Microstructural and microbiological relationships in matured Serra da Estrela cheese

Freni K. Tavaria, Patrícia M. Reis and F. Xavier Malcata

Escola Superior de Biotecnologia, Universidade Católica Portuguesa,

Rua Dr. António Bernardino de Almeida, P-4200-072 Porto, Portugal

www.esb.ucp.pt

Introduction

Owing to unique organoleptic characteristics, Serra da Estrela cheese is considered the most popular traditional cheese in Portugal. In the last years, an enormous amount of information has been gathered aimed at the full characterization of this product. This type of studies are essential toward future attempts to design and use well-defined non-starter cultures during manufacture, so as to satisfy legal measurements. Scanning electron microscopy (SEM) was used successfully by many investigators to unfold the development of cheese structure. In this study we report the use of SEM to examine the differences in the microstructure between cheeses made with or without refrigerated milk. These results, combined with microbiological data, provide a comprehensive view of the distribution of micro-organisms in the ripened Serra da Estrela cheese.

Methodology

Microbiological analyses In order to quantify the various microbial groups in our cheese samples, 10 g of cheese were homogeneized for 2 min with 90 mL of a sterile 2% (w/v) sodium citrate solution. One-milliliter samples were decimally diluted in sterile 0.1% peptone water, and plated on a range of selective media (Tavaria and Malcata, 2000).

Scanning electron microscopy SEM was performed on cheeses from several dairy farms. Parallelepipeds, (1 x 1 x 2 cm pieces) were cut from the interior of the cheese and fixed in formal saline, as described by Dean *et al.* (1959) for at least one month. The samples were subsequently dehydrated in a graded ethanol series, and directly examined in a JSM-5600LV (JEOL, Tokyo, Japan) scanning electron microscope operated at 15 kV.

Results and Discussion

As shown in Fig 1, the numbers of LAB (lactic acid bacteria) in 60 d-old cheeses manufactured from non-refrigerated and refrigerated milks were similar, and both relatively high (7.2-8.8 log units/g cheese). Significant differences could be seen in the numbers of staphylococci, yeasts and *Enterobacteriaceae,* which were much lower for cheeses manufactured from refrigerated milk at this time (60 d); however, by 180 d these differences were not significant.

In Fig 2 the SEM micrographs of Serra da Estrela cheese show a rather homogeneous protein matrix, with void open spaces (believed to entrap fat globules before sample preparation for observation), as well as populations of elongated yeasts and cocci unevenly distributed throughout the cheese mass in well-defined colonies.

Refrigeration resulted in cheeses with less void spaces, thus reflecting a more proteinacous matrix, probably a consequence of a higher lipolytic activity from the proliferating psychrotrophic microflora at 180 d; by 90 d of ripening, cheeses manufactured from refrigerated milk show a rather "desorganized", less compact matrix with larger void spaces. This may be due to the fact that refrigeration affects the colloidal phase of the milk by partially solubilizing the β-casein, which results in a less compact coagulum.

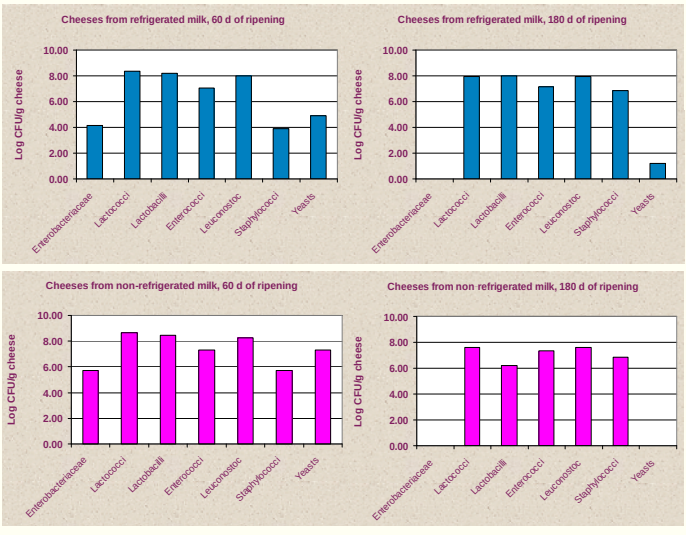

Figure 1. Microbial viable numbers in cheeses manufactured with refrigerated and non-refrigerated milks, after 60 d and 180 d of ripening.

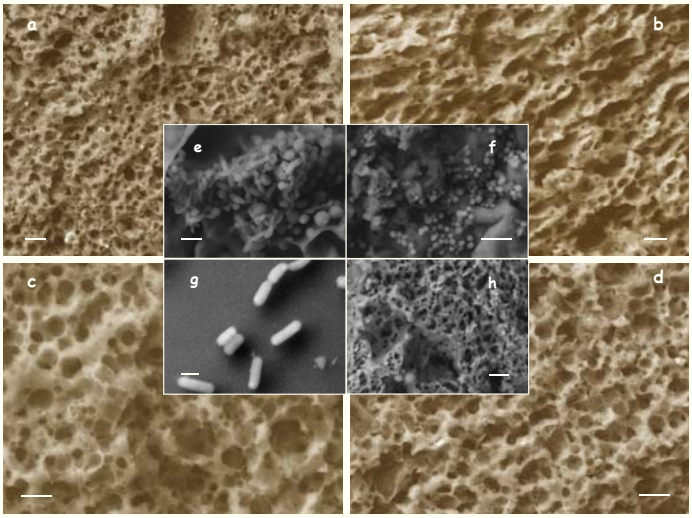

Figure 2. Scanning electron micrographs of Serra da Estrela cheese made with non-refrigerated (a,c) and refrigerated (b,d) milk, matured during 90 d (a,b) and 180 d (c,d) respectively. Micrographs (e) and (h) show large spherical and elongated cells (yeasts), within a colony (e) and embebbed in the cheese matrix (g). Micrograph (f) shows cocci-shaped bacteria dispersed in the casein matrix while in (h) bacilli from cheese are observed from a cell suspension. Scale-bar (a-d) = 10 μm; scale-bar (e and f) = 5 μm; scale-bar (g) = 1 μm; scale-bar (h) = 15 μm.

Conclusion

➔ The differences observed in the microbiological data between different dairies are significant, as would be expected due to the highly heterogeneous quality of the raw materials used in its manufacture, namely milk, coagulant and amount of salt used, as well as manufacturing practices themselves. These differences are also apparent in the SEM micrographs pertaining to cheeses manufactured in different dairies with the same maturation time (Reis and Malcata, 2001).

➔ The high microbial numbers of lactic acid bacteria shown by the traditional plate count methodology are confirmed by 'in situ' observations of sliced cheese, thus suggesting that the main microorganisms seen here belong to the LAB group owing to their high numbers and persistence; these microorganisms are likely responsible for the main metabolic events that take place during ripening.

References

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