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Health aspects of cheese

Symposium in Drøbak

Norway

6-8th of October 2009

Programme
Abstract book
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Programme

Tuesday 6th of October

12.00 – 14.00 Registration
13.00 – 14.00 Lunch

14.00 – 14.20 Symposium opening
Siv Skeie, Coordinator of the NordOst network
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Session A
14.20 – 17.40: Probiotic microorganisms in cheese
Chairman: Siv Skeie, Norwegian University of Life Sciences, Norway.

Key note speaker:

14.20 - 15.05	Probiotic cheese, Dr. Vesa Joutsjoki, MTT, Finland.
15.05 – 15.15	Discussion

Oral presentations:

15.15 – 15.30	Probiotic cheese with *Lactobacillus GG.* Hanna Jatila, Valio Ltd R&D, Finland.
15.35 – 15.50	Assessment of *In vitro* methods for the evaluation of the probiotic potential. Professor Judith Narvhus, Norwegian University of Life Sciences, Norway.
15.55 – 16.25	**Coffee break**
16.25 – 16.40	Elaboration of *L. plantarum* TENSIA Comprising Probiotic Estonian Cheese. Merle Rätsep, Bio-Competence Centre of Healthy Dairy Products Ltd, Estonia.
16.45 – 16.55	Summing up
17.15 – 19.15	Guided tour on the island (10 – 15 pr groups)
20.00 -	Dinner at Forpleiningen

Wednesday 7th of October

Session B:
9.00 – 12.45 Functional compounds in cheese

Chairman: Rimantas Venskutonis, Kaunas University of Technology, Lithuania.

Key note speaker:

09.00 – 09.45 Bioactive Compounds in Swiss Cheese.
Dr. Barbara Walther from Agroscope Liebefeld-Posieux Research Station (ALP), Switzerland.

Oral presentations:

0945 – 10.00 Combinations of strains of *Lactobacillus helveticus* and *Lactobacillus delbrueckii* modify the anti-hypertensive activity in Swiss-type cheeses.
Dr. Valerie Gagnaire, INRA-Rennes, France.

10.05 – 10.20 ACE inhibition potential of Gammelost during ripening.
Tahir Mahmood Qureshi, Norwegian University of Life Sciences, Norway.

10.25 – 11.00 Coffe break& poster session

11.00 – 11.15 Cheese Powder of Matured Cheeses as Natural Flavour Enhancer.
Dr. Camilla Varming, University of Copenhagen, Denmark.

11.20 – 11.35 Peculiarities of Proteolysis in Finnish and Estonian Open Texture Cheeses.
Anastassia Taivosalo, Tallinn Technical University, Estonia.

11.40 – 12.00 Poster presentations

11.40 – 11.45 Increasing cheese functionality with *Rosacea* extracts: effects on growth and survival of lactic acid bacteria.
Milda Pukaslskiene, Kaunas University of Technology, Lithuania.

11.45 - 11.50 First steps in the development of functional products by using plant origin bioactive components in white Lithuanian cheese.
Danute Venskutoine, Kaunas University of Technology, Lithuania.

11.50 – 11.55 Effect of copper in the microflora and in the final quality of Finnish Emmental cheese.
Dr. Lourdes Mato Rodríguez. University of Helsinki, Finland.

11.55 – 12.00 Effects of Seasonal Variation in Milk Composition on the Quality of Cheese.
Kell Andersen, Århus University, Denmark.

12.05 – 12.15 Summing up

Session C:

12.15 – 17.00 Reduced component cheese (Low fat and low salt)

Chairman: Hilde Østlie, Norwegian University of Life Sciences, Norway.

1st Key note speaker

12.15 – 13.00 The roles of fat and salt in cheese.
Professor Paul McSweeney, University College Cork, Ireland.

13.00 – 14.00 Lunch

2nd Key note speaker

14.00 – 14.45 Scandinavian research in forefront of low-fat cheese development.
Professor Siv Skeie, Norwegian University of Life Sciences & Professor Ylva Ardö, University of Copenhagen, Denmark.

14.45 – 14.55 Discussion

Oral presentations:

14.55 – 15.05 Experimental design: Reduced fat Cheddar cheese
Kim Marius Moe, Norwegian University of Life Sciences, Norway.

15.05 – 15.20 Dynamics of microbial population during the ripening of reduced fat Cheddar cheese.
Aleksandra Stjepanovic, Norwegian University of Life Sciences, Norway.

15.25- 15.40 Modified electron microscopy techniques for the examination of the microstructural properties of low-fat Cheddar cheese made with different adjunct cultures and addition of skim-milk or butter-milk powders.
Dr. Ehab Romeih, University of Cairo, Egypt and Norwegian University of Life Sciences, Norway.

15.45 – 16.15 Coffee break & poster session

Session D:

16.15 – 17.15 **Too low fat cheese for health?**

Chairman: Inga Ciprovica, Latvian University of Agriculture, Latvia.

Key note speaker

16.15 – 17.00 Health aspects on milk fat in cheese
Professor Tine Tholstrup, University of Copenhagen, Denmark.

17.00 – 17.15 Discussion

17.15 – 17.50 **Poster presentations: Session C & D**

17.15 – 17.20 Use of Lactic Acid Bacteria and Enzymes to Improve Flavour and Texture of Low-Salt Cheese.
Kirsten Kastberg Møller, University of Copenhagen, Denmark.

17.20 – 17.25 A comparison of enzymatic activities of *Lactobacillus helveticus* and *Lactobacillus casei* strains with potential to improve ripening of low fat cheese.
Marie Penderup Jensen, University of Copenhagen, Denmark.

17.25 – 17.30 The Ability of Dairy Lactococci and Lactobacilli to Utilize Milk Fat Globule Membrane Carbohydrates.
Kim Marius Moe, Norwegian University of Life Sciences, Norway.

17.30 – 17.35 Effect of Milk Fat Content on Rennet Coagulation Properties and Whey Drainage.
Wiem Bel Hadj Hmida. University of Food Industries of Tunis, Tunisia.

17.35 – 17.40 Are reduced fat cheeses a risk for health?
Tiina Ritvanen, Finnish Food Safety Authority Evira, Finland.

17.45 – 17.55 Summing up Session C & D

19.00 Dinner at the Fort

Thursday 8th of October

Session E:

09.00 – 12.00 Maturation of cheese varieties of the Nordic Countries

Chairman: Ylva Ardö, University of Copenhagen, Denmark.

Oral presentations:

0900 – 0915	Ripening of semi hard Norwegian cheese varieties Hilde Kraggerud, Tine BA & Norwegian University of Life Sciences, Norway.
0920 - 0935	Characterisation of proteolysis during ripening of Grevé Ulrika Rehn, University of Copenhagen, Denmark.
0940 – 0955	Cheese eye formation measurement with laser camera Hanna Jatila, Valio Ltd R&D, Finland.
1000 – 10.15	Microbial Characterization of Finnish and Estonian Open Texture Cheeses: Manufacturing and Ripening Irina Stulova, Tallinn Technical University, Estonia.
10.15 – 10.45	**Coffebreak**
10.45 – 11.00	Quality of Latvian semi-hard cheeses. Alla Miķelsone, Latvian University of Agriculture. Latvia.
11.05 – 11.20	Traditional Lithuanian Cottage Type Cheese: Factors Affecting Texture. Professor Daiva Leskaukaite, Kaunas University of Technology, Lithuania.
11.25 – 11.40	Summing up
11.40 – 12.00	**Summing up of NordOst: What did we achieve? - Related to the objective in the project?** Professor Siv Skeie, Norwegian University of Life Sciences, Norway.

12.00 – 13.00 Lunch

Session A: Probiotic microorganisms in cheese

Key Note Lecture

Probiotic Cheese

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Cheeses offer at least the following advantages over freshly fermented milk products as carriers of probiotic micro-organisms:

- higher pH
- high buffering capacity
- almost anaerobic environment
- a more stable medium to support long-term survival
- a relatively high fat content
- growth-promoting nutrients (short peptides, amino acids)

The probiotic microbes used in cheese manufacture are mainly *Lactobacillus* and *Bifidobacterium* stains. Some trials have also been performed with *Enterococcus faecium*. In general, lactobacilli have a fairly good viability in all milk products. This is because they

- grow in a microaerophilic encironment
- are proteolytically active and can so get nutrients from milk
- are used as starters an are members of NSLAB
- tolerate low pH values and are viable in a wide temperature range

There are several ways to incorporate a probiotic microbe into cheese matrix. The simplest and the most common way is to add a strain as an adjunct of the starter. For this method, the technological features of the probiotic strain must be satisfactory.

In most cases, maintaining the viability of probiotics in cheese needs some optimization of the cheese making technology.

- Very often manufacturing and ripening temperatures must be altered
- The combination of a starter and probiotic must be optimized
- Viability of the probiotic strain may be enhanced by microencapsulation or spray-drying
- The way of addition of the probiotic must be optimized; can it be added with the starter or is separate fermentation needed?

Because of the changes in manufacturing technology or the technological traits of the probiotic strain, effects on cheese composition or sensory properties are often encountered. These may be caused by

- enhanced proteolysis (especially secondary proteolysis)
- increased concentration of free fatty acids
- increased production of organic acids
- fast consumption of lactose as a result of increased □-galactosidase activity

Depending on study, improved/unchanged/impaired sensory properties of the end product have been reported.

The other important bacterial genus with probiotic properties in addition to *Lactobacillus* is *Bifidobacterium*. Bifidobacteria originate from gastrointestinal tract and have an important

role in gastrointestinal health. They are also associated with several potential therapeutic benefits. Because of their natural habitat, bifidobacteria have some very specific growth requirements. For this reason, there are limitations in incorporation of bifidobacteria to freshly fermented milk products, which usually have low pH and aerobic conditions. Usually, some process improvements are necessary to enhance the viability of bifidobacteria. These may include for example

- addition of protein hydrolysates for nutrients
- modulation of cooking, ripening and storage temperatures
- reduction of salt concentration
- careful design of starters in single-stage fermentations
- two-stage fermentation, when sigle-stage fermentation is not possible
- microencapsulation or freeze-drying of the *Bifidobacterium* strain before addition into cheese matrix

Because of their specific characteristics, bifidobacteria often have an effect on cheese manufacturing process. They may

- increase the concentration of organic acids, especially acetic acid
- increase the concentration of free fatty acids
- increase the amount of free amino acids by secondary proteolysis
- increase β-galactosidase activity

In addition to the probiotic effects of live microbial cells, indirect or biogenic effects are mediated by metabolites of the living cells. In some cases both types may be yielded by a same probiotic strain. The best characterized bioactive metabolites in dairy products are

- conjugated linoleic acid (CLA)
- antimicrobial compounds
- bioactive peptides (peptides with hormone- or drug-like activity)

The proteolytic system of lactic acid bacteria is in key role in the conversion of bioactive peptides. Ripened cheeses are usually a rich source of bioactive peptides because of the proteolytic activity during ripening.

In recent years, development of DNA sequencing technology has made possible the sequencing of entire genomes of living organisms. The genome of a commonly used mesophilic starter *Lactococcus lactis* was sequenced almost ten years ago. To date, the genomes of all relevant dairy starters have been resolved. Genome sequence information has been applied to design DNA-microarrays, which allow the monitoring of genome-scale gene expression. DNA-microarrays can be applied to

- comparison of the properties of different strains
- screening of optimized starters
- detailed examination of probiotic or other specific characteristics
- characterization of complex microbiota (e.g. gastrointestinal tract)

The possibility for genome-based primary selection of strains for specific applications reduces the need of time-consuming and expensive process simulations or therapeutic trials.

Session A: Probiotic microorganisms in cheese

Oral presantations

Probiotic cheese with *Lactobacillus GG*

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Lactobacillus rhamnosus GG is a probiotic bacterium that has well documented effect on human health with over 400 scientific publications. The mechanisms maintaining health include supporting the defences of the mucosal barrier in the gut and enhancing immune response. LGG also alleviates atopic excema, reduces the risk for respiratory tract infections, dental caries and intestinal infections and when occurring, shortens the duration of acute diarrhoea.
In order to be effective, a probiotic bacterium usually needs to be in viable state when entering the gut. When included into a food, it needs to survive food processing, shelf life and stomach acidity. However, it should not have an adverse effect on food sensory properties or shelf life.

The effect of food matrix on oral and intestinal survival of LGG was studied in a 9 week clinical trial with 36 persons. The subjects were given a mixture of probiotics including LGG, *L. rhamnosus* Lc705, bifidobacterium and a propionibacterium. either vegetable capsules, yoghurt or cheese. All the subjects carried the probiotic strains during the intervention. During the follow-up, LGG had mean gastrointestinal survival time of 14.5 days. After the follow-up, 29% of the subjects still carried LGG. The product form did not affect the survival of LGG.

The survival of LGG in cheese was studied in low fat propionic acid fermented cheese. Viability and total amount of bacteria in cheese was studied with cultivation and qPCR. LGG survived until the end of 201 days' storage at the level of 10^7 cfu/g.

The effect of LGG on cheese sensory properties and LGG survival was studied in a cheese trial. The cheese with LGG added did not differ from cheese without LGG when evaluated by trained panellists. LGG counts stayed at the level of 10^7 during ripening.

In conclusion, cheese is a suitable matrix for ingesting LGG. It survives well the cheesemaking process and shelf life, does not change the cheese sensory properties and survives well in GI tract also.

Keywords: Lactobacillus GG, probiotic, survival, sensory quality

Assessment of *in vitro* methods for the evaluation of probiotic potential.

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Despite a number of very well-documented probiotic bacteria cultures on the market, the hunt is still on for even more strains. This is driven by two main goals – an altruistic desire for better health of the general population brought about by natural methods, and a commercial desire from the producers of probiotic products to find the super probiotic strain that will capture a large part of this expanding and profitable market.

The ultimate proof of probiotic effectiveness is only obtainable through exhaustive and stringently controlled clinical trials. Clinical trials are complicated and extremely costly to carry out. Some commercial strains are backed up by many scientific articles showing a variety of beneficial health effects in clinical trials. However, no applications to use health claims for probiotic bacteria have so far passed through the eye of EFSA's needle.

Probiotic strains may be isolated, for example, from the healthy human gastrointestinal tract (GIT). However the GIT in one person may contain over a thousand different microorganisms and even different clones of the same species may have more or less potential probiotic properties. Due to their assumed GRAS status, lactic acid bacteria (LAB) and bifidobacteria have been targeted for potential probiotics. However other members of the GIT microbiota, such as *E. coli* have been used as probiotics in a few trials. LAB are universally found in the human GIT, but several different species may be found even in one individual. These types of bacteria are also known to be present in other commercial fermented products and reports of negative effects in healthy people are virtually unknown. LAB from various fermented food products have also been investigated for their probiotic attributes. It may in the future be easier to add LAB originating from a food, rather than from the GIT.

The reservoir of potentially probiotic bacteria is vast and following isolation of new possible probiotic strains it is necessary to have a battery of *in vitro* tests that can be used to subtract strains that are unlikely to reach the small intestine and colon in a viable state. Further, tests have been devised that attempt to show positive effects such as adhesion to colon epithelial cells and mucus, induction of cytokine production in enterocytes. Other potentially positive effects including production of bacteriocins and inhibition of pathogens are also assessed in *in vitro* tests.

With the rationale that probiotic bacteria must be able to pass through the first line defences of the GIT in a viable state – the very low pH in the stomach and the presence of bile salts in the duodenum - tolerance to acid and bile are the first tests that are usually performed on new isolates. They are simple and quickly done and do not require advanced expertise or expensive equipment. However there is considerable variation in the methodology used in different articles, making it difficult to compare results obtained. Reports also use a variety of ways to express the tolerance to these conditions. Other *in vitro* tests also use a variety of methodology. There is a need for a consensus of opinion concerning the way to carry out *in vitro* testing and a correlation of results obtained with the results of *in vivo* assessment of potentially probiotic strains.

Keywords: probiotic properties, Lactobacillus, Bifidobacterium, acid and bile resistance, GI tract

Elaboration of *L. plantarum* TENSIA Comprising Probiotic Estonian Cheese

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Cheese is used as a probiotic vehicle to a less extent than fermented milk products.The problem caused in cheese by non starter lactobacilli (NSLAB), which appear during cheese ripening and can cause unwished proteolysis, appearance of various undesirable compounds and loss of commercial quality of cheese. Lactobacilli of human origin adapt poorly in cheese environment rich in protein and fat and poor in carbohydrates, which is atypical for them. Therefore, only few probiotic lactobacilli comprising cheeses are available

Material and methods: Novel probiotic strain *Lactobacillus plantarum* TENSIA (DSM 21380) has been previously isolated from a healthy Estonian child (Mikelsaar *et al.,* 2002; Annuk et al., 2003). The strain belongs to the Bio-Competence Centre of Healthy Dairy Products Ltd. *L. plantarum* TENSIA is an antimicrobial probiotic that produces several antimicrobial substances and possesses plantaricin encoding genes

Probiotic cheese, based on the Estonian cheese was developed in cooperation with Dairy Cooperative E-Piim. Altogether six industrial scale experimental batches of cheese (1.3 tons each) were prepared - three regular cheeses (controls) and three cheeses comprising 0.04% TENSIA as an adjunct starter. Before testing the cheese ripened at 12°C for 30 days at relative air humidity 80-85%.

The pH was measured directly from cheese by five parallel measurements from different parts of a cheese block. The SCFA profile of cheese was detected by gas chromatograph according to Innocente et al., (2000)

The LB profile and survival of TENSIA in cheeses were analyzed by conventional ten-fold serial dilutions. Lactobacilli isolates were identified with the API 50CHL System**.** The survival of TENSIA was confirmed by AP-PCR according to Matsumiya *et al*. (2002).

The biogenic amines and polyamines content in cheese were detected by gas chromatograph by modified method of Nakovichi (2003).

Results: No significant differences in pH dynamics in TENSIA comprising cheese and regular cheese throughout the ripening and storage were found. Towards the end of ripening the concentration of acetic (0.6±0.2 *vs* 0.09±0.1; p=0.03) and lactic acid (11.9±0.7 *vs* 9.9±2.0; p=0.03) in TENSIA comprising cheese were significantly higher than in regular cheese. Other SCFA were found only in traces of in both cheeses.

Formation of most biogenic amines incl. cadaverine and histamine into cheese during ripening and storage was not established. Only Tyramine was found in detectable amounts in all batches of cheeses. However, no significant difference in tyramine concentrations in TENSIA comprising cheese in comparison with control cheese was detected. Putrescine contents were found to be higher in TENSIA comprising cheese.

The *L. plantarum* counts were 10 000 times higher in *L. plantarum* TENSIA cheese to the day 28 in comparison with the control cheese. While in control cheese homofermentative lactobacilli, *L. casei* and *L. buchneri* strains were present, all aforementioned species were missing in *L. plantarum* TENSIA comprising cheese.

Conclusion: Thus the strain *L. plantarum* TENSIA possesses the ability to inhibit cheese NSLAB and suppress the cheese contaminating microbiota. Strong antimicrobial activity against enteropathogens of the strain could figure additive value to the elaborated Estonian cheese variety.

Keywords: probiotic, lactobacillus, cheese

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Session B: Functional compounds in cheese

Key Note Lecture

Bioactive Compounds in Swiss Cheese

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The complex microflora and the strong proteolysis contribute to the formation of an important number of peptides during cheese ripening. Some of these peptides are known to be biologically active and may have a positive impact on health. Among them the best investigated bioactive peptides are those showing an antihypertensive effect.

The contents of two such antihypertensive peptides namely Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) were determined in 10 different Swiss cheese varieties using HPLC with subsequent triple mass spectrometry. In individual samples, the total concentration of VPP and IPP varied between 1.6 and 424.5 mg/kg. In most cheeses VPP was present at greater concentrations than IPP. Key factors such as milk pretreatment, cultures, scalding conditions, and ripening time were identified to influence the concentration of these two naturally occurring bioactive peptides in cheese.

In another study the angiotensin-converting enzyme (ACE)-inhibitory activity as well as the concentration of the two ACE-inhibiting tripeptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) were studied during cheese ripening in seven Swiss cheese varieties. Good correlation was found between the ACE-inhibitory activity and the total concentration of VPP and IPP at advanced ripening stages. In most of the investigated varieties the ACE-inhibitory activity as well as the concentrations of the two tripeptides initially increased during ripening time. The chemical characterization of the investigated cheeses revealed that qualitative differences in the proteolysis pattern rather than the quantitative differences in the degree of proteolysis are responsible for the observed variations in the concentrations of VPP and IPP. The presence of *Lactobacillus helveticus* in the starter culture was also associated with elevated concentrations of VPP and IPP.

In an in vivo study the effect of cheese containing either high or low contents of VPP and IPP and corresponding concentrations of synthetic tripeptides on blood pressure of spontaneously hypertensive rats (SHR) were investigated using a telemetry system. Unlike the findings of several other studies using tail-cuff systems, neither cheese nor synthetic lactotripeptides had a significant effect on blood pressure in SHR.

Unlike the positive effects of bioactive peptides, the release of free amino acids and their further degradation to biogenic amines during cheese ripening is associated with adverse effects on human health. In cheese usually histamine, tyramine, cadaverine and putrescine can be found in elevated concentrations. In healthy subjects these amines are enzymatically degraded in the intestinal mucosa. However, patients sensitive to biogenic amines may react with circulatory disturbance or other health problems after consumption of foods with moderate to high concentrations of biogenic amines. Cheeses made from raw milk are often reported to contain high concentrations of biogenic amines. In recent work several varieties of Swiss origin were investigated for their content of biogenic amines. The results indicate that usual levels are rather low but that exceptions may occur. The factors influencing the development of biogenic amines are not fully understood, but it seems that raw milk flora and/or persistent flora in cheese factories are responsible for the formation of unusual high concentrations of biogenic amines in cheese during ripening.

Keywords: Bioactive compounds, cheese, ACE inhibiting peptides, biogeniic amines

Session B: Functional compounds in cheese

Oral presantations

Combinations of strains of *Lactobacillus helveticus* and *Lactobacillus delbrueckii* modify the anti-hypertensive activity in Swiss-type cheeses

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Lactic acid bacteria are potential producers of bioactive peptides, which may exert different activities *in vivo*, affecting the cardiovascular, endocrine, immune, digestive and nervous systems [1]. The aim of our study was to determine how different combinations of lactobacilli used as starters, can modify the bioactive peptide content in cheese regarding *in vitro* anti-hypertensive activity and how technological parameters can modulate this content.

Selected strains of the three species encountered in Swiss-type cheeses were inoculated with combinations of different levels: *Streptococcus thermophilus* (ST, 2 levels of the same strain), *Lactobacillus helveticus* (LH, 2 strains H1 and H2 and none H0), *Lactobacillus delbrueckii* (LD, 2 strains D1 and D2 and none D0). 24 model cheeses were then manufactured under identical controlled conditions, except the combinations of thermophilic starters. Microbial and physico-chemical analyses were performed on cheeses at different times of process and ripening.

The different starter combinations gave different acidifying curves and thermophilic strain growths during the process. Proteolysis patterns of cheeses varied quantitatively and qualitatively with the type of starters used during ripening regarding global proteolytic index variations and peptide profiles. Among the peptides produced, some exhibited *in vitro* anti-hypertensive activity expressed as concentration that inhibits 50 % of angiotensin I converting enzyme activity, i.e. IC_{50}. Levels and dynamics of this anti-hypertensive activity varied according to the combination of lactobacilli inoculated: an effect of LH strain was observed at all ripening stages, the effect of LD was mainly due to interactions between LH and LD, the combination H1D1 leading to the highest values IC_{50} in the course of ripening. Twenty-one peptides with potential anti-hypertensive activity were identified: 15 from β-casein, 4 from α_{s1}- and 2 from α_{s2}-caseins. Two particular anti-hypertensive peptides, VPP and IPP, were specifically quantified showing a strong correlation between their content and the IC_{50}: the higher content, the lower IC_{50}, depending on the lactobacilli strain combinations used.

Two combinations of strains with two different and extreme levels of anti-hypertensive activity, ST-LH1-LD1 and ST-LH2-LD2 were then chosen to test the modulation of the activity by technological parameters (milk origin and cooking temperature). The starter combination gave a specific fingerprint that varied more with changes of temperature during manufacture than with changes in milk origin and composition.

This work showed that the choice of the combinations of strains of thermophilic lactobacilli: i) is essential to enhance anti-hypertensive activity in the cheeses at the end of ripening time and ii) the technological parameters used can only slightly modified the fingerprint of the strains.

Keywords: bioactive peptide, lactobacilli, Swiss-type cheese, anti hypertensive activity

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ACE Inhibition Potential Of Gammelost During Ripening.

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The Angiotensin Converting Enzyme (ACE) is a carboxydipeptidyl metallopeptidase which is predominantly expressed in a membrane bound form in the vascular endothelial and epithelial cells in the brain, but it may also be found in blood and numerous body fluids [1]. ACE promotes the activation of the vasodepressor bradykinin of which an increase causes hypertention. Bioactive peptides from cheese have been shown to have ACE inhibitory properties [2-16]. If ACE inhibitory peptides are present, angiotensin I is converted to the vasopressor angiotenisn II instad of to the vasodepressor bradykinin. In a previous study it was shown [11] that the traditional Norwegian cheese Gammelost had a ACE inhibitory potential which was 4 times higher than a Duch type cheese (Norvegia). It has been shown that the amount of ACE inhibitory peptides varies during cheese ripening depending on the ripening system of the cheese [6].

Gammelost is a low fat cheese (0.5 % fat) ant it is made from skimmed milk which is acid and heat presipitated. The the moisture of the fresh cheese curd should be 48 – 50 %. The curd is casted, and the curd is cooked (90-95°C) in whey for 1-2 h. The cheese is then cooled, the mould removed, the cheese is dried. The cheese is then sparyed with a suspension of *Mucor Mucedo* which grows quite fast, and the cheese is ripened after 10-14 days. It has then turned from a white unripened cheese mass to a brown cheese with a strong flavour. The protelysis is rather strong in this cheese, and the soluble nitrogen totals approx. 90 % of the total nitrogen (TN), and the amount of NH_3 is about 10 % of the TN [17].

The development of ACE inhibitory peptides of different N fractions was studied during ripening of Gammelost. The ACE inhibitory avctivity was around 80 % in the water and water-ethanol soluble fraction, while the ACE inhibitory activity of the citrate and the citrate-ethanol soluble fractions was between 45 and 55 %. No significant change in the ACE inhibitory activity was found during ripening.

Keywords: ACE inhibitive paptides, Gammelost

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Cheese Powder of Matured Cheeses as Natural Flavour Enhancer

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Cheese powders are used as functional and natural cheese flavour ingredients in various industrial food applications, where they can be more convenient, in ease of use and storage, than regular cheese. In addition, cheese powders produced from well matured cheeses have the potential, at low dosage (0.5-2%), to replace taste and flavour enhancers such as mono sodium glutamate (MSG) and yeast extracts; and give a balanced flavour profile and rich mouth feel. Cheese powders produced from well matured cheeses may be used in applications such as ready meals, biscuits, sauces, dips, processed cheese and savoury snacks, and cheese powders used as flavour enhancers, aids in producing foods that are low fat, low salt, as well as natural without any additives.

Cheese powder contains overall the same flavour components as the cheeses it is made from, modified by some changes taking place during the cheese powder processing. Odour is provided by volatile aroma compounds, and taste by water soluble substances such as amino acids, short chain carboxylic acids, peptides and salts. In addition, taste- and flavour enhancing effects might be provided by glutamic acid, some peptides, short chain carboxylic acids, salt, nucleotides as well as interactions between those.

In the present study cheese powder was produced from a combination of selected well matured cheeses such as hard type, smeared type and blue type cheese (Lactosan A/S (Ringe, Denmark). Aroma compounds in the cheese powder were analysed by dynamic headspace sampling GC-MS, and amino acids and carboxylic acids were analysed by HPLC. By sensory profiling the effects of adding the cheese powder (1%) to a béarnaise sauce were evaluated relative to a reference béarnaise sauce.

Compared to the reference the béarnaise sauce added cheese powder was more yellow in colour, had a richer taste with a more well-balanced character, a less sourish taste, and a note of cheese flavour. Of the aroma compounds identified in the cheese powder particularly some sulfur compounds, aldehydes, methyl ketones and ethyl esters have the potential to impart odour. Among the amino acids, glu contributes directly to taste enhancement of the béarnaise sauce, and GABA, asp, ala, leu, met and ile were all present in the cheese powder at concentrations above their taste threshold levels. Acetic acid, propanoic acid, lactic acid and butanoic acid found in the cheese powder are likely contributors to the fullbodied taste of the béarnaise sauce.

In summary, the cheese powder had a significant effect on the sensory properties of the béarnaise sauce. The composition of flavour active components in the cheese powder has been elucidated, and it can explain the observed effect. Exactly how the cheese powder interacts with the components of the béchamel sauce and how the individual components relate to the sensory parameters do, however, need further investigation.

Keywords: flavour enhancer, cheese powder, aroma compounds, amino acids, béarnaise sauce

Peculiarities of Proteolysis in Finnish and Estonian Open Texture Cheeses

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Introduction: Sensory analysis of open texture cheeses made in Finland and Estonia have shown that there are differences in aroma and taste of those cheeses, although technological processes were quite similar. Throughout the ripening of cheese, proteolysis is considered to be most complex and important transformation to develop aroma, flavor and texture. During the ripening of the cheese, the caseins are hydrolyzed initially by residual coagulant activity retained in the curd and by plasmin to a range of large and intermediate-sized peptides which are hydrolyzed by proteinases and peptidases from starter LAB, NSLAB and perhaps secondary microflora to shorter peptides and amino acids (Fox, 2004). Therefore, the objectives of the present work were: 1) to apply capillary electrophoresis (CE) and ultra performance liquid chromatography (UPLC) as methods for determination and quantification of cheese casein fractions and free amino acids (FAA); 2) to monitor and compare the hydrolysis of caseins during ripening of cheeses and try to find the cause of differences in proteolysis.

Materials and methods: Milk and cheese samples for control trials were obtained from the Estonian and Finnish dairies. Cheese samples were taken from each vat (4) after 0, 10, 30, 45, 60 and 90 days of ripening for analysis. The samples and the running buffers for the analysis of milk and cheese protein fractions by CE were prepared as described by Recio and Olieman (1996).

CE analyses were carried out with a Beckman P/ACE MDQ Capillary Electrophoresis system controlled by 32 Karat ^{TM} Software, version 8.0. Separations were performed using a neutral capillary (Agilent) with dimensions 60.2 cm x 50 μm i.d. and a slit opening of 100 x 800 μm**.** Separations were carried out at 45°C and a linear voltage gradient from 0 to 25 kV in 3 min was used, followed by constant voltage at 25 kV for 47 min. The sample introduction was achieved by pressure injection for 20 s at 0.5 psi. UV detection was performed at 214 nm. Identification was done using the milk protein standards (Sigma) and by comparison of the separation pictures with literature ones. To achieve quantitative results from CE, the relative concentration of each protein fraction was used in milk samples (Heck et. al, 2008). In cheese samples, the moles of each casein fraction per gram of cheese were calculated.

To determine the free amino acids (FAA) content in cheese samples by UPLC, 2.5 g of grated cheese were homogenized with 25 mL of MilliQ water, heated for 10 min at 75°C and centrifuged. Supernatant was diluted with isopropanol at ratio 1:1 and centrifuged prior derivatization. Derivatization of cheese samples was done as described in Waters manual (1993). Free amino acids analysis was performed on a Waters Acquity UPLC system connected to Waters Empower^{TM} 2.0 software. The 21 amino acids containing standard solution was used to quantify the free amino acids in cheese.

Results: The CE results showed that there were significant differences in casein hydrolysis during ripening of Finnish and Estonian cheeses. The α_{s1}-casein was more intensively

degraded and less β-caseins were hydrolyzed during maturation of both cheeses. Quantitatively much more α_{s1}-caseins were hydrolyzed in Finnish cheeses, whereas more pronounced degradation of β-caseins took place in the Estonian ones. The results of FAA analysis showed that there were more FAA in Estonian cheeses compared with Finnish cheeses. It means that more intensive lysis of starters had occurred in Estonian cheeses and their intracellular peptidases proceeded degradation of smaller peptides to free amino acids.

Conclusions: Our study has shown that CE is an efficient method for monitoring the hydrolysis of caseins in cheese during ripening. UPLC was successfully applied as a rapid, sensitive and accurate method for determination FAA in cheese. Comparison of technological processes used for production of these cheeses showed that differences in proteolysis could be caused by peculiarities of vat period (e.g. "scalding" time and temperature) that lead to differences in cheese pH and consequently can regulate the activity of plasmin, rennet and bacterial enzymes in cheese which are responsible for casein degradation during ripening. More intensive proteolysis in Estonian cheeses could be one of the reasons of faster maturation of Estonian open texture cheeses.

Keywords: capillary electrophoresis (CE), casein, Estonian and Finnish cheeses, free amino acids, proteolysis, ultra performance liquid chromatography (UPLC).

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Session B: Functional compounds in cheese

Poster presantations

Increasing cheese functionality with *Rosacea* extracts: effects on growth and survival of lactic acid bacteria

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Aromatic, medicinal herbs and spices are a good source for natural compounds possessing various health effects and therefore their application for the increasing nutritional value of various foods has been in the focus of numerous studies. Although thousands of compounds were isolated and characterized in numerous plants, there are still many less investigated species. For instance, the publications on bioactive compounds in such Rosaceae family plants as *Agrimonia eupatoria*, *A. procera, Filipendula ulmaria, F. vulgaris* and *F. palmate* are rather scarce. This fact encouraged us to select these plants for a more comprehensive study with the final aim of the assessment of their application in the production of valuable functional ingredients, which might be used in various foods to increase their health benefits.

The extracts from the above mentioned species were prepared by using different extraction procedures and various solvents. Some of these extracts were shown to possess strong radical scavenging properties. *A. eupatoria* and *A. procera* extracts, isolated from the over ground parts, were able to scavenge free radicals, radical scavenging capacity (RSC) varied: 9.1–97.5% in $DPPH^{\cdot}$ and 6.7-79.5% in $ABTS^{\cdot+}$ reaction depending on the polarity of the solvent used to obtain the extract [1,2]

This study was aimed at screening their influence on the growth and survival of lactic acid bacteria. Such information is necessary to assess the possibilities of using plant origin components in the production of the fermented dairy products, including various types of cheese.

To the best of our knowledge, no information has been published on the influence of the selected plant extracts on the growth of lactic acid bacteria. The isolates of lactic acid bacteria from Lithuanian and Norwegian cheeses were isolated and identified by API 50CH identification. The effects of *Rosaceae* ethanol extracts on the growth and survival of bacteria were tested by a micro dilution broth method using microtiter plates. The minimal inhibitory concentration (MIC) was calculated by determining the concentration of extracts that suppressed bacteria growth as measured by optical density of the microtiter plate wells. Bacteria composition of different Lithuanian cheeses varied in a wide range, however, only three non starter species (NSLAB) *Lb. fermentum, Lb.paracasei and Lb. brevis* were found, while the composition of Norwegian (Nokkelost) cheese consisted of two NSLAB species, *Lb. plantarum* and *Lb. paracasei* which did not change during ripening. The MIC of ethanol extracts against all tested strains ranged from 15 to 3.8 mg/ml while indicating that addition of such extracts into the cheese are not likely to interfere with NSLAB during cheese ripening. To assess the possibilities of using selected plant extracts for increasing cheese functionality further studies will be focused on the effects of plant extracts on milk pathogenic bacteria and moulds as well as on the sensory properties of the final product.

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First steps in the development of functional product by using plant origin bioactive components in white Lithuanian cheese

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Plants biosynthesize a great variety of bioactive compounds, which may be used in the formulation of nutraceuticals and foods. Addition of plant bioactive constituents, generally referred as phytochemicals, to traditional foodstuffs is one of the main ways to develop functional foods. Cheese may also be considered as a matrix for the enrichment with plant bioactives; however, they should be compatible with other cheese components and traditional sensory requirements. This study was aimed at the assessment of the possibilities to increase the functionality of white Lithuanian curd cheese by raspberry and strawberry extracts. The main objectives in achieving this aim are to characterize plant extracts and to evaluate their compatibility with cheese, particularly its sensory quality.

Firstly, some important properties, such as radical scavenging capacity (RSC), the content of phenolic compounds and antimicrobial activity of the extracts were determined. The effective concentration of $DPPH^{\bullet}$ radical scavenging (EC_{50}) of raspberry press-cake extracts isolated from various plant cultivars by hexane, acetone, methanol and water was 2.07–7.70, 0.02–0.22, 0.04–0.49 and 0.18–0.30 %, respectively (the lower EC_{50} means the higher RSC). The following concentrations of the extracts were equivalent to the activity of 1 mmol of Trolox (reference synthetic antioxidant) solution as measured in $ABTS^{\bullet+}$ reaction: acetone 0.02-0.10 %; methanol 0.06-0.37 %; water 0.12–0.21 %. Total content of phenolic compounds was 2.4–156.1 mg gallic acid equivalents (GAE) in 1 g of extract. The minimum inhibitory concentration of extracts on the growth of *Esherichia coli* and *Staphylococcus aureus* varied from 1.41 to 3.75 and from 1.41 to 5.63 μg/ml, respectively.

The EC_{50} of different cultivars of strawberry leaf extracts varied from 0.023 to 0.043 %, while the concentrations of extracts equivalent to the activity of Trolox solution were 0.03–0.054 %. Total content of phenolic compounds depending on the solvent of extraction and plant variety was from 228 to 405 mg GAE /g extract. The extracts isolated with polar solvents contain high amounts of ellagic acid, which is considered as a phenolic compound possessing various health benefits.

All these findings indicate that the extracts may be considered as promising additives in cheese: (i) they may increase antioxidant properties of cheese by retarding its lipid oxidation and providing defence in human body as exogenous scavengers of excessive radical species; (ii) they may increase the content of bioactive components and to provide additional health effects (e.g. ellagic acid was reported as anticancer agent).

The extracts were added to the white Lithuanian curd cheese at various concentrations (1, 2, 3, 5, and 7 %). It was found that from the sensory point of view the acceptable dose of raspberry press-cake extracts may be up to 3 %, while the concentration of strawberry leaf extracts in cheese is limited to 1 %. The concentration of phenolic compounds and ellagic acid was remarkably increased at the applied doses; however, to prove possible improvements of health benefits by the extract addition more comprehensive *in vivo* studies are required.

Keywords: curd cheese, raspberry press-cake extract, strawberry leaf extract, functional ingredient

Effect of copper in the microflora and in the final quality of Finnish Emmental cheese

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Background: In Switzerland, Emmental cheese is traditionally made in copper vats, and accordingly, copper (Cu) levels typically lie between 7.6 and 16.5 ppm. Cu ions leached from the vat are mainly bound by casein proteins and are therefore transferred into the cheese; many cheese makers believe that Cu has a beneficial effect in Swiss cheese quality (Sieber *et al*, 2006). However, Emmental cheese-processing plants in many countries currently use stainless-steel vats and Cu is added or not depending on the particular manufacturing practices. In Finland, where stainless-steel vats are use for manufacturing Emmental cheese, a $CuSO_4$ salt solution is added into the cheese milk in order to increase the Cu concentration of the milk from less than 0.1 to 1.3 ppm. This supplement brings the Cu level in Finnish Emmental cheese close to the traditional Swiss-made Emmental, but still less than 15 ppm, which is the highest acceptable Cu level. In manufacture of organic Emmental cheese, the addition of Cu salt supplement is not allowed but the same type of vats are used.

Because of the essential but toxic nature of Cu, microbial organisms have mechanism to regulate intracellular Cu concentration (O'Halloran, 1993). There are few isolated reports on the effect of Cu on physiological and biochemical activities of lactic acid bacteria (LAB), and propionic bacteria (Kiemer *et al*. 1961; Maurer *et al*. 1975; Lee *et al*. 2005 a, b). Very few microbial physiological data are available on those conditions and factors in ripening Emmental cheese matrix promoting or preventing clostridial spore germination and vegetative growth; copper is one external factor which could influence the risk of clostridial cheese spoilage and consequently the quality of Emmental cheese. Published data on this subject turned out to be very limited despite of the economical importance of late blowing defects in Emmental and other semi-hard and hard ripened cheeses.

Since the effect of Cu on the microbial community of this type of cheese, as well as its total role in its final quality has been poorly investigated, the aim of our study was to elucidate the effects of Cu supplemented in growth medium on growth and viability of strains used as starters and adjunct cultures for Emmental cheese manufacture, and further to elucidate possible variability in copper resistance at strain and species levels; to investigate the role of Cu on spore germination, vegetative growth and sporulation of *Clostridium tyrobutyricum,* which is capable to cause texture and flavour defects in ripened cheeses like Emmental cheese; and finally the effect of Cu in the microbial community and in the quality of Emmental cheeses manufactured in the dairy pilot plant facilities of the of Food Technology Department at the University of Helsinki.

Materials & Methods: Thirteen strains belonging to *Lactobacillus delbrueckii, Lact. helveticus, Lact. rhamnosus, Streptococcus thermophilus* or *Propionibacterium freudenreichii* species were exposed to various copper concentrations in the proper growth medium at relevant growth temperatures and effects of supplemented copper on bacterial growth and cell viability were determined by optical density and pH measurements, and by plating's. Spore suspensions from three *C. tyrobutyricum* strains were used in two experimental set-ups. The first set-up studied the effects of supplemented (0 to 30 ppm) copper during spore germination and vegetative growth processes of *C. tyrobutyricum* as measured by plating's. The second set-up studied the effects of copper (0 to 30 ppm) during growth and sporulation processes of *C. tyrobutyricum* as measured by optical density at 550 nm and plating's after heat treatment

of the samples respectively. In order to investigate the effect of Cu in the quality of Emmental cheese produced in Stainless-steel cheese vat, cheeses with and without addition of Cu salt solution into the cheese-milk were produced. Cheeses were examined looking at microbiologycal analysis, total solids, pH, degree of proteolysis, amount of certain organic acids and Cu content at the different sampling points.

Results: When the effect of Cu was measured with pure strains in laboratory growth media conditions, among the species considered, *L. delbrueckii* was the most Cu resistant and *St. thermophilus* the most sensitive one. There was also a considerable amount of variation in Cu resistant at strain level (Mato and Alatossava, 2007). Inhibition of germination, vegetative growth and sporulation processes by copper was strain-dependent. Both sporulation and germination were more sensitive than vegetative growth of *C. tyrobutyricum* to the inhibitory effects by copper. There were not signifficative differences in the microbial counts at the different sampling points when comparing cheeses where Cu was added or not to milk during manufacturing. The pH and solid content were also not showing significative differences. Cheeses without Cu addition shown more lactic acid consumption and propionic acid production after 30 days ripening period compared with those where Cu was added. Only a tinny difference can be seen in the proteolysis after 60 days ripening between the two set of cheeses.

Conclusions: The preliminary studies in the effect of Cu on the growth and viability of starters and adjunct cultures used in the manufacture of Emmental cheese, as well as the effect of Cu in the cycle of life of *Cl. tyrobutyricum*, suggest that the addition of Cu, at the levels it is found in Emmental milk-cheese and cheese, may regulate the growth and viability of the bacterial community during Emmental cheese manufacture. The higher consumption of lactic acid with the concomitant production of propionic acid in cheeses without Cu addition in milk-cheese, after 30 days ripening, suggets a regulation in the propionic bacteria activity due by the presence of Cu. A little more degree of proteolysis could be seen in cheeses without Cu addition after 60 days ripening.

Keywords: Copper, Emmental cheese, lactic acid bacteria, propionibacteria, *Clostridium tyrobutyricum.*

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Effects of Seasonal Variation in Milk Composition on the Quality of Cheese

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Coagulation of cheese milk is very much affected by parameters such as fat and protein content. In order assure good coagulation results the cheese milk is usually standardized to a certain protein to fat ratio (Skeie, 2007). Such a ratio can be achieved several ways, including mixing whole milk with skimmed milk or by removal of some fat. Although cheese milk is standardized according to a desired protein to fat ratio, seasonal variation in the quality of cheese is still observed (Lindmark-Monsson *et al.,* 2003) This indicates that other factors besides the protein/fat are of importance for good coagulation results. This project attempts to identify the components in milk which give rise to this variation. Using several methods such as GC/MS, LC/MS, FTIR and NMR we identify many individual milk components and their concentration. This includes content of lactose, proteins and metabolites. These data are then correlated with the coagulation properties of the cheese milk. Using advanced chemometric models we strive to identify the components which influence coagulation of cheese milk. Once the important parameters have been identified it is our aim that the these parameters can be analyzed on site at individual plants using standard instrumentation, such that prior to cheese making the milk can be adjusted to good coagulation conditions. Milk samples are collected from spring 2009 to summer 2010 from two Danish dairies.

Keywords: milk, cheese, seasonal variation

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Session C: Reduced component cheese (Low fat and low salt cheese)

Key Note Lectures

The Roles of Fat and Salt in Cheese

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Increased affluence in Western societies has lead to excessive intake of calories and Na^+. Consumer concern over the levels of fat and salt in cheese has lead to the development of low-fat and low-salt variants of specific varieties. The consumption of low-fat cheese has remained low which has been attributed to poor consumer perception of the products based on taste and texture. To produce low-fat cheese with improved organoleptic properties, it is necessary first to understand the roles played in cheese by fat. The level of fat influences many aspects of cheese, including composition, biochemistry, microstructure, yield, rheological and textural properties. In addition, the fat's melting point and state (non-globular, homogenised, globule size distribution, solid-to-liquid ratio) have a major impact on the rheology, flavour and cooking properties of cheese. Fat also contributes directly to flavour and indirectly *via* lipolysis and metabolism of the resulting fatty acids. Western diets contain twice or three times the amount of Na^+ that is necessary for heath and excessive intake of sodium can have undesirable physiological effects, particularly hypertension and increased excretion of calcium. Usually, cheese is a relatively minor contributor to total dietary salt intake; however, there has been interest in producing low-salt cheese, usually by maintaining its ionic strength by partial replacement of NaCl with KCl. The level of NaCl in cheese varies from~0.7-~6 % (w/w) and the principal effects of salt in cheese are influencing ripening by controlling microbial growth and activity, controling enzyme activity, assisting in the later stages of syneresis (and thus influencing moisture and hence cheese texture), causing physical changes to the cheese proteins which influence texture, protein solubility and probably protein conformation. NaCl regulates the a_w of cheese and significantly affects its composition and flavour. The role of NaCl in cheese flavour is direct (salty taste) and indirect, though its influence on ripening). Finally, maintaining an adequate ionic strength of the aqueous phase of cheese is critical to avoid the development of bitterness. Usually, the ionic strength of cheese is governed largely by its NaCl content and reducing salt can thus lead to bitterness by increasing the degradation of β-casein by residual chymosin leading to the production of short, hydrophobic and extremely bitter peptides, including β-CN (f193-209).

Scandinavian research in the forefront of low-fat cheese development

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Reduced-fat cheese has a long tradition in the Nordic countries. In earlier days, the butter was the most valuable dairy product from bovine milk, and cheese was made of the about half-skimmed milk that was left over. The introduction of the effective alfa separator during late 1800s was detrimental for the cheese quality in Scandinavia because their fat content decreased considerable.

For ages, low-fat cheese was considered to be the poor man's food or diet food and no ambitions were made to make it delicious. After the two large wars in Europe, the development of several high quality normal-fat cheese varieties with 25 – 28 % fat was intense in the Nordic countries. Reduced-fat cheeses of the same varieties with 15 – 17 % fat was developed to fulfil the consumers demands for lower fat intake, because it had became obvious especially during the sixties that the traditionally high fat consumption in the Northern European countries was not compatible with our modern way of living. During the beginning of the seventies, researchers had found by correlating health statistics with food consumtion, a relation between heart diseases/ increased cholesterol in blood and a too high intake of dairy fat. This initiated a demand for cheeses with even lower fat content (10 %). However, the connection between dairy fat consumtion and these diseases has later been difficult to prove and there are indications that cheese might even be preventive towards these diseases. During this time also a lot of efforts were made to find methods to accelerate cheese ripening with the purpose of saving money by shorten the ripening time. The first high quality semi-hard low-fat cheese with only 10 % fat was the result of a concept that combined the results from development of low-fat cheese and accelerated ripening (Ardö et al, 1989). In this concept the deep breakdown in the cheeses of peptides into amino acids and further to aroma compounds was accelerated without changing the rate of the initial casein breakdown (where a to high degradation may introduce bitter flavour in semi-hard cheese).

Initially, heat treated *Lactobacillus helveticus* strains were used successfully, because of their high and broad activity of amino acid release, and the temperatures needed to inactivate glycolysis (to not interfeer with acidification) and general proteolytic activity were sufficiently different from those that should inactivate the enzyme activities that should remain active (Ardö et al., 1989). Flavour as well as texture of low-fat cheese may be improved by a considerably increased total amount of amino acids (Ardö et al., 2002; Skeie et al., 1995). Several cheese ripening cultures have then been developed for similar use by the starter culture companies, which contain selected strains with high and broad aminopeptidolytic and low or no proteolytic activity. This concept has been successfully used in the development of several low-fat cheeses in Sweden with only 10 % fat and some with 5 % fat.

For some years now it has been a sport for the dairy companies to decrease the fat content as much as possible, and several Danbo type cheeses with a fat content of only 5 – 6 % are now found on the Danish market. They have a characteristic flavour from the surface flora, a high moisture content and rather short shelf life. These cheeses are expensive to produce, because more milk are needed for the same amount of cheese, and the process is sensitive to small changes and needs to be efficiently controlled manually.

Because of their ability to catabolise amino acids, propionic acid bacteria (PAB) are also interesting microorganisms when it comes to the flavour development of low fat cheese. However, as the low fat cheese has high moisture content the metabolic activity of the cheese might be difficult to control. Use of such cultures might therefore be a real challenge for the cheesemaker.

Development of the cheese structure is critical in low-fat cheese production, because fat is replaced by not only protein, but also water. Optimally the moisture to fat-free cheese should not change when the fat content is decreased, but that is not always possible in cheeses with only 10 % fat or less. The free moisture may however be bond by free amino acids. This requires a considerable increased amount of free amino acids (to the level of a long-time ripened cheese) in a rather young cheese with a limited casein breakdown (Ardö et al., 2002; Skeie et al., 1995). Another concept is to add microparticulated whey proteins to a low-fat or even no-fat cheese. When the whey proteins are microparticulated they may act as fat globules in the casein matrix of the cheese. Such low-fat cheese varieties are produced commercially in Scandinavia.

The next trend has been to combine low-fat with other healthy properties in cheeses. Low-fat cheese with probiotic bacteria has been developed in Finland (Ryhälä et al., 2001), and low fat cheeses fortified with vitamin D is also available on the market.

Production and accumulation of bioactive peptides have been studied in low-fat cheese (Ardö et al., 2009).

Keywords: low fat cheese, increased peptidolysis, cheese flavour, cheese structure

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Session C: Reduced component cheese (Low fat and low salt cheese)

Oral presantations

Experimental design: Reduced fat Cheddar cheese

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Cheddar cheese was made with starter culture *Lactococcus (Lc.) lactis* ssp. *lactis* ML8 in a two factorial experiment at two levels: (1) Adjunct culture; *Lactobacillus (Lb.) paracasei* INF448 or *Lb. paracasei* INF456, (2) Addition; butter-milk powder (BMP) or skim-milk powder (SMP) and cream. Uniform fat and protein contents were achieved in all cheese treatments. The experiment was made in three replicate blocks. Microbial and microstructure results from this experiment will be explained in the coming two presantations.

Dynamics of microbial population during the ripening of reduced fat cheddar cheese

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Abstract

Constantly increasing knowledge on the importance of the healthy diet for the improvement of the overall wellbeing has lead to the development of the food products with reduced fat content. The aim of this study was to asses the microbial diversity in reduced fat Cheddar cheese by employment of culture-depended methods supported by molecular based methods for the purpose of species and strain typing. Cheddar cheese was made in a two factorial design, with increased content of buttermilk as the first factor and with two adjunct lactobacilli as the second factor. The adjuncts were *Lactobacillus paracasei* INF448 and *Lactobacillus paracasei* INF456. The experiment was made in three replicate blocks. Following enumeration 16 colonies were picked from each batch and each cheese. Isolates were sampled at the beginning (0 week old cheese) of the ripening and from 4, 10 and 24 week old cheese. Out of, in total 993 isolates from all of the cheeses, 321 were selected for further analysis. Results obtained by phenotypic methods showed that all the isolates were homo-fermentative lactobacilli. Furthermore, 100 isolates have been subjected to 16S rRNA gene sequencing which revealed that 3 isolates belonged to *Lactobacillus brevis*, 2 to *Lactobacillus rhamnosus*, while the rest of the isolates belonged to *Lactobacillus paracasei* or *Lactobacillus casei*. Typing of the selected isolates has been performed using Repetitive sequence-based polymerase chain reaction (rep-PCR). Rep-PCR fingerprinting, using the REP1R-Dt and REP2R-Dt primers, has revealed significant diversity among the strains though ripening of the examined cheeses, especially in the beginning of the ripening process.

Key words*: reduced fat Cheddar cheese, microbial diversity, Rep-PCR*

Modified electron microscopy techniques for the examination of the microstructural properties of low-fat Cheddar cheese made with different adjunct cultures and addition of skim-milk or butter-milk powders.

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The microstructure attributes of low-fat Cheddar cheeses (7% fat) were investigated using a scanning electron microscopy technique (SEM) as well as confocal scanning laser microscopy technique (CSLM), for monitoring the localization of fat and bacteria within the cheese matrix. The cheese was made with starter culture *Lactococcus (Lc.) lactis* ssp. *lactis* ML8 in a two factorial experiment at two levels: (1) Adjunct culture; *Lactobacillus (Lb.) paracasei* INF448 or *Lb. paracasei* INF456, (2) Addition; butter-milk powder (BMP) or skim-milk powder (SMP) and cream. Uniform fat and protein contents were achieved in all cheese treatments. The microstructure examination showed a slight structural change as a function of the addition of adjunct cultures as compared to the control cheeses without adjunct culture. The cheese treatments with adjunct cultures had a similar homogeneous/continuous appearance of the protein matrices. Moreover, a large variation in cheese microstructural properties was observed between low-fat Cheddar cheese made with SMP and that of BMP. Addition of SMP leads to an increase in the protein folds and bigger protein aggregates. Thus, inhomogeneous voids, which appeared in the SEM surface technique as cavities, were obvious in low-fat cheddar with SMP, whereas the addition of BMP caused a homogenous, compacted fusion and dense structure with less coarse and voids compared to SMP cheese. The fat globules were more pronounced and scattered throughout the protein matrix of the cheeses with BMP. The starter bacteria were located within the protein networks in a shape of clusters distributed homogeneously allover the cheese matrix in all cheese treatments as observed by the modified SEM technique. Although, the adjunct culture bacteria were extremely difficult to visualize in the cheese matrix, however when found, they appeared in a shape of a huge massy cluster. The SEM technique also showed the presence of probable exopolysaccharides (EPS) in all cheeses with adjunct cultures. The CSLM technique confirmed the microstructural attributes observed from the SEM techniques; however, CSLM was neither able to explore the adjunct culture bacteria nor the EPS within the cheese matrix. In conclusion, addition of BMP to low-fat Cheddar cheese altered the structural networks with a trend of much finer, smoother and promoted arrangements of protein matrices, the fat in the BMP cheeses was more distributed within the protein matrix compared to that of SMP cheeses.

Keywords: Low-fat Cheddar, cheese microstructure, scanning electron microscopy, confocal scanning laser microscopy.

Session C: Reduced component cheese (Low fat and low salt cheese)

Poster presantations

Use of Lactic Acid Bacteria and Enzymes to Improve Flavour and Texture of Low-Salt Cheese

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Many epidemiological studies have demonstrated that high sodium intake is associated with an increased risk of adverse health effects in particular raised blood pressure and bone demineralization (osteoporosis) (INTERSALT Cooperative Group, 1988; Lin *et al*., 2003). Most modern diets contain more than twice the amount of sodium as compared to the average consumption recommended by WHO (< 2 g of sodium/day). Consequently, health authorities and retailers put a significant pressure on the entire food processing industry to direct product development towards low- or reduced-sodium alternatives. (INTERSALT Cooperative Group, 1988; WHO/FAO, 2003).

The cheese making industry is no exception to this trend as salt (NaCl) has been traditionally added in varying amounts as a functional ingredient to the vast majority of cheese types. In practice, however, this challenge is not easily met due to the fact that salt plays a central role in controlling the growth and metabolic activity of both desirable and undesirable microorganisms and enzymes in cheese (Guinee & Fox, 2004; Johnson *et al.*, 2009). Thus, salt is on the one hand indirectly responsible for balanced peptidolysis, lipolysis and amino acid catabolism during ripening, which are all critical pathways for the development of normal cheese flavour and texture. On the other hand, salt contributes directly to preserving the cheese. In addition, salt itself acts as a global flavour enhancer in cheese (Man, 2007).

The best current approach to reducing the sodium content of cheese is to replace sodium salts with sodium/potassium blends. However, the use of potassium salts is limited by the development of metallic, bitter and other off-flavours (Fitzgerald & Buckley, 1985).
This PhD project investigates the influence of salt reduction on the biochemistry of cheese ripening focusing on flavour and texture related differences developing as compared to a normal-salted cheese. The central aim of the project is to deal with the challenge of developing overall solutions based on microorganisms and/or enzymes to remedy such differences.

Keywords: cheese, low-salt, ripening, microorganisms, enzymes

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A comparison of enzymatic activities of *Lactobacillus helveticus* and *Lactobacillus casei* strains with potential to improve ripening of low fat cheese

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Low fat cheeses are in high demand on the market; however, their flavour and texture are difficult to control. One way of improving flavour and texture of low fat cheese is to use adjunct cultures. Two species used as adjuncts are the mesophilic *Lactobacillus casei*, part of the non-starter lactic acid bacteria group, and the thermophilic *Lb. helveticus*, part of the starter culture group. Several enzymes are involved in the development of flavour and texture during ripening and the balance between these enzymatic activities is important for the quality of the mature cheese. The aim of this study was to compare selected enzymatic activities of *Lb. helveticus* and *Lb. casei* involved in amino acid release and amino acid catabolism.

Six thermophilic *Lactobacillus helveticus* and six mesophilic *Lactobacillus casei*, all cheese related, were grown to mid-exponential phase in MRS broth and cell free extracts were prepared. The cell free extracts were assayed for different enzyme activities. General aminopeptidase (PepN and PepC) and X-prolyl dipeptidyl aminopeptidase (PepX) activities were examined using the chromogenic substrates Lys-, Arg-, Arg-Pro- and Gly-Pro-*p*-nitroanilide (Exterkate 1975). Aminotransferase activity was determined by measuring the production of Glu from α-ketoglutarate mixed with Phe, Leu, Asp or Met using the colorimetric Glu assay kit of Boehringer Mannheim (Thage *et al.* 2004). Hydroxyacid dehydrogenase activity was determined by measuring the rate of consumption of NADH during the conversion of β-phenylpyruvate (from Phe) or α-ketoisocaproate (from Leu) into their corresponding hydroxy-acids (Smit *et al.* 2004).

Lb. helveticus and *Lb. casei* both had PepN, PepC and PepX activity. The difference in activity level was considerably larger between the two species than the strain variation within each species, though the activity level also was highly strain dependent. For instance, one *Lb. casei* strain had a high level of PepX activity whereas the other five strains had not. In all cases, the activity level of *Lb. helveticus* was more than ten times higher as compared to *Lb. casei*, and *Lb. helveticus* should therefore be more efficient in releasing amino acids during ripening likely resulting in improved texture and enhanced background flavour. The aminotransferase activity against Phe, Leu, Asp and Met was detected in both species. The species dependent pattern in the activity level was more pronounced than the strain dependent pattern. The *Lb. helveticus* strains had about twice as high activity against Phe than the *Lb. casei* strains, which on the other hand had more than five times as high activity against Leu, Met and Asp. The high activity against Phe gives *Lb. helveticus* a larger potential to introduce floral notes during ripening while *Lb. casei* could introduce malty and cheesy notes through Leu catabolism, sulphur notes through Met catabolism and buttery notes through Asp catabolism (Ardö 2006). Interestingly, one *Lb. helveticus* strains had no activity against Met and Asp. The hydroxyacid dehydrogenase activity against β-phenylpyruvate, the α-ketoacid from transamination of Phe, was higher for *Lb. helveticus* as compared to *Lb. casei*, while the opposite was observed against α-ketoisocaproate, from transamination of Leu. This species dependent hydroxyacid dehydrogenase activity pattern resembled that found for the aminotransferase activity. Hydroxyacids are not considered important aroma compounds (Ardö 2006), why the α-ketoacids produced by the high aminotransferase activity against Phe

for *Lb. helveticus* and against Leu for *Lb. casei* might not be entirely converted into aroma compounds.

The variation found between species as well as strains offers the possibility to select combinations of the species and the strains to achieve a specific balance of enzymatic activities required for a desired flavour and texture development in low fat cheese.

Keywords: lactic acid bacteria, aminopeptidases, aminotransferases, hydroxyacid dehydrogenases, cheese

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The Ability of Dairy Lactococci and Lactobacilli to Utilize Milk Fat Globule Membrane Carbohydrates.

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The utilization of carbohydrates associated with the bovine milk fat globule membrane by selected lactobacilli strains isolated from Norwegian semi-hard cheese and starter *Lactococcus* sp. were studied. Growth of the lactic acid bacteria were studied in a carbohydrate-restricted media, supplied with one of seven carbohydrates: D-glucose, *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, *N*-acetyl-D-neuraminic acid, D-galactose, L-fucose and D-mannose. Growth was measured by optical density (OD_{620}) and enumeration on MRS- or M17 agar. Three growth descriptors were devised to differentiate statistically between growths of the bacteria on the different carbohydrates. The selected cheese isolates were able to utilize several of the monosaccharides found in the milk fat globule membrane. The ability to utilize *N*-acetyl-D-galactosamine for growth differed between the studied lactobacilli. None of the lactobacilli studied were able to utilize fucose or *N*-acetyl-D-neuraminic acid for growth. Growth of lactobacilli on the acylated aminosugars *N*-acetyl-D-glucosamine or *N*-acetyl-D-galactosamine induced a decrease in the number of viable bacteria at an earlier stage than when grown on any of the other monosaccharides utilized by the studied lactobacilli. The cheese starter bacteria *Lactococcus lactis* subsp. *lactis* ML8 was able to utilize all of the currently identified monosaccharides found in the milk fat globule for growth except *N*-acetyl-D-neuraminic acid.

Keyword: Carbohydrate utilization, lactic acid bacteria, milk fat globule membrane

Effect of Milk Fat Content on Rennet Coagulation Properties and Whey Drainage

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During the last years, the production of low-fat cheeses has increased. However, there is little information on the influence of the fat contents on the coagulation properties of milk.

The aim of the present study is to investigate the effect of milk fat content on the rennet clotting properties and whey drainage.

Three milk samples (skimmed 0.3%; partially skimmed 1.5% and whole 3.2% (w/v)) have been studied. The follow-up of rennet coagulation was carried out by the means of both turbidimetric and conductivimetric methods. The syneresis, the rate of draining and yield of cheese were evaluated. A physicochemical characterization of milks, curds and serums were also carried out for a better comprehension of the phenomena.

The physicochemical characterization has shown that the full-fat curd had the highest dry matter content, the most important fat recovery and the highest casein loss in the whey compared to the other reduced-fat samples. The examination of the turbidimetric signal obtained during the enzymic coagulation has shown that whole milk was characterized by the shortest hydrolysis phase compared to its low-fat counterparts (partially skimmed and skimmed). The higher the milk fat content was, the longer the aggregation phase lasted. The examination of the conductivimetric signal has revealed that the conductivity of milk is inversely proportional to its fat content. Reducing the milk fat content had a negative effect on cheese yield and increased syneresis rate.

Keywords: milk fat, rennet coagulation, turbidimetric method, syneresis

Session D: Too low fat cheese for health?

Key Note Speaker

Cheese and cardiovascular disease

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Although it has often been postulated that the consumption of dairy products is associated with risk of coronary vascular disease study results have been conflicting. It is well known that milk fat due to its high content of saturated fats increases cholesterol concentration in the blood. It is also agreed that increased blood cholesterol is a risk factor in regard to cardiovascular disease. However, interestingly we and others have demonstrated that a high intake of cheese contrary to butter of equal fat content does not increase total and LDL cholesterol. The observation of a neutral effect of cheese in regard to plasma cholesterol is new, and may explain at least partly why several observational studies over recent decades have described cheese as being an exception. This oral presentation will include recent findings on dairy products and risk markers of coronary heart disease. Data on association between cheese intake and coronary heart disease will be summarized together with results from human intervention studies. Possible mechanisms for the different effect of cheese will be discussed.

Key words
Cheese, coronary heart disease, blood cholesterol.

Session D: Too low fat cheese for health?

Poster presantations

Are reduced fat cheeses a risk for health?

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Objectives: We hypothesized that different production conditions may influence to the fatty acid profile of reduced/low fat cheeses. The main object of this study was to compare fatty acid profiles between regular and reduced/low fat cheeses in the market, in order to detect if the quality of the fat is dependent on fat content. Also the method for fatty acid analysis was validated from flame ionisation detector (FID) to mass selective detector (MSD).

Methodology: Hard, matured cheeses were purchased from local market. Cheeses were categorized to three classes according to their fat content. Fat was analyzed by Schimd-Bondzynski-Ratzlaff principle. Fat for fatty acid analysis was separated by DPS detergent method. Methyl esterification was done by base-catalyzation. FAMEs were analyzed by GC-MS. Statistical handling included ANOVA, Pearson correlation and PCA-analysis.

Results: The ANOVA test revealed that neither reduced fat cheeses nor low fat cheeses differ ($p>0.05$) from full fat cheeses in saturated, monounsaturated, polyunsaturated, *trans* fatty acids or conjugated linoleic acids. However, some correlations between fat content and individual fatty acid content were remarkable.

Session E:
Maturation of cheese varieties of the Nordic Countries

Oral presantations

Ripening Of Semi-Hard Norwegian Cheese Varieties

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The influence of the ripening temperature on the sensory attributes during maturation of cheese was studied in two different semi-hard, washed curd, commercial Norwegian cheese varieties with similar gross composition. Multivariate models derived from sensory attributes of cheese demonstrated that ripening temperature and maturation time had systematic, independent effects on the sensory properties of cheese of the varieties examined. There is a demand for a non-destructive method to monitor eye formation in cheese during ripening. A simple method based on existing equipment in the dairy industry is demonstrated. Images were acquired using a conventional, low resolution online X-ray instrument. Image processing methods for detecting eyes of cheese and measuring volume and size distribution were developed. The method was found promising for quality control as it will make possible a non-destructive monitoring of the eye formation of the cheese throughout the ripening period.

Characterisation of proteolysis during ripening of Grevé

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Propionic acid bacteria (PAB) contribute to eye and flavour formation in cheese. They are added to both hard cheese varieties (such as Emmental) and to semi-hard cheese varieties (such as Jarlsberg, Maasdamer and Grevé) (Fröhlich-Wyder and Bachmann, 2004; Ardö, 2004). Ripening of hard cheese with PAB involves activities of added thermophilic lactic acid bacteria (LAB), while semi-hard cheese with PAB is made with mesophilic starter bacteria. The differences in technology and microbiology between these types of cheese impact ripening. Ripening of hard cheese with PAB has been broadly investigated (Fröhlich-Wyder and Bachmann, 2004), while the aim of this work was to characterise proteolysis during ripening of a semi-hard cheese with PAB, Grevé.

Cheeses from two different producers of Grevé were analysed from production and until approximately four months of ripening. Enumeration of PAB and LAB was performed with agar plating (sodium lactate agar, SLA, and MRS agar, respectively). Caseins were analysed with capillary electrophoresis (CE) and peptides and amino acids were analysed with RP-HPLC.

The ripening conditions of Grevé include a warm room period where the PAB grows to high numbers. PAB were found at a level of approximately log 8-9 CFU/g cheese after the warm room period and they dominated the microbial flora from this time point.

The casein degradation was characterised by rennet and plasmin activities and the peptide formation was influenced mainly by plasmin, rennet and mesophilic starter activities. Peptides derived from β-casein by plasmin activity accumulated during ripening, as seen in semi-hard cheese with mesophilic starter without PAB (Ardö et al., 2006). The relative content of Asp, Ser and Lys (mol% of the total content of amino acids) decreased and the relative content of Glu and Leu increased specifically during the warm room period, indicating a relation to PAB activity. Higher levels of Pro could be correlated with higher numbers of PAB.

Proteolysis in Grevé was shown to be influenced mainly by rennet, plasmin and starter activities, but the contents of some amino acids were indicated to correlate with PAB activities.

Keywords: proteolysis, propionic acid bacteria, semi-hard cheese, cheese ripening

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Cheese eye formation measurement with laser camera

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The large round eyes of Emmental cheese are the distinctive characteristics of this cheese type. The eyes are formed during warm room ripening due to carbon dioxide formation in propionic acid fermentation. The propionic acid fermentation and eye formation are affected by several variables, e.g. cheese pH, salt concentration, ripening temperature and amount of eye nuclei in cheese before warm room. Due to different conditions inside cheese block, there is also variation in the eye formation in the cheese block. However, the cheese packing plants, retailers and consumers expect even quality and appropriate eye formation from every Emmental piece they get.

In this work the eye formation of Finnish Emmental was monitored with a laser camera by scanning the cheese cuts at the cheese packing line. The camera image was transformed into numerical data and interpreted with statistical methods. The variation in the amount of cheese eyes was studied inside a cheese block, between the cheese blocks from the same cheese vat, between the cheese vats made from the same milk lot and between different milk lots.

The results show that there was difference in the amount of eyes between the cheeses made from different milk lots. There was also some variation in the amount of eyes in different parts of the cheese. The amount of eyes of the cheeses from the same cheese vat was quite homogeneous.

The laser camera method is a good tool for product improvement concerning the Emmental eye formation. As the data collection is automatized, it is possible to monitor vast amount of cheese, which makes precise statistical analyses and even modelling the cheese eye formation possible.

Keywords: Emmental, eye formation, laser camera, statistics

Microbial Characterization of Finnish and Estonian Open Texture Cheeses: Manufacturing and Ripening

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Introduction: Microorganisms are essential components of all natural cheese varieties and play important roles during both cheese manufacture and ripening. They can be divided into two main groups; starters and secondary flora. The starter flora is responsible for acid development during cheese production. The secondary flora is composed of complex mixtures of bacteria, yeasts and moulds, and is generally specifically associated with particular cheese varieties. The secondary flora may be added in the form of defined cultures, but in many situations is composed of adventitious microorganisms gaining access to the cheese either from ingredients or the environment (*Beresford et al., 2001)*.

The composition and bacteriological quality of milk used in cheese manufacture have a significant influence on both the yield and the quality of the cheese produced.
Sensory analysis of open texture cheeses (Tilsiter-type) produced in Estonia and Finland has shown differences in aroma and taste of those cheeses, although technological processes were quite similar. The objective of the present study was to investigate the possible influence of raw milk and adventitious microflora (sporeformers and nonstarter lactobacilli) on the ripening processes and development of microbial communities in the cheeses.

Materials and Methods

Sampling
Milk samples were collected from the raw bulk milk tank, the pasteurized milk tank and the vat milk in cheese making plants, minimum amount of milk sample was 50 ml. Cheese samples were taken from raw cheese after pressing (before brining) and then after 10, 30, 45, 60 and 90 days of ripening. Cheese samples were taken from 4 parallel vats at each sampling time. Cheese samples taken during ripening were refrigerated and stored at - 40°C.

Enumeration of microorganisms
Milk and cheese samples were diluted according to the IDF standard (Anonymous, 1996). Total bacterial counts were measured by the pour plate technique with plate count agar (PCA) and incubated at 30°C for 72 h. Samples for the psychrotrophic count were spread on PCA and plates were incubated at 7°C for 7 days (IDF Standard 101A:1991). For enumeration of aerobic spore-forming bacteria aliquots of milk were heat-treated at 80°C for 10 minutes. After being cooled in an ice bath, the samples were immediately plated on PCA and incubated at 30°C for 72 h. Lactococci were determined on M-17 agar, incubated at 30°C for 72 h, and lactobacilli in MRS agar (De Man, Rogosa and Sharpe), incubated at 30°C for 72 h.

Identification
Dynamics and composition of cheese microflora (especially NSLAB) was characterized using different molecular-genetic techniques (PCR, DGGE) using characteristic colonies isolated from plates and total DNA isolated from samples. For identification of *Pseudomonas fluoresens,* species specific primers of *Pseudomonas fluorescens* and *Pseudomonas putida* control strains were used.

Results: Our result showed that microbiological quality of the milk used for cheesemaking was satisfactory. The counts of total mesophilic bacteria and psychrotrophic bacteria were higher in raw milk produced in Finland, *Pseudomonas fluorescens* was dominating in both dairies. Aerobic spore-forming bacteria counts were low in both cases, and disappeared rapidly during ripening, probably due to the inhibitory effect of decreasing pH. High counts of mesophilic LAB were present in Estonian and Finnish raw milk, with predominance specie being cocci. Similar counts of lactococci and lactobacilli were found in curd samples. A sharp decrease (about one-two log units) was recorded in LAB counts at the 10th day of manufacture and after this date the further decrease was relatively slow.
According to RAPD and especially DGGE analysis of cheeses it became evident that the microbiota of Finnish cheeses was more diverse than of Estonian cheeses. Dominating bacteria identified in both cheeses belong to the genus *Leuconostoc.* Some unusual species were also found – *Bacillus pumilus* in Finnish cheeses and *Aerosphaera taetra* in Estonian cheeses, their sources being unknown.

Conclusions: This work was the first comparative microbiological study of Finnish and Estonian open texture cheeses by means of culturing and PCR-DGGE analysis. To analyze the microbiological diversity a polyphasic approach was used – combining microbial enumeration in selective culture media, randomly amplified polymorphic DNA (RAPD) analysis of isolates and denaturing gradient gel electrophoresis of the total bacterial DNA extracted from cheese samples. DGGE proved to be better method than PCR for identification and monitoring the dynamics of lactic acid bacteria during cheese ripening as it was less time consuming and more informative.
Revealed differences between bacterial composition and interactions of starters with adventitious microbes could be possible reasons of flavor differences in studied cheeses.

Keywords: open texture cheese, Estonian and Finnish cheeses, cheese microbiology, raw milk quality

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Quality of Latvian semi-hard cheeses

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Background: Krievijas and Holandes are the most popular semi-hard cheeses among the broad varieties of similar cheeses in Latvia. At least one of these classical cheeses is produced at almost each cheese dairy in Latvia. The fact that local purchasers have their preferable brands, always denotes a distinction between cheese sensory properties (as flavour, taste and appearance) and it depends on many factors starting with the initial step of cheese making – milk quality and following appropriate treatment and finishing with cheese ripening conditions.
To avoid the cheese defects (off-flavours, texture and taste defects) during ripening which may lead to commercial loses, the majority of cheese makers processing local milk into cheese elevate the pasteurization temperatures of milk, add more starter culture, do not add hot water to mix the curd and whey before heating, longer curd treatment and decreased ripening temperatures of cheese (instead of average 12° C, 6-8° C are broadly used). Alterations in the mentioned technological steps automatically predict inferior properties of cheeses. For this reason, sensory evaluation of classical cheeses was performed.

The aim of this work was to investigate the volatile profile and the NSLAB diversity in Latvian semi-hard cheeses with inferior sensory properties from different manufacturers.

Materials & methods: Eight commercials samples either Krievijas or Holandes (Table 1) were randomly chosen for sensory evaluation and analyses of the volatile profile. NSLAB identification and counting were made for cheeses with inferior flavour.

Table 1. The characteristic of analysed cheese samples

Manufacturer	Cheese brand name	Protein content, %	Fat content, %	Fat content in dry matter, %	Salt content, %
JSC 'Smiltenes piens'	Krievijas	17	28.2	50	1.5-2.5
	Holandes	17	26.0	45	1.5-3.0
JSC 'Rīgas piena kombināts'	Limbažu (Holandes)	23	26.8	45	1.5-3.0
	Limbažu (Krievijas)	23	29	50	1.3-1.8
JSC 'Trikātas piens'	Krievijas	23	29	50	1.3-1.8
	Holandes	26	26.8	45	1.5-3.0
LTD 'Mālpils piensaimnieks'	Holandes	25	25.2	45	1.5-3.0
JSC 'Cesvaines piens'	Holandes	26	26.8	45	1.5-3.0

Sensory properties such as flavour, structure and colour were measured using a line scale method. The samples were evaluated by 27 panellists.

Serial dilution of the cheese samples with inferior flavour (1:1000 and 1:10 000) in saline was made. NSLAB were cultivated using MRS media. Strain identification was performed by the API 50 CHL system (BioMerieux, Marey l'Etoile, France). Detetcion of volatiles was conducted using solid phase GC/MS.

Results: The typical unpleasant flavours in commercial Krievijas cheeses were bitterness and acidity; whereas Holandes cheese did not have any notable differences except saltiness. The majority of the cheeses regardless of brand were recognized as more firm compared to cheeses (also used in sensory tests) which gained first place in the latest evaluation of dairy products in Latvia.

Bitterness in Krievijas cheeses should be considered as a complex condition between milk treatment and ripening temperatures associated with the use of mesophilic starter cultures. According to Lemieux (1991) these strains possess deficiency of proteolytic enzymes capable of hydrolyzing bitter primary breakdown products of cheese proteins. According to Fox et.al. (2004) more hydrofobic peptides were found in Cheddar made from pasteurized milk, which influence not only on bitterness but is a possible factor that could explain increased firmness. In each Krievijas cheese with inferior flavour *Lactobacillus (Lb.) curvatus* was detected, and from one cheese *Lb. plantarum* was also isolated. The concentration of *Lactobacillus spp.* in Krievijas cheeses varied from 4.69 to 5.39 log10 cfu ml^{-1}.

28 aroma compounds were identified in Krievijas cheeses. Identified groups included four organic acids (acetic, butanoic, hexanoic and octanoic acid), four alcohols (ethanol, 3-methyl butanol, 1-pentanol, 2-methyl-3-pentanol), three aldehydes (acetaldehyde, 3-methylbutanal, nonanal), four benzene derivatives (benzaldehyde, phenylacetaldehyde, naphthalene, toluene), two esters (ethylacetate, ethylbutyrate), two lactons (delta decalactone, delta-dodecalactone), seven ketones (2-propanone, 2-butanone, 2-pentanone, 2,3-butanedione, 2-heptanone, 2-butanone-3-hydroxy, 2-nonanone) and two sulphur compounds (trisulfide dimethyl, methional). The origin of the compounds does according to Alewijn M. (2006) imply on protein and fat catabolism without dominance of volatiles from any particular component. Naphthalene and toluene are not typical cheese compounds, but presumably originate from milk as contaminants.

In Holandes cheese the isolated NSLAB were *Lb. paracasei* subsp*. paracasei* and *Lb. rhamnosus* and the colony forming units enumerated in this sample was log 6.32 cfu ml^{-1}. 26 aroma compounds were identified in Holandes cheese. The identified range consisted of six organic acids (acetic, butanoic, hexanoic, octanoic, nonanoic and decanoic acid), three alcohols (ethanol, 3-methyl butanol, 2-methyl-3-pentanol), two aldehydes (acetaldehyde, nonanal), three benzene derivatives (benzaldehyde, phenylacetaldehyde, naphthalene), one ester (ethylbutyrate), two lactons (delta decalactone, delta-dodecalactone), seven ketones (2-propanone, 2-butanone, 2-pentanone, 2,3-butanedione, 2-heptanone, 2-butanone-3-hydroxy, 2-nonanone) and two sulphur compounds (trisulfide dimethyl, methional). Theoretical analysis of the origin of the identified compounds suggests that the majority of volatiles were derived from fat and protein catabolism.

Conclusions: Bitterness and acidity were the most common unpleasant flavours in Krievijas cheese. Naphthalene was an atypical cheese volatile with a tar flavour was found in both Krievijas and Holandes cheeses. Medium chain fatty acids were observed only in Holandes cheese.

Key words: API, SPME, GC-MS, Krievijas cheese, Holandes cheese

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Traditional Lithuanian Cottage Type Cheese: Factors Affecting Texture

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Lithuanian cottage type cheese – traditional cheese made for hundreds of years in Lithuania. This cheese is manufactured from cows' milk by coagulation with lactic acid bacteria to the isoelectric pH (4.6) of casein. This produces a curd that is cooked at 50 ^{o}C, put into triangular shaped cheese bag and pressed to remove whey. The most common defects of the texture of Lithuanian cottage type cheese are the following: crumbly/short; corky; mealy; pasty (sticks to the palate and fingers); weak (breaks down too quickly when cut by knife or taken from the bag). The aim of this study was to identify the main factors affecting the textural properties of Lithuanian cottage type cheese.

Cheeses of with different protein, fat and moisture content, as well as cheeses made from milk pre-treated by transglutaminase (TG) were the subject of this study. The textural properties of cheeses were analysed by quantitative creep and stress relaxation tests and force compression tests between parallel plates (Instron Universal Testing Machine). Texture profile test was used to determine fracture force, cohesion and gumminess of cheeses (TA.XT2 Texture Analyser).

The results showed the relationship between quantity and properties of individual constituents (proteins, fat and moisture) and textural properties of cheese. As the concentration of proteins in the cheese increased the product displayed greater elasticity and was more difficult to deform. TG induced cross-linking of proteins resulted firmer structure of cheese with higher σ_f than that made from milk without TG. The contribution of fat to the textural properties of Lithuanian cheese was shown to depend on the temperature. At low temperatures when milk fat was predominantly solid, high fat cheeses were more elastic than low fat cheese. At higher temperature (20-25 ^{o}C) E and σ_f were relevantly constant in the range of fat content 6-22 g/100g. The fracture strain was maximum at fat content 0 g/100g and then decreased sharply in the range of fat content - 13-22g/100g. The increase in moisture content of Lithuanian cheese decreased E and σ_f as well as the firmness of the product.

The milk treatment by TG resulted in markedly increased cheese yield due to the enhanced serum binding of the gel network stabilized by additional intermolecular bonds. Consequently, textural properties of Lithuanian cottage type cheese were found to be different from the control cheese produced without TG. Pre-treatment of milk by 2 U/1g proteins TG at 40 ^{o}C for 20-60 min prior pasteurisation resulted in cottage type cheese with lower fracture force, higher cohesion and lower gumminess.

The results of this study showed that the textural properties of Lithuanian cottage type cheese can be improved by combining the quantity and properties of the major constituents of cheese: proteins, fat and moisture.

Keywords: Lithuanian cottage type cheese, textural properties.


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