

Abstract Preview - Step 3/4

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Topic: C) State of the Art techniques for the analysis of biodiversity and microbial interactions of foodborne microbes

Title: YEAST DIVERSITY IN TRADITIONAL PORTUGUESE SOFT CHEESE BY CULTURE DEPENDENT AND INDEPENDENT DNA APPROACHES

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Text: Serpa is an artisanal ripened Portuguese cheese granted the Protected Designation of Origin (PDO) label. It is produced from raw ewe's milk, using flowers of *Cynara cardunculus* L. as rennet and without the addition of a starter culture, therefore, the complex microbial community present during the ripening strongly contribute to the final quality and safety of the cheese. Most of the microbiota present in raw milk cheese are lactic acid bacteria and their importance is well known. Additionally, yeasts are considered member of the secondary microbiota, where they play an important role during the ripening. Thus, considering their importance, yeast species must be identified to determine their influence on the maturation and deterioration of cheese. In this context, the aim of the present study was evaluated the yeast community present in traditional Portuguese Serpa cheese by different DNA approaches.

Cheese samples were taken at the end of the ripening from five different dairy industries. Three industries belonged to PDO "Queijo Serpa", while the other two were non-PDO registered. The yeast community was investigated by culture dependent techniques using PCR-RFLP analysis of ITS region and sequencing of the 26S rRNA and culture independent approach by HTS of the ITS rRNA.

The results obtained showed that yeast counts ranged between 4.2-5.66 log CFU/g. The species identified by culture dependent methods, mainly corresponded to *Debaryomyces hansenii* and *Kluyveromyces marxianus*, with *Candida* spp. and *Pichia* spp. present to a lesser extent. The culture-independent results confirmed the prevalence of *Debaryomyces* spp. and *Kluyveromyces* spp. but, also, that *Galactomyces* spp. was relevant for three of the five producers, which indicates its importance during the early stages of the cheese ripening process, considering it was not found among the dominant viable yeast species. In addition, the differences between yeast species from PDO and non-PDO registered industries, showed that the lack of regulation of the cheese-making practices may influence the final yeast microbiota. In conclusion, the prevalent of yeast isolates from *D. hansenii* and *Kluyveromyces* spp., indicate that may have an important role during cheese ripening and in the final sensorial characteristics. Thus, the study of their technological and functional properties could be relevant, in the development of an autochthonous starter culture, to ensure final quality and safety of the cheese.

Keywords: Cheese, PCR-RFLP, High-Throughput Sequencing, Yeast community

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