Volatile organic compounds produced in milk by *Enterococcus faecalis*

G. Battelli*, T. Silvetti, M. Decimo, M. Brasca,
Institute of Sciences of Food Production, National Research Council (ISPA-CNR), via Celoria 2
*giovanna.battelli@ispa.cnr.it

Abstract
Enterococci can contribute positively to the development of flavour during cheese ripening as their presence is consistently reported in many raw milk cheeses. They can influence flavour taste and texture of cheeses as they produce several enzymes that interact with milk components, thus promoting important biochemical transformations. For this survey, *40 Enterococcus faecalis* strains, collected in different valleys in northwest Italy, were inoculated in milk and submitted to head-space solid-phase-micro- extraction gas chromatography-mass spectrometry analysis. The major volatile compounds detected were: ethanol, diacetyl, acetoin, acetic and benzoic acids. The variability was huge, demonstrating a very different enzymatic activity among strains. Apart from other important phenological parameters for characterizing strains that can be used as starter cultures, it seems useful to also give some information about their capability of enhancing flavour characteristics in the cheese.

Keywords: *Enterococcus faecalis*, Flavour, SPME, milk, enzymatic activity

Introduction

Enterococci are ubiquitous LAB which constitute a component of indigenous microflora in both the raw and pasteurized milk. As NSLAB, they are present in many types of cheeses (Giraffa, 2003). This work tries to provide more information on aroma compounds produced by *E. faecalis* present in NSLAB for improving cheese flavour.

Material and methods

Bacterial strains and growth conditions**.** Forty *Enterococcus faecalis* strains, from different northwest Italy regions belonging to the bacterial collection of ISPA-CNR, were used in this study. The strains, previously cultured overnight at 37°C in M17 broth, were inoculated in 5 ml of UHT whole milk in 20 mL sterile head-space glass vial sealed with PTFE/silicone, obtaining a final concentration of approximately 10^8 CFU/mL of milk. After incubation at 37°C for 48 h, the vials were immediately stored at -20°C. Prior to the analysis 3.5 g NaCl were added to the vials. Volatile compound analysis was performed by means of a Head-Space Solid Phase Micro Extraction module (Combi-Pal automated sampler CTC Analytics, Zwingen, Switzerland) coupled to a gas chromatograph- mass spectrometer (Agilent Technologies, Inc., Wilmington, DE, USA). Conditioning: 10 min at 50°C; stirring, 250 rpm; fiber, DVB/CAR/PDMS 50/30 µm (Supelco, Bellefonte, USA); exposition, 40 min at 50°C maintaining stirring; desorption at 260°C for 10 min in the injection port of the GC. Chromatographic conditions were previously described (Revello et al., 2010). VOCs analysis was also performed on control milk containing M17 broth.

Results and discussion

The principal VOCs (in terms of level) found in all milk samples were acetaldehyde, benzaldehyde**,** 2-heptanone, 2-nonanone, 2-undecanone, butyric, hexanoic, octanoic and decanoic acids plus other VOCs to a lesser extent (Fig. 1). In milk inoculated with the *E. faecalis* strains, ethanol, diacetyl, acetoin, acetic acid, and benzoic acid were also found. Consequently, only these volatiles were considered, assuming that they derive from the microbial metabolic activities in milk.

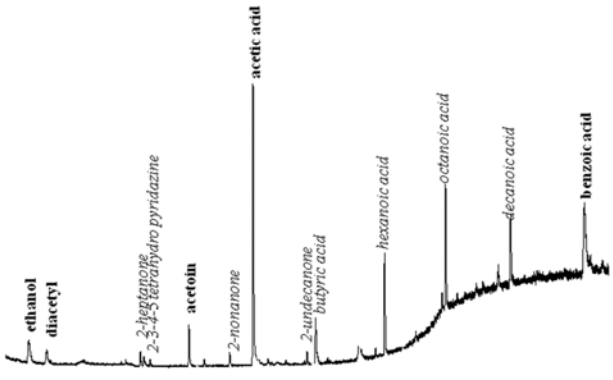

Fig. 1. Total ion chromatogram of the volatiles in the headspace of a milk inoculated with an E. faecalis strain (AA8) extracted by means of SPME. In bold, compounds present mainly in inoculated milk, in italic compounds present also in control milk.

Ethanol possesses limited influence on cheese aroma, but it contributes to the formation of esters (Vítová *et al*., 2006). Enterococci production of ethanol is related to citrate metabolism (Sarantinopoulos *et al*., 2001; Serio *et al*., 2010). In *E. faecalis*, ethanol is also produced through the partial reduction of acetyl phosphate needed to maintain the redox balance during the heterolactic fermentation of gluconate (Axelsson, 2004). Diacetyl and acetoin are important flavour compounds, responsible for the creamy, buttery aroma in many dairy products, being diacetyl perceivable at a very low concentration. Both compounds derive from the catabolism of citrate representing an important technological characteristic of

many LAB. Even though citrate metabolism has been exhaustively documented in strains of *Lactococcus*, *Lactobacillus*, and *Leuconostoc*, more limited and sporadic information concerning citrate metabolism in *Enterococcus* strains are available (Foulquié Moreno *et al*., 2006). For the *Enterococcus* genus, production of acetic acid was already observed, ascribing its origin to various sources (Freitas *et al*., 1999). It is well-documented that milk products can contain natural benzoic acid as some LAB are able to convert hippuric acid or tyrosine into benzoic acid (Garmiene *et al*., 2010). However, this work represents the first demonstration of benzoic acid production in milk by *E. faecalis*.

The data regarding the 5 principal compound detected in the inoculated milk, were standardised and inserted in a radar plot in order to compare the resulting bouquet of the strains from the different valleys (Fig. 2). Strains from Val d'Aosta and Cuneo Valleys produced less benzoic acid than the strains from Lombardy, moreover, they were capable to produce more diacetyl and acetoin.

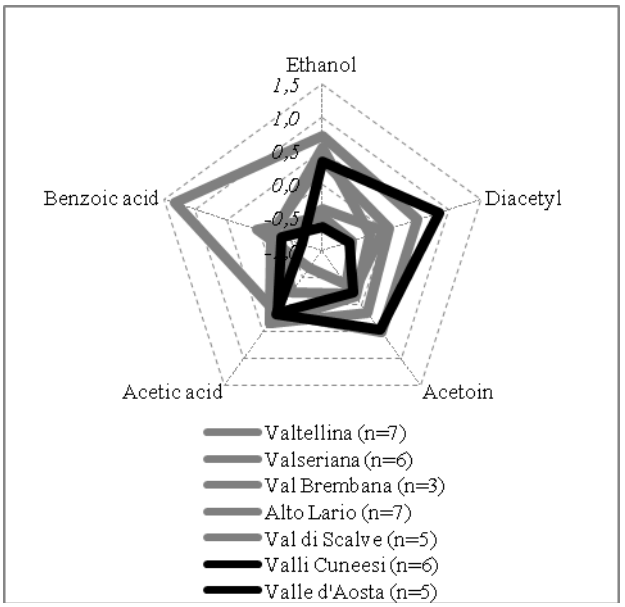

Figure 2. Radar plot of the mean values for the main VOCs extracted by means of SPME-GC-MS of milk inoculated with the E. faecalis strains collected in different mountains valleys. Numbers in brackets refer to the number of strains isolated from milk curd or cheese of the different valleys.

Conclusion

While aroma components formed by bacteria during cheese production have been extensively investigated, the general capability of microorganisms for the production of volatiles has not been thoroughly explored. The results of this study represent the first step in understanding the relevant role of Enterococci in flavour development in raw milk cheeses. Apart from other important phenological parameters for characterizing strains that can be used as starter cultures, it seems useful to also give some information about their capability of enhancing flavour characteristics in the cheese. To complete these data, a sensory evaluation would be necessary to investigate the real impact of VOCs produced by strains on the resulting aroma of dairy products.

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