

## THE STUDY OF ATTENUATED STARTERS IN HOLANDES CHEESE RIPENING

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### Abstract

Ripening is a slow, and consequently an expensive process. Acceleration of cheese ripening has received considerable attention in the scientific literature (Fox et al., 1996, El Soda et al., 2003, Wilkinson, 1993, Upadhyay and McSweeney, 2003). Certain approaches have been used to accelerate the ripening of cheese, including the use of an elevated ripening temperature, addition of attenuated starters, and use of adjunct cultures. The aim of the paper is to investigate the role of attenuated starter to formation of Holandes cheese sensory properties.

Holandes cheeses (Edam type cheese) from one manufacturer with/without attenuated starter culture were chosen for experiments. Cheese samples were ripened for 60 days at 6 °C and 12 °C. Cheeses were analyzed during ripening (60 days) for chemical (aroma compounds), physical ( $a_w$ , pH and elasticity) and microbiological (colony forming units of lactic acid bacteria) composition. Mesophilic lactic acid bacteria were enumerated on MRS agar at 37 °C 72 h. Volatiles were detected using solid phase GC/MS, the extraction of components was carried out using of 75  $\mu$ m CAR-PDMS fibre. pH,  $a_w$  and elasticity of cheese samples were measured using appropriate standards procedures.

Attenuated starter has impact on the sensory properties of Holandes cheese. The starter intensifies the flavour of cheese and accelerates proteolysis without necessarily reducing of ripening time. Modification of the ripening temperature influences the rate of flavour and structure development in Holandes cheeses. In spite of attenuated starter the slow development of flavour are observed in cheeses ripened at 6 °C.

Attenuated starter accelerates ripening, intensifies flavour and controls the growing of non starter lactic acid bacteria in Holandes cheese.

**Key words:** Holandes cheese, attenuated starter, aroma compounds, ripening conditions

### Introduction

Cheese ripening is a slow and expensive process. Acceleration of cheese ripening has received considerable attention in the scientific literature (Fox et al., 1996, El Soda et al., 2003, Wilkinson, 1993, Upadhyay and McSweeney, 2003). Various approaches have been used to accelerate the ripening of cheese, including the use of an elevated ripening temperature, addition of exogenous enzymes or attenuated starters, use of adjunct cultures, use of genetically modified starter bacteria and high-pressure treatments.

The simplest and the most successful approach to accelerate ripening studied to date is an elevated ripening temperature (Folkertsma et al., 1996). Modification of the ripening temperature is used to control the rate of flavour development in hard cheeses, and ripening at an elevated temperature results in the rapid development of flavour, although problems can occur with texture.

Finally, recent advances in the genetics of lactic acid bacteria (LAB) and a greater understanding of the role of specific enzymes in the generation of volatile flavour compounds in cheese during ripening will facilitate the development of starter genetically modified to enhance flavour development.

The aim of this paper is to investigate the role of attenuated starter to formation of Holandes cheese sensory properties.

### Materials and Methods

Unripened Holandes cheese (Edam type) samples with and without attenuated starter cultures were chosen from one cheese manufacturer in Latvia. Cheese has 45% of fat content in dry matter and not greater than 44% of moisture content according to the branch standard LPCS 11:2001 "Holandes siers".

Cheese samples were ripened for 60 days at 6 °C and 12 °C for evaluation of the influence of attenuated starter on cheese ripening during the different temperature regimes. The ripening

temperature at 6 °C was selected according to ordinary practice in Latvian cheesemaking and at 12 °C was intended to achieve the classical sensory properties of Holandes cheese.

Cheese samples were analyzed during ripening for chemical, physical and microbiological indices. Mesophilic LAB were enumerated on MRS agar at 37 °C 72 h.

Volatile compounds were detected using solid phase GS/MS (Clarus 500 GC/MS, PerkinElmer Inc.), the extraction of components was carried out using of 75 µm CAR/PDMS fibre.

pH was measured using 3520 pH Meter – JENWAY (Barloworld Scientific Ltd., Essex, UK). The compression force for measurement of cheeses elasticity was determined by TA.XT Plus Texture Analyser (Stable Microsystems Ltd., Surrey, UK). A spherical probe (P/1S – Ball Stainless) was used for compression of sample. Test speed, distance and trigger force were 2 mm s<sup>-1</sup>, 5 mm and 0.0493 N, respectively.

Water activity was determined by Meter AquaLab LITE (Decagon Inc, USA).

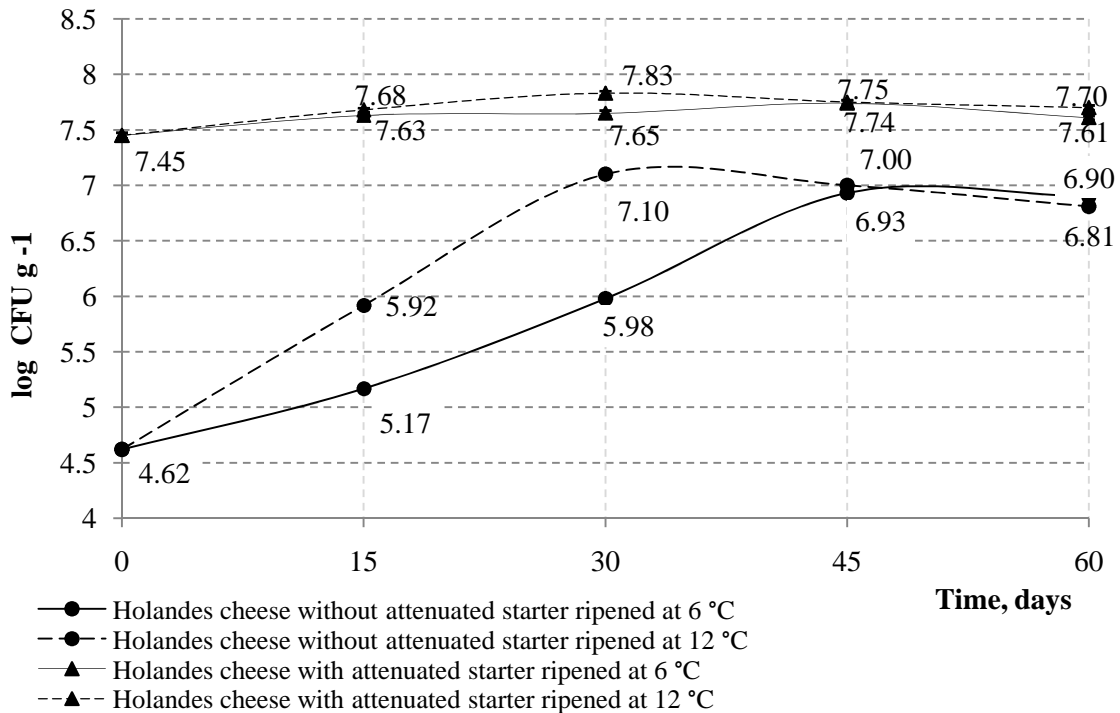
All parameters were determined in triplicate or more according to the method of procedure.

Analysis of variance (ANOVA) was performed in order to identify significant differences in pH and elasticity between cheese samples with and without attenuated starter.

**Results and Discussion**

The quality and intensity of cheese flavour cannot be predicted precisely during ripening. There is an economic incentive for the development of methods for the acceleration of cheese ripening, provided that the flavour and texture can be maintained and characteristic of the variety (Fox et al., 2000). Acceleration of cheese ripening can be achieved by various practices among them could be mentioned elevated ripening temperatures and usage of attenuated starter. The influence of above mentioned factors on ripening Holandes cheese have been analyzed.

Colony forming units of non starter lactic acid bacteria in analysed Holandes cheese samples were presented in Figure 1.



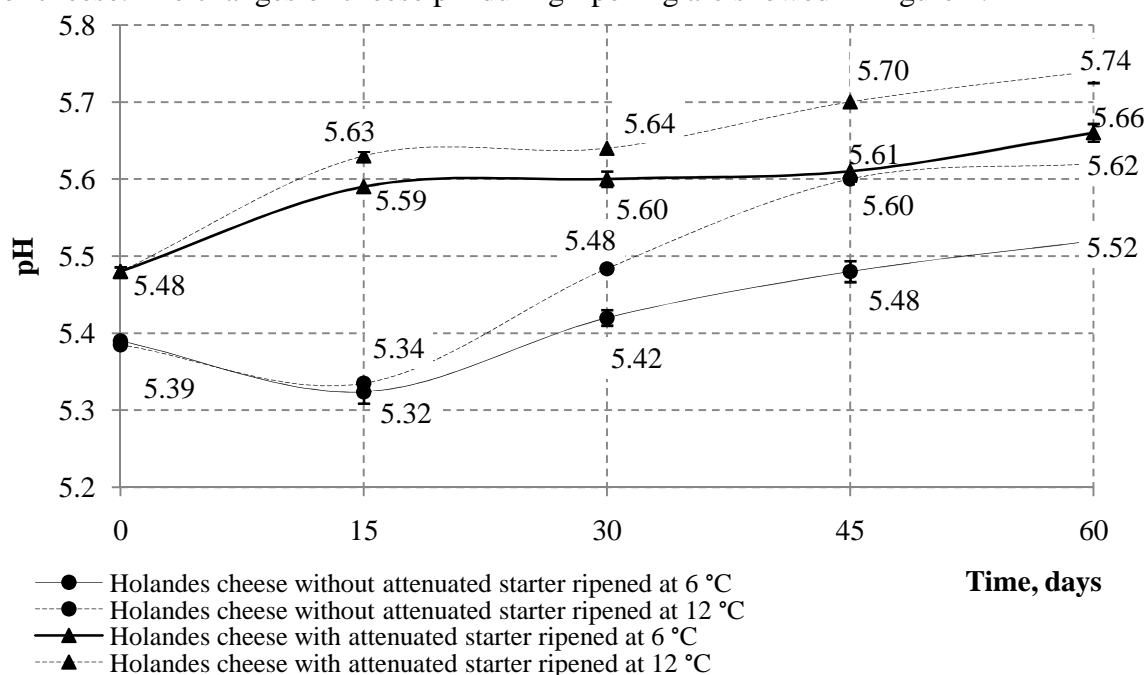
**Figure 1. The dynamic of colony forming units of non starter lactic acid bacteria in Holandes cheeses with/without attenuated starter during ripening**

The differences in non starter lactic acid bacteria colony forming units were observed in cheeses with and without attenuated starter from the beginning of ripening. The highest value of colony forming units was observed in Holandes cheese samples with attenuated starter ( $7.45 \log \text{CFU g}^{-1}$ ) comparing with Holandes cheese samples without attenuated starter ( $4.62 \log \text{CFU g}^{-1}$ ).

On 30 day of ripening the growth of colony forming units was  $7.83$  and  $7.10 \log \text{CFU g}^{-1}$  in comparison with initial numbers, reaching peak value for cheese ripening at  $12^\circ\text{C}$ . The obtained results showed that the lowest growing rate of non starter lactic acid bacteria was detected in Holandes cheese samples with attenuated starter during ripening. Cheese samples matured at  $6^\circ\text{C}$  the peak value of colony forming units reached after 45 days of ripening. Ripening at  $6^\circ\text{C}$  reduces lactic acid bacteria growth rate. However, successful ripening at elevated temperatures requires careful control of cheese composition and microflora.

During ripening enzymes such as residual chymosin, residual plasmin and enzymes from the starter bacteria and cheese microflora are still active and metabolise residual lactose, protein and fat till different compounds, also loss of water by evaporation is observed. Salt content, lactate concentration, amino acids, small peptides and calcium phosphate are important for changes of water activity from  $0.995$  at the beginning till  $0.971$  at the end of ripening in cheese. Water activity influences the proliferation of microorganisms in cheeses. There are find differences in the growing rate of microorganisms between samples with and without attenuated starter during ripening.

The growth rate and population density of non starter lactic acid bacteria are affected by pH of cheese. The changes of cheese pH during ripening are showed in Figure 2.



**Figure 2. The dynamics of pH in Holandes cheeses with and without attenuated starter during ripening**

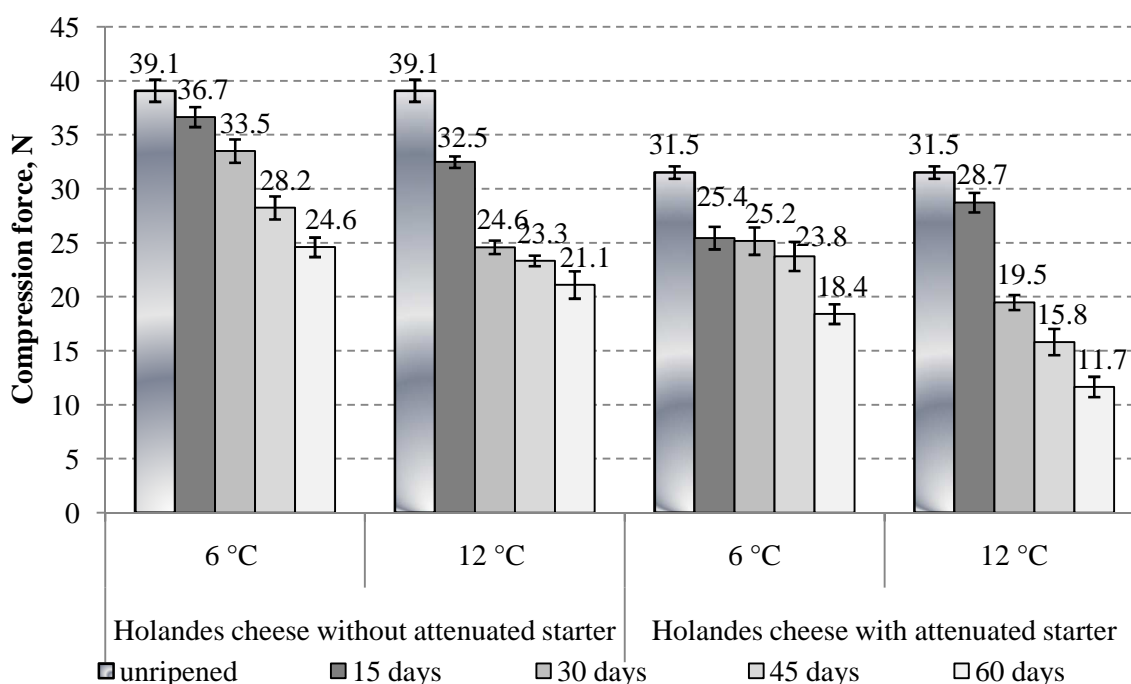
The ripening temperature influences the rate of proteolysis, lypolysis, cheese microflora, texture and aroma of cheese and as a result observes more rapid elevation of pH curves. Also higher pH was determined in Holandes cheese samples with attenuated starter in the beginning of ripening. It could be explained by origin of alkaline products of proteolysis due to proteolytic and peptidolytic activity of attenuated starter (Cogan et al., 2002). Also, attenuated starters can be defined as lactic acid bacteria which are unable to produce

significant levels of acid during cheesemaking, but which provide active starter enzymes that are important for cheese ripening and flavour development. There are observed some similarities in the dynamics of pH between cheese samples with and without attenuated starter. Due to higher initial pH of samples with attenuated starter the increasing of pH continues during ripening, reaching pH 5.74 at ripening temperature of 12 °C and pH 5.66 at ripening temperature of 6 °C. This differs significantly ( $p < 0.05$ ) from cheeses without attenuated starter, respectively, pH 5.62 at 12 °C and pH 5.52 at 6 °C.

The development of volatile compounds in Holandes cheese samples have been studied throughout ripening period. 24 compounds, each with a distinctive aroma character, have been identified in cheese, and these provide the largest contribution to diversity of cheese flavour. Compounds identified in cheeses include fatty acids (acetic, butanoic, hexanoic, octanoic, nonanoic, decanoic and dodecanoic acids), ketones (2-pentanone, 2-undecanone, diacetyl, acetoin, 2-octanone, 2-undecanone, 2-nonanone, 2-heptanone), alcohols (3-methyl butanol, 1-pentanol, 2-methyl propanol), aldehydes (nonanal, octadecanal, 3-methyl butanal, benzaldehyde), lactones ( $\delta$ -dodecalactone) and D-limonene.

The same volatile compounds were present in each type of cheese. Differences were observed in the levels of 3-methyl butanol, 1-pentanol, 2-pentanone, acetoin, 2-octanone, 2-nonanone, 2-undecanone, nonanal, 3-methyl butanal, benzaldehyde. In cheeses with attenuated starter have the higher levels of mentioned components than in cheeses without attenuated starter. The influence of cheese microflora on volatile compounds production was observed. Moreover, the levels of all compounds increased in cheese at a ripening temperature of 12 °C, compared with 6 °C.

The influence of ripening on Holandes cheese texture properties was showed in Figure 3.



**Figure 3.** The changes of texture in Holandes cheeses with and without attenuated starter during ripening

The changes of cheese elasticity were observed in cheese samples. Some authors (Bertola et al., 2000) were found that texture development was accelerated by increasing the ripening temperature. According to Lawrence (1987) data, the cheese texture changes

markedly during the first two weeks of ripening and exactly ripening temperature has significant impact on cheese texture during all ripening.

Holandes cheese with attenuated starter was softer ( $p < 0.05$ ) and the tendency was maintained during ripening at 6 and 12 °C. It indicated on higher proteolysis rate in cheeses with attenuated starter due to increase of water soluble nitrogen fraction that contribute to water binding and texture softening (Waagner, Nielsen, 2004). Besides softer texture, differences in the flavour profile of cheese can be observed analyzing the volatiles in cheeses. The flavour of the ripened cheese samples is richer and more complex when attenuated starter is used in cheese production. It could be explained with different volatile compounds, each with a distinctive aroma character, have been identified in cheese sample, and these provide the largest contribution to the diversity of cheese flavour.

### Conclusions

1. Attenuated starter accelerates ripening, intensifies flavour and controls non starter lactic acid bacteria in Holandes cheese.
2. Attenuated starter is unable to produce significant levels of lactic acid during cheesemaking.
3. The higher levels of 3-methyl butanol, 1-pentanol, 2-pentanone, acetoin, 2-octanone, 2-nonanone, 2-undecanone, nonanal, 3-methyl butanol and benzaldehyde are established in cheeses with attenuated starter than in cheeses without attenuated starter.
4. In spite of attenuated starter the slow development of flavour are observed in cheeses ripened at 6 °C.

### References

1. Bertola, N.C., Califano, A.N., Bevilacqua, A.E., Zaritzky, N.E. (2000). Effects of ripening conditions on the texture of Gouda cheese. *International Journal of Food Science and Technology*, 35(2), pp.207-214.
2. Cogan T.M., Beresford, T. (2002) Microbiology of hard cheese. In: *Dairy Microbiology Handbook*, 3<sup>rd</sup> ed., Robinson, R.K. (ed.), Wiley-Interscience, New York, pp.515–560.
3. El Soda, M., Ahmed, N., Omran, N., Osman, G., Morsi, A. (2003) Isolation, identification and selection of lactic acid bacteria cultures for cheesemaking. *The Emirates Journal of Agricultural Sciences*, 15 (2), pp. 51–71.
4. Folkertsma, B., Fox, P.F., McSweeney, P.L.H. (1996) Accelerated ripening of Cheddar cheese at elevated temperatures. *International Dairy Journal*, 6, pp.1117–113.
5. Fox, P.F., Guinee, T.P., Cogan, T.M., McSweeney P.L.H. (2000) *Fundamentals of Cheese Science*. Gaithersburg, MD: Aspen, p.587.
6. Fox, P.T., Wallace, J.M., Morgan, S., Lynch, C.M., Niland, E.J., Tobin, J. (1996) Acceleration of cheese ripening. *Antonie van Leeuwenhoek*, 70 (2-4), pp. 271–297
7. Lawrence, R.C., Creamer, L.K., Gilles, J. (1987) Texture development during cheese ripening. *Journal of Dairy Science*, 70, pp. 1748–1760.
8. Upadhyay, V.K. and McSweeney, P.L.H. (2003) Acceleration of cheese ripening. In: *Dairy Products: maximizing Quality*, Smit, G.(ed.), Woodhead Publishers, Cambridge. pp. 419–447.
9. Waagner Nielsen, E. (2004) Principles of cheese production. In: *Handbook of Food and Beverage Fermentation Technology*. Hui, H., Goddik, L.M., Hansen, A.S., Josephsen, J., Wai-Kit Nip (eds), New York, Basel: Marcel Dekker, pp. 257–283.
10. Wilkinson, M.G. (1993) Acceleration of cheese ripening. In: *Cheese: Chemistry, Physics and Microbiology*, 2<sup>nd</sup> ed., Fox, P.F.(ed.), Chapman&Hall, London. pp. 523–556.