



## SPATIAL DISTRIBUTION OF THE BACTERIAL COMMUNITY IN COTIJA CHEESE ANALYZED BY FISH

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**Key words:** Fluorescence in situ hybridization; microbial community; artisanal cheese.

**Introduction.** Cotija cheese is produced in an area between Jalisco and Michoacán, Mexico. It is manufactured from raw milk, and ripened, by at least three months, by native microbiota (1). In our group, we have identified some genus and species that are present in the ripened cheese, as well as in different stages of its manufacture (2). This work describes the microbial distribution (analyzed by a culture independent method, FISH) that may result from a differential physicochemical environment inside it (3).

**Methods.** A three-month ripened cheese was divided in 5 zones from center to surface (Fig. 1). Physicochemical analysis included: pH, acidity,  $[Cl^-]$ ,  $[Ca^{2+}]$ , water activity ( $a_w$ ), redox potential (Eh), fatty acid composition and PAGE protein profiles. 16S rRNA was the target for FISH probes. In order to select specific sequences for bacterial genus that had been already found in Cotija cheese, public data bases (GenBank and RDP) were consulted. A universal probe was used to quantify the total bacterial population. Cell permeabilization (3) and probes specificity were tested with pure cultures before experimenting with the cells extracted from the different cheese zones.

**Results.** Higher salt,  $Ca^{2+}$  concentration and pH values were observed in the surface than in the central zone. While acidity and  $a_w$  had the opposite behavior. The Eh value was more negative in the center than in the surface, in any case it showed an environment with low oxygen content (Fig. 1).

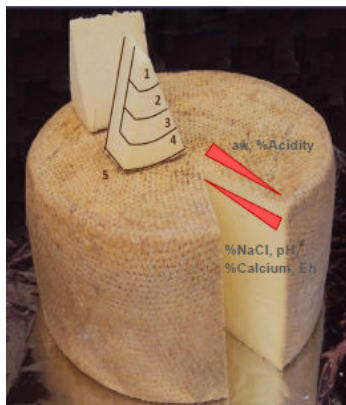


Fig.1. Sampling and gradients present in cheese.

As for enzymatic activity, proteolysis occurs homogeneously inside the cheese matrix; while there was a higher activity in the rind. Lipolysis was observed in all zones, with preference for the hydrolysis of C13:0 and C18:2 fatty acids. In accordance to the enzymatic activity results, a uniform distribution of the bacterial genus responsible for these activities (*Staphylococcus*, *Bacillus* and *Enterococcus*) was found, no dominance of any of them was observed (Fig. 2).

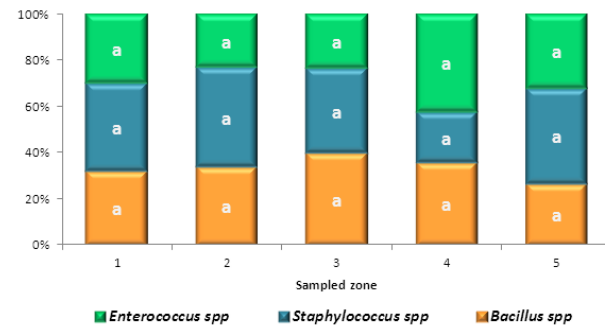


Fig.2. Proportion of bacterial genus in each zone. Same letter indicates no significant difference with  $\alpha = 0.05$ .

**Conclusions.** The physicochemical gradients observed could be caused to the gradual moisture loss during ripening, rather than to bacterial metabolic activities. In spite of the big size of the cheese, chemical gradients inside it did not show enough difference in order to select bacterial populations, which are reflected in similar enzymatic activities inside the product.

The three bacterial genera studied in this work represent only 11% of the total bacterial population with no dominance of any of them.

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