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## Short Communication

# Fungal profiles of Spanish country-cured hams

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The fungal population of Spanish country-cured hams was analyzed. For 160 surface samples from 40 hams yeast counts between  $10^4$  and  $3 \times 10^5$  cells per g and filamentous fungi from  $5 \times 10^2$  to  $3 \times 10^4$  colony-forming units per g were found. The yeasts isolated belonged to the species *Debaryomyces hansenii* and were capable of growth at 16% of NaCl. The molds isolated belonged to the genera *Eurotium*, *Penicillium* and *Trichoderma*. 70% of the isolates were identified as *Eurotium repens* the species most frequently isolated. Five species of *Penicillium*: *Penicillium expansum*, *P. cyclopium*, *P. viridicatum*, *P. brevicompactum* and *P. simplicissimum*, represented about 25% of the isolates.

Key words: Molds; Yeasts; Hams

## Introduction

Country-cured hams are dry cured meats commonly produced and consumed in Spain. The best known and most important factory in Spain is Sánchez Romero Carvajal, Jabugo, S.A. Its name comes from a small village in the south west of Spain, Jabugo, in the province of Huelva, near Portugal. The country-cured hams in this factory are produced from 'Iberian breed pigs'. A traditional characteristic of this breed is its customary feeding habits; 40% of total body weight is accounted for by extensive feeding on acorns (*Quercus ilex* subsp. *rotundifolia*) and forage. The total weight of the animal should be above 140 kg 'in vivo', to obtain hams of optimal size (Najarro and Salazar, 1981).

Abundant mold growth is often observed on the surface of country cured hams and is associated with a long period of aging, Ayres et al. (1980). Several authors, mainly in the United States, have described the mycoflora of country-cured hams; the molds usually found are *Penicillium*, *Aspergillus*, *Cladosporium* and *Alternaria* (Sutic et al., 1972). In some cases the production of several toxins were reported (Escher et al., 1973; Wu et al., 1974).

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The presence of yeasts mainly *Debaryomyces* and *Candida*, (Draughon et al., 1981) have also been reported.

In Italy, *Aspergillus*, *Penicillium* and *Trichoderma* have been described in Parma and San Daniele hams, (Dragoni et al., 1980). In Spain no systematic work on the fungal flora of country-cured hams has been reported although some data about fungal profiles in salami have been described (Sesma Vea, 1975). As the fungal flora could be responsible for the particular taste of the Jabugo hams, this study was undertaken to elucidate their fungal flora.

## Materials and Methods

Variations in humidity and temperature were recorded in the factory with a thermohygrometer (T 1150-58 CASELLA, London). The salt content of the hams was determined according to Grau (1965). The pH was estimated by introducing an electrode coupled to a pH-metre directly into several points of the ham (E-488 METROHM, Herison, Switzerland).

Surface samples ( $1 \text{ cm}^2 \times 0.7 \text{ cm}$  thickness) from 40 hams (160 samples) cured for 6 to 24 months in the factory were taken aseptically, weighed and homogenized with 10 ml of sterile water; serial dilutions were made in sterile water and platings (in triplicate) were carried out on GAE (glucose, 1%; asparagine 0.1%; yeast extract 0.05%;  $\text{KH}_2\text{PO}_4$  0.05%;  $\text{FeSO}_4$  0.001% and  $\text{MgSO}_4$  0.05%), Czapek-Dox (sucrose 3%;  $\text{NaNO}_3$  0.2%;  $\text{KH}_2\text{PO}_4$  0.05%;  $\text{MgSO}_4$  0.05%,  $\text{KCl}$  0.05%;  $\text{FeSO}_4$  0.005%) and YEPD (yeast extract 1%, peptone 2%, glucose 2%). For solid media was added agar 1.5%. Plates were incubated for 96 h at  $28^\circ\text{C}$  before colonies were counted.

The effect of  $\text{NaCl}$  on growth of *Debaryomyces marama* was investigated in Erlenmeyer flasks containing 300 ml of YEPD inoculated with 1.6 mg (dry weight) of cells and incubated in a New Brunswick G10 incubator-shaker (250 rpm) at  $28^\circ\text{C}$ .

Identification was performed by standard methods currently used in taxonomy for yeast: Barnett et al. (1979), Van der Walt (1970), Kreger-van Rij (1970) and Wickerham (1946); Penicillia: Raper and Thom (1949) and Aspergilli: Raper and Fennell (1965) and Domsch et al. (1980).

## Results and Discussion

One of the main factors that determines the composition of the microbial flora on the surface of the ham is climate. In Jabugo the summers are short and mild, the winters are long and generally wet, its rainfall is about 1000 liters/ $\text{m}^2$ /year. The temperature ranges between  $37^\circ$  in August and  $-3^\circ\text{C}$  in December to February. In the main Bodega, 'Fundadores', where the hams are cured the temperature ranges between  $17-21^\circ\text{C}$  in the month of July and  $13-17^\circ\text{C}$  in the month of November (year 1981). The variations in humidity in the bodega are shown in Fig. 1.

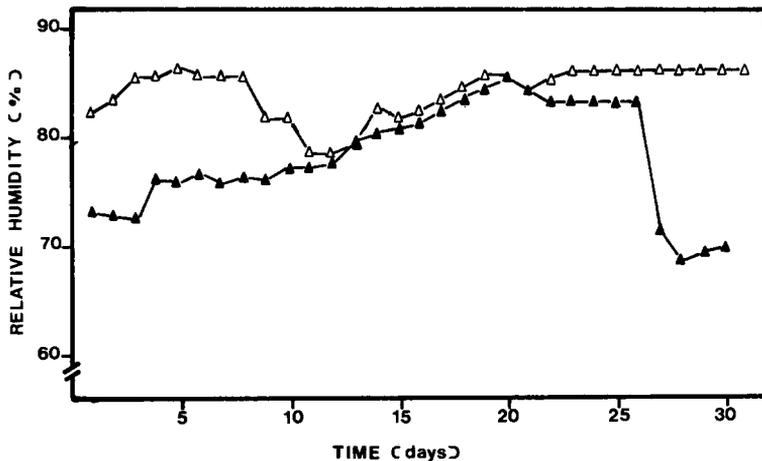


Fig. 1. Variations of the relative humidity in the cellar 'bodega Fundadores'.  $\Delta$ , July;  $\blacktriangle$ , November, year 1981.

Surface NaCl concentrations of the hams ranged between 2.5 and 3% (w/w) whereas inside they were 0.9–1.1% (w/w). Surface pH was in the 5.7–5.9 range.

For the 160 samples examined the number of yeast ranged from  $10^4$  to  $3 \times 10^5$  per g. The number of colony forming molds varied from  $5 \times 10^2$  to  $3 \times 10^4$  per g.

All yeast isolates identified (in total 140) were characterized by not being able to ferment glucose, galactose, maltose, sucrose, lactose, cellobiose, trehalose, melibiose, melezitose and raffinose. The following carbon compounds were assimilated: glucose, D-arabinose, galactose, salicine, fructose, sucrose, maltose, cellobiose, trehalose, melezitose, raffinose, inulin, soluble starch, glycerol, erythritol, D-mannitol, sorbitol, ethanol and succinic acid. D-xylose, D-arabinose, L-rhamnose, L-sorbose, D-glucosamine, lactose, melibiose, galactitol, meso-inositol, methanol and DL-lactic acid were not assimilated and assimilation of potassium nitrate was negative. Growth on yeast extract agar with 50% (w/w) glucose and growth at 37°C was not demonstrated for any of the isolates.

From the above results and the morphology of the cells and of the ascospore, it is suggested that all isolates belong to the genus *Debaryomyces*, species *Debaryomyces marama*.

The strains of *Debaryomyces marama* isolated were able to grow in high NaCl concentrations as shown in Fig. 2. This agrees with results described by other authors for different species of *Debaryomyces* (Hobot and Jennings, 1981).

All mold isolates (i.e. 132) from 40 samples of country cured hams were identified as members of the genera *Eurotium*, *Penicillium* and *Trichoderma*. The predominant group isolated were members of the *Eurotium glaucus*, species *Eurotium repens* (93 isolates). 20 isolates, almost half of the *Penicillia*, belonged to *Penicillium viridicatum*. Other *penicillia* isolated were *P. expansum*, *P. cyclopium*, *P. brevicompactum* and *P. simplicissimum*. The *Trichoderma* species isolated were identified as *T. viride* (three isolates).

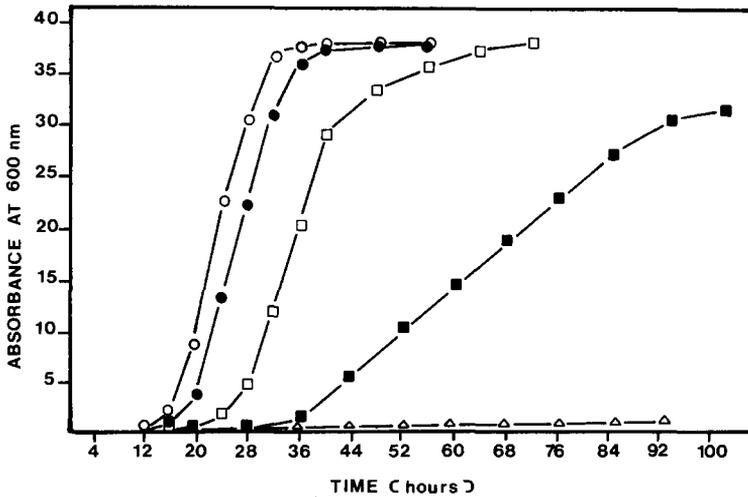


Fig. 2. Effect of NaCl concentration on the growth of *Debaryomyces marama*. ○, without and with 4%; ● 8%; □ 12%; ■ 16% and △ 20% NaCl.

The curing and ripening processes of Jabugo hams are different to those described for several types of country-cured hams, but the dominating yeasts and fungi which develop on the surface of Jabugo hams, i.e. *Debaryomyces*, *Eurotium* and *Penicillium* appear to be similar to those described for other types of country cured hams (Moreau and Moreau, 1959; Leistner and Ayres, 1968; Sutic et al., 1972; Moreau, 1979; Dragoni et al., 1980 and Draughon et al., 1981).

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