51st International Congress of Meat Science and Technology
August 7-12, 2005 – Baltimore, Maryland USA

INHIBITORY EFFECT OF SODIUM BENZOATE, POTASSIUM SORBATE AND METHYL P-HYDROXYBENZOATE ON FUNGI RECOVERED FROM TWO TYPES OF PORTUGUESE SMOKED DRY SAUSAGES

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Key Words: Portuguese smoked dry sausages; Spoilage fungi; Preservatives; Inhibitory effect; Modified atmosphere package; Shelf-life

Introduction

Mould spoilage in Portuguese smoked dry sausage (chouriço) packaged in modified atmosphere package (MAP) may occur, reducing the shelf life and causing substantial financial losses to manufacturing companies (Matos et al., 2003a). Presence of moulds also could constitute a potential hazard to human health considering that some mould strains might be toxigenic (Sweeney & Dobson, 1998). In Portugal, as in the E.U., benzoates, sorbates and the esters of p-hydroxy-benzoic acid may be used in surface treatments of dry sausages casings. However, resistance of fungi to these preservatives depends on factors such as pH, type and strain, preservative concentration, inoculums level, product composition, water activity, other additives, physical treatment of processing and smoke application, storage temperature, length of storage, storage atmosphere, and type of packaging. (Jay, 2000; Asehraou et al., 1997; Sofos, 1989; Hotchkiss, 1989; Lueck, 1980). It is therefore, interesting to investigate the effect of these additives at pH 6.5 on the spoilage fungi of these meat products.

Objectives

Mainly goals of this work were to identify some important spoilage fungi isolated from two types of Portuguese chouriço after producer-defined shelf life period (120 days at 20±5ºC) in MAP (55% N2 / 45% CO2), which might decrease storage period and compromise product safety and, to study the effect of potassium sorbate, sodium benzoate and methyl p-hydroxybenzoate on the growth rate of representative mould isolates in vitro conditions.

Methodology

The experiments were conducted at a commercial meat plant and the study was based on 12 samples (each sample was composed by a mixture of three sausages, a total of 36 sausages) randomly drawn from two batches. Representative sausages of each experiment were produced as outlined by Matos *et al*. (2003b) and were packed separately in modified atmosphere (55% N_2/45% CO_2) and stored at 20±5ºC for 120 days (shelf-life period). Identification of fungi was performed according conventional

mycological methods based on morphological and physiological characterization using taxonomic tables.

Each mould strain was tested in triplicate in peptone water (PW):g l^{-1}): 30 g of bacto proteose peptone (Difco, 0120-17-6) per l of distillate water, pH of 6.5 and incubation at 25ºC for 5 days. Salt solutions were prepared in distillate water with addition of 0.1 g, 0.5 g, 1.0 g, 1.5 g and 3.0 g 100 ml^{-1} salt in order to reach the final concentration in the microwell of 0.01, 0.05, 0.1, 0.15 and 0.3% (a ten fold dilution (v/v) corresponding to 25 ìl /250 ìl), respectively. From each spore suspension, 25 ìl was dispensed into wells of a 96-microwell plate containing 200 ìl of peptone water (PW) and 25 ìl of each salt solution to give a final volume of 250 ìl in each well. In the positive control salt solutions were replaced by sterile water and, in the negative control the corresponding volume of spore suspension was replaced by PW-medium. Microplates were covered with special lids, to avoid evaporation of water during incubation period.

Growth curves of the mould batches in each well of the microplates were obtained by reading optical density at 650 nm (OD_{650}) twice a day for 5 days on an EL 808 – Ultra Microplate Reader (Bio-Tek Instruments, Inc. Winooski, VE). The specific growth rates, μ, were calculated as the slope of the linear part of the ln-plots, representing the exponential growth phase. Results are presented as mean ± standard deviation (n=3) of the growth rate (ì h^{-1}). Negative control was subtracted from each result.

Results & Discussion

Identification of moulds yielded 11 groups: *Penicillium terrestres* (43.4%), *Penicillium* sp. (13.3%), *Fusarium* sp. (10%), *Aspergillus glaucus* (10%), *Aspergillus versicolor* (6,8%), *Monilia fruticola* (3.3%), *Absidia* sp. (3.3%), *Cephalosporium* sp. (3.3%), *Rhizopus stolonifer* (3.3%) and *Fusarium tricinctum* (3.3%). Differences founded between identified species in our study and from those reported by several authors (Andersen, 1995; Kivanc *et al*., 1992; Mutti *et al.*, 1992; Dragoni *et al*., 1991) could be related with the applied processing and packaging technology and also with the house ambient, which is the main source of moulds (López Díaz *et al*., 2001). In our case, mould fermentation is not promoted and, the species reported here, were isolated after shelf life period in MAP.

The methodology used to quantify *in vitro* fungal growth was quite different from the one traditionally applied for studies of fungal/mould growth. In the present study, we observed mycelium growth on top of liquid media to be an exponential function of time when registered as OD_{650} values. We were thus able to quantify growth of fungal mycelia using classical microbiological growth equations, whereby the evaluation of inhibitory effects of the tested salts was straightforward. This approach has to our knowledge not been reported before.

MHB showed greater inhibitory effect than SB and PS in all fungi isolates with exception in *Aspergillus glaucus* [Tm30(A)], in which the inhibitory effect of MHB was similar to PS (Figure 1). At 0.05% (w/v) all fungi were inhibited with MHB with exception for *Rhizopus stolonifer* [Tm25(A)] which, only with a concentration higher than 0.1% started to decrease the growth rate. In what concerns PS and SB, PS was more effective to inhibit mould growth than SB, with exception in *Absidia* sp [Tm16(R)], in which both presented similar inhibitory effect.

Conclusions

Although contributions from other fungi than the ones isolated can not be excluded, Penicillium terrestres (43.4%), Penicillium sp. (13.3%), Fusarium sp. (10%), Aspergillus glaucus (10%), Aspergillus versicolor (6,8%), Monilia fruticola (3.3%), Absidia sp. (3.3%), Cephalosporium sp. (3.3%), Rhizopus stolonifer (3.3%) and Fusarium tricinctum (3.3%) were found in varieties Alentejano and Ribatejano of Portuguese chouriço after 120 days at 20±5ºC in MAP.

Addition of MHB at 0.1% (w/v) could represent an alternative to be considered in order to assure the safety of the final product and to reduce the substantial financial losses felt by manufacturing companies. At pH 6.5, MHB showed greater inhibitory effect than SB and PS in all fungi isolates with exception for Aspergillus glaucus [Tm30(A)], in which the inhibitory effect of MHB was similar to PS. In what concerns PS and SB, PS was more effective to inhibit mould growth than SB, with exception in Absidia sp [Tm16(R)], in which both presented similar inhibitory effect. However, at pH 6.5, PS and SB did not exhibit their entire antifungal properties, which are effective antifungal agents only at pH=6. In this way, before more specific conclusions could be drawn, mycotoxin formation and its inhibition should also be investigated and, further studies performed in the product, should be carried out.

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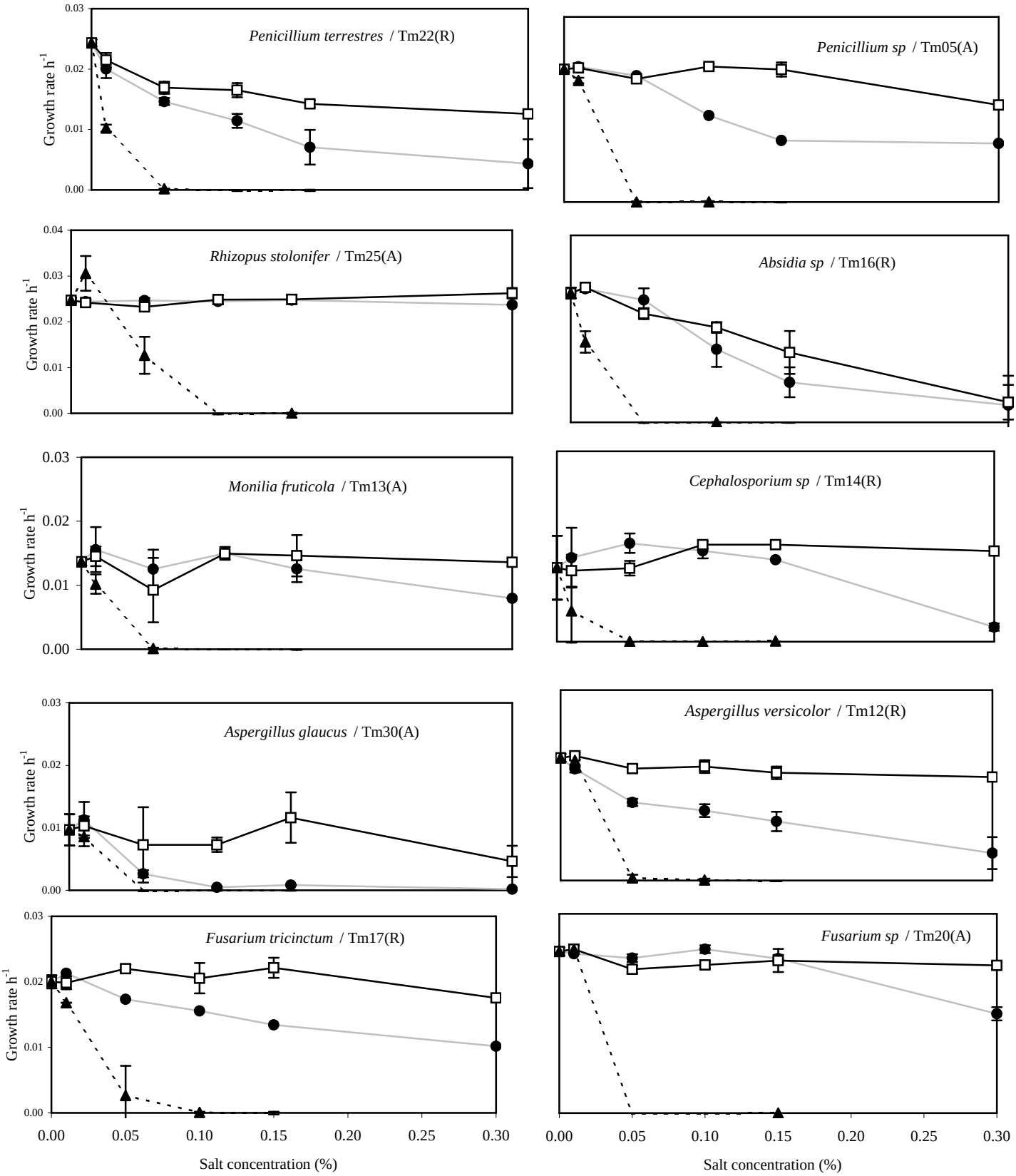
Tables and Figures

Figure 1. Growth rate (mean values ± SD, n=3) of representative mould isolates recovered from chouriço type Alentejano (A) and from type Ribatejano (R) in PW medium (pH of 6.5 and incubation at 25ºC) with addition of potassium sorbate (?) and sodium benzoate (?) at five different concentrations and of methyl *p*-hydroxybenzoate (?) at four different concentrations. Growth rate h^{-1} was determined twice a day for 5 days (growth period). For each salt concentration, values of growth rate with the same letter do not differ significantly (p>0.05).


Penicillium terrestres / Tm22(R)
Penicillium sp / Tm05(A)
Rhizopus stolonifer / Tm25(A)
Absidia sp / Tm16(R)
Monilia fruticola / Tm13(A)
Cephalosporium sp / Tm14(R)
Aspergillus glaucus / Tm30(A)
Aspergillus versicolor / Tm12(R)
Fusarium tricinctum / Tm17(R)
Fusarium sp / Tm20(A)
Growth rate h-1
Salt concentration (%)