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## Exploring the influence of variable NaCl content on microbial viability, autolysis and cell distribution during maturation of semi-hard cheese

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*Publication date:*  
2014

*Document version*  
Early version, also known as pre-print

*Citation for published version (APA):*

Ryssel, M., Søndergaard, L., Arneborg, N., & Jespersen, L. (2014). *Exploring the influence of variable NaCl content on microbial viability, autolysis and cell distribution during maturation of semi-hard cheese*. Poster session presented at 11th International Symposium on Lactic Acid Bacteria, Bergen, Netherlands.

# Exploring the influence of variable NaCl content on microbial viability, autolysis and cell distribution during maturation of semi-hard cheese

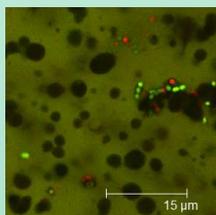
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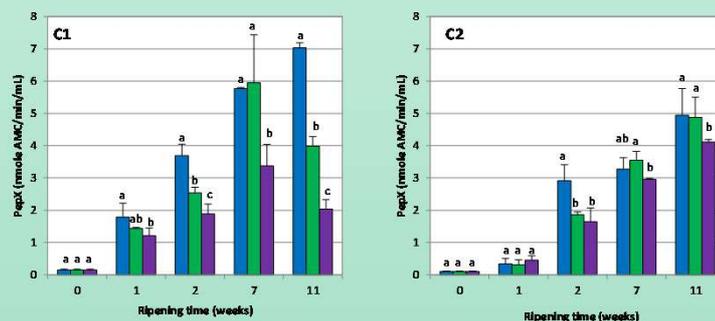
## Abstract

Salt is essential for human health but too much can be harmful leading to high blood pressure, heart disease and even strokes. Reducing NaCl in cheese is a major challenge for the dairy industry. NaCl influences the cheese structure, texture, flavour and the microbial stability, which is all of importance for the matured cheese.

The aim of this study was to investigate how variable NaCl content influenced the starter culture in a semi-hard cheese. Cheeses were made with 0.0, 1.3 and 1.8 % (w/w) NaCl using two different starter cultures (C1 and C2). The cheeses were analysed during production and at 1, 2, 7 and 11 or 12 weeks of maturation. They were analysed with respect to viable count, level of cell autolysis and cell distribution using confocal laser scanning microscopy (CLSM) combined with LIVE/DEAD staining.



**Figure 1.** Example of a CLSM picture from the internal part of the cheese with C1 after 2 weeks of ripening with 1.3% NaCl. Cells are stained with LIVE/DEAD staining. Green cells have intact cell membranes (alive) and red cells have permeabilized membranes (dead).



**Figure 3.** Activity of X-prolyl dipeptidyl aminopeptidase (PepX) (nmole/min/mL cheese extract) in semi-hard cheese during 11 weeks ripening. Cheeses were produced with target NaCl levels of 0.0 % w/w (■), 1.3 % w/w (■), and 1.8 % w/w (■) and with two starter cultures (C1 and C2). Week 0 is samples taken before salting.

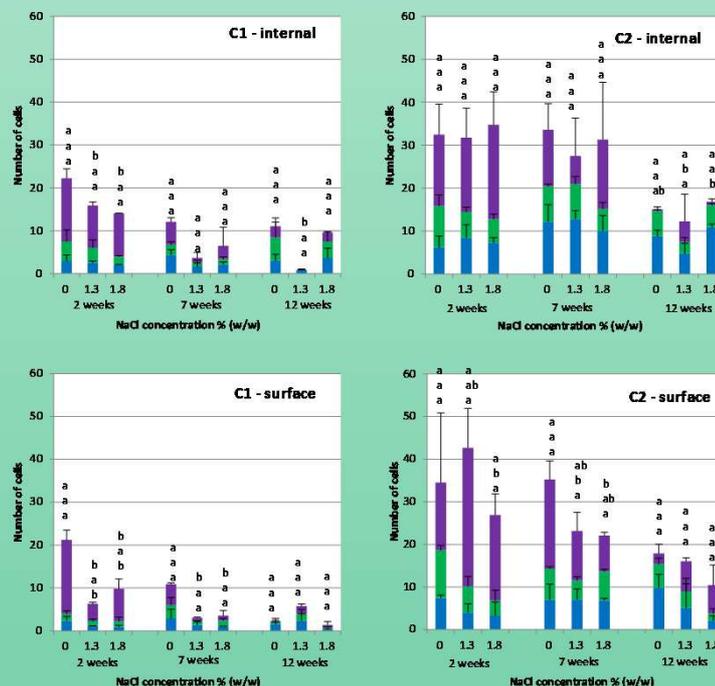
## Results

The NaCl content in the cheese did affect the microbial development of the starter culture. The impact on the microbial development was larger in C1 compared to C2.

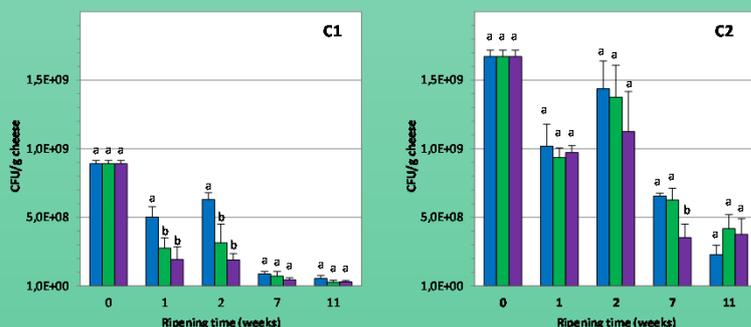
An example of a CLSM picture from a 2 week old cheese made with C1 are shown in figure 1.

The first two weeks of maturation, the cell number of C1 declines faster in the samples with NaCl than in the sample with 0% NaCl (fig. 2). However, after 7 weeks there were no differences between the samples. For C1, autolysis of the cells was significantly affected by the NaCl content. A decrease in NaCl content lead to an increase in autolysis. This can be seen in figure 3. The same tendency was observed by CLSM where cell dead was more pronounced with 0% NaCl (data not shown). Also the tendency of the cell counts (fig. 2) could be confirmed by CLSM where fewer live cells could be observed in the samples with NaCl (fig. 4).

For the starter culture C2 the cell number was not affected by NaCl in the same degree as for C1 (fig. 2). During ripening there was a tendency to observe more autolysis in the sample with 0% NaCl, but not to the same extent as for C1 (fig. 3). For both starter cultures the number of cells in micro colonies is declining faster than the number of single cells and cells in groups of 2-3 cells (fig. 4).



**Figure 4.** Cell number and distribution of live cells on internal and surface semi-hard cheese samples during 12 weeks ripening, measured with confocal laser scanning microscopy. Cheeses were produced with target NaCl levels of 0.0 % w/w, 1.3 % w/w, and 1.8 % w/w, and with two starter cultures (C1 and C2). Cells were divided into groups with single cells (■), 2-3 cells (■), and ≥ 4 cells (■).



**Figure 2.** Cell count (CFU/g) in semi-hard cheeses during 11 weeks ripening. Cheeses were produced with target NaCl levels of 0.0 % w/w (■), 1.3 % w/w (■), and 1.8 % w/w (■) and with two starter cultures (C1 and C2). Growth was analysed on LM 17. Day 0 is samples taken before salting.

## Conclusion

This study shows that starter cultures are affected in different ways by a decrease in NaCl. This knowledge is important when designing specific starter cultures for cheeses with reduced NaCl content.

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