Lactic microflora in Serpa raw ewes' milk cheese - a culture dependent approach

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INTRODUCTION

Serpa cheese exemplifies a ripened traditional Portuguese cheese made from raw ewes' milk, without addition of starters and using as rennet dried flowers of the plant *Cynara cardunculus*. It is known as a high quality cheese produced in Alentejo, in an established geographical area (Figure 1), with Protected Designation of Origin (PDO), as provided for in Regulation (EEC) 2081/92 of the European Commission. Due to its specificity Serpa cheese is unique among traditional Portuguese cheeses and is renowned and appreciated (1-3).

The absence of any standardizing thermal process and starter microorganisms means that its quality and characteristics depends mainly on the endogenous flora. Bacteria, yeasts, and molds are presented and it is recognised that lactic acid bacteria (LAB), contributes strongly to sensorial qualities and safety of this type of product (4). Nevertheless, the study of its microbial diversity and benefits that can be achieved from this knowledge is limited. Particularly LAB diversity may give various attributes to cheese such us technological aspects and probiotic strains that contribute to consumer health (5).

In order to contribute to the preservation of this Portuguese dairy heritage and also for the rational optimization of their production and quality, the aim of this study was to characterize the LAB communities at the end of ripening. This will be done through a combination of conventional cultivation and molecular techniques, in order to establish and characterize the most influential strains.

Figure 1 – Established geographical area of production (pink) of Serpa DOP chesse (Regulation Decree n.º 39/87).

MATERIAL AND METHODS

The sample consisted of twelve units of thirty days ripened DOP cheeses from three dairies (A, G, C) located in the geographical area of production. In each dairy were collected, two samples produced in spring and two in winter. Additionally, they were also studied four cheeses, obtained in two uncertified dairies (V, B), making a total of sixteen samples.

Total mesophilic bacteria*, Enterobacteriaceae, E. coli, Staphylococci,* lactic acid bacteria and yeasts were counted on PCA, VRBG, TBX, Baird Parker and RBC agar, respectively. Five colonies from highest dilution of MRS agar were identified at species level by sequencing of 16S rRNA. Sequences were compared with the EMBL and GenBank database using the BLAST algorithm. The identities at species level of the isolates were determined on the basis of highest score (4). Moreover pathogens detection of of *Salmonella* spp. and *Listeria monocytogenes* were carried out according to the International Organization for Standardization protocols (ISO 6579, 2002; ISO 11290-1, 2004).

RESULTS AND DISCUSSION

No pathogenic bacteria were found in any producer in both seasons and the flora was dominated by LAB (Figure 2). The counts revealed no significant differences between samples of the different producers or seasons. In MRS agar were around 8.5 log cfu/g. These counts were similar to that reported for the same type of cheese (8-10) and other kinds of cheese manufactured with raw ewes' milk (1, 4).

However, differences in LAB diversity were observed when the isolates were investigated for their identification. A total of 77 isolates of LAB were identified and predominant LAB in raw cheese at the end of ripening belong to 3 different genera, *Lactobacillus* (79%) , *Enterococcus* (12%) and *Leuconostoc* (9%), with six main species identified, *Lactobacillus paracasei* (24%) and *casei* (22%), *Lactobacillus plantarum* (14%), *Lactobacillus brevis* (11%), *Leuconostoc mesenteroides* (9%) and *Enterococcus faecium* (6,5%) (Figure 3). Other LAB identified present in minor proportion (1 to 4%) were *E. faecalis*, *E. hirae*, *L. pentosus*, *L. curvatus*, *L. crustorum*, *L coryniformi* and *L. brevis* (Figure 3). These results reflect the rich and diverse microbiota of Serpa cheese and normally featuring traditional cheeses (5).

The most prevailing species in cheeses from the three dairies (A, G, C) were *L. paracasei* and *L. casei*, representing between 40 and 60% of the species present. These two species and *Leu. mesenteroides* were presented in the three DOP industries (Figure 4). Among seasons, *Leu. mesenteroides* were not detected in winter samples (Figure 5). In addition, although similar species were identified in both seasons, the percentages of the LAB species range between seasons.

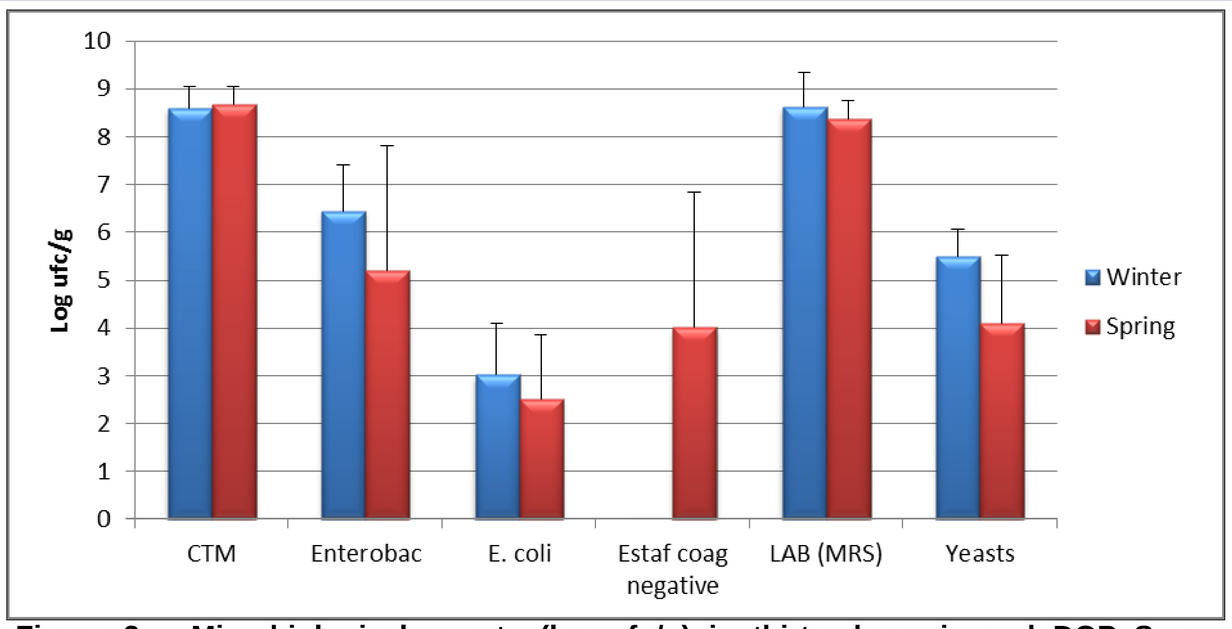

Figure 2 – Microbiological counts (log cfu/g) in thirty days ripened DOP Serpa cheeses from three dairies (A, G, C).

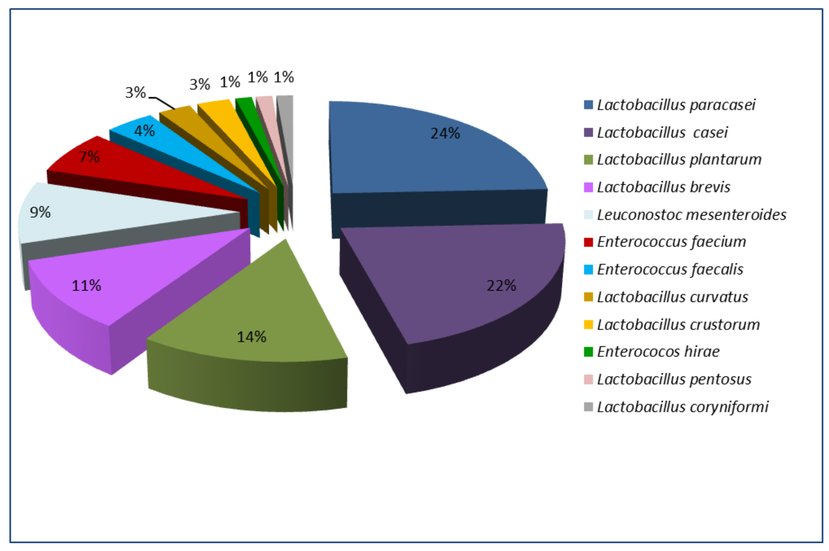

Figure 3 – Prevalence of LAB species in thirty days ripened DOP Serpa cheeses.

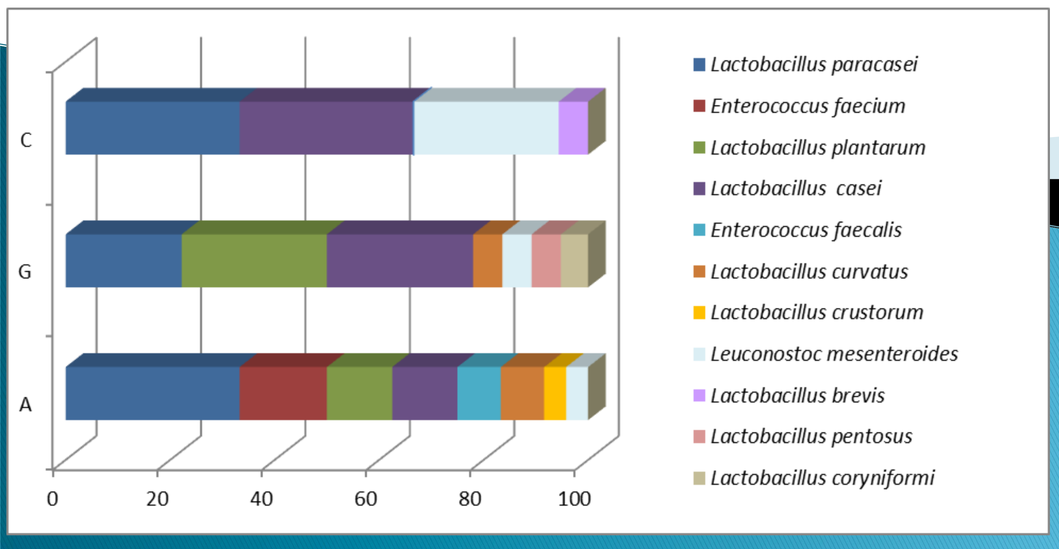

Figure 4 – Prevalence of LAB species in thirty days ripened DOP Serpa cheeses from three dairies (A, G, C).

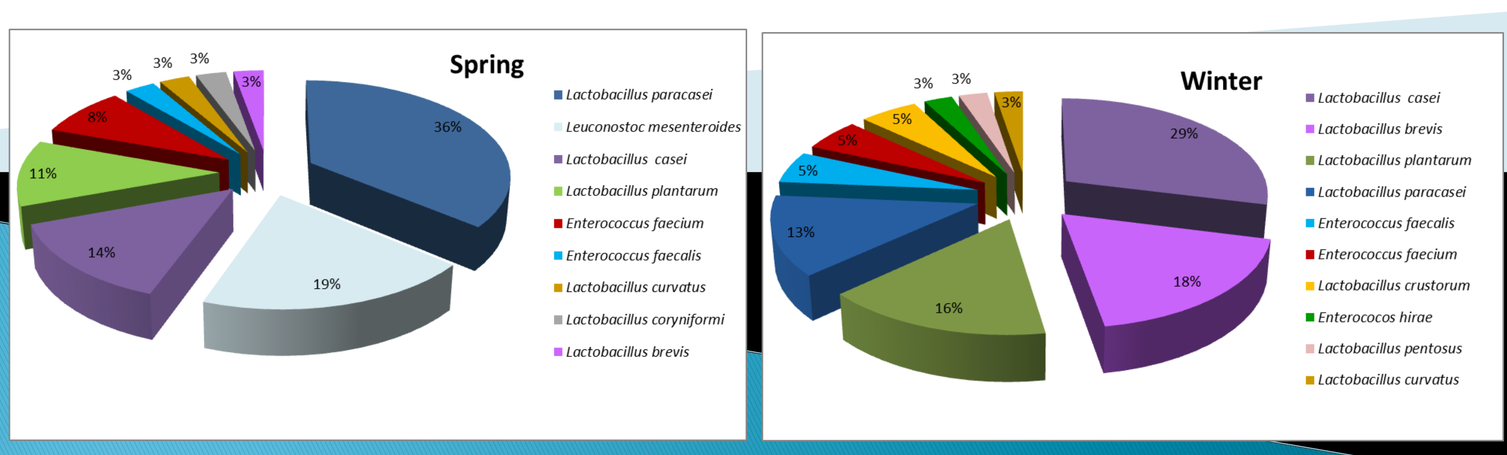

Figure 5 – Prevalence of LAB species in thirty days ripened DOP Serpa cheeses obtained from three dairies (A, G, C), produced in winter or spring.

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CONCLUSIONS

Serpa cheese, showed high prevalence and wide diversity of LAB, mainly *Lactobacillus* spp., *Enterococcus* spp. *and Leuconostoc* spp. In addition differences among the LAB species identified were found between producers and seasons.

This study of this traditional "Serpa" cheese microflora might contribute to the selection of autochthonous microbial strains for their used as probiotic or starter cultures with the aim to improve its safety and functional characteristics