

TWO CASE STUDIES OF ANTI-LISTERIA EFFECTS IN TRADITIONAL ALPINE CHEESES AND THEIR ENVIRONMENT: COMPOUNDS PRESENT IN CHEESE AND BIOFILMS PRESENT ON CHEESE RIPENING WOODEN SHELVES

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Introduction. The contamination incidence of certain cheeses by *Listeria monocytogenes* is lower than that foreseen by some predictive microbiology models based on physico-chemical parameters such as temperature, pH, a_w and salt content. This suggests that microorganisms present in cheeses and their production environment inhibit the growth of pathogenic bacteria by various mechanisms. In the present work, we focused on two case studies of anti-*Listeria* effects: its growth inhibition by (i) the microbial consortia present on wooden shelves used for the ripening of Reblochon cheese (a French, raw milk, pressed curd cheese) and (ii) by water-soluble extracts (WSEs) of Asiago cheese (an Italian, raw milk, semi-cooked pressed curd cheese) curd.

Methods. (i) Wooden shelves were sampled out at the end or at the beginning (i. e. after having been brushed with water and subsequently dried) of a cheese ripening cycle. Native or autoclaved (125°C, 15 min) wooden shelves were then inoculated with two *L. monocytogenes* strains. Their growth was monitored for 12 days at 15°C and 99% relative humidity. (ii) WSEs (prepared as in [1]) from Asiago cheeses ripened for 6, 12 or 18 months were ultrafiltrated onto 10 kDa cut-off membranes to remove proteins, dialysed with 100 Da cut-off membranes to remove salts and organic acids and freeze-dried. Lyophilisates were then resuspended in Brain Heart Infusion (BHI) broth, and *L. monocytogenes* growth monitored in BHI broth with or without lyophilisate.

Results and discussion. (i) Fig.1 revealed that the microbial consortia present on cheese ripening wooden shelves (mainly cheese surface microflora such as *Geotrichum candidum* [2]) inhibit *L. monocytogenes* growth. The strength of the inhibitory effect was dependent on the *Listeria* strain tested and the cleaning state of the shelves, but not on the farmhouse origin of the cheeses ripened on these shelves. pH (from 6.8 to 8) and a_w (>0.91) values were not the dominant parameters involved in the inhibition of *L. monocytogenes* that was observed. The contributions of nutrient competition and/or production of antimicrobial metabolites by the microorganisms present on wooden shelves were assessed.

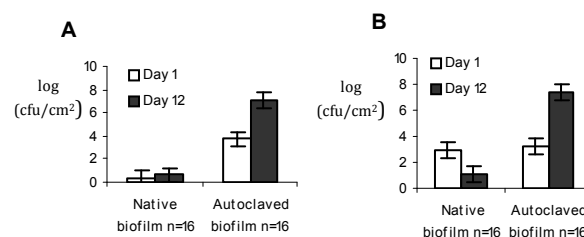


Fig.1 *L. monocytogenes* (strains 162 (serotype 1/2a) (A) and 481 serotype (1/2b) (B) from Aerial collection) population, 1 and 12 days (means for 4 farmhouse origins and 2 repetitions) after inoculating with 10^2 UFC.cm⁻² on native and autoclaved wooden shelves sampled out at the beginning of Reblochon cheese ripening cycles. No significant differences were found between the 4 farmhouse origins. Error bars indicate confidence intervals ($P < 0.05$).

(ii) WSEs of Asiago cheeses strongly inhibited *L. innocua* (LRGIA01 strain) growth in BHI broth at 30°C. WSEs of cheeses ripened for 12 months presented a higher inhibitory activity than those from cheeses ripened for 6 and 18 months. WSEs of Asiago cheese also inhibited, although to a lesser extent, the growth of *L. monocytogenes* (strain 102 from AERIAL collection). This inhibition might be due to antimicrobial peptides such as bacteriocins or fragments of caseins resulting from proteolysis accompanying cheese ripening.

Conclusions. Inhibition of *L. monocytogenes* growth by micro-organisms present on Reblochon cheese ripening wooden shelves (i) and Asiago cheese curd water soluble compounds (ii) were demonstrated. Further research concerning the anti-*Listeria* effect in several strains of *L. monocytogenes* is necessary to explore the complex and probably multi-parameter mechanisms of inhibition by the microorganisms present in cheese and their environment.

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