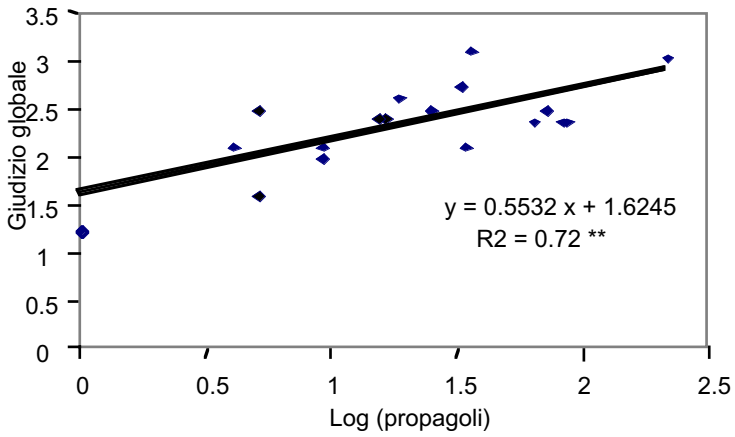

Fig. 1. Relationship between the rate of fungal colonization of 18 salami and the panel test results.

Tests on artificial inoculation of salami by selected isolates of *P. aurantiogriseum* and *P. nalgiovense* and on production of mycotoxins either *in vitro* or *in vivo* are in progress.

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