IDENTIFICATION OF IMPORTANT SPOILAGE BACTERIA AND FUNGI FROM TWO TYPES OF PORTUGUESE SMOKED DRY SAUSAGES AFTER SHELF-LIFE PERIOD IN MODIFIED ATMOSPHERE PACKAGE

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Background

The microbiology of dry smoked sausages is variable and complex and the rate of spoilage of these meat products can reduce the shelf life and cause substantial financial losses to manufacturing companies. Several studies were conducted to identify the bacterial populations in fermented dry sausages, however, despite increasing interest for modified atmosphere package (MAP), the effect of MAP on the spoilage bacteria and fungi of these meat products is not yet clear. Besides identifying the bacterial and fungal contamination during the manufacture processing, it is further important to identify the spoilage micro-organisms that will remain or proliferate during the storage period in MAP, and which might decrease shelf life and compromise product safety.

Objectives

The purpose of this study was to determine important spoilage bacteria and fungi in two types of Portuguese dry smoked sausages (chouriços) packaged in modified atmosphere (45% CO_2 and 55% N_2), which do not yet show clear spoilage changes at the end of the producer-defined shelf life period (120 days at 20±5ºC).

Materials and methods

Samples studied for this project were "chouriços" type Alentejano (A) and Ribatejano (R) produced by a large factory in Portugal as outlined by Matos et al. (2003). Identification and characterisation of bacterial species were based on phenotypic [cell morphology and fermentation profile according Jensen and Jorgensen (1994) and Jensen et al. (1995)] and genotypic (16S rDNA sequencing) characters according Knarreborg (2002). Identification of fungi was performed according conventional mycological methods based on morphological and physiological characterisation using taxonomic tables. The near-full-length sequences of the isolates have been deposited in GenBank under accession numbers AY587776 - AY587843. The phylogenetic relationship of the different bacterial species was determined by comparative sequence analysis of their 16S rDNA genes.

Results and discussion

A total of 30 fungi isolates were obtained from Alentejano (12) and from Ribatejano (18) types of smoked dry sausage, and the distribution of recovery from the CRB media is given together with the viable counts in Table 1. In Fig. 1 are shown microscopic photographs of five representative mould isolates. Fig. 2. shows the 16S rDNA-based tree reflecting the relationship of bacterial species. A total of 77 bacterial isolates were obtained from Alentejano (35) and from Ribatejano (42) types of sausage. In both types of sausages, and among the mould strains identified, *Penicillium*, *Fusarium* and *Aspergillus* species constituted the predominant mycoflora, with 50%, 16.6% and 16.6% for Alentejano variety and with 61.1%, 11.1% and 16.6% for Ribatejano sausage, respectively. Although contributions from other bacteria than the ones isolated can not be excluded, *Enterococcus faecium* (24.7%), *Bacillus subtilis* (23.4%), *Staphylococcus epidermidis* (14.3%), *B. cereus* (7.8%), *Pediococcus acidilactici* (6.5%) *Bacillus pumilus* (6.5%), *Clostridium bifermentans* (3.9%), *Bacillus licheniformis*, and *Enterococcus faecalis* (2.6%) were found in varieties A and R of Portuguese chouriço after 120 days at 20±5ºC in MAP. Major differences observed between the two varieties of chouriço were the absence of *Pediococcus* species and the presence of high numbers of isolates identified as *Staphylococcus epidermidis* in product A and, the high incidence of *Bacillus* species in sausage type R. Considering that MAP applied to both types of product was constituted

by the same percentage of gases and, the storage conditions, maturation period and smoke treatment were the same, differences found between products may be due to spices formulation in each type of product, the type of natural casings used and, the hygienic quality of the raw meat.

Conclusions

The presence of aerobic *Bacillus* related species and fungi in these MAP chouriços, where the air in the package was replaced by a specific mixture of carbon dioxide and nitrogen (45% CO_2 and 55% N_2), can be explained considering that oxygen is not always completely removed and may also permeate through the packaging material (Vermieren et al., 1999). In fact, the level of residual oxygen in MAP packs may be due to factors such as the oxygen permeability of the packaging material, the ability of the food to trap air, poor heat sealing ability of the packaging material causing air to leak in ineffective evacuation and/or ineffective gas flushing (Smith et al., 1986), the storage period and temperature (Smiddy et al., 2002). Further studies must be carried out to confirm the results obtained in this work in order to establish the spoilage population which might decrease shelf life and compromise product safety.

References

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Table 1.
Identified fungi, distribution of recovery from CRB media and viable counts obtained for each type of Portuguese dry smoked

Identified fungi[a]	Log CFU g^{-1}	
	Alentejano chouriço 1.77 ± 0.52[b]	Ribatejano chouriço 1.61 ± 0.30[b]
	Number of isolates	
Penicillium terrestres: [Tm15(A), Tm18(A), Tm23(A), Tm01(R), Tm02(R), Tm03(R), Tm06(R), Tm08(R), Tm09(R), Tm21(R), Tm22(R), Tm26(R), Tm27(R)]	3	10
Penicillium sp : [Tm04(A), Tm05(A), Tm19(A), Tm28(R)]	3	1
Fusarium sp: [Tm07(A), Tm20(A), Tm24(R)]	2	1
Fusarium tricinctum: [Tm17(R)]	0	1
Aspergillus glaucus: [Tm30(A), Tm29(A), Tm10(R)]	2	1
Aspergillus versicolor: [Tm11(R), Tm12(R)]	0	2
Rhizopus stolonifer: [Tm25(A)]	1	0
Monilia fruticola: [Tm13(A)]	1	0
Absidia sp: [Tm16(R)]	0	1
Cephalosporium sp [Tm14(R)]	0	1
Total	12	18

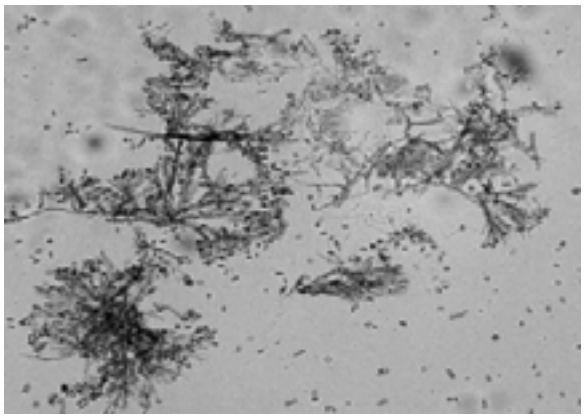
[a] Mould isolates obtained from Alentejano (A) and Ribatejano (R) sausages.
[b] CRB media (Oxoid; CM549), mean value ± standard deviation (n=6).

LEGENDS:

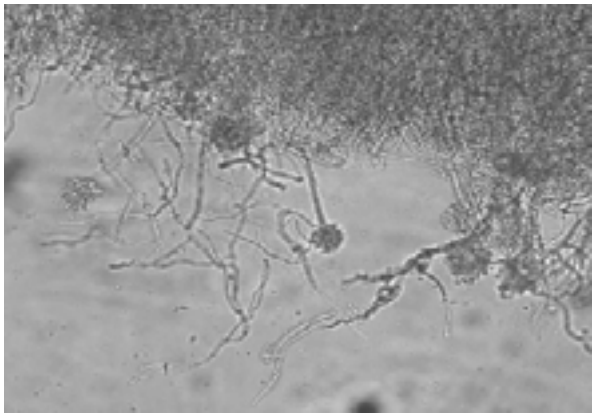
Figure 1. Microscopic photographs of five representative mould isolates: a) *Monilia frutícola* [Tm13(A)]; b) *Aspergillus glaucus* [Tm10(R)]; c) *Fusarium tricinctum* [Tm17(R)]; d) *Fusarium sp* [Tm07(A)]; e) *Penicillium sp* [Tm28(R)].

Figure 2. 16S rDNA-based tree reflecting the relationship of species. The length bar indicates 10% estimated sequence divergence.

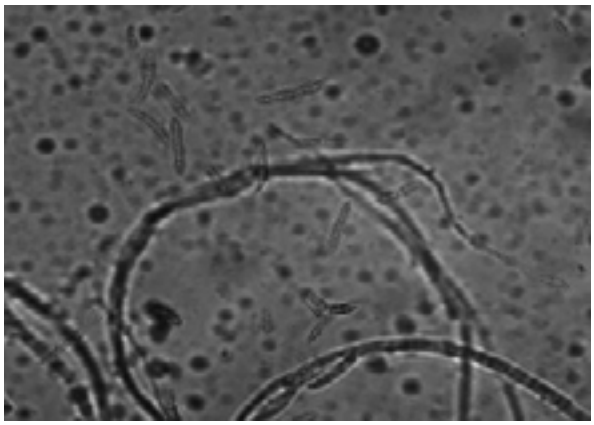
a)



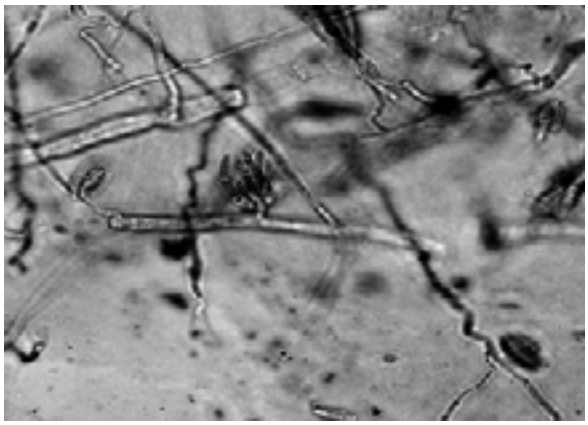
b)



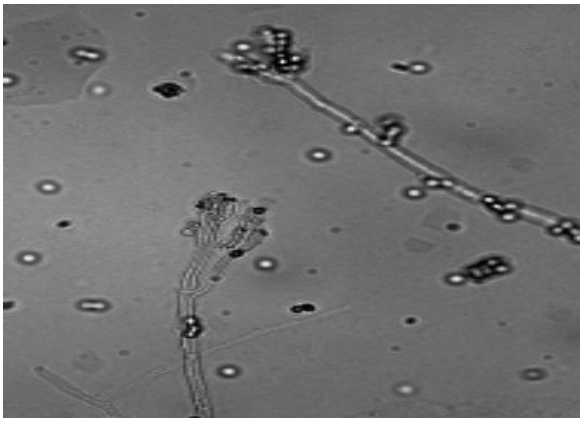
c)

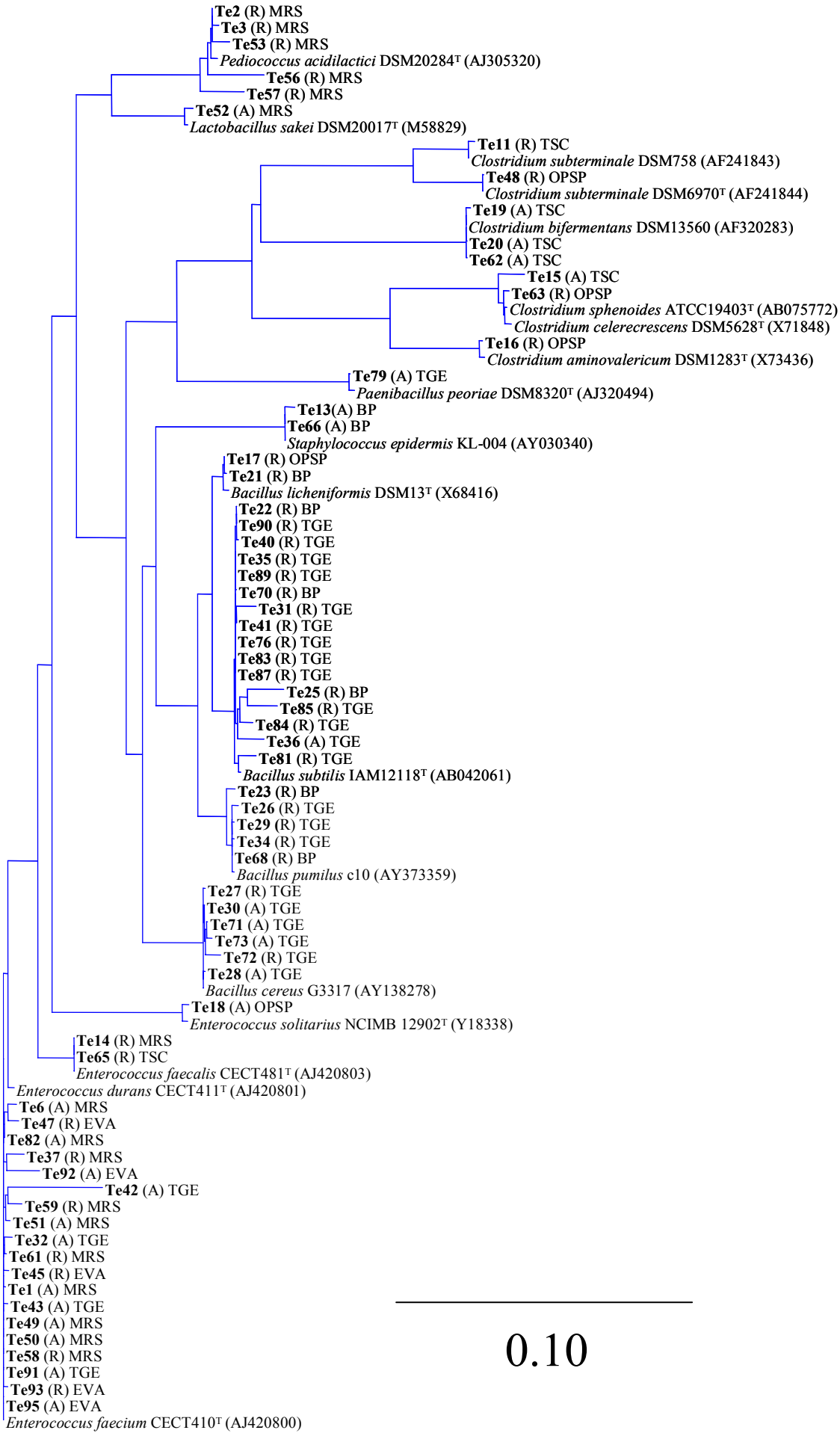


d)



e)




Te2 (R) MRS
Te3 (R) MRS
Te53 (R) MRS
Pediococcus acidilactici DSM20284T (AJ305320)
Te56 (R) MRS
Te57 (R) MRS
Te52 (A) MRS
Lactobacillus sakei DSM20017T (M58829)
Te11 (R) TSC
Clostridium subterminale DSM758 (AF241843)
Te48 (R) OPSP
Clostridium subterminale DSM6970T (AF241844)
Te19 (A) TSC
Clostridium bifermentans DSM13560 (AF320283)
Te20 (A) TSC
Te62 (A) TSC
Te15 (A) TSC
Te63 (R) OPSP
Clostridium sphenoides ATCC19403T (AB075772)
Clostridium celerecrescens DSM5628T (X71848)
Te16 (R) OPSP
Clostridium aminovalericum DSM1283T (X73436)
Te79 (A) TGE
Paenibacillus peoriae DSM8320T (AJ320494)
Te13(A) BP
Te66 (A) BP
Staphylococcus epidermis KL-004 (AY030340)
Te17 (R) OPSP
Te21 (R) BP
Bacillus licheniformis DSM13T (X68416)
Te22 (R) BP
Te90 (R) TGE
Te40 (R) TGE
Te35 (R) TGE
Te89 (R) TGE
Te70 (R) BP
Te31 (R) TGE
Te41 (R) TGE
Te76 (R) TGE
Te83 (R) TGE
Te87 (R) TGE
Te25 (R) BP
Te85 (R) TGE
Te84 (R) TGE
Te36 (A) TGE
Te81 (R) TGE
Bacillus subtilis IAM12118T (AB042061)
Te23 (R) BP
Te26 (R) TGE
Te29 (R) TGE
Te34 (R) TGE
Te68 (R) BP
Bacillus pumilus c10 (AY373359)
Te27 (R) TGE
Te30 (A) TGE
Te71 (A) TGE
Te73 (A) TGE
Te72 (R) TGE
Te28 (A) TGE
Bacillus cereus G3317 (AY138278)
Te18 (A) OPSP
Enterococcus solitarius NCIMB 12902T (Y18338)
Te14 (R) MRS
Te65 (R) TSC
Enterococcus faecalis CECT481T (AJ420803)
Enterococcus durans CECT411T (AJ420801)
Te6 (A) MRS
Te47 (R) EVA
Te82 (A) MRS
Te37 (R) MRS
Te92 (A) EVA
Te42 (A) TGE
Te59 (R) MRS
Te51 (A) MRS
Te32 (A) TGE
Te61 (R) MRS
Te45 (R) EVA
Te1 (A) MRS
Te43 (A) TGE
Te49 (A) MRS
Te50 (A) MRS
Te58 (R) MRS
Te91 (A) TGE
Te93 (R) EVA
Te95 (A) EVA
Enterococcus faecium CECT410T (AJ420800)
0.10