# Acidifying and Aromatic Properties of *Enterococcus* Strains in Ovine and Bovine Milks

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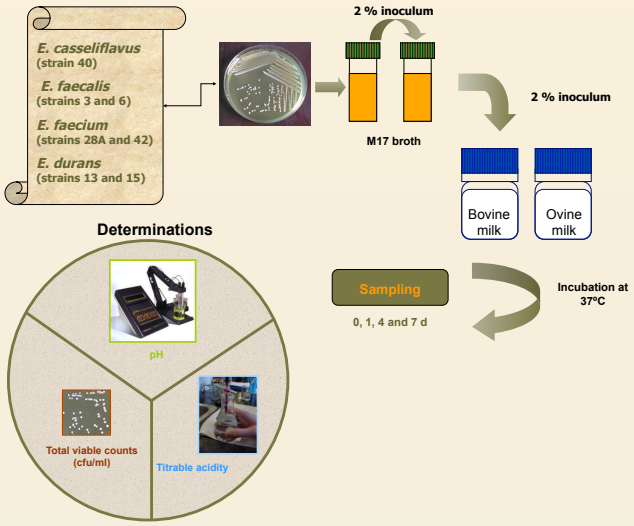
## INTRODUCTION

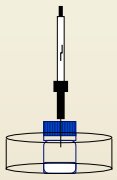
Enterococci are lactic acid bacteria (LAB), which can occur in a variety of cheeses, especially artisan cheeses produced in Southern Europe from raw or pasteurised milks. The positive effects of enterococci on cheese are related to specific biochemical traits, such as lipolytic activity, citrate utilisation, and production of aromatic volatile compounds (Giraffa, 2003).
The ability of starter LAB to produce acid contributes to the rapid reduction of pH, which enhances whey expulsion of curd and reduces moisture content. Furthermore, the additive effect of low pH and low moisture content in cheese reduces the risk of microbial contamination (Pérez *et al*., 2003). Additionally, the aromatic compounds obtained from metabolic activity of *Enterococcus* contributes to the final sensorial properties of cheese.
The aim of this work was the study the role of selected enterococci on ovine and bovine milks, in terms of acidifying properties and aroma potential.

## MATERIALS AND METHODS




**Volatile Compounds Analysis**

**Solid-Phase- Microextraction (SPME) - GC/MS**



- Fibre: Stable Flex Divinilbenzene/Carboxen/PDMS, 50/30 µm
- Sample: 20 g of milk weighted in a 100 ml-Shott flask, mixed with 100 µl of 3-octanol (internal standard - IS)
- Fibre was inserted in flask through septum provided in the cap, and exposed for 60 min in a 45ºC water bath, under stirring
- Fibre conditioned at 250ºC for 30 min in a GC injector, before and between analyse
- Helium used as carrier gas, at a rate of 1 ml/min
- For thermal desorption, fibre remained in injector for 10 min.
- GC-MS analysis was carried out using FFAP column (0.25mm x 0.39 mm)
- Peak identification was based on comparison of mass spectra of pure compounds
- Results were obtained via the ratio between area of peak and area of internal standard

## RESULTS AND DISCUSSION

The seven strains under study showed similar growth patterns, either in bovine or ovine milks, and reached levels of $10^8$-$10^9$ cfu/ml by 1 d of incubation (except *Enterococcus durans* 13, which exhibited a better growth in bovine than ovine milk) (see Fig. 1). An important decrease in pH was observed for all strains in both types of milk, by 1 d. The most important reduction was observed for *E. casseliflavus* 40 in bovine milk, and for *E. faecium* 42 in ovine milk, and for *E. durans* strains 13 and 15 in both milks (Fig. 2). A good correlation was obtained between pH and titrable acidity.

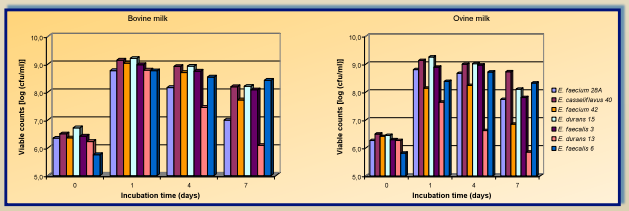



Figure 1: Total viable counts obtained in inoculated bovine and ovine milks throughout incubation at 37ºC

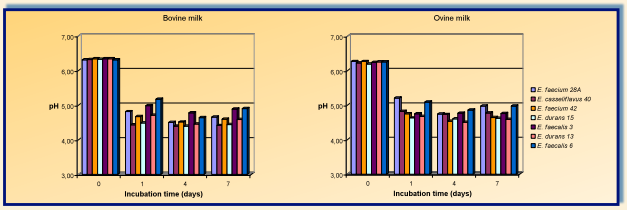



Figure 2: pH evolution in inoculated bovine and ovine milks throughout incubation period at 37ºC

In control milks with (without) inoculum one detected the presence of ketones and small amounts of fatty acids and absence volatile organic acids. The presence of acetic acid was observed after 1 d of incubation, as a result of glicolytic activity. An increase in fatty acid (caprylic, capric and lauric acids) concentrations resultant from microbial lipolysis was also reported. *Enterococcus faecium* 42, *E. durans* strains 13 and 15 showed the highest production of acetic acid either in bovine or ovine milks (Fig. 4a, b). These results validate the lowest pH obtained for those samples (seen Fig. 2). The quantity of fatty acids present in bovine milk samples is higher than in ovine milk (Fig. 4b, d)

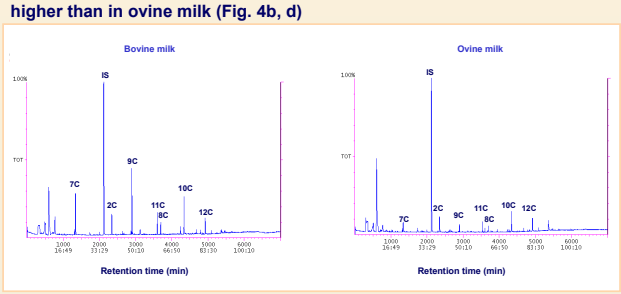



Figure 3: GC-MS chromatograms of volatile compounds and the internal standard (IS) 3-octanol obtained for *E. faecium* 28A in bovine and ovine milks using SPME technique. Peaks: 7C, 2-heptanone; 2C, acetic acid; 9C, 2-nonanone; 11C, 2-undecanone; 8C, caprylic acid; 10C, capric acid; 12C, lauric acid.

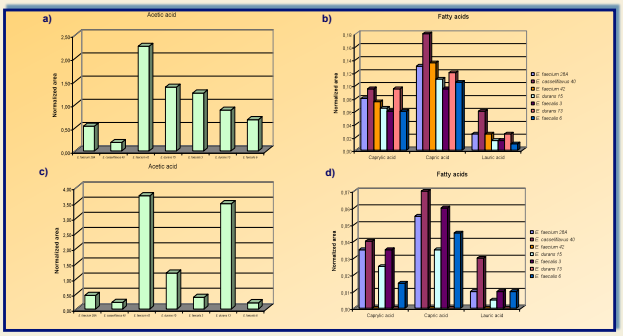



Figure 4: Normalized area of acetic acid (a,c) and fatty acids (b,d) for enterococci after 7 days of incubation at 37ºC in bovine (a,b) and ovine milks (c,d)

In general, all samples exhibited an important decrease in ketones throughout incubation in relation to the control (Fig. 5).

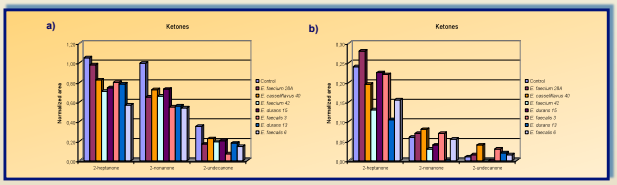



Figure 5: Normalized area of ketones for samples with enterococci incubated for at 37ºC in bovine (a) and ovine (b) milks. Control represents milk samples with no inoculum

***E. faecium* 42 and *E. durans* 13 and 15 showed to be good acidifier strains via production of high levels of acetic acid, hence leading to flavor implications. In general, all strains exhibited some extent of lypolitc activity.**

## ACKNOWLEDGEMENTS

Financial support for the first author was provided by a Ph.D. fellowship (SFRH/BD/7000/2001) granted by the Portuguese government through program PRAXIS XXI (Fundação para a Ciência e Tecnologia, Portugal). Financial support for experimental work was provided by project POCTI/1999/AGR/36165 *ENTEROCOCOS: deepening the knowledge on the role of enterococci, from manufacture through maturation of traditional cheeses*, funded by Fundação para a Ciência e Tecnologia, Program POCTI. Dr. Freni Tavaria is hereby gratefully aknowledged for help with SPME/GC-MS analysis.

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