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Characterization of surface mycoflora in Nebrodi hams

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Abstract. Within the framework of a three-year project, the screening of spontaneous fungal species grown on hams produced with "Nebrodi black pig" meat and seasoned in different environments of the Nebrodi area has been carried out, in order to characterize mycological population of such typical products and to keep under the presence of unexpected changes due to fungal contamination. In most of the aitchbones examined, the prevailing fungal species isolated resulted to be xerotolerant or xerophilic, due to their peculiar ability of adaptation to surface a_w and to thermohygrometric conditions applied in ripening rooms. With regard to the *Eurotium* strains isolated, *Eurotium herbariorum, Eurotium rubrum* and *Eurotium cristatum* have been detected. With regard to the *Penicillium* strains isolated, *Penicillium nalgiovense*, *Penicillium chrysogenum, Penicillium griseofulvum, Penicillium olsonii,* and *Penicillium aurantiogriseum* have been detected. In any of the aitchbones examined, undesirable *Penicillium* species such as *P. commune* or *Penicillium solitum* (which are considered responsible for the production of the so-called "phenol defect" in hams) and *P. nordicum* (which proved to be one of the greatest Ochratoxin A producer in hams and in protein-based foods) have not been detected.

Keywords. Phenol defect – Hams – Moulds – *Penicillium solitum* – *Penicillium nordicum.*

Caractérisation de la flore fongique de surface dans les jambons de Nebrodi

Résumé. *On a étudié la mycoflore sur les jambons produits avec la viande de porcins de races autochtones appartenant aux types génétiques Nebrodi et séchés dans les environnements de la région de Nebrodi, afin de caractériser la population fongique sur ces produits locaux et de surveiller la présence de changements anormaux dus à la contamination des moisissures. Dans la plupart des os du bassin («quasi») examinés, les espèces fongiques prédominantes ont été xérotolérantes ou xérophiles, grâce à leur capacité caractéristique de s'adapter à l'*a_w *superficielle et aux paramètres hygrothermiques utilisés dans les environnements de vieillissement. En ce qui concerne les espèces* Eurotium *isolées, on a constaté la présence de* Eurotium herbariorum*,* Eurotium rubrum *et* Eurotium cristatum*. En ce qui concerne les espèces de* Penicillium *isolées, ont été trouvées* Penicillium nalgiovense*,* Penicillium chrysogenum*,* Penicillium griseofulvum*,* Penicillium olsonii, *et* Penicillium aurantiogriseum*. Dans aucun des os du bassin examinés, n'ont été trouvées d'espèces indésirables de* Penicillium*, comme* P. commune *ou* P. solitum *(qui sont responsables du dénommé "défaut de l'acide phénique" dans les jambons) et* P. nordicum *(qui s'est avéré être l'un des plus grands producteurs d'ochratoxine A dans les jambons et les produits à base de protéines).*

Mots-clés. *Défaut de l'acide phénique – Jambons - Flore fongique – «Quasi»* - Penicillium solitum - Penicillium nordicum.

I – Introduction

Among factors influencing air-borne contamination of foods, microbial population in environmental air (Heldman, 1974) play a great role. In general, fungal spores proved to represent the prevailing part (56%) of the air-borne microflora (*Aspergillus*, *Penicillium*, *Rhizopus*, *Cladosporium*, *Fusarium*), while *Bacillus* spores, Gram-positive and Gram-negative bacteria represent the remaining one. Nevertheless, in industrial environments where foods such as meat derivatives are produced, fungal population can reach 70% of the total microbial population (Singh *et al.*, 1986).

In aged meats, moulding is directly connected with physico-chemical parameters recorded in productive environments (Baldini *et al*., 2000). In ripened meats, maturing techniques applied usually allow for the rapid and distinctive colonization by a great number of mycetes, which are considered fundamental to impart both desirable appearance and good organoleptic characteristics to these meats. On the contrary, in dry-cured meats maturing techniques are more and more pointed to obtain products where no or little surface mycoflora has grown: only autochthonous moulds indicating that ripening process is shaping up good and proving to compete and then prevail over undesired species should be tolerated. In general, in hams unexpected changes can occur and even persist in the final product in case thermo-hygrometric parameters reach high values during the resting and dehydration process is not carried out correctly.

A first example of such changes is represented by the so-called "phenol defect", a detrimental effect due to a fungal colonization of the aitchbone area by *Penicillium commune* (Spotti *et al*., 1988) or by *Penicillium solitum* (Spotti, personal communication) during seasoning in case salt content on ham surface results lower than 17.5% (saturated salt solution) during salting and Relative Humidity (RH) values result higher than 85%. In fact, this area represent the wettest part of the ham and it could be more subjected to spoilage by *Penicillium* species, while the muscle portion, where dehydration occurs in a faster way, is usually more subjected to spoilage by *Eurotium* species, which tend to prevail because of their xerophily (Baldini and Spotti, 1995). To avoid "phenic acid defect" it should be taken into account that: (i) a satured salt solution on the cut surface allows salt penetration in the aitchbone area during salting; (ii) fast dehydration is essential within the first 15 days of resting; (iii) the control of thermo-hygrometrical parameters such as RH is fundamental to avoid fungal growth; (iv) an increasing in dehydration could be applied in case fungal spoilage occurs during resting and pre-ripening.

A further example of the above-mentioned changes is represented by the presence in seasoned hams of ochratoxin A (OTA), a strong, nephrotoxic, secondary metabolite that can be produced in meat products by some fungal species such as *Aspergillus ochraceus* and *Penicillium nordicum* and that can persist in the finished product. The moulds responsible for OTA production can develop on the surface of aged products and start producing OTA in case some variations in thermo-hygrometric parameter occur, so their presence must be always kept under control by means of periodical laboratory tests.

Within the framework of the above-mentioned project, the aim of this work was to screen spontaneous fungal species grown on aitchbones from hams produced with "Nebrodi black pig" meat and seasoned in different environments of the Nebrodi area, in order to characterize mycetical population of such typical products and to keep under the presence of the above-mentioned unexpected changes due to fungal contamination.

II – Materials and methods

The screening of the mycoflora grown on aitchbones from hams seasoned in traditional environments of the Nebrodi area has been carried out by using sterile swabs, since they allow to scratch out a significant amount of conidia from any of the aitchbones assessed. After collecting the conidial mass, each swab has been plated on Malt Extract Agar (MEA) and on Dichloran 18% Glycerol Agar (DG18). Petri Dishes have been incubated at 25°C for seven days, in order to allow sporification of the fungal species isolated. Fungal identifications have been carried on selective media, according to the methods proposed by Pitt (Pitt and Hocking, 2009) and by Samson (Samson *et al*., 2004):

- Malt Extract Agar (MEAB), 25% Glicerol Nitrate agar (G25N), Creatine Sucrose Neutral Agar (CSN), CY20S, Yeast Extract Sucrose Agar (YES) incubated at 25°C for seven days;
- Czapek Yeast extract Agar (CYA) incubated at 5°, 25° and 37°C for seven days.

III – Results and discussion

The screening of the mycoflora grown on aitchbones from products seasoned in traditional environments of the Nebrodi area has been carried out on hams at different stages of their long-term ripening, since thermo-hygrometric parameters recorded in ripening plants proved to be greatly influenced by environmental outdoor conditions. In most of the aitchbones examined, yeasts have been isolated. In fact, they usually can colonise surface layers of cured meats for most of the seasoning time, both contributing to the development of the final typical aroma and avoiding oxidative processes on the ham surface (Martin *et al*., 2006).

In all the aitchbones examined, (Table 1) the prevailing fungal species isolated resulted to be xerotolerant or xerophilic (capable of growing at water activity (a_w) values lower than 0.85). In particular, the presence of *Eurotium* species and the growth of more xerotolerant fungal species belonging to *Penicillium* can be both due to their peculiar ability of adaptation to surface a_w and to thermo-hygrometric conditions of these plants where RH values range from 85 to 92% and where temperatures range from 10° and 20°C.

Table 1. Prevalence of fungal species isolated on aitchbones at the end of the seasoning from Nebrodi hams, at different stages of their long-term ripening

Species isolated	Frequency of contaminated aitchbones (%)		
	Year 2005	Year 2006	Year 2007
Eurotium herbariorum	85.7	63.6	38.5
Penicillium gladioli	71.4	18.0	46.0
Penicillium griseofulvum	71.4	27.0	7.6
Penicillium olsonii	57.0	9.0	23.0
Penicillium chrysogenum	0	9.0	7.6
Penicillium nalgiovense	0	9.0	7.6
Penicillium aurantiogriseum	0	27.0	23.0
Eurotium rubrum	0	9.0	30.7
Eurotium cristatum	0	18.0	7.6
Aspergillus candidus	0	9.0	0
Aspergillus versicolor	0	9.0	7.6
Penicillium glabrum	0	0	7.6
Aspergillus sydowii	0	9.0	0
Hyphopichia burtonii	0	9.0	0
Yeasts	85.7	72.7	100.0

With regard to the *Eurotium* strains isolated, *Eurotium herbariorum, Eurotium rubrum* and *Eurotium cristatum* have been detected. Their presence can be explained by the fact that: they're markedly xerophiles (so they're able to grow within a wide range of temperature and a_w values); they tend to form fast-growing colonies that usually prevail over *Penicillium* ones; they reproduce themselves in both a vegetative (via darkish-coloured conidia) and a sexual (via yellowish-coloured cleistotecia containing ascospores) way. Such species, as well as *Aspergillus* ones, can frequently occur on ripened and dry-cured meat, as the surface a_w is markedly lower on these kind of products.

With regard to the *Penicillium* strains isolated, *Penicillium nalgiovense*, *Penicillium chrysogenum, Penicillium griseofulvum, Penicillium olsonii,* and *Penicillium aurantiogriseum* have been detected. In literature, such species are frequently mentioned as those occurring in seasoning environments, so their presence has been well tolerated.

Among *Penicillium* species, *Penicillium gladioli* has been also detected. As it was isolated yet

within the first part of the project on Nebrodi salami and it was focused on because of its properties as autochthonous "starter" culture, its presence has been well tolerated too.

In addition to this, it must be underlined that some undesirable *Penicillium* species such as *P. commune* or *Penicillium solitum* (which are considered responsible for the production of the so-called "phenic acid defect" in hams) and *P. nordicum* (which proved to be one of the greatest Ochratoxin A producer in hams and in protein-based foods) have never been detected on the examined aitchbones. This means that variations in thermo-hygrometric parameter haven't occurred and that seasoning have been carried out correctly in hams from Nebrodi area.

With regard to other environment-contaminating moulds, contamination by *Cladosporium, Aureobasidium* and *Mucor* species has been only rarely detected. The growth of these moulds should be avoided both on hams and salami, as they don't allow homogeneous drying of the product in the first steps of the process and they can form darkish spots on casings of salami or on surface of hams.

Ultimately, *Hyphopichia burtonii* has been detected just once. This species is attributed to Filamentous Fungi and indicated as "yeast-like mould" because of its morphological and reproductive characteristics which enable it to grow widely on the surface of solid media with a_w values ranging from 0.85 to 0.90. Its presence is usually tolerated as it proved not to produce toxic metabolites, to compete with undesired species and to partially inhibit OTA production (Spotti *et al*., 2009). For the above-mentioned reasons, its use as possible "competitor" of any undesired *Penicillium* species has been at present taking into account.

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