

## 476. Developing DNA-based methods to detect the presence of adulterant milk in Serra da Estrela Cheese

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The Serra da Estrela (SE) cheese is a dairy product with Protected Designation of Origin (PDO), made of raw autochthonous sheep milk (from SE and/or Churra Mondegueira breeds), locally harvested flowers of *Cynara cardunculus* L., and salt. The traditional manufacturing practices and local sourcing of the ingredients are not only compulsory, according to its PDO, but also determinant to the distinct organoleptic profile of the final product. Fraudulent production may, however, include milks from cheaper sources. Food fraud in dairy products is commonly detected with DNA-based methods, like PCR, Real-time PCR, multiplex PCR and PCR-RFLP[1]. This project' goal is to explore analytical methods, including the previously mentioned examples, and develop methodologies that are capable of detecting adulterant milk, specifically in SE cheese.

After analysing the electrophoretic pattern RAPD-PCR with different combinations of arbitrary primers, it was possible to distinguish milk samples from SE and Mocha breeds. Furtherly, primers were design to target the distinctive fragment in the RAPD pattern, resulting in a more easily interpreted method: Sequence Characterized Amplified Region PCR (SCAR-PCR)[2].

The control region of the mitochondrial DNA (mtDNA) is a good target since it is more stable than genomic DNA. PCR amplification of the control region of the mtDNA (D-loop) can detect cows' milk in a mixture with milk from SE sheep. An undergoing bioinformatic analysis of the D-loop region, that started with a populations genetics approach to increase our understanding on the relatedness between the mtDNA of different sheep breeds, is now being focused in theorizing new analytical approaches.

By tackling the problem from different angles, we can now detect milks from Mocha sheep and cow, in mixtures with SE milk. Moreover, we are currently focused in using sequencing data to catalyse the further development of molecular techniques with higher sensitivity and accuracy for fraud detection in SE cheese.

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### **References**

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[2]Cunha et al. (2016) *RAPD and SCAR markers as potential tools for detection of milk origin in dairy products: Adulterant sheep breeds in Serra da Estrela cheese production. Food Chemistry* 211: 631-636.