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BOOK OF ABSTRACTS

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Session I

Nutritional and Health Aspects / Bioactive Components

Cheese in Nutrition and Health

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Cheese has a long history in the human diet. Recent advances in nutrition science have highlighted the contribution of cheese to nutrition and health.
The types and amounts of cheese consumed are high in Europe, USA and Canada whereas in South American, African and Asian countries the significance of cheese in nutrition is rather low although increasing.
In ancient times, cheese was primarily a concentrated form of milk with the benefit of a prolonged shelf life. The high content of fat and protein in cheese made it an energy-rich and nutritious food that was adapted to our hard working ancestors.
In recent times, diet has been linked to various diseases like diabetes, obesity, cardiovascular disease, osteoporosis and cancer, and the focus of nutrition research has shifted towards specific food ingredients contributing to nutrition and health.
Cheese is a rich source of essential nutrients, in particular amino acids, bioactive peptides, fatty acids, vitamins and minerals.
The high concentration of essential amino acids in cheese contributes to growth and development.
There is evidence to suggest that two bioactive tripeptides VPP and IPP found in sour milk fermented with *L. helveticus* lower blood pressure. These peptides were also detected in specific cheese varieties in significant quantities.
Conjugated linolenic acid and sphingolipids present in cheese may have anticarcinogenic properties.
Despite the presence of a remarkable amount of saturated and trans-fatty acids, there is no clear evidence relating the consumption of cheese to any disease.
The high concentration of calcium in cheese is well known to contribute to the formation and maintenance of strong bones and teeth, but also shows a positive effect on blood pressure and helps in losing weight in the case of low-energy diets.
Cheese is an important dairy product and an integral part of a healthful diet thanks to its substantial contribution to human health.

Keywords: cheese, nutrition, health, proteins, bioactive peptides, TFA, SFA, CLA, sphingolipids, calcium

Application of Probiotic *Lactobacillus gasseri* K7 in Cheese Production

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A human isolate *Lb. gasseri* K7 produces bacteriocins with wide range of inhibition and has some other probiotic properties established *in vitro* and *in vivo*, such as inhibition of pathogens, inhibition of adhesion of *E. coli* and *S. aureus* and good survival in a pig GI. The possibility to apply K7 strain in cheese was first examined in a semi-hard cheese production. While attempts to produce cheese with *Lb. gasseri* as a single starter culture were unsuccessful, a probiotic cheese with good organoleptic properties was obtained in a combination with *Str. thermophilus*. The ability of *Lactobacillus* K7(Rif^r) to inhibit clostridia was studied in a semi-hard cheese artificially inoculated with *Cl. tyrobutyricum* (2.5 x 10^3 spores mL^{-1} of milk). The appearance of late blowing as well as the concentration of butyric acid was reduced in cheeses with added *Lb. gasseri* (Rif^r) (1.43 vs. 0.70 g kg^{-1} of 6-weeks old cheeses). The gassericin K7 A gene was detected by PCR in cheeses with added K7(Rif^r) strain as well as in the colonies grown on MRS agar with rifampicin (250 μg mL^{-1}). Although we failed to detect bacteriocins directly in cheese we suppose that they contributed to the antagonistic activity against clostridia, since pH and concentration of organic acids did not differ significantly in cheeses with or without addition of K7 strain. In addition, inhibition of mesophilic non-starter lactobacilli in K7(Rif^r) - supplemented cheeses might be explained by bacteriocin activity. Whether or not the gassericin K7 A genes are actually expressed *in situ* in cheeses, will be evaluated by quantitative Reverse Transcriptase PCR using gassericin K7 A specific oligonucleotide primers. We have already succeeded to optimise the Real-time PCR for quantification of gassericin K7 A genes in the total DNA of cheese samples with added K7 cells.

Keywords: *Lactobacillus gasseri,* bacteriocins, *Clostridium tyrobutyricum,* cheese

Some Factors Influencing Tyramine Content in Dutch-type Semi-hard Cheese

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Tyramine content was measured during ripening (until 176 days) in the core (C) and edge (E) samples of Dutch-type semi-hard cheese produced from pasteurized milk by two dairies (R, H) in two levels of fat content (30 and 45 %) using two different starter cultures (Y, L), respectively.

Tyramine content (y, mg kg^{-1}) increased ($P < 0.001$) with increasing time of ripening (x, days) in the cheeses of both producers (R: $y = 0.88x – 31.4$, $R^2 = 0.30$; H: $y = 0.50x – 6.3$, $R^2 = 0.18$), and its content was higher ($P < 0.01$) in E-samples in comparison with C-samples. Time of ripening, part of the cheese and starter culture accounted for ($P < 0.01$) 67, 28 and 4 % of explained variability of tyramine content in the cheese, respectively. Enterococci counts were significantly higher ($P < 0.01$) in E-samples than in C-samples, indicating secondary enterococci contamination from an ambient environment of each dairy during cheese production.
Based on the testing in the decarboxylase screening medium (DCM), tyrosine-decarboxylase positive lactic acid bacteria (LAB) isolates constituted 6 – 32 % of an aliquot of total LAB isolates from the cheeses of both producers; tyrosine-decarboxylase positive enterococci were present only in R-cheeses (4 – 26 % of an aliquot of total isolates). However, using the PCR method, tyrosine-decarboxylase gene sequence (*tyrdc)* was found only in 13 and 42 % of the DCM-positive LAB and enterococci isolates, respectively. Moreover, *tyrdc* was found in *Lactobacillus helveticus* strain, a component of the Y-culture. *L. curvatus* subsp. *curvatus* and *Enterococcus durans*, *E. faecalis* and *E. casseliflavus* were identified as *tyrdc*-positive LAB and enterococci in the cheeses, respectively.
Secondary contamination of Dutch-type ripening cheese with adventitious tyramine-producing bacteria during production seems unavoidable; PCR method is suitable for early detection of these bacteria.

Keywords: tyramine, tyrosine-decarboxylase gene, lactic acid bacteria, enterococci

Determination of Biogenic Amines in Different Cheese Varieties using Ultra Performance Liquid Chromatography (UPLC)

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Proteolysis is the principal and most complex biochemical event occurring during maturation of most cheese varieties, being of high significance for the development of texture and flavour of the final product. Furthermore, proteolysis plays an important role in the liberation of substrates for secondary catabolic changes (e.g., decarboxylation, deamination, transamination). Biogenic amines are produced in cheese by enzymatic decarboxylation of amino acids. High levels of biogenic amines can result in food poisoning, and cases of histamine intoxication have occurred subsequent to the consumption of cheese.
The objective of this study was to establish a pre-column derivatization method to determine biogenic amines in different cheese varieties. AccQ-Fluor derivatizing reagent (6-Aminoquinolyl-N-hydroxysuccinimidyl carbamate) was used to analyze primary and secondary biogenic amines by ultra performance liquid chromatography (UPLC™). A reliable method was developed for the separation of 19 biogenic amines in cheese within nine minutes.
Commercial cheese samples from retail outlets in Vienna were analyzed referring to their biogenic amine content using UPLC™. The biogenic amine content varied to a great extent, depending not only on the type of cheese (fresh, soft, semi-hard, hard, very hard), but also within a certain cheese variety. About 25 % of samples had a histamine or tyramine content above 100 mg kg^{-1}. The highest concentration of histamine was found in Tiroler Almkäse (1920 mg kg^{-1}) and Vorarlberger Bergkäse (657 mg kg^{-1}), the highest amount of tyramine was found in Cantal, Olmützer Quargel and Tiroler Almkäse (about 470 mg kg^{-1}). Moreover, 11 % of samples had a putrescine or cadaverine content higher than 100 mg kg^{-1}. The highest concentrations were detected in Olmützer Quargel (956 mg putrescine and 1282 mg cadaverine per kg). Thus, the total concentration of biogenic amines in some samples was about 2800 mg kg^{-1}.
In conclusion, the fast and reliable UPLC™ method to determine biogenic amines in cheese represents a valuable tool to ensure quality of dairy products in future.

Keywords: biogenic amines, cheese, UPLC, 6-Aminoquinolyl-N-hydroxysuccinimidyl carbamate

Effect of Ripening on the Fate of Some Pathogenic Bacteria in PDO Pecorino Romano Cheese

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Pecorino Romano is an Italian Protected Designation of Origin (PDO) cheese. It's specifications establish that this cheese must be made from raw or thermized whole sheep milk and ripened at least for 5 months. This cheese, largely exported to the USA and Canada (about 20'000 tons per year), is the most popular ovine cheese produced in Italy. The safety of the cheese made with non-pasteurized milk is discussed by the health authorities of several countries (USA, Australia, etc.).
The aim of this study was to investigate about the ability of some pathogenic bacteria (*Salmonella* spp, *Listeria monocytogenes*, *Staphylococcus* coagulase + and *Escherichia coli* O157:H7) to grow and to survive during the manufacture and the ripening of PDO Pecorino Romano cheese.
A total of 12 cheese making trials (3 replicates x 4 pathogens) were performed with raw milk inoculated with the pathogenic test strains to obtain about 10^6 CFU mL^{-1} in milk vat. Cheese composition and pathogens presence were evaluated in inoculated milk, in cheese after 1, 90 and 150 days of ripening.
Physico-chemical parameters of cheeses (pH, moisture, A_w and salt content) were within the range of the commercial Pecorino Romano cheese. Consequently, the behavior of the studied pathogens in experimental cheese can be extended to the commercial one. After 90 days of ripening, long before the commercial ripening, all cheeses were free from the inoculated pathogens.
In conclusion, when Pecorino Romano is produced under PDO specifications, the effect of a fast acidification together with an intense dry salting and a long ripening time preclude the possibility of growth and survival of the tested pathogens.

Keywords: ram milk cheese, Pecorino Romano, pathogenic bacteria, cheese safety

Session II

Influence of Milk Production Conditions on Milk and Cheese Quality

Cows' Feeding and Milk and Dairy Products Nutritional and Sensory Properties: A Review

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This review summarises the recent knowledge established on the relationships between the diet of animals and the sensory and nutritional quality of cattle milk and dairy products. It starts by a rapid overview of the digestive and metabolic pathways involved in the secretion of milk components that play a major role on milk and dairy products nutritional and/or sensory properties. A specific attention is given to fatty acids (FA) composition, carotenoid, retinol and tocopherol content as well as plant secondary metabolites like terpenoids and phenolic compounds and milk endogenous enzymes. The literature data confirms the wide plasticity of these milk components related to cows' nutritional factors. Feeding dairy cattle with pastured grass by comparison with diets based on concentrate or maize silage leads to more yellow and softer cheese and butter because of the respective increase in β-carotene and unsaturated FA like oleic, vaccenic and to a lesser extend linolenic and rumenic acids, to the detriment of the 10 to 18 carbons saturated FA. The raw milk cheeses issued from pasture are also generally characterised by their stronger flavour but this effect seems to be cancelled when the milk is previously pasteurised. Within the grass based diets, major differences in sensory and nutritional characteristics of milk and derived products are also observed according to the preservation of the grass (pastured vs conserved). Conversely, the influence of the grass preservation mode concerns mainly the dairy products yellow colour and carotene content (higher when grass is preserved as silage, by comparison to hay) and also the cheese flavour in the case of large size cheese models. Several recent experiments showed a significant effect of grass botanical composition mainly on milk FA composition and on cheese texture and flavour. In addition, dietary supplements of plant oil or oilseeds, proposed to increase the nutritional value of dairy fat, have effects similar to pastured grass on FA composition and dairy products texture, even sometimes more marked, but they simultaneously increase other trans isomers of 18:1 and 18:2 and sometimes are responsible for off-flavours in milk or cheese because of unsaturated FA oxidation. The off-favour formation seems to vary according to the lipid nature and presentation (oil or oilseeds) and dietary antioxidants.

Keywords: Cows' Feeding, Milk, Dairy Products, Nutrition, Sensory Properties

Seasonal Changes of Volatile Profiles of Bitto PDO Cheese

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Bitto is a P.D.O. raw-milk cheese that has remote origin rooted in the Alpine area of Valtellina (Italy) where it is produced in the period June 1st - September 30th. Presence of extensive pastures in this area causes the seasonal transhumance of herds from intermediate elevations to the highest ones in the late spring and summer. To date, no studies have been carried out on the volatile profile of Bitto cheese as affected by changing of grazing pasture during transhumance of cows.
In this study, 25 Italian Brown cows were fed grazing from May to October 2006 and moved from 1200 to 1400, 2000 till 2200 meter altitude (and *vice versa*). The botanical composition of pasture was determined as well as the volatile profile of pasture and fat from milk and the derived 70-d ripened Bitto cheese. Desorbed volatiles were measured by P&T-GC-MS.
Terpene composition of milk and cheese samples varied dramatically depending on the botanical composition of pastures. As expected, the most complex terpene profile was recorded in milk and related cheese produced at the highest locations and presence of δ-3-carene was positively related to grazing altitude. In general, terpene profile of cheeses reflected those of the original milks. Volatiles derived from cheese ripening (acids, esters, alcohols, ketones) mainly varied as a consequence of the variability of milk characteristics. On these bases, it was not possible to point out a characteristic terpene profile for Bitto cheese as well as to define a specific "*bouquet*". In this regard, obtained data confirm how noticeable is the influence of mountain farming conditions on cheese volatile profile which, therefore, can represent a marker of the type of cow feeding rather than a tracer of the manufacturing area.

Keywords: Bitto cheese, volatile profile, alpine pasture, GC-MS

Interactions between Bacteria Type of Intramammary Infection and Physico-Chemical Properties of Cheese

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Clinical mastitis, although causing direct economic loss to farmers, does not affect the quality of the bulk milk and thereafter cheese, since those animals are milked separately. In contrast, milk from sub-clinically infected udders is milked into the bulk milk tank, thus having a direct influence on the milk quality for industrial uses.
Comparing milk from sub-clinically glands infected by one of the udder pathogens: *Staphylococcus aureus, Escherichia coli, Streptococcus dysgalactiae* and *Streptococcus chromogenes* causing similar somatic cell counts and similar cell distribution resulted in significant differences in milk quality and curd yield.
Clotting time in milk coming from infected glands was longer (1294 vs. 3663 sec) and curd firmness measured by Optigraph (A30) was lower (10.38 vs. 2.26 V). No differences were found in total fat, protein and casein between the control and infected milk. Moreover, in the first two weeks of ripening, 2-3 times more whey drained from the cheese of the infected quarters, resulting in ~ 4 % less curd. The strength of the cheese as determined by the TA XT2 Texture analyzer was significantly higher in the first 2 months and significantly lower at 4 months in the control cheese vs. cheese from the infected quarters.
In parallel, size-fractionation of the milk's proteose-peptone was achieved by FPLC Gel Filtration. Lyophilized fractions were added to fresh bacteria-free milk, which resulted in a significant negative effect of the lower molecular weight fraction that increased clotting time by 3-5 times in infected milk.
The reported results suggest that there is a direct influence of bacterial enzyme/s and/or the host immune system response to the various bacteria that cause sub-clinical mastitis, which has a detrimental effect on the quality and yield of cheese made from that milk, with no relation to the effect on the milk itself, i.e., increased somatic cell count and fat and casein content of the milk.

Keywords: mastitis, cheese yield

Proteolysis and Glycolysis in Model Portuguese Cheeses, Manufactured from Various Milk Sources Throughout Ripening

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Portuguese traditional cheeses are usually manufactured from raw whole milk, which brings an indigenous microflora – mostly lactic acid bacteria (LAB), to play a role in the cheesemaking process. This fact constitutes a source of variability of the final organoleptic as well as the overall quality of the product, because of a number of poorly uncontrolled changes promoted thereby. Hence, standardization of manufacturing practices, along with use of well-defined (yet specific) starter cultures are in order. Addition of tailor-made starter cultures aids in uniformity in all stages of manufacture and ripening via mainly conversion of lactose to lactic acid and breakdown of caseins into flavour precursors, thus contributing to unique characteristics of each traditional cheese.

Hence, the main objective of this research work was to evaluate glycolysis and proteolysis in Portuguese cheeses – via development of a food model system that mimics traditional cheesemaking practices as far as possible. Toward this purpose, sterile cheeses were produced from sterilized ewe's, goat's and cow's milk, using either animal or plant rennet, and following the conventional procedure: strain inoculation, addition of rennet, incubation at 30ºC for clotting, cutting and removal of whey. Inoculation was carried out with strains previously isolated from traditional Portuguese cheeses: *Lactobacillus plantarum*, *Lactobacillus brevis* and *Lactococcus lactis*, either as single or as mixed culture of lactococci and lactobacilli. Cheeses were then kept up to 60 d at 7ºC – in attempts to simulate the typical cheese ripening environment. Glycolysis and proteolysis of the various model cheeses were then assessed throughout ripening. The evolution in content of organic acids and sugars, as well as in ripening extension and depth indices throughout time was somewhat dependent on the various experimental conditions tested – but distinct patterns were actually observed. *Lactococcus lactis* exhibited the fastest acidification capacity among strains, irrespective of milk type.

Keywords: lactic acid bacteria; casein breakdown; organic acids; ewe's, goat's and cow's milk, cheese

Session III

Starter and Ripening Organisms

Cheese Cultures: From just Acidification to Complex Functionalities

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The many activities of starter and ripening cultures are central for quality, safety, and potential health benefits of cheese. Lactic acid bacteria are currently almost always added to cheese milk to produce lactic acid from lactose and contribute to biochemical changes during ripening and develop the characteristic attributes of cheese. On the other hand a large variety of other microorganisms originating from many sources (milk, equipment, ripening rooms, human) may participate in the ripening of cheese, to produce organoleptic and biochemical changes from within or on cheese surface, but also impact cheese safety. Due to general improvements in the hygienic quality milk, application of heat and tight controls of the processing environment, natural inoculation of cheese is more difficult to achieve and control for optimum cheese quality. Therefore a wealth of research has been done recently to better understand the specificities and many effects of complex cheese microbiota and the roles of leading organisms on cheese quality and safety, in the aim to develop biodiversity, and improve composition and functionality of ripening cultures.
The latest scientific findings on characterization, preparation and handling of secondary cultures with important activities during cheese ripening will be presented. Furthermore cultures with new functionalities that can support and complement more conventional cheese cultures will be discussed. Particular focus will be placed on antimicrobial properties for the development of protective cultures to prevent growth of contaminants and pathogens in high risk cheeses Furthermore the potential of probiotic cultures for functional cheese production, and the impact of cheese microorganisms and their metabolic products on other health benefits of cheese will be addressed. Finally the prospect for innovative technologies to model, study and produce complex cheese cultures at industrial scale will be discussed, with particular reference to cell immobilization technology.

Keywords: ripening culture, cheese ecology, technology, functionality

The Variability of *Lactobacillus delbrueckii* Growth Dynamics During Cheese-Making Generated a High Diversity within Swiss-Type Cheeses

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This study investigates the growth dynamics of *Lactobacillus delbrueckii,* a thermophilic lactic acid species used in the manufacture of numerous cheese varieties. Dynamics were studied during the moulding of Swiss-type cheeses manufactured from clean raw milk inoculated with different thermophilic starter species.
24 cheeses – 18 different combinations of starters and 6 replicates – were manufactured in similar and controlled conditions. The cheeses were inoculated with one strain of *Streptococcus thermophilus* – added at a low or a high level, *Lactobacillus helveticus* – strain 1, 2 or not, and *Lactobacillus delbrueckii* – strain 1, 2 or not. The strains 1 and 2 differed by their acidifying and proteolytic potentials evaluated in milk (high for both strains 1). The results showed a great variability of *L. delbrueckii* growth dynamics during cheese-making. We were able to relate statistically the growth dynamics of *L. delbrueckii* with some variables linked to the growth and metabolism of the other thermophilic bacteria. We also highlighted that different growth dynamics of *L. delbrueckii* generated different characteristics in the 1-day-old and ripened cheeses. Finally, we observed that the acidifying activity of the strain *L. delbrueckii* 2 in cheese was not predictable according to its potential evaluated in milk.
During the moulding of Swiss-type cheeses, the growth dynamics of *L. delbrueckii* were influenced by the other thermophilic bacteria, which led to think that some interactions took place between some species of thermophilic lactic acid bacteria. These interactions and resulting *L. delbrueckii* growth dynamics have been found determinant for the characteristics of the ripened cheeses.
Interactions between thermophilic bacteria have been pointed out. Like *L. delbrueckii*, growth dynamics of other thermophilic lactic acid bacteria could generate variability in ripened cheese, so these bacteria could be used as a mean to maintain diversity within Swiss-type cheeses.

Keywords: Swiss-type cheese, *Lactobacillus delbrueckii*, thermophilic lactic acid bacteria, growth dynamics.

Caseinolytic Activity of *Lactobacillus helveticus* Studied in a Cheese Model

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Lactobacillus helveticus are widely used as adjunct cultures in different kinds of cheeses to accelerate ripening and enhance aroma production. They are chosen because of their high and broad intracellular peptidolytic activities. However, a too high extracellular proteolytic activity has the potential to produce bitter tasting peptides that may accumulate in cheese. To date proteolytic activity of *Lb. helveticus* have in many cases been assessed using synthetic chromogenic substrates such as succinyl-Ala-Ala-Pro-Phe-*p*Na. However, we found that this substrate is probably more specific towards intracellular endopeptides than extracellular proteinases since the activity is inhibited by EDTA and not by a serine protease inhibitor. At present there are no reliable method to analyse proteolytic activity in *Lb. helveticus* without interference from other enzyme activities.
We decided to evaluate the proteolytic effect of six different *Lb. helveticus* cultures in a cheese model. The *Lb. helveticus* cultures were cultivated in milk or MRS prior to inoculation to study the influence of the growth media on proteolysis. A two week old cheese with dry-matter adjusted to 40 % was melted and upon cooling inoculated with the different *Lb. helveticus* cultures. After two and four weeks of incubation at 20°C protein and peptide profiles were analysed using CE and HPLC respectively.
One new peak appears in all CE chromatograms though most obvious when the cultures had been pre-grown in MRS. All cultures were able to degrade peptides over time as revealed by HPLC with the extent of degradation being strain dependent. Pre-cultivation in milk gave rise to a lower peptidolytic activity compared to pre-cultivation in MRS, indicating less lysis of these cells in the cheese model.

Keywords: *Lactobacillus helveticus,* Proteolysis, Cheese Model

Monitoring the Composition and Succession of Smear Cheese Surface Microflora During Ripening

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The ripening of smear cheese is characterized by the succession of populations of bacteria, yeast and sometimes also moulds on the cheese surface. The dynamics of this complex microbial bioceonosis plays an important role in the development of sensorial as well as hygienic characteristics of the cheese.
16S rRNA gene based terminal restriction fragment length polymorphism (tRFLP) fingerprinting was performed to generate culture-independent community fingerprints for monitoring succession of bacterial populations on the cheese surface during ripening. In parallel pure cultures were isolated from the same samples and identified by 16S rRNA gene sequencing. *In silico* restriction analysis was performed to achieve the assignment of the detected tRFs to certain bacterial genera. Furthermore, 16S rDNA sequence analysis of pure cultures allowed the identification of typical cheese surface bacteria like members of *Arthrobacter*, *Corynebacterium* or *Staphylococcus*. However, although rather unusual for cheese gram-negative bacteria like *Marinomonas* spp. or *Psychrobacter* spp. were also isolated frequently. On the basis of their 16S rDNA sequences the isolated pure cultures could be assigned to the respective tRFs, thus enabling monitoring of the bacterial succession during cheese ripening.
Succession of yeasts and moulds was investigated by amplification and subsequent fragment length analysis of the ITS-2 region within rRNA operons as well as by isolation and identification of pure cultures. A thorough identification of the detected yeasts and moulds was hampered by insufficient resolution within the ITS-2 region. But on the basis of ITS-2 fragment length analysis we could demonstrate a succession of yeasts and moulds which was in line with the macroscopic observation of moulds during ripening.

Keywords: smear cheese, microbial succession, yeast, mould, bacteria, tRFLP

Advances in Cheese Surface Starter Development

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For more than 10 years cheese surface smear cultures are being studied by a BFEL working group in Kiel. The aim was to replace old-young smearing by the use of defined smear cultures in order to reduce the contamination of smear cheeses with moulds, enterobacteria, enterococci and pseudomonads. For semi-hard cheese, such as Tilsit, 5 species are essential: *Debaryomyces hansenii, Staphylococcus equorum, Brevibacterium linens, Microbacterium gubbeenense* (or Arthrobacter nicotianae) and *Corynebacterium casei*. Smeared soft cheese requires *Geotrichum candidum* additionally, *C. casei* is of lesser importance. Acid curd cheese requires different yeasts, *Kluyveromyces marxianus* and *Candida krusei*. The validity of these concepts was confirmed for semi-hard cheese in a demonstration project funded by the EU. Green cheeses provided by industrial partners developed typical aroma and a surface flora consisting essentially of culture strains. For one cheese type the choice of the *B. linens* strain was important. This showed that "production secrets" might contribute more to the final properties of smear cheese than generally expected. The definition of acid curd cheese cultures was also confirmed at pilot scale in a project funded by a German industrial partner.
Further improvement of cultures concentrates on studies of anti-listerial activities of yeasts and staphylococci, which are early ripening microorganisms and thus are ideal candidates for better protection of cheese from contamination with *Listeria monocytogenes*. Recently *S. equorum, C. krusei* and *Pichia norvegensis* strains were isolated which reduced *L. monocytogenes* counts in model systems by 4 - 7 magnitudes. The protective potential of these strains is currently investigated using experimental semi-soft, soft- and acid curd cheeses artificially contaminated with L. monocytogenes.
Another focus of the current work on surface cultures is the nature of the colour development (red, orange, yellow, brown and pink colours) on the surface of different smear cheese varieties. New results on interactions between pigmented coryneforms, pigmented staphylococci, yeasts, moulds and bacterial contaminants (enterococci, enterobacteria and pseudomonads), which are currently obtained in a cheese agar model system, are presented.

Keywords: surface culture, anti listeria culture

Contribution of *Lactococcus lactis* and *Streptococcus thermophilus* to Cheddar Cheese Ripening : A Proteomic Approach

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Lactococcus lactis is the traditional starter culture for Cheddar cheese manufacture. However, addition of *Streptococcus thermophilus* has recently been included in the starter mix to counter possible lactococcal phage attack. We have recently shown that inclusion of low levels of *St. thermophilus* in the starter mix or the use of *St. thermophilus* as a single starter alters the secondary non-starter flora and impacts on flavour development. Elucidation of the complex interactions which occur throughout ripening between individual strains of the Cheddar cheese flora, through the release of numerous proteins, including various enzymes into the cheese when cells die and lyse would greatly enhance our understanding of Cheddar cheese ripening process. The aim of this study was to obtain a proteomic view of the different groups of proteins released in Cheddar cheese at 1 day, 1, 2, 4 and 6 months of ripening by *L. lactis* subsp. *lactis* 303 and an industrial strain of *St. thermophilus* TH4 used as single starters or in combination. The aqueous phase of Cheddar cheese at each time point was prefractionated by size-exclusion chromatography and gel filtration to separate bacterial and milk proteins before separation by 2D-PAGE. Two hundred individual proteins were excised from gels, digested with trypsin and characterised by tandem mass spectrometry (MALDI-Q-TOF) prior to sequence homology database search. Comparison of the proteins involved in stress response, glycolysis, and proteolysis detected as a function of time and cheese microflora will be discussed. Data regarding the origin and nature of the enzymes released into the cheese arising from the different starters and their possible effect on flavour development measured in the same cheeses will also be discussed.

Keywords: Cheddar cheese, starter culture

The Use of PCR-DGGE as a Culture-Independent Approach to Study the Diversity of Lactic Acid Bacteria in Flemish Artisan Raw-Milk Gouda-Type Cheeses

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PCR-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) is a molecular fingerprinting technique that relies on the sequence-based separation of PCR-amplified fragments of the 16S rRNA or other genes. It already demonstrated to be successful in assessing the diversity and dynamics of bacterial populations in many areas of microbial ecology, including food microbiology. In this study, PCR-DGGE was evaluated as a culture-independent alternative for conventional culture methods to study the diversity of lactic acid bacteria (LAB) in two Flemish artisan raw milk Gouda-type cheeses ripened for 8 and 12 weeks. In parallel, conventional isolation was performed using four different selective media and appropriate incubation conditions, the isolates being identified using $(GTG)_5$-PCR DNA-fingerprinting and sequence analysis of the 16S rRNA and *pheS* genes in case $(GTG)_5$-PCR did not provide a (reliable) identification result. In addition, analysis of DGGE profiles of bulks of cells, obtained by harvesting the colonies from the 10^{-1} dilution from different media, was performed to profile the cultivable community.
Both PCR-DGGE and conventional culturing revealed small differences in overall species diversity between the two cheeses and batches, highlighting the typical and unique characteristics of artisan products. Diversity of 8 and 12 week-old cheeses was relatively similar. Based on conventional isolation, samples were mainly found to contain members of *Lactococcus lactis* subsp. *lactis*, *Lactobacillus paracasei*, *Lb. plantarum*, *Lb. brevis*, *Lb. rhamnosus* and *Pediococcus pentosaceus*. In addition, occasionally *Lb. curvatus*, *Lb. perolens*, *Weissella* sp. and *Streptococcus* sp. were found. Additionally, DGGE on total cheese DNA and on the cultivable community revealed the presence of *Lb. parabuchneri*, *Lb. gallinarum* and *Enterococcus faecalis*. Our results also indicate that some isolated species could not be retrieved by PCR-DGGE. Consequently, we recommend the integrated use of culture-dependent (by means of cultivable community analysis) and culture-independent approaches to study the diversity of LAB in Gouda-type cheeses.

Keywords: PCR-DGGE, Gouda, culture-independent, community analysis

Session IV

"Omics" Techniques

Use of 'Omic' Approaches to Study Cheese Manufacture and Ripening

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In recent years, use of the suffix 'omics' has become common in biological science. Its use implies that the totality of a biological system is being studied. The most common use of the suffix is in genomics where it pertains to studies of complete genomes. While there has been a very rapid expansion in the availability of complete genomes, the genome sequence on its own does not provide an exhaustive knowledge of a particular organism or biological system. However, the genome sequence provides the basis, on which a range of related omic disciplines, including transcriptomics, proteomics, metabolomics become possible. The technology that has driven these new areas includes high throughput DNA sequencing, DNA and protein microarrays and mass spectrometry. The rapid accumulation of data has required parallel growth in the field of bioinformatics to facilitate data analysis and interpretation.

Cheese can be regarded as a biological system composed of milk (protein, fat and lactose), exogenous enzymes (rennet), a range of microorganisms and their metabolic by-products. These components interact during manufacture and ripening to produce the variety of cheeses that differ in terms of key quality attributes that are available on the market today. Traditionally, cheese research has tended to focus on the impact of individual process variables or particular biochemical pathways on product quality. The opportunity now exists to move beyond this approach and embrace the holistic methodologies provided by omic technologies. Complete genome sequences of the cow, sheep and goat are becoming available and these are being further distilled to define the 'milk genome'. Genomes of the primary cheese starter cultures, including *Lactococcus lactis, Streptococcus thermophilus* and more recently *Lactobacillus helveticus* are publicly available. Methods to study the cheese proteome have been developed and mass spectrometer based techniques are being applied to defining its metabolome. It is expected that such approaches will facilitate a greater understanding of the biological processes that occur during cheese manufacture and ripening. They will assist in starter and adjunct strain selection, identification of key enzymes involved in cheese manufacture and ripening and will facilitate a full definition of the compounds that provide the taste and nutrition value associated with cheese.

Keywords: cheese, genomics, proteomics, metabolomics

Proteome and Metabolic Analysis of *Lactococcus lactis* in a Cheese Model during Ripening

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Lactococcus lactis, commonly used as a starter bacterium in cheese manufacture, is considered as a model among lactic acid bacteria. The availability of three complete genome sequences of *L. lactis* and several lactococcal plasmids enables the development of post-genomic studies. Proteomic and transcriptomic approaches have already been used to study gene and protein expression in different stress conditions encountered during cheese-making process. However, these studies have generally been carried out in liquid media (milk or synthetic media) and with a laboratory strain.
The aim of the present work is to characterize the functional diversity and the behaviour of *L. lactis* in cheese during ripening. To do that, we combined proteome and metabolic analysis with three genetically different strains of *L. lactis* in UF-cheese model (made with ultrafiltrated milk) containing fat, after 1 and 7 days of ripening at 12°C. Enzyme activities and metabolites involved in cheese flavour and texture development were particularly investigated.
We developed a rapid methodology to recover bacterial cells from cheese to avoid changes in proteome during cell preparation. Cell extracts were prepared from the bacteria and successfully analysed by 2D gel electrophoresis. Cytoplasmic proteins separated in a 4-7 pH gradient were identified by mass spectrometry and quantified. Enzyme activities were also measured in the same cells after lysis either with lysozyme or by mechanic disruption according to the different enzyme stabilities. To complete the analysis, metabolites produced in cheese will be analyzed by both HPLC and GC-MS.
This global approach will undoubtedly provide new insights for understanding lactic acid bacteria behaviour in cheese and to establish relationships between genome, proteome, enzymatic activities and metabolite production.

Keywords: *Lactococcus lactis*, proteomic, metabolomic, cheese

Naturally Occurring Genetic Markers in Bacteria and their Use for Authentication of Emmentaler PDO Cheese

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A general method for the detection and identification of specific strains of bacteria is described. The assay is based on the observation that insertion sequences (IS) in different strains of bacteria occur at diverse loci on the bacterial genome. Exclusive PCR primers can be selected for a particular strain where one of the primers is specific for a particular IS element and the other is specific for the adjacent DNA sequence in the genome. Only bacterial strains containing the IS element at the particular point on the genome will yield an amplicon of the correct size after PCR. We have illustrated this method by selecting primers for the detection of bacteria used in the manufacture of Swiss Emmentaler PDO cheese. Using this method we were able to differentiate Emmentaler cheese manufactured in Switzerland from Swiss type cheeses made in other European countries.

Keywords: Genotyping, insertion sequences, PCR, lactic acid bacteria, facultatively heterofermentative lactobacilli, emmental cheese, authenticity, geographic origin

Whole Genome Expression of *Lactococcus lactis* in Cheese

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The exploration of bacterial expression *in situ* in cheese is essential to understand ripening process, limiting factors, and general ecosystems dynamic. New performant methods of total RNA extraction from dairy products have been recently developped (Ulvé et al., submitted; Monnet et al., submitted). The aim of this study was to explore the overall expression of *L. lactis*, in a model experimental cheese: i) during growth in cheese from 10^7 to 10^9 cfu g^{-1}; ii) with or without the presence of fat, and iii) as function of the cell distribution (number of colonies for the same total biomass).
L. lactis LD61 (10^7 cfu mL^{-1}) was inoculated in a cheese made from ultrafiltration retentate prepared from microfiltrated milk (i.e. with less than 10 cfu mL^{-1} of bacteria) and incubated at 30°C. Rennet was added at pH 5.2 and the curd was stored for several weeks at 12°C. Samples of cheese were collected at 2 h, 8 h, 24 h and 7 days. Total RNA was extracted as described in Ulvé et al.. Quantity and quality of the RNA extract were checked before analysis by microarrays. In parallel, the proteom of the cells extracted from the matrix was established at 8 h, 24 h and 7 days.
Preliminary results of transcriptomic and proteomic analyses indicate that the presence of fat , as well as bacterial cell distribution, can modify the *in situ* gene expression of *L. lactis* LD 61.

Keywords: genome expression, cheese, *Lactococcus lactis*

Session V

Flavour Development Measured with Sensory and Instrumental Methods

Cheese Flavour Measured with Sensory and Instrumental Methods: A Review

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The state of the art and current trends in the flavour and sensory analysis are reviewed. This review examines the applications of sensory and instrumental methods in cheese analysis. A brief history of the development of sensory analysis is included and this is illustrated by descriptions of the different types of techniques utilized in cheese testing. Most of the instrumental methods to extract (SPME, SD, Purge and Trap, RAS, etc..) and test (GCO, MS,) flavour content in cheese are also illustrated. Applications described include identification and classification of flavour and aroma and other measurements of quality of cheese using MS-based Electronic Nose. Advantage and weakness of the different techniques are reported. As pattern recognition techniques are widely used to analyze the data obtained from these multi techniques, a discussion on principal components analysis and correlation between human and instrumental potentiality is essential. The present review includes, also, as examples combined study using sensory and instrumental methods of some traditional Sicilian cheeses: the evaluation of Ragusano PDO cheese aroma, the influence of native pasture in the diet of cow's milk used to produce different Ragusano cheese, the classification of Piacentinu cheese produced with traditional and industrial tools as well as with natural and commercial saffron and produced with raw and pasteurized milk, the identification of different quality correlated to a different production area (Cru Area) for Sicilian Pecorino PDO cheese.

Keywords: cheese, flavour, aroma, sensory, instrumental methods, GC, MS, olfactometry,

Flavour Production of Stilton Blue Cheese Microflora

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Stilton is an internally mould-ripened semi-soft blue cheese variety granted the status of a protected designation origin (PDO) by the European Commission. The microflora and aroma profile in Stilton was studied regarding its three main parts: blue veins, white core and outer crust. Increased emphasis was placed on the yeasts because of their presence in the blue cheeses without being part of the starter flora. Numerous studies have investigated the role of the yeasts in other blue cheeses to determine whether they should be used as part of the starter culture or not. Several yeasts isolated from the blue cheeses have strong lipolytic and proteolytic properties suggesting they may enhance maturation. They were also found responsible for inhibition of the sporulation of *Penicillium roqueforti* and potentially reducing the formation of blue veins.
Yeasts from the three different parts of the cheese were isolated and identified by using and comparing a series of molecular techniques. The flavour profiles of the three cheese parts were analyzed by Atmospheric Pressure Chemical Ionisation (APCI) and Gas Chromatography Mass Spectrometry (GC-MS). There was a clear spatial distribution of the yeasts and flavour compounds in Stilton cheese. Yeast combinations with the starter *P. roqueforti* were grown outside the cheese matrix and were studied regarding the microbial growth interactions and flavour production. Principal Component Analysis (PCA) was used to present and plot the relation of the aromas within the three regions and the different growth combinations.
Yeast communities in the three sections of Stilton consisted of combinations of five species common in blue cheeses. The three parts showed distinct flavour profiles suggesting each section contributes a particular character to the final aroma. Analysis showed a strong correlation of the flavour compounds produced in different sections of the cheese and yeast/mould combinations studied. The approach followed in this study allows for insight into the interactions between the microbial communities that might take place locally and form these flavour profiles.

Keywords: blue cheeses, dairy yeasts, flavour, interactions

Development of an Integrated Low-Cost Fiber Optic Based Spectrofluorimeter for Measuring the Quality of Cheeses

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Rapid screening techniques to determine quality characteristics of cheeses are of great interest for both industry and consumers. The dairy industry, like the food processing industry in general, has come under increasing pressure to deliver products of high and constant quality into the market place. The chemical determination of cheeses is a very important task, which is classically undertaken by different physico-chemical methods to determine pH-value, fat and calcium contents, nitrogen fractions, etc. These methods require trained staff, multi-step procedures of sample processing, and are not suitable for large-scale screenings.
Nowadays, there is a need for the cheese processing industry to have tools available for real time control of production lines to check whether in-process material, during a given processing step, meets the necessary compositional or functional specifications to reach a predetermined quality standard in the final product. In this context, fluorescence spectroscopy could be considered as fast, relatively low-cost and provide a great deal of information with only one test. It is sensitive, non-destructive, rapid, environmentally friendly and non-invasive, making it suitable for on-line or at-line process control and appropriate for process control.
Nevertheless, all the studies demonstrating the ability of fluorescence spectroscopy for characterizing cheese quality were, so far, used with a standard laboratory-based spectrofluorimeter. Up-to date, no portable instrument is available on the market. Recently, we have developed an integrated and portable spectrofluorimeter comprising an integrated PC with a touch-screen for recording and evaluation of data, a light emitting diode (LED) for excitation, a spectrometer and a fiber optic. The accuracy and effectiveness of this portable spectrofluorimeter to predict some physico-chemial parameters of 12 French Saint Nectaire cheeses belonging to four brands was assessed by using partial least squares (PLS) regression. Simple-to-use, this portable fluorimeter provides a fast and accurate analysis in 1 sec, while 2 min was needed to acquire the same spectrum using the standard laboratory-based spectrofluorimeter.

Keywords: cheese, Physico-chemical, portable spectrofluorimeter, patial least squares regression

Identification of Peptides and Amino Acids in Cheddar Cheese with Savoury Flavour

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Amino acids and low molecular weight peptides are significant to Cheddar cheese flavour and contribute specifically to taste, but their precise role remains unclear. Degradation of caseins by proteases and peptidases during ripening leads to the formation of these important water-soluble taste contributors. A good balance between proteolysis and peptidolysis is known to prevent development of bitterness and ensure formation of the background flavour of Cheddar cheese towards a desirable brothy and savoury flavour.
Umami, the Japanese word for savoury or delicious, is an important sensory taste attribute for mature Cheddar cheeses because of the naturally high content of glutamic acid. However, there are most likely more components in Cheddar cheese that contribute to this sensation. Hydrophilic di- and tri-peptides, in particular those containing glutamic acid and other hydrophilic amino acids have been described as umami- and savoury-like. These compounds occur in Cheddar cheese but limited work has been done on identification and their taste properties are contradictory discussed in the literature. Besides peptides and amino acids, organic acids and salts have also been identified as potent taste compounds or taste enhancers in cheese.
Current research is focussed on the identification of peptides and amino acids in the complex water-soluble extract of commercially available mature Cheddar cheeses selected for being described as savoury. The water-soluble extract from the cheeses were fractionated by preparative gel permeation chromatography, and a tentative sensory evaluation revealed selected fractions with savoury taste properties. The content of amino acids and peptides were analysed using different HPLC techniques and hydrophilic peptides were especially abundant in the key savoury fractions. Further fractionation and identification of these peptides are planned in near future. Such detailed knowledge on the peptide and amino acid composition of savoury cheese fractions is important for product development of cheeses with a selected taste profile.

Keywords: Savoury flavour, Peptide profile, Amino acids, Cheddar

Session VI

Characeristics of Traditional, Regional Cheese Varieties

Characteristics of Traditional Regional Cheese Varieties of East-Mediterranean Countries

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Traditional cheeses represent a heritage and are the result of accumulated empirical knowledge passed on from generation to generation. The pedoclimatic conditions in most parts of East-Mediterranean countries (Balkans, Turkey, Near-East) are characterized by relatively small and irregular precipitations, hot and dry summers and a largely hilly terrain. Such environmental conditions are not very favourable for cattle but suitable for sheep and goat. Thus, the majority of traditional cheeses in these countries were - and most of them still are - made from the milk of these two animal species.

The relatively high ambient temperature, the lack of refrigeration facilities and the fact that most of the cheeses were produced in family enterprises or in small artisanal units led the cheese market to be dominated (> 50 %) by "white brined cheeses" (WBC), which are ripened and stored under brine until consumption (e.g. Feta). WBC have no rind, no gas holes and are semi-soft to semi-hard with an acidic (pH ~ 4.5), salty and, some of them, piquant flavour. To improve keeping quality, the drained curd of some WBC is additionally scalded at very high temperatures between 80 and 100°C (e.g. Halloumi).

Traditional cheeses of the region include also pasta filata semi-hard cheeses (e.g. Kashkaval), the curd of which after draining and a usually natural acidification (pH ~ 5.2) is scalded and kneaded at ~ 75°C. They usually have a flat-cylindrical shape, no holes, straw-yellow colour (light yellow when made from cow's milk). Some hard cheeses (e.g. Kefalotyri, Ras, Mihalic) are also traditionally made in the region and are characterised by salty, piquant flavour and good frying properties.

Whey cheese production (e.g. Myzithra, Manouri, Lor, Anari) was developed very early in this area, since the whey from sheep and goat milk cheese-making is very rich in protein. The yield can be improved if milk of these small ruminants and/or cream is added to the whey.

Keywords: East-Mediterranean cheeses, goat milk cheeses, sheep milk cheeses, White brined cheeses

Microbiological and Proteolytic Aspects of Parmigiano Reggiano Cheese Ripening

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Parmigiano Reggiano is a typical Italian hard cooked cheese, aged for at least 12 months, produced from raw cow's partially skimmed milk with the aid of a natural whey starter mainly composed of thermophilic lactobacilli which largely dominate the bacterial population during the first phase of ripening. During the ripening, milk curd caseins undergo to an extensive degradation due to the action of rennet enzymes and milk/bacterial proteases.
Sixteen twin-wholes have been produced at the same time, in the same dairy, with the same milk and using the same natural whey starter. Accurate and laborious sampling allowed to analyse the cheese during the production and through all the 24 months ripening. Different zones of cheese have been considered.
The evolution of starter and non-starter lactic acid bacteria were followed using traditional and innovative cultural media containing whey, curd and ripened cheese. When innovative media were used, an increase of the microbial cultivable population higher than in traditional media was observed. The cell viability was checked using epifluorescence microscope. More than three hundred strains isolated during the production and the ripening were characterized by RAPD-PCR using UPGMA cluster analysis. Specific species PCR and 16S rDNA sequencing was conducted to confirm some bacterial identities. Microbial peptidases activities found in the extract of cheese, free from cells, were set up and evaluated.
Moreover, using originally developed extraction techniques and LC/MS methodologies, the most abundant oligopeptides (<10 kDa), deriving from α_{S1}- and β–casein, have been identified. Indications on the preferential cleavage sites of the enzymes present in cheese and on their activity at different stages of the ripening have been obtained. In particular, it has been found that the peptide pattern is dynamically evolving during the ageing time in a strict relationship with the different enzymatic activities displayed by the different bacteria.

Keywords: Parmigiano Reggiano cheese, proteolysis, ripening, peptidases activity, oligopeptide

Influence of Calcium on Melting Properties of Raclette Cheese

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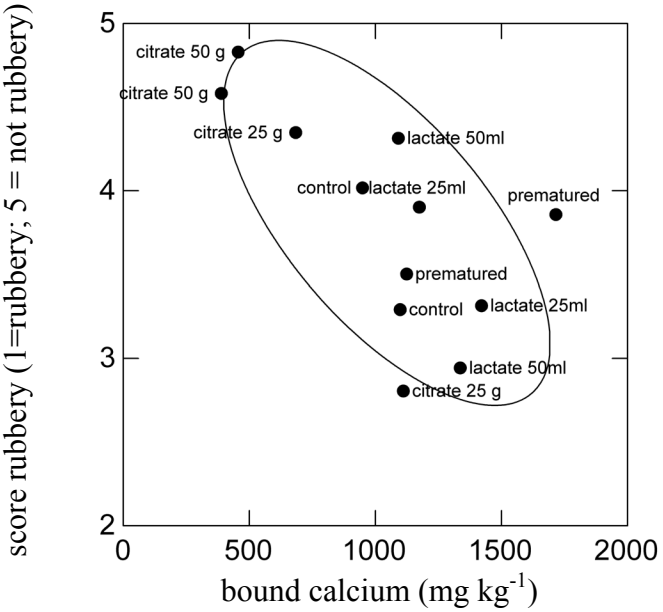
Raclette, as the name implies (French *racler* = to scrape [the melted cheese] off), is a cheese mainly consumed in the melted state. A wide variety of ALP studies have shown that calcium plays an important role in the melting of raclette: Lower contents of total and lower shares of insoluble calcium have always been associated with improved melting properties. ALP therefore conducted further studies with experimental raclette cheese made from pasteurised milk to obtain a better understanding.
The aim of the studies was to examine the influence of the type, extent and time of pH reduction during cheese manufacture on the soluble and total calcium content. The pH-reducing measures applied were pre-ripening of the vat milk and addition of different concentrations of citric and lactic acid to the curd washing water. It was shown that the time and pattern of pH reduction were crucial to the extent of calcium loss with the whey. Good calcium dissolution from the casein matrix was obtained when the pH value was reduced dramatically after curdling. Then again, significantly less calcium was dissolved with lactic acid than with citric acid, although the pH values obtained in the whey were comparable.
The complexing of the citric acid with the calcium seems to be a key effect favouring the solubilisation of calcium. However, not only the loss of calcium with the whey, but also the state of calcium in the ripe cheese was affected by the addition of citric acid: the proportion of dissolved calcium was dramatically increased, a consequence of not only the lower pH, but also of the complexation. Sensory analysis of the melted cheeses confirmed that the addition of citric acid to the curd washing water had the biggest effect. In these cheeses the melting properties were very significantly superior across practically all parameters apart from taste (see figure). This verified the important role of calcium – the less calcium and the lower the share of insoluble calcium in the curd, the better the melting properties.

Keywords: raclette cheese, melting properties, calcium, citric acid



Sensory and Rheological Traits of Mexican Queso Chihuahua

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Traditionally, Mexican Queso Chihuahua has been made from raw milk, but as food safety issues increase for cheeses sold with minimal aging, there is concern that pasteurization of the cheesemilk will alter the sensory traits (flavor and texture) unique to this cheese. An international study was developed to characterize the sensory traits of young cheese made with raw or pasteurized milk and to determine rheological changes that occurred with aging.

Multiple brands of Queso Chihuahua were obtained within days of manufacture from the Chihuahua region of Mexico. Descriptive analyses of flavor and texture were conducted with panelists trained to a universal or product specific Spectrum™ intensity scale, respectively. Microbial analyses were conducted prior to testing to ensure product safety. Four brands were selected and obtained at 3 different times of the year (early winter, mid-spring, and summer) and aged at 4°C for up to four months. Rheological properties were measured every 4 weeks using texture profile, small amplitude oscillatory shear, and torsion analyses.

Results showed that the most prominent attributes in the young cheeses were salty, sour, diacetyl, cooked, whey, bitter, and milkfat flavors with raw milk cheeses having more intense sour and bitter notes compared to the pasteurized milk cheeses. Many cheese texture attributes were similar, but raw milk cheeses were perceived as softer than the pasteurized milk cheeses. Rheological results supported that the raw milk cheeses were softer and their properties more variable than the pasteurized milk cheeses. Aging affected the rheology of the cheeses more than the seasonality of the cheesemilk with the seasonality impacting the rheology of the raw milk cheeses more than the pasteurized milk cheeses.

As the demand for Hispanic-style cheeses increases, defining and understanding the sensorial and rheological attributes of traditionally-made Mexican cheeses provides guidance as new ways are explored to improve the production and shelf life of the cheese.

Keywords: sensory, rheology, cheese, Chihuahua

Changes of Free Fatty Acid Contents and Sensory Properties of "Kaşar" Cheese During Ripening

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In Turkey, 50 – 150 traditional cheese varieties are known. Kaşar cheese is very popular and economically the second most important, with an annual production of about 41'000 tons. It is produced from raw and heat-treated ewe's milk, or a blend of cow's and ewe's milk and mostly from cow's milk. In t
his study, the effect of added "pregastric lipase" on accelerated ripening is investigated. Marketing wants to shorten the ripening time and to reduce the costs of production.
Four batches of cheese were produced with 0 (control), 5, 8, and 11 g pregastric lipase preparations per 100 L cow milk. Sensory analysis was performed and yield, dry matter, fat, salt, total nitrogen, titratable acidity, pH, acid value, total volatile fatty acids and free fatty acids were determined at 1, 15, 30, 45, 60 and 90 days of ripening.
During ripening of Kaşar cheese, the addition of pregastric lipase preparations did not affect yield, dry matter, fat in dry matter, salt in dry matter, total nitrogen in dry matter, and titratable acidity values, but influenced the pH value ($p < 0.05$). Addition of pregastric lipase preparations and ripening time showed effects on acid values, total volatile fatty acids and free fatty acids by interaction. Cheese with 5 and 8 g added pregastric lipase had best sensory quality, no rancid or foreign off-flavour was observed during ripening until 90 days.

Keywords: Kaşar cheese, Pregastric lipase; Free fatty acids, Sensory properties

Session VII

Process Analysis and Control

Process Analysis and Control in Cheese Production – How to Control and Optimize the Process for Quality and Economy

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Most cheese is produced in large plants (24 hours -7days a week). T secure a high quality and good economy it is important to use the right process control tools. But to do this without loosing the special characteristics of the different cheese types we have to know which parameters to control and how to do it. There are many process control systems around but the dairy industry as a whole have been rather slow to implement them, because cheese production is often regarded as very "special". But we can see the cheese production as any other process.
The control of the process can be done, by using rather simple methods like Statistical Process Control (SPC), which focuses on single important parameters and their variation, or more complex methods like Multivariate SPC or similar multivariate methods. The focus should be on the variation and how to minimize it. To optimize a process that is not in control is not very efficient so a good start is SPC and use basic parameters like temperature, flow, time etc. In the optimization it is better to use the more complex methods. To really improve the cheesemaking process, it will be necessary to use new measurements and parameters so that the data appears in real-time. This can include in-line IR, NIR, microwaves and others.
Focus on the variation in the process, not the product, minimize this variation and then optimize. To do this one needs tools (statistical tools, management tools, organization) that put the production process in focus. Understanding variation is the key to managing chaos.

Keywords: cheese, variation, process analysis, statistical process control

Similarity of Norvegia and Präst Produced by Different Dairy Plants and Identification of Attributes for Prediction their Quality Development

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The objective of this work was to identify the variation in cheese composition between cheese plants producing the same cheese variety and to identify attributes that could be suitable for prediction of cheese quality. This has been made by analysis of microbial and chemical composition and sensory properties during ripening of two commercial varieties of cheese (Norvegia and Präst) produced at different dairy plants. The microbial development has been described previously [Østlie et al., 2004; Østlie et al., 2005]. These two cheese varieties are brine salted with the same type of mesophilic starter (DL), however several steps in the production technology differs and thereby also their sensorial properties. For this experiment each cheese variety was delivered from three different dairy plants geographically spread in the countries 7 times from February until September.
The statistical analysis showed higher variation on cheese composition between the dairies producing Präst than those producing Norvegia. This was related to a much higer salt content in Präst cheese from one of the dairies, which most probably influenced the ripening and thereby the composition of free amino acids (FAA) organic acids and volatiles of the cheese.
As expected, the effect of age significantly ($P < 0.05$) influenced both the content of FAA, volatile components, the content of various microorganisms and the content of organic acids in Präst and Norvegia. The dry matter content was significantly ($P < 0.05$) influenced by age in Präst which is a cheese with rind, while as expected the dry matter of the rindless Norvegia was not influenced by age.
The cheeses could be clearly separated by their constituents by principal component analysis (PCA) already after 30 days of ripening. The FAA composition of Präst and Norvegia after 30 and 270 days of ripening showed that the cheeses clustered after 30 days of ripening and kept their clustering throughout ripening, and the variation explained by the first two PCs were similar at all ripening stages. The Präst cheeses could be separated due to season of production by the PCA analysis, with a clear separation between cheeses produced during spring (February –May) and the grazing season (June – September). By PCA analysis of the organic acids, the cheeses could be clearly separated by their content of organic acids at all stages of ripening, but with less of the variation explained by the first two principal components as ripening progressed. Grouping of volatile compounds by PCA were evident already after 30 days of ripening with the methyl aldehydes grouped closely throughout ripening. While the quality of Präst improved during ripening and obtained the best sensory quality grading after 270 days of ripening, Norvegia obtained a significantly ($P<0.05$) better sensory grading after 180 days of ripening than after 270 days of ripening.
The levels of the FAA are most probably the best predictor for cheese quality in both Norvegia and Präst. This assumption is based on the fact that most of the variation was explained both early and late during ripening by the free amino acid composition, and that the distribution was uniform. The correlation between development of FAA and sensory quality needs to be better explored in the future.

Østlie et al. (2004). *Int. J Food Microbiol.*, **94**(3), 287-299
Østlie et al. (2005). *Int. Dairy J*, **15**(6-9), 911-920.

Keywords: Cheese quality, ripening, principal component analysis

Autocatalytic Multistage Structure Formation Reaction in Dairy Based Systems

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A multistage structure formation process is observed in systems such as fresh cheese or processed cheese. It therefore was the objective of this study to identify and to assess the influencing factors driving the reaction. These findings are used to develop an in-depth understanding of the structure formation mechanism.
The investigations are carried out with a rheometer to track the structure formation course online during the processing. It was found that the structure formation reaction follows a typical time course through four different phases. The shape of the viscosity curve can be influenced by processing and compositional factors. Shear intensity, temperature and an upstream homogenisation of the fat phase were investigated as processing factors. The experiments showed that higher shear intensities result in an acceleration of the structure formation process. This can be explained by the intense mixing of the ingredients leading to an increasing contact probability of the proteins. Furthermore, it was assessed that a processing temperature of 70°C and higher is necessary to induce the structure formation. An upstream homogenisation of the fat phase, which influenced the incoming droplet size of the fat globules, leads to a faster reaction.
Regarding composition, content of protein, fat level, rework addition and pH were varied. The experiments showed that the presence of fat is indispensable, without fat the reaction hardly takes place. Besides, it was found that with increasing protein content the structure formation takes place earlier. As shown in figure 1, the rework addition affects the structure formation regarding it leads to a speed up of the structure formation course. Therefore, it seems that rework acts as a starter in an autocatalytic reaction.

Keywords: multistage structure formation, processed cheese, emulsion, rework

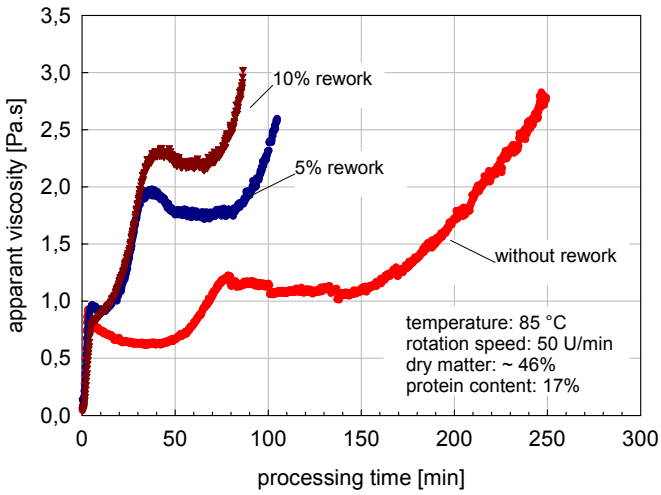

Figure 1: Effect of rework addition on the course of viscosity as function of processing time in a dairy protein model system

Characterisation of Different Blue Cheeses Using Custom-Design Multispectral Imager

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The quality of cheese can be measured directly by sensory methods or indirectly by chemical or mechanical measurements. However, many of these methods are unsuitable to be used or adopted by the cheese industry for rapid analysis. Spectroscopic methods are attractive analytical techniques for determining the quality of dairy products. Spectroscopy on heterogeneous materials such as cheese may however suffer from inaccuracy since macroscopic structural information is lost. In order to get information at the macroscpopic level, image analysis in the visible, fluorescence, infrared and magnetic resonance ranges have been developed. These methods are generally suited to distinguish and quantify structures in the images.
A "Multiway Imager" (MUWI) has been developed for characterising the spatial organisation of food products. This system is able to record multivariate images with more than 20 independent spectral conditions, the available channels (plans) of the image are obtained from UV (350 nm), visible or Near Infrared (850 nm) illumination conditions. In this work, the effectiveness of this MUWI system for characterising 35 retailed pre-packaged AOC blue cheeses (Bleu d'Auvergne (12), Fourme d'Ambert (23)) was assessed. The recorded images are "cubes" with two spatial dimensions and one spectral dimension. The image texture analysis was carried out using custom-designed algorithms programmed in Matlab (The MathMorks Inc., MA, USA). The parameters related to the image texture were extracted from each image and the tables of the descriptors (such as "Area", "Major axis length", "Eccentricity", "Solidity", …) were built for each type of image. The descriptor tables were then subjected to multidimensional statistical treatments. The organization of blue fungi at the surface of cheese slices according to the type of blue-cheese and the types of producer and retailer was investigated either using unsupervised methods such as Principal component analysis (PCA) or supervised ones such as Partial least square discriminant analysis (PLS-DA).

Keywords: Blue-cheese, characterisation, multivariate image, chemometrics

Sampling and Online Measurement of the Water Content in Hard Cheese

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The goal of Process Analytical Technology (PAT) is to understand and control the manufacturing process, and consistently ensure a predefined quality at the end of the manufacturing process. Gains in quality and efficiency are likely to come from preventing rejects and re-processing, facilitating continuous processing to improve efficiency, manage variability, increasing capacity and reducing production times by using on-, in-, and/or at-line measurements and controls.
During the first year of working with PAT, in relation to cheese production, it became obviously that many of the quality variables collected was affected by a high sampling error.
To map the sampling errors generated when taking out a sample form a cheese production it was deiced to carry out a total investigation of the variation within a batch of hard cheese. In the investigation the main focus was on the variation in the water content (a stabile water content is important in relation to salt uptake and ripping of the cheese). The investigation was based on the "Theory of Sampling" (TOS), formulated by Pierre Gy.
After the investigation it became evident that the variation within a batch of hard cheese was far greater then expected. A large part of this variation was systematical and could be related to the pressing of the cheese. In order to minimize the variation and thereby optimize the salting and ripening of the cheese it was necessary to install an online technique for measuring the water content.
In the investigation it also became clear that there is a large variation in the water content within one block of cheese (15 kg). It was therefore necessary to find an online measurement technique that was scanning the entire cheese block. It was found that the best technique was a microwave measurement. An online microware technique has therefore been installed.
After installation of the online technique it has been documented that the pressing of the cheese can be optimized based on the results form the online measurement. The variation in the water content within one batch of cheese has been reduced by up to 30%, which give a more even salt uptake and ripening of the produced cheeses.

Keywords: PAT, Sampling and online microwave measurement, moisture, cheese

Session VIII

New Cheese Making Technologies (incl. Low-Fat Cheese and Innovative Cheese Products)

Developments in Pre-Treatments for Cheese-Milk

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Classically, very few pre-treatments are applied to milk for cheese-making, with some cheese varieties being simply made from raw whole milk, but most being made from pasteurised milk, the composition (e.g., fat:protein ratio) of which may have been adjusted by standardisation. However, there has been consistent interest in more sophisticated strategies for manipulation of the milk processing stage of the cheese-making process. Approaches taken include use of alternative processing technologies (e.g., membrane filtration, high-pressure treatment, homogenisation at normal or high pressures, more severe heat treatments) or addition of sources of protein or milk solids (e.g., milk powders, whey protein products). The reasons for such alterations to the milk processing stage can be divided into a number of classes. Firstly, it may be of interest to control the microbiology of the raw milk and the resulting cheese more than may be possible by pasteurisation (e.g., removal of spores, control of non-starter lactic acid bacteria). Secondly, there is always economic interest in increasing the yield of cheese, for example through heat- or pressure-induced incorporation of whey proteins, or direct addition of denatured microparticulated whey proteins. Thirdly, the target of processing may be manipulation of cheese ripening, e.g., inactivation of enzymes to reduce likelihood of off-flavour development, of accelerating ripening through increasing enzyme-substrate interactions; the impact of processing on texture and functional properties of cheese, such as melting, may also be significant. Finally, the considerations for manufacture and ripening of different cheese varieties, or sub-classes of specific varieties (e.g., low-fat cheese) will clearly differ and add to the complexity of the technological options available. This presentation will review key principles for pre-treatment of cheese-milk, as summarised briefly herein.

Keywords*:* milk; cheese; heat treatment; new technologies

Increased Cheese Yield Through Application of Phospholipase: Elucidating the Mechanism and the Influence of Adding Extra Phospholipids to Cheese Milk

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Phospholipases, i.e. enzymes with specific activity towards phospholipids, can be applied in cheese milk in order to obtain increased product yield with unchanged functionality. The phospholipids in milk are present primarily in the milk fat globule membrane, and lyso-phospholipids and free fatty acids released by the action of phospholipase are surface active compounds.
Hydrolysis of milk phospholipids with phospholipase A1 prior to renneting has been shown to significantly increase yield of mozzarella through better moisture and fat retention during whey drainage and stretching (Lilbæk et al, 2006) as well as influencing the surface properties of milk and whey (Lilbæk et al, 2007). The observed yield improvement is presumably a result of improved emulsification and water-holding capacity as a consequence of the lysophospholipids present in the curd. No significant differences in the cheese microstructure during processing or during the first weeks of storage was found, nor any differences in functionality (melting, stretching, and browning), indicating that these factors were largely unaffected by phospholipid hydrolysis.
We will present results from experiments combining the use of phospholipase with enrichment of cheese milk with buttermilk phospholipids and further elucidate the mechanism behind the observed improved cheese yield.

H.M. Lilbæk, M.L. Broe, E. Høier, T.M. Fatum, R. Ipsen, N.K. Sørensen (2006). *J Dairy Sci*., **89,** 4114-4125
H. Lilbæk, T. Fatum, R. Ipsen, N.K. Sørensen (2007). *J Agri. Food Chem.*, **55**, 2970-2978

Keywords: phospholipase, phospholipids , cheese yield

Enhancement of Proteolysis and Flavours in Cheddar Cheese using Encapsulated Recombinant Aminopeptidase of *Lactobacillus rhamnosus* S93

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Addition of enzymes into milk homogeneously while elevating their retention in Cheddar cheese matrix appears to be the simplest and cheapest method to accelerate cheese ripening. Alginate has been used as an immobilization matrix for biomolecules and microorganisms, but very little works have been reported on its application for accelerating cheese ripening. The objective of this study was to investigate the effect of recombinant aminopeptidase (PepN) from *Lactobacillus rhamnosus* S93, in an alginate-chitosan immobilized form, to accelerate Cheddar cheese ripening.
After the enzyme-polymer mixture was extruded from the 300 μm nozzle of an encapsulator (Inotech, IE-50 R), hardened in a $CaCl_2$ (0.15 M)-chitosan (0.1 %) solution for 10 min, the free and encapsulated enzymes were added into the curd (200 L milk vats) during the renneting or salting stage, respectively. Proteolysis was determined by measuring water soluble nitrogen (WSN), the nitrogen soluble in phosphotungstic acid (PTA-SN) as well as amino acids. Flavour compounds were evaluated using GC/MS-SPME. Sensory and scanning electron microscopy data were also gathered.
The encapsulation efficiency was increased from 10 to 90 % in the presence of chitosan. The enzyme lost in the whey accounted for less than 1 %, indicating the stability of the beads during the manufacturing stages. The superior flavour characteristics and higher proteolysis were observed in the experimental cheeses, as compared to those of control.
This study presents a method to enhance the proteolysis and flavours using the recombinant PepN in encapsulated form, and also shows the incorporation of the immobilized PepN into the cheese matrix at a rate of about 99 %.

Keywords: Cheddar cheese, proteolysis, flavour, enzyme encapsulation, *Lactobacillus rhamnosus* S93

Alternatives to Improve the Quality of Low-Fat Prato Cheese, a Brazilian Semi-Hard Cheese

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Currently in Brazil, the demand for dairy products with low-fat content has been fast growing. However, cheeses with reduced fat content are generally characterized as having an undesirable rubbery texture and lack of flavor and/or presence of off-flavors that are atypical compared to their full-fat counterparts.
In this research, the ripening of low-fat Prato cheese using *Lactobacillus casei* as adjunct culture or fastuosain, a proteolytic enzyme from unripe fruits of *Bromelia fastuosa* was studied.
Seven treatments were carried out: one using only *Lactococcus lactis* ssp *lactis* and *Lactococcus lactis* ssp *cremoris* as starter culture (treatment A, control) and six by modified methods. Three of them were fermented by the microorganisms listed above with the addition of *Lactobacillus casei* as adjunct culture, in the following proportions: 20, 30 and 40 % (treatments B, C and D, respectively). To the other three treatments 4, 20 and 12 mg enzyme per L of milk of fastuosain were added (treatments E, F and G, respectively).
In cheese manufactured with adjunct culture, the ripening indices were higher than in the control cheese. The proportion between traditional (70 %) and adjunct culture (30 %) resulted in more intense proteolysis during ripening and consequently better texture characteristics than control cheese. The enzyme addition promoted, since the first day, acceleration of cheese ripening. In samples of modified cheeses of treatments E and F, the time of ripening could be reduced by 15 days. The addition of 4 g L^{-1} enzyme (treatment E), resulted in more increased proteolysis and improvement of the physicochemical, sensory and texture characteristics of the cheeses.
The addition of the adjunct culture or fastuosain enzyme had a positive influence on cheese ripening, being feasible alternatives to improve the quality of cheeses with reduced fat content.

Keywords: ripening, adjunct cultures, proteolytic enzyme, quality, Prato cheese

Effect of Ultra-High-Pressure Homogenisation of Goats' Milk on Textural, Microstructural and Colour Characteristics During Cheese Ripening

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Goat cheeses were made from pasteurised (PA; 72ºC, 15 s), homogenised and pasteurised (PH; 18+2 MPa and 72ºC for 15 s) and ultra-high-pressure homogenised (UH; 300 MPa at 30ºC inlet temperature) goats' milks in order to compare their textural, microsturctural, and colour characteristics during ripening. Texture, microstructure and colour were evaluated by uniaxial compression test, confocal laser scanning microscopy and Hunter colorimetry, respectively.
Both PA milk and PH milk cheeses were more cohesive during ripening than the UH milk cheeses. In all the ripening steps evaluated UH milk and PH milk cheeses were firmer than PA milk cheeses. Although all cheeses showed a loss of elastic characteristics with ageing, PA milk cheeses initially presented the most elastic behaviour, and the highest decrease thereafter.
In relation to the microstructural analysis, UH milk cheeses showed that their fat globules appear completely integrated into the protein matrix, resulting in a total different microstructure when compared with either PA or PH milk cheeses. PH milk cheeses showed an intermediate microstucture (in size fat globule and protein matrix) between PA and UH milk cheeses.
Colour evaluation showed that UH and PH milk cheeses had higher lightnesses than UH milk cheeses and presented the smallest *b* index values.

Keywords: ultra-high-pressure homogenisation, goat milk, cheese texture, colour

Session IX

Cheese as a Food Ingredient

Cheese as a Food Ingredient

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Over the past few decades, cheese has been a growing in commercial importance in the food industry for its use as an ingredient. Its use as a food ingredient includes as a topping on pizza, filling in appetizers, slices on hamburgers, and sauces in pasta dishes. Traditionally cheeses such as Mozzarella, process and imitation cheeses were the main ingredient cheeses but now there are a growing number of other cheese types being used as ingredients, e.g. in the US these include pizza (i.e. cheese not meeting the standard of identity for Mozzarella), Hispanic and cream cheeses. For ingredient cheeses such as Mozzarella, considerable research has been conducted on the impact of altering milk composition, manufacturing conditions and the type of starter cultures on their functional properties that are important for its use as an ingredient, e.g. slicing, shredding and melting properties.

It is now well recognized that insoluble calcium associated with the caseins in cheese plays a critical role in cheese texture including melting properties. The total calcium, as well as the insoluble calcium, content can be controlled by altering the critical pH values during manufacture, e.g. at coagulation or whey draining. Post-manufacture changes in the proportions of insoluble/soluble calcium are primarily responsible for most of the early changes in cheese texture and not proteolysis as was commonly believed until recently. End-users of cheese in various foods routinely demand consistent functionality over a long shelf-life and the cheese has to meet very specific performance targets. Research will be shown that illustrates various approaches to modify the insoluble calcium content in cheese, studies on the performance of cream cheese as an ingredient and strategies to extend the acceptable performance window for cheese. A framework will be presented that allows cheese texture and functional properties to be described in terms of specific molecular interactions of the caseins.

Keywords: functionality, insoluble calcium, rheology, texture

Surface Properties of Cheese Related to Industrial and Oral Processing

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Responding to the consumer's desire, an increasingly large part of cheeses nowadays is sold as slices. Adhesion of the cheese to the knives during cutting and of cheese slices to each other in the package is a major drawback of this development. Adhesive properties of cheese also play an important role in the oral cavity during consumption of cheese. Adhesiveness can range from a desired cohesiveness on the one hand to excessive stickiness/tackiness, which makes clearance of the oral cavity difficult. In this presentation several techniques are introduced to quantify the complex surface properties of cheese: a roller/slider stickiness measurement, a blade cutter experiment and a surface angle measurement of liquids deposited on cheese surface. Semi-hard cheeses differing in their degree of ripening, fat and moisture content as well as processed cheese were examined by these techniques. Microstructural observations and sensory analysis complemented the investigations. The influence of composition, processing and microstructure on the adhesive properties will be shown and correlations between instrumental and sensory parameters will be deduced. New insights into the surface properties of cheeses will aid in designing strategies to improve the processability.

Keywords: adhesiveness, sliceability, stickiness, instrumental evaluation

Influence of Low Molecular Weight Emulsifers on the Texture and Baking Properties of Nonfat Process Cheese

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Stirred curd cheese bases were manufactured by acidification of nonfat milk to pH 5.6 using citric acid. Various levels (0 to 4 %) of a mono-/diglyceride emulsifier blend were added to cheese base during nonfat processed cheese (NFPC) manufacture. Cheese was stored at 4°C for 7 days before analysis. Functionality was assessed using UW-Meltprofiler, texture profile analysis (TPA), dynamic low-amplitude oscillatory rheology and baking on a pizza. A trained sensory panel evaluated the baked pizzas for skinning, force to stretch, chewiness, cohesiveness of mass and hardness. The composition of all NFPC samples were similar. These NFPC had a reduced sodium content since no sodium-based emulsifying/melting salts were used. Hardness, determined by TPA, increased in NFPC when glycerides were added. When NFPC was heated, extent of flow (EOF) increased whilst viscosity decreased in cheeses containing > 1 % glyceride. However, upon cooling from 70 to 45°C, the addition of glyceride resulted in a faster rate of increase in viscosity. Maximum loss tangent (LTmax) values decreased in NFPC containing glyceride levels > 0.1 %. At high temperatures, storage modulus (G') values for the NFPC containing 4 % glyceride were slightly higher than G' values for all other NFPC probably indicating that some protein rearrangements took place during heating. The NFPC made with glyceride were white in color when heated and cooled and were not sticky. Skinning, chewiness and hardness were significantly reduced when NFPC containing 4 % glyceride were baked on pizzas, indicating a softer structure in these cheeses. Microstructural analysis of the NFPC using transmission electron microscopy revealed that the added glycerides modified the density of the casein matrix and increased the size of serum pockets. In conclusion, NFPC with greatly improved baking properties, such as, reduced skinning, reduced stickiness, and white color, were developed by the addition of glyceride in NFPC manufacture.

Keywords: nonfat processed cheese, cheese functionality, low molecular weight emulsifiers

Processed Cheese Texture and Manipulation of Ingredient Interactions

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Some two thirds of Fonterra's natural cheese production is destined for the process kettle. This ingredient cheese is tailored to meet the needs of the processed cheese manufacturer and falls into two categories, "functional" and "flavour". Functional cheese melts in the kettle and has a high level of intact casein to provide good "body" or texture in the finished processed cheese. Much focus has been on preserving the caseins – identifying key manufacturing steps and storage conditions that slow the rate of proteolysis. The two key enzymes are rennet and plasmin - each has a different effect on the caseins and a different set of levers to control its activity. More recently, effort has been focussed on identifying the key caseins in processed cheese manufacture. This work has demonstrated that the caseins are not equal in their ability to deliver "functionality" to processed cheese. The focus of this presentation is on manipulation of processed cheese texture by altering interactions between the caseins and other ingredients incorporated in the processed cheese.

Keywords: processed cheese, ingredients, cheese texture

Use of Hexose Oxidase to Improve the Functionality of Pizza Cheese

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The demand for pizza cheese increases annually: Along with this the demand for improved cheese functionality also increases. Shorter and higher cooking times employed in advanced pizza cook processes lead to issues with over browning due to residual galactose. The application of Hexose Oxidase to grated pizza cheese has been shown to effectively counter this problem.
Further there is now also a demand for improved micro-biological protection against opportunistic yeasts and moulds. Hexose oxidase can also help here being especially effective as an oxygen scavenger when used in conjunction with re-sealable packs which for example which are increasingly employed for end user home and catering convenience convenience.

Keywords: oxidase, pizza cheese

Grated Grana Padano Cheese: New Hints on how to Control Quality and Recognize Imitations

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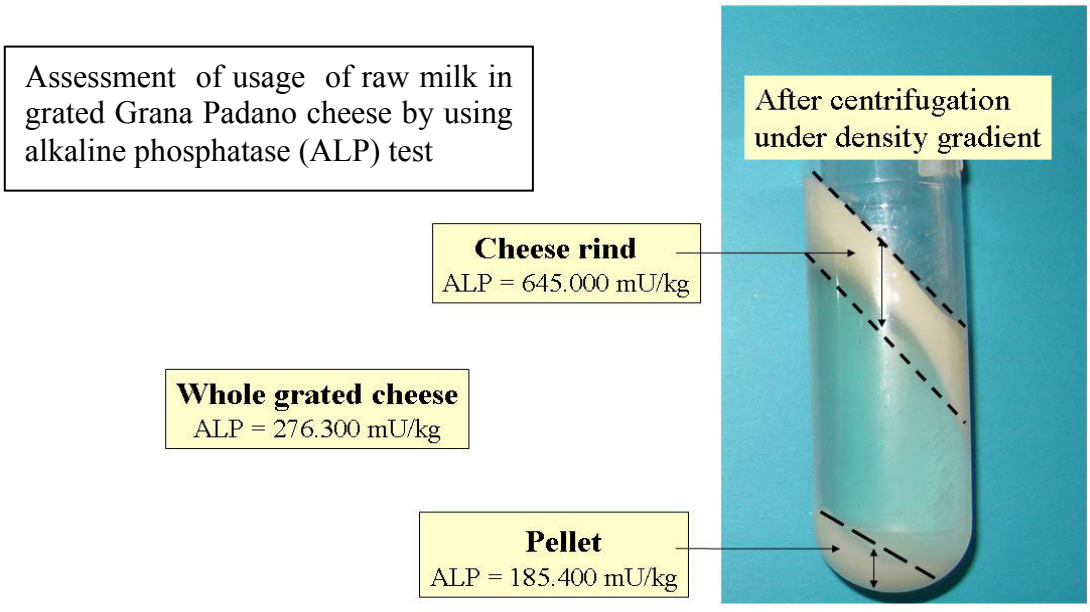
Evaluation of the sensorial and chemico-physical characteristics defined by the product specification for the PDO cheese Grana Padano (G.P.) is not a realistic option for the assessment of correspondence of marketed cheeses to this denomination, particularly for the grated products. When the rind is still attached to the cheese wedge, it is easy to recognize the authentic G.P. and to check the overall quality. When cheese is grated, things become more difficult. Therefore, beside inspective controls by the *Consorzio di Tutela* along the production chain, a specific strategy for quality control of grated G.P. on the market has been developed.
Usage of raw milk can be assessed by measuring alkaline phosphatase activity in the outermost layer of the cheese. A suitable extraction method, based on density gradient centrifugation of solubilized cheese (figure), has been developed to separate the rind present in grated cheese.
Grated G.P. must be obtained by grating the whole wheel, without adding extra rind. Due to the very slow progress of proteolysis in the rind, compared to the cheese body, specific peptides were identified by CZE which enable to control whether extra rind is present in the grated product.
The content of free amino acids on cheese protein basis and the relative contents of 6 single amino acids allow to evaluate whether the cheese is properly aged with respect to extent and typicalness of proteolysis.
In addition, the determination of lysozyme content is useful to evaluate the correct usage of this technological aid in cheesemaking.
As of end 2006, an extensive investigation on marketed grated G.P. was undertaken during which about 400 samples are planned to be checked for the aforementioned parameters. Results obtained up to present demonstrate that the simultaneous application of these parameters allows to rule out all of the low-quality and imitation grated cheeses.

Keywords: grated Grana Padano cheese, quality control, imitation cheese



Session X

Packaging

Putting Packaging First

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Packaging of cheeses has evolved from being used for protection and transportation of the product from the cheese shop or local dairy to the homes to being a multifaceted task involving not only chemical, physical, microbiological, and sensory aspects but also elements such as marketing and sales, legislation, environmentally sound production schemes, and consumer-related parameters (e.g. openability and overall convenience). Convenience aspects and long distribution times have resulted in increased use of modified atmosphere packaged products, the application of active packaging concepts, and market introduction of products which are grated, cubed, sliced, or otherwise manufactured to provide easy-to-use consumer products.
The introduction of new packaging concepts has been substantiated to some extent by research. However, the somewhat empirical pack-and-pray approach still dominates commercial usage of many aspects of cheese packaging technology.
Several food producers underestimate the effects of the packaging on quality deterioration. In order to preserve product quality, it is of paramount importance to thoroughly understand and focus on the interactions taking place between the packaging and the product. Only by applying this knowledge for further tailoring of packaging to the individual types of cheeses will the cheese producers secure their cutting edge positions.
The presentation highlights the major quality deteriorative processes in cheese and how these may actually be prevented or minimized by packaging. Focus will be on present and future cheese packaging concepts, and the presentation will include both scientific and market-oriented aspects when putting packaging first!

Keywords: Packaging, cheese, quality

Effect of Olive Oil and Glycerol on Physical Properties of Whey Protein Emulsion Films

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Olive oil was incorporated into whey protein through emulsification to produce films. Whey protein films were prepared by dispersing 100 g kg^{-1} protein in distilled water and plasticized with different levels of glycerol (glycerol: protein = 0.5, 0.6 and 0.7).
Olive oil was added at different levels (oil/protein = 0.0, 0.2, 0.3 and 0.4). The emulsion films were evaluated for mechanical properties, water vapor and oxygen permeability and opacity. It was observed that water vapor permeability for the film decreased with increasing olive oil content in the film, but the mechanical properties also decreased.

Keywords: whey protein, edible films, olive oil, emulsion, water vapor permeability, mechanical properties, gas permeability

Comparison of Nisin Addition in the Food Bulk and Nisin Incorporated in an Antimicrobial Plastic Film in Fresh Cheese Preservation: Study of Physicochemical Parameters and anti-*Listeria monocytogenes* Effect

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To control food contamination by undesirable and pathogenic microorganisms, the bacteriocin nisin was used as food preservative. Two ways of nisin addition were compared: nisin incorporation in the bulk cheese, and nisin adsorption onto a polyethylene food grade plastic film deposited onto the surface of the fresh cheese product. The main benefit of either approach is to extend the food shelf-life by inhibiting negative and pathogenic microbial growth. In the case of antimicrobial packaging, the bioactivity is based on the release of the inhibiting molecule from the film and diffusion into the food. Active films may have different advantages: (i) reduce the addition of larger quantities of antimicrobials incorporated into the bulk of the food (ii) gradual release of nisin from the package and maintain of a higher surface concentrations of antimicrobial agents.
In this study, the antimicrobial activity was tested against food pathogenic bacteria namely *Listeria monocytogenes* L0203. The nisin was added in the cheese preparation at 20°C after periods of 4 h and overnight for lactic ferments acidification and milk-clotting enzyme activity steps respectively. Counts of aerobic bacteria, lactic flora (*Lactococcus* and *Lactobacillus*), *L. monocytogenes*, concentrations of proteins, pH and dry matter were measured. Preparation without nisin was used as a control. As a result, the preparation of cheese with nisin significantly improved the microbial stability of cheese at 4°C for at least 21 days. The effectiveness of antimicrobial packaging in suppressing the microbial growth was proved to be distance dependent: the antimicrobial effectiveness was higher for low distances from the film and the inhibitory activity decreased as the distance from the film increased. The physico-chemical parameters (pH, concentration of protein and dry matter) were determined and were the same with or without nisin application.

Keywords : active packaging, nisin bioassay, antimicrobial, cheese

Poster Session 1

Determination of Biogenic Amines in Grated Parmesan Cheese

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Biogenic amines are organic bases produced by the metabolic activity of microorganisms. The presence of these amines in cheese is usually related to the decarboxylation of amino acids during ripening. Biogenic amines are vaso-active and/or psycho-active compounds, and are involved in the so-called "cheese reaction". A total content of 500 mg kg^{-1} of biogenic amines in any given foodstuff is considered to be a critical amount. Their formation and accumulation is related to the hygienic conditions of industrial/artisanal cheese making, and a result of the decarboxylating activity of some starter and non-starter bacteria. For this reason biogenic amines are considered a potential biomarker of hygienic conditions during cheese production.
In the present work some biogenic amines and their precursor amino acids were determined in commercial samples of grated Parmesan cheese, using an ion-pair HPLC method. Some of the samples analyzed carried a PDO label (Parmigiano Reggiano PDO and Grana Padano PDO) while other samples did not have that label, because they are produced exclusively as grated cheeses.
Biogenic amines data correlated with: compositional parameters (proteins, pH, ash, and NaCl concentration), as well as with proteolysis parameters such as the Ripening Index (WSN/TN) and the electrophoretic patterns of caseins.
Among the free precursor amino acids, phenylalanine was found to be the most prevalent (up to 3000 mg kg^{-1}), suggesting that an elevated proteolysis of these cheeses had been confirmed by a very high ripening index (up to 56 %).
Among biogenic amines, histamine (from 11 to 679 mg kg^{-1}) and tyramine (10-78 mg kg^{-1}) were most prevalent. The variability of biogenic amines content in the different cheeses suggests that amine formation and accumulation could be prevented in this cheese. Further investigations to determine the conditions giving rise to the increased biogenic amine content are necessary.

Keywords: histamine, tyramine, proteolysis, Parmesan cheese

Screening of *Lactobacillus plantarum* and *Lactobacillus paracasei* Strains for their Capability to Reduce Cholesterol in Synthetic Medium and Milk

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During the last two decades several attempts to reduce the cholesterol level in synthetic media were carried out using a large variety of lactobacilli, lactococci, and bifidobacteria strains, mainly in the presence of bile salts and in anaerobiosis conditions. Some dairy thermophilic cultures and probiotics, isolated from human gut, were also tested by some authors for the ability to decrease cholesterol in milk.
In this work 8 *Lactobacillus plantarum* and 5 *Lactobacillus paracasei* strains of food origin, belonging to the collection of the Department of Exploitation and Protection of the Agricultural and Forestry Resources (Di.Va.P.R.A.), were screened in order to test their cholesterol lowering-action in de Man, Rogosa, Sharpe (MRS) broth, supplemented with cholesterol; a commercial strain of *Lactobacillus acidophilus* was used as positive control since the ability of this species to assimilate cholesterol was widely established in MRS with added bile salts and in anaerobiosis. The synthetic

medium was inoculated at 2 % for 24 hours at 37°C; spent broth and uninoculated control were assayed for their cholesterol content by enzymatic analysis.
Among all tested strains, two *L. plantarum* and three *L. paracasei* strains gave rise to a significant reduction of the cholesterol level in MRS broth; in particular *L. plantarum* strains lowered the cholesterol content by an average of 19.4 %, while *L. paracasei* strains by an average of 6.8 %. The two *L. plantarum* strains possessing relatively high cholesterol-lowering activity in MRS broth were also tested in UHT whole homogenized milk following the same procedure. Results showed that *L. plantarum* strains maintained this activity also in milk; in fact after 24 hours the decrease of the cholesterol ranged from about 5.0 % to 8.2 % without significant variations between the two strains.

Keywords: cholesterol, *L. plantarum*, *L. paracasei*, milk

Lactic and Propionic Acid Bacteria Survive Gastrointestinal Transit of Healthy Volunteers Treated with Amoxicillin-Clavulanic Acid Consuming Raw Milk Cheese

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Raw milk cheeses are characterised by a large diversity in microflora which is mainly responsible for the high diversity of the sensorial properties. In addition, raw milk cheeses could have positive effects on human health. As previously observed, the consumption of hard-cooked raw milk cheese reduced the emergence of amoxicillin-resistant enterococci in faeces of healthy volunteers treated with amoxicillin-clavulanic acid (amoxiclav). What could be involved in this observation is the interaction between the dominant microbial cheese populations, *i.e.* lactic and propionic acid bacteria, and intestinal enterococci. This explains why the survival of lactic and propionic acid bacteria of cheese has been examined after their passage through the gastrointestinal tract of 3 volunteers.
Lactic and propionic acid bacteria were enumerated with 3 specific media in one eaten cheese and faecal samples at different periods: before, during and after amoxiclav treatment with concomitant consumption of cheese. About 1000 isolates were collected and characterized at species and strain levels using the PCR technique and the IRTF spectroscopy for certain.
The eaten hard-cooked raw milk cheese contained about 10^6 cfu g^{-1} of thermophilic lactobacilli (*Lactobacillus delbrueckii*), 5 x 10^7 cfu g^{-1} of mesophilic lactobacilli (*Lb. paracasei* and *Lb. rhamnosus*), 5 x 10^6 cfu g^{-1} of pediococci (*Pediococcus acidilactici*), and 10^6 cfu g^{-1} of propionibacteria (*Propionibacterium freudenreichii*). During the consumption of cheese, lactic acid bacteria and propionibacteria counts ranged respectively from 10^4 to 10^7 cfu g^{-1} of faeces and from 10^4 to 10^6 cfu g^{-1} of faeces, the counts differing from subject to subject. The lowest levels were obtained during amoxiclav treatment. Some strains of lactic and propionic acid bacteria of the hard-cooked raw milk cheese were found in the faeces of the 3 volunteers, even during amoxiclav treatment. In conclusion, they could potentially interact with intestinal enterococci and could be considered as probiotics.

Keywords: human trial, raw milk cheese, lactic acid bacteria, propionic acid bacteria, enterococci, intestinal microflora, probiotics

Production of Conjugated Linoleic Acid (CLA) by Lactic Acid Bacteria Isolated from Italian Traditional Cheeses

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Conjugated Linoleic Acid (CLA) is a mixture of positional and geometric isomers of linoleic acid (C18:2) in which double bonds are conjugated. Studies with animal models have demonstrated that CLA consumption and particularly of *cis*-9*, trans*-11 (c9t11-18:2) and *trans*-10*, cis*-12 (t10c12-18:2) isomers inhibits the initiation of carcinogenesis and tumorigenesis, reduces body fat content and increases muscle mass, decreases atherosclerosis, improves hyperinsulinemia and enhances the immune system. The CLA are produced through the isomerization of linoleic acid or vaccenic acid by animal, but various studies show that they can be also synthesized by microrganism in milk or in different cultural substrates.
The aim of this work was to select lactic acid bacteria strains able to synthesize CLA and useful as starters or adjunct cultures for the development of yogurt and cheese with potential health or nutritional benefits.
This research was performed with about fifty strains of *Lactobacillus plantarum*, *Lactobacillus paracasei, Lactobacillus casei, Lactococcus lactis*, *Lactococcus lactis* spp. *cremoris*, *Lactococcus lactis* spp. *lactis* isolated from traditional cheeses of Piedmont region (North-West, Italy).
Among these strains, the *Lactobacillus plantarum* 110-C9.10.2 and the *Lactobacillus paracasei* 37-B8.7 have shown, on synthetic medium (MRS broth) added with free linoleic acid, the highest CLA production (13 and 27 mg L^{-1} of medium, respectively).
Further studies are in progress to evaluate the activity of these strains on milk during yogurt and cheese production.

Keywords: conjugated linoleic acid, lactic acid bacteria, milk

Effect of the Physiological State of *Lactococcus lactis* on Growth of *Staphylococcus aureus* During Cheesemaking

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Lactococcus lactis is widely used in cheesemaking, for its production of lactic acid and organoleptic molecules. *L. lactis* also plays an important role in inhibiting growth of spoilage microorganisms or pathogenic bacteria, and inhibiting toxin production, especially for *Staphylococcus aureus*. In France, *S. aureus* has been identified as the prevailing agent of collective food poisoning due to dairy products. To control these infections and reduce the production of enterotoxins, it is first necessary to limit the development of *S. aureus* in cheeses. The aim of this work was to study the incidence of the physiological state of two strains of *L. lactis* ssp. *cremoris*, SK11 and Wg2, on growth of *S. aureus* RN6390 in semi-hard cheese, which involves a high staphylococcal risk cheesemaking technology.
The physiological states of the *L. lactis* strains were evaluated during their growth in pure culture in milk, by using a fluorescent probe combined with flow cytometry. Two physiological states (A and B) were retained for each *L. lactis* strain, based on their fluorescence value. Cells prepared in both states were tested for their properties as cheese starter inocula. For cheesemaking, microfiltered

milk was inoculated with a culture of *L. lactis* (10^6cfu mL^{-1}) in A and B physiological states, in the presence of *S. aureus* (10^3cfu mL^{-1}). We observed enhanced acidification kinetics, acceleration of lactococcal growth, and decreased growth of *S. aureus*, when using the *L. lactis* inoculum having physiological state A compared with physiological state B.
These results suggest that one determining factor in the development of *S. aureus* during cheesemaking is the physiological state of the lactic starter, which can be easily evaluated by flow cytometry. This study should be extended to other species of lactic acid bacteria and other strains of *S. aureus*. Our results will be useful for dairy industrials in controlling microbiological risks.

Keywords: *Lactococcus lactis*, *Staphylococcus aureus*, physiological state, cheese

Results Illustrating how Semi-Hard Cheese can act as an Ideal Vector for Delivery of Probiotic Strains

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Population studies on a range of European semi-hard cheeses have shown several positive aspects for using cheese as a means of delivering probiotic cultures. Results are presented here for Gouda made with several diverse probiotic cultures
Based on work performed using 10 L model cheese vats it is clear that the probiotic cultures exhibit strain dependant characteristics which allow some strains to multiply in the cheese milk and / or cheese curd during ripening. It has also been confirmed that the probiotic cultures are also preferentially incorporated into the curd matrix, rather than lost in the cheese whey. These factors act synergistically to multiply the number of viable cells present in the end product. Further the probiotic cultures also exhibit exceptional long term viability within the cheese matrix over the entire shelf life of the product.

Keywords: probiotic, viability

Effects of Probiotics Cultures and Salt Reduction on the Characteristics of UF White Brined Cheeses

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UF cheeses in brine have a very important place in the production and consumption of dairy products on the Serbian market.
The objective of the present study was to determine the effects of *Lactobacillus acidophilus* LAFTI L10 and *Bifidobacterium lactis* LAFTI B94, as adjunct cultures, on the composition, proteolysis and sensory properties of UF cheeses and to investigate the survival of probiotic bacteria during ripening. Probiotic cheeses were salted at different salt level (2.0 and 4.0 % salt in moisture) in order to investigate influence of salt reduction on cheese characteristics and probiotic viability during ripening.
The population of *L. acidophilus* survived to numbers > 10^7 cfu g^{-1}, which is necessary for positive effects on health. *B. lactis* was maintained on the high level during 10 days of ripening and than decreased to 6 log cfu g^{-1}. Different salt content did not significantly influence on the count of starter and probiotic strains. The composition and the pH value were not significantly different

between cheeses. The rate of proteolysis of cheeses with probiotic bacteria was slightly higher than that in control cheese, probably as a consequence of their different proteolytic activity. Levels of water soluble nitrogen (WSN/TN), as well as levels of phosphotungstic acid soluble nitrogen (PTA/TN) increased significantly with time and the rate being inversely proportional to the salt level of cheeses.
Sensory evaluation showed that probiotic cheeses, with both salt content, had higher sensory scores than control cheese, without probiotic strains (about 90 and 70 % of max. possible quality scores, respectively).
These results showed that probiotic strains can be successfuly used in production of UF brined cheeses.

Keywords: UF cheese, probiotic bacteria, salt content, composition, proteolysis

A Chromatographic Procedure to Isolate Caseinphosphopeptides from Cheeses

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Caseinphosphopeptides (CPPs) are polyphosphorylated fragments of caseins that are released as a consequence of proteolysis during cheese ripening. Their polyphosphorylation confers them the ability to sequester divalent cations such as calcium: they have therefore been proposed to prevent tooth enamel demineralization and enhance calcium passive absorption following ingestion of dairy products. In the present study, their affinity for cations was exploited to isolate them from the water-soluble fraction of cheeses by immobilized metal affinity chromatography (IMAC) (following a chromatographic procedure described by Lund & Ardö, 2004). Since CPPs have a molecular weight in the 600-10'000 Da range, the IMAC fractions containing CPPs were analysed by gel filtration chromatography (with a calibrated column). This chromatographic analysis was applied to 3 samples of a non cooked pressed curd cheese (produced in Savoie, France) ripened for 1 (control), 35 (young cheese) and 55 days (medium cheese) in an attempt to quantify their CPPs content. It revealed that not only CPPs but also molecules with a lower molecular mass interacted with the IMAC chromatographic support. Further analyses are needed to identify these molecules (CPPs and molecules such as phosphoserine?). The quantity of molecules isolated by IMAC with a molecular weight in the range of CPPs was minimal in the sample ripened for 35 days. On one hand, it is hypothesized that CPPs accumulate along cheese ripening since multiphosphorylation prevents the direct action of proteolytic enzymes, on the other hand, phosphatases present in cheese curd might dephosphorylate these peptides and thus allow the action of proteolytic enzymes. It is thus of interest to develop methodologies to quantify and characterize CPPs along cheese ripening, since, besides the direct potential nutritional interest of CPPs, they might (in conjunction with phosphatase and proteolytic activities assays in cheese curds) allow to assess the role of casein dephosphorylation on proteolysis.
M. Lund, Y. Ardö (2004). *J. Agric. Food Chem*., **52**, 6616-6622

Keywords: caseinphosphopeptides, chromatographic analysis, cheese ripening

Probiotic Cheese Quality

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Hard cheese is an attractive product for probiotic delivery: The cheese matrix protects the bacteria from oxygen, and also the moderate pH in cheese and high buffering capacity contribute to probiotic viability in cheese. In addition, cheese matrix protects the probiotic bacteria in human gastrointestinal tract, which boosts their viability and functionality in gut.
In order to have an effect in human body, the probiotic bacteria have to survive in relatively high numbers during the long shelf-life of the hard cheese. It would be ideal to use a bacterium capable of multiplying in cheese, but the probiotic should not have an effect on the cheese consumer quality by altering the flavour, texture or shelf life.
In these trials we tested the effect of probiotic addition on the sensory quality of the low fat, propionic acid fermented cheese made at Valio Ltd. Joensuu cheese factory. The probiotic was added as either concentrate or freeze-dried powder at the level of $5*10^{11}$ cfu in 10 000 L milk. The cheese was evaluated by experts using the triangular test and quality score points. The cheese composition, short chain fatty acids, lactic acids and LGG counts were analysed. The confirmation trial was made with LGG concentrate addition.
Trained hard cheese panellists could not differentiate the cheeses made with or without LGG powder or concentrate in triangular test. Both trial cheeses were given equal quality score points compared to the reference cheese without LGG addition.
The cheese with LGG concentrate had higher amounts of acetic and propionic acids and lower amount of lactic acid than the cheese with LGG powder and reference cheese. The cheese with LGG concentrate had also higher LGG count.
In a three vat -confirmation trial with LGG concentrate, the panellists did not find the difference between the trial and reference cheeses in triangular test. The quality score points of the trial cheese were similar to the reference cheese.
The trial cheeses had lower concentrations of acetic and propionic acids and higher concentrations of lactic acids.
The LGG counts in cheese at the beginning of sales period have been followed for 5 years. The LGG count has remained at the level of $2x10^7$ cfu g^{-1} throughout the years.
Lactobacillus rhamnosus LGG survived in cheese during ripening, and it did not have adverse effect on the sensory quality of the cheese. The LGG counts in cheese have been on constant high level during the years of production.

Keywords: Low fat cheese, sensory quality, viability, *Lactobacillus rhamnosus* LGG

Influence of Sodium Chloride on the Quality of Cheddar Cheese

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Salt is essential in our diet, but it is well established that excessive levels can lead to disease. The average current daily salt intake is more than double the recommended level. The contribution of cheese to dietary sodium intake varies depending upon type and volume consumed. Sodium chloride is added primarily as a preservative, but also impacts on processability and flavour. This study was undertaken to determine the impact of variable salt levels on Cheddar cheese quality. Cheeses were manufactured in triplicate with 0.5, 1.25, 1.80, 2.25, 2.50 and 3.00 % (w/w) added salt and ripened at 8°C for 224 days. Differing salting levels had a significant impact on the key compositional parameters. Salt in moisture levels significantly decreased with decreasing salt levels, and moisture of non-fat substances significantly increased with decreasing salt levels. Starter lactic acid bacteria levels were highest and non-starter lactic acid bacteria grew more rapidly in the

lowest salt cheeses. Lysis was most rapid in the high salt cheeses. Proteolysis was greatest in low salted cheeses, due to increased water activity and enhanced chymosin/microbial activity. Degradation of α_{s1}- casein increased with reduced salt levels. Lipolysis was not influenced by salt addition rates. Firmness, fracture stress and fracture strain decreased with decreasing salt content. Descriptive sensory analysis found that the 0.5 and 1.25 % cheeses had off-flavours and significant sensory differences were found between the 1.80, 2.25, 2.50 and 3.00 % cheeses. All cheeses were found to be bitter and bitterness was found to increase with decreasing salt content.

Keywords: Sodium chloride, Cheddar cheese

Anti-Listeria Effect of Water-Soluble Extracts of Asiago Cheese

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Cheese ripening involves a complex series of biochemical events that leads to the characteristics taste, aroma and texture of each cheese variety. The most complex of these biochemical events, proteolysis, is caused by milk, milk-clotting, starter and secondary flora enzymes. During cheese ripening, peptides generation mainly results from caseinolysis. Some of these peptides are bioactive: they exert biological activities such as immunomodulatory, antithrombotic and antibacterial activities.
Asiago is a "Protected Denomination of Origin" (PDO) cheese of the North-Eastern region of Italy produced in two different varieties according to the length of ripening. *Asiago d'Allevo* is the variety produced with skimmed raw milk and ripened for periods varying from 6 to 18 months.
The main goal of this research was to evaluate the presence of antibacterial peptides in *Asiago d'Allevo* cheese toward two bacterial strains. The samples analysed were produced in alpine farms of Asiago plateau (Vicenza, Italy).
The presence of antimicrobial peptides was assessed in water-soluble extracts (WSE) of Asiago cheese ultrafiltrated onto 10 kDa cut-off membranes to remove proteins and dialysed in 100 Da cut-off dialysis bags to remove salt and organic acids. The antimicrobial activity of these WSEs was tested on *Listeria innocua* first. The samples that presented an inhibition were subsequently tested on *Listeria monocytogenes.* Antimicrobial activity was assayed by a micro-method using 96-well microplates and a microplate reader to determine microbial growth inhibition.

Keywords: antibacterial, *Asiago, Listeria,* peptides

Application of Multiple-Locus Variable-Number Tandem-Repeat Analysis (MLVA) to Type *Staphylococcus aureus* Isolated from North Italian Dairy Products

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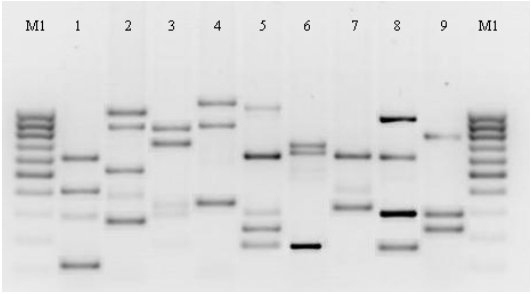
Staphylococcus aureus is a known major cause of foodborne illnesses, and milk and dairy products are often contaminated by enterotoxigenic strains of this bacterium.

A number of typing techniques are available to help trace the source and transmission rates of *S. aureus* from foods. Such techniques include restriction fragment length polymorphism analysis (RFLP), pulse-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST).
Recently, multiple-locus variable-number tandem-repeat (MLVA) was developed as a new generation protocol to subtype food-borne pathogens.
The aim of the paper was to evaluate, using MLVA, the genetic differences among *S. aureus* isolated from raw milk and dairy products of North Italy.
The study employed a total of 101 *S. aureus* strains isolated from 19 different provinces in 7 regions (Emilia Romagna, Liguria, Lombardia, Piemonte, Veneto, Trentino Alto Adige and Valle d'Aosta). The strains were from different raw milk and dairy products, and came from different animal species (79 isolates from cow and 22 from goat) with no evident problems of mastitis.
The MLVA profile (combination of the six primers *spa*, *clfA*, *clfB*, *sdr*, *sspA* and *coa*) of *S. aureus* isolates produced 4 to 7 bands, approximately between 100 and 1.200 bp.
The genomic variability in the *S. aureus* strains was considerable; in fact MLVA analysis revealed a high number (91) of MLVA patterns obtained for the 101 isolates studied here.
Cluster analysis showed that with a 30% similarity cut-off value, 5 major clusters and 27 strains do not fall into any cluster.
MLVA polymorphism was found to be a useful technique to discriminate several genetic variants in the *S. aureus* isolates, but no correlation was observed between the clusters obtained and the strains isolated from the different regions.
The present study is the first in Italy to use MLVA to study the polymorphism of *S. aureus* isolated from different dairy products.



Example of agarose gel of MLVA patterns generated from *S. aureus* isolates. M1: 100 bp DNA step ladder.

Keywords: *Staphylococcus aureus*, typing, MLVA

Total Antioxidant Capacity of Sheep's Milk

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Total antioxidant capacity in sheep's milk samples was measured by the spectrophotometric method of Re et al (1999) as modified by Chen et al (2003). This method is based on the scavenging of the radical cation $ABTS^{\cdot+}$ (2,2'-azinobis[3-ethylenbenzothiazoline-6-sulphonic acid]) by Trolox® as a reference antioxidant, monitored at 730 nm. The radical cation is produced by the overnight reaction (12 - 16 h) of ABTS (7 mM) with potassium persulfate (2.45 mM) in 10 mL deionized water, in the dark, at room temperature. The working solution is prepared by diluting it with saline phosphate buffer, pH 6.7, until the absorbance at 730 nm is 0.7 ± 0.02. To 2.0 mL working solution various amounts of Trolox (or milk samples) are added and the absorbance is read after 10 minutes at 25ºC. Under these conditions, a 25 μM Trolox concentration provides 100 % scavenging of $ABTS^{\cdot+}$. Trolox calibration curves were linear. The total antioxidant capacity of milk (TAC) is given as the equivalent amount of Trolox (μmol mL^{-1} milk).

Milk samples were diluted 10 to 15 times to avoid excessive turbidity, even at pH 6.7. Under the conditions described, values for percent scavenging were linear with the amount of diluted sample used. Milk fractions studied were: whole milk, skim milk and whey.
Sheep's milk samples were obtained from 9 commercial flocks throughout the lactating period, from February until July. Sheep were fed forage and concentrate until March and allowed to graze outdoors from early April, receiving some concentrate as supplement. Antioxidant capacity in milk samples was associated with the skim milk fraction (22 μmol mL^{-1}), with no differences observed between whole and skim milk. No differences were observed between milk samples before and after outdoor grazing, probably due to the fact that this method determines antioxidant capacity of water soluble compounds. Sheep's milk antioxidant capacity was about 50 % higher than that of cow's milk.

Keywords: antioxidant capacity, sheep milk, pasture, ABTS

Production of a Healthier Goat Cheese from PUFA-Enriched Milk

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A principal dietary sources of CLA are milk and dairy products. A feeding trial was designed with the aim to enhance the CLA content in goats' milk fat under field conditions and to investigate the extent of the changes and consequences for milk processing and cheese quality. Thus, a commercial supplement enriched in linseed (SEL) was incorporated into the goats' diet. Several industrial cheesemaking trials (9000 L), using both milk from goats' herds fed SEL and milk from control diet, were carried out. Fatty acid profile in milk and cheese fat was thoroughly monitored.
In the conditions assayed, minimum effects on the bulk milk composition were observed but the incorporation of SEL into the goats' diet markedly affected the lipid profile of milk. An increase in total CLA was found (0.62 *vs.* 1.27 %) and *cis*9-*trans*11-C18:2 was the principal isomer, accounting for as much as 82 % of the total CLA as revealed the Ag^{+}-HPLC analysis. In addition to enhancing CLA content, other FA potentially beneficial for human health, such as *trans*11-C18:1 and *omega-3* fatty acid *(C18:3n-3)*, also increased in the milk fat of goats fed SEL diet (1. 30 *vs.* 3.25 % and 0.31 *vs.* 1.26 % respectively). Furthermore, a noticeable reduction in saturated fatty acids level (67.3 *vs.* 58.5 %), especially of those fatty acids considered to be cholesterol-raising (C12:0, C14:0 and C16:0), was also detected.
The PUFA (CLA and *omega-3*) content after cheese processing and storage was comparable with the starting material, and no losses of potentially healthful FAs were found. Finally, although textural and sensorial analysis showed that cheeses made with milk from SEL diet were slightly softer than control, they exhibited similar taste and flavour characteristics and also good consumer acceptance. In conclusion, feeding goats with SEL could be an alternative way of obtaining dairy products with added health and nutritional value.

Keywords**:** CLA, PUFA, goat cheese, linseed

Effect of Milk Fermentation on Global Gene Expression in Human Blood Cells

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The immunostimulatory effect of yogurt has been studied in humans but, mostly, in vitro and in animal models. The evidence suggesting an enhanced immune responsiveness following ingestion of yogurt remains, however, limited. Also, the components responsible for these effects and the mechanism by which these components exert their immunological action are not completely understood.
In this approach our goal is to study the transient changes in human gene expression as a result of short period of exposure to bioactive components in GDL-milk (milk coagulated with glucono delta-lactone) and yogurt by using microarray technology. On the basis of this study we expect to identify key genes which are specifically and differentially regulated as well as to define gene expression signatures composed of genes that are coordinately expressed in humans, in response to the bioactive components present in these dairy products.
To this end we carried out a randomised, single blinded, controlled, crossover study with six healthy human volunteers by providing yogurt and GDL-milk and by examining the blood cell transcriptome at different time points following yogurt and GDL-milk ingestion. Our preliminary analysis of microarray data based on rank difference analysis method (RDAM) and combinatorial clustering analysis allowed us to identify genes that were differentially expressed in humans after ingestion of yogurt and milk. These genes are discussed in the context of the metabolic and immunological properties of dairy fermented products

Keywords: Yogurt, milk, immunostimulatory, microarray, transcriptome.

ACE-Inhibiting Tripeptides VPP and IPP in Different Cheeses of Swiss Origin

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The content of the two antihypertensive peptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) was determined in a total of 101 samples from 10 different Swiss cheese varieties using high performance liquid chromatography with subsequent triple mass spectrometry (HPLC-MS^3). In the category of extra-hard and hard cheeses the PDO cheeses Berner Alpkäse and Berner Hobelkäse, L'Etivaz à rebibes, Le Gruyère, Sbrinz, Emmentaler (organic and conventional) and in the category of semi-hard cheeses the varieties Tilsiter, Appenzeller ¼ fat and full fat, Tête de Moine and Vacherin fribourgeois were screened in the study. The average concentration of the sum of VPP and IPP in the screened cheese varieties varied to a large extent but substantial variations were also obtained within the samples from the individual cheese varieties (Figure 1). The lowest average concentration of the two tripetides was found in L'Etivaz à rebibes (n = 3) at 19.1 mg/kg whereas Appenzeller ¼ fat (n = 4) contained the highest concentration at 182.2 mg/kg. In individual samples the total concentration of VPP and IPP varied between 1.6 and 424.5 mg/kg. With the exception of a sample of a 10 year old cheese, VPP was always present at higher concentrations than IPP. Milk pretreatment, cultures, scalding-conditions as well as ripening time were identified as the key factors influencing the concentration of these two naturally occurring bioactive peptides in cheese. The results of the present study show that various traditional cheese varieties contain on average

similar concentrations of the two antihypertensive peptides to the recently developed fermented milk products with blood-pressure lowering property. This may serve as a base for the development of a functional cheese with blood pressure lowering property.

Keywords: ACE-inhibiting peptides; VPP; IPP; Swiss cheese varieties

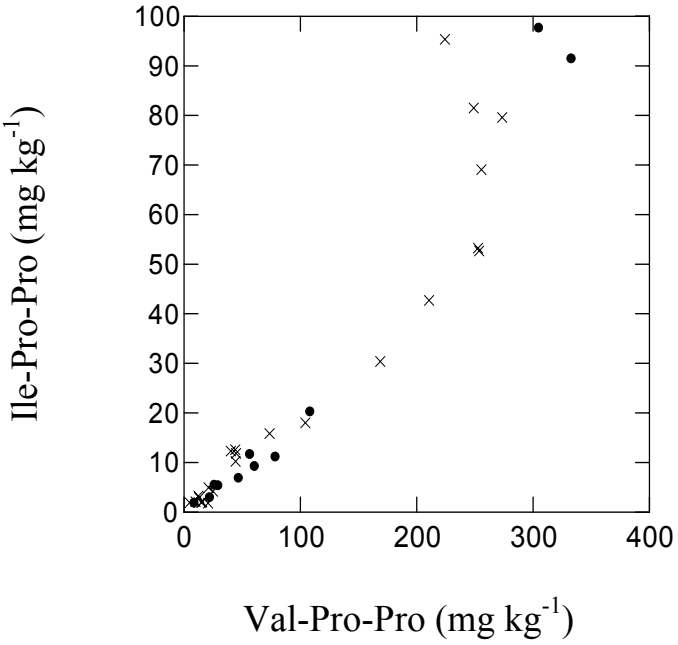

Figure 1: Val-Pro-Pro and Ile-Pro-Pro in • Berner Alpkäse (n=11) and x Berner Hobelkäse (n=23)

ACE-Inhibiting Tripeptides VPP and IPP During Ripening of Appenzeller ¼ Fat Cheese

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Recent studies have shown that the two angiotensin-converting enzyme (ACE)-inhibiting tripeptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) can occur in traditional cheese varieties at concentrations similar to those found in fermented milk products, which have been shown to lower blood pressure in hypertensive subjects. Milk pretreatment, cultures as well as scalding and ripening are important factors that determine the concentrations of VPP and IPP in cheese. In order to investigate the concentrations of VPP and IPP in Appenzeller ¼ fat cheese, a semi-hard cheese of Swiss origin, three loaves were purchased at the age of 90 days from three manufacturers and further ripened in a pilot plant under the same conditions. The concentration of the two ACE-inhibiting tripeptides VPP and IPP was measured at monthly intervals during the usual consumption period of Appenzeller ¼ fat cheese until the age of 10 months. Although similar trends were obtained for the corresponding concentrations of VPP and IPP in individual cheeses, large differences were obtained among the three loaves in the total concentration of VPP and IPP, ranging from 8-235 mg/kg (Figure 1). In two cheeses made partially with the same combination of cultures the concentrations of VPP and IPP increased continuously during ripening. In contrast to this, the concentrations of VPP and IPP remained at rather low level in the third cheese made with other cultures. The results of the present study indicate that maximal concentrations of VPP and IPP in cheese are mainly determined by the type of cultures used for cheesemaking. Further studies will

have to show the impact of selected cultures on the achievable concentrations of VPP and IPP in cheese.

Keywords: ACE-inhibiting peptide; VPP; IPP; cheese ripening

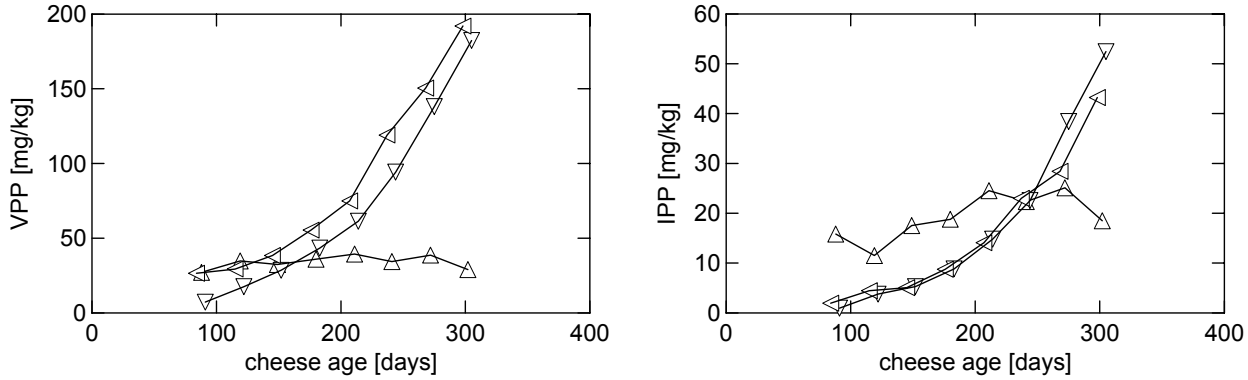

Figure 1: Concentrations of VPP and IPP during ripening of three Appenzeller ¼ fat cheeses

Behaviour of the Probiotic Strain *Lactobacillus gasseri* K7 in Ripened Semi-Hard Cheese

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Two strains of *Lactobacillus gasseri* were tested for their suitability as probiotic additives to cheese. They were added separately along the processing of semi-hard cheese (Tilsit type and Swiss type cheese, maximal warming temperature 38°C and 42°C respectively) together with the starter cultures. Samples were drawn from the centre region in defined intervals during the ripening phase to determine the survival of the probiotic strains. Growth of both strains was evidenced in the first 24 hours of cheese processing but only the strain K7 showed an ability to remain in concentrations above 10^6 cfu/g during the entire 90-day ripening period. The starter culture MK401, consisting mostly of *Lactobacillus delbrueckii* subsp. *lactis, Streptococcus salivarius* subsp. *thermophilus* and *Lactococcus lactis* subsp. *lactis*, was not affected by the bacteriocinogenic activity of K7. No compromising effects were observed regarding acidification and flavour development by the addition of *Lactobacillus gasseri* strains.

Keywords: cheese ripening, *Lactobacillus gasseri*, probiotic, flavour

Goat Cheese Production Naturally Enriched in Fatty Acids with a High Nutritional Value Using Different Feeding Systems

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In this study, the objective was to evaluate the influence of different feeding systems on the fatty acid profile in both goat milk and cheese.
Twenty-four Sarda goats in early lactation were divided in three homogeneous groups on the basis of DIM (71 ± 5; mean ± SE), milk yield (1320 ± 71 mL), LW (46.7 ± 1.48 kg). Two groups, supplemented with 300 g $head^{-1}$ day^{-1} of commercial concentrate were allowed to browse for 24 hrs daily on 2 plots characterised by a different cover proportion of woody and herbaceous species. These proportions were 90 and 10 % in one plot (Low herbage cover - LH), 70 and 30 % in the

second plot (High herbage cover – HH). The third group (Control - C) was stall-fed with hay (1.2 kg head^{-1} day^{-1}, 50 % alfalfa and 50 % ryegrass) and a commercial concentrate (0.6 kg head^{-1} day^{-1}). In three occasions the milk of each experimental group was collected and processed into cheese. Cheese was ripened at 24 hours and 2 months. Fatty acids composition was determined in milk and cheese samples.
Feeding system influenced significantly milk fatty acids composition. Between short chain fatty acids, butyric acid, characterised by a high anticarcinogenic effect, was significantly higher in the milk from pasture groups (HH, LH) than in the milk from control group (C). On the contrary the aterogenicity index (C12:0+4xC14:0+C16:0)/(unsaturated fatty acids), lied to the cardiovascular diseases appearance in human, was lowest in both grazing goat's milks. Milks from grazing goats were also characterised by the highest levels of fatty acids with a high nutritional value, such as polyunsaturated fatty acids (PUFA) in particular α-linolenic acid and ω-3 acids (EPA and DHA). A similar trend was observed in both unripened and ripened cheeses.
These results suggest that the grazing system improves nutritional milk and cheese quality for the advantage of the health consumers

Keywords: goat, milk, cheese, fatty acids

Calcium Levels and Chessemaking Aptitude of Raw Milk from Sheep Flocks under Part Time Grazing

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Calcium content and rheological properties of ewes´ raw milk from 10 commercial flocks of the Basque Country were measured throughout the milking season from February to late July. Flock sizes ranged between 200 and 500 heads of *Latxa* breed and all of them belonged to the Denomination of Origin Idiazabal Cheese.
Flocks´ diets consisted of concentrates and forages (hay and silage) until late winter. Around the middle of March Part Time Grazing (PTG) started, which means that ewes were turned out to pasture for a few hours and then housed again. Progressively, as season went further, grazing hours growed to the detriment of concentrate and forage supply.
A light increase in calcium levels was observed at the end of the milking period; 1689 ± 75.29 mg L^{-1} was measured at the beginning, whereas 1911 ± 170.28 mg L^{-1} value was obtained for the latest milks. We noticed higher data dispersion as we moved forward in the milking period. This might be due to a greater variability introduced by pasture based diets that are less homogeneous than concentrate and forage rations.
Curd firmness, measured as NIR transmission (%) in a Gelograph, and gel firming rate (Δfirmness/Δtime) showed a linear decrease, which was consistent with the results obtained for the curd resistance to compression. A relation between calcium content and technological aptitude of the milk was found.
This work was supported by grants from UPV/EHU (9/UPV 00042.125-15317/2003), UNESCO 05102 and INIA (RTA 2006-00100-C02-02). E. Abilleira acknowledges a predoctoral fellowship from the Basque Government.

Keywords: calcium, sheep milk, technological properties, grazing

Volatile Terpene Analyses in Milk and Cheeses by Solid-Phase Microextraction Technique

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Terpenes are lipophilic aliphatic compounds involved, as secondary metabolites, in plant pollination, in plant resistance to predation and infection. These compounds abound in certain species, particularly dicotyledons and, if ingested by herbivores, could be subsequently found in associated milk, cheese and meat. Terpenes have then recently attracted interest both for their possible impact on cheese sensory properties and potential terroir-biomarkers in milk and cheese. Various techniques were used for their determination including dynamic headspace gas chromatography-mass spectrometry or purge-and-trap but also headspace solid-phase microextraction (HS-SPME) was suggested for its low costs and easy use.
The aim of this work was the development of a HS-SPME method with a three-phasic fiber to analyse these compounds in milk and cheese that could allow to detect a higher number of compounds with respect to the other fibers generally used in literature. The better extraction method defined, both from the qualitative as well as the quantitative point of view, included a treatment of sample (milk or cheese) with a NaH_2PO_4 solution and an absorption in the headspace for 1 h at 53 °C.
During a research supported by Pro-Alpe Project of Italy Government, this method was applied to analyse milks and cheeses produced by cows fed in diversified grassland. This method allowed to isolate and identify many monoterpenes, as limonene, β-pinene and *p*-cymene, and also sesquitertenes as β-caryophyllene and *t,t*-farnesol. Therefore, the proposed method could satisfactorily perform qualitative and quantitative analyses of terpene compounds and be suitable to define terpene profile in dairy products and their link to grazing management.

Keywords: SPME, terpenes, milk, cheese

Persistency of Conjugated Linoleic Acid (CLA) in White Cheese from High CLA Milk

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The *cis*-9, *trans-11* 18:2 (CLA) presents several healthy properties demonstrated in experimental models. Being the cheese an important vehicle of this fatty acid for human being, this study was carried out to test the persistence of 9-*cis* 11-*trans* (CLA) on white cheese elaborated from high CLA raw milk. Milk containing 3.54 g CLA $100g^{-1}$ FA was obtained from Holstein (Holando Argentina) cows grazing an oat pasture (11 kg pasture DM cow^{-1}) and receiving a supplement composed by corn grain, corn silage, sunflower oil, fish oil and sunflower meal. After a 10 days period, individual milk samples were collected, analysed and transformed into white spreadable cheese reproducing industrial technology.
Fatty acid composition was analyzed by gas chromatography and comparison between raw milk and cheese were evaluated using the T-test for paired observations.
Aterogenic Index (AI) value resulted in 2.32 in basal milk, while it lowered to 1.45 in high CLA milk. The vaccenic acid (VA = *trans*-$11C_{18:1}$, CLA precursor) and *cis-9 trans*-11-CLA values were 2.29 and 1.04 g $100g^{-1}$ of FA in basal milk, respectively. The supplementation allowed to increase

these concentrations in + 4.66 g $100g^{-1}$ in VA and + 1.89 g $100g^{-1}$ FA in CLA. No differences in $C_{20:5\ n3}$ (EPA) and in $C_{22:6\ n3}$ (DHA) milk concentrations were detected.
In our study, the transformation of high CLA concentration milk into cheese didn´t significantly modified the different fatty acids in the product. In general, recovery was high and that of CLA in particular resulted in 101 %.
These results, which agree with other authors reports, show no negative metabolic or kinetic reactions of the starter or heating processes on final CLA content in the products. It is necessary to replicate these trials over different cheesemaking technologies integrating potential effects of other starter bacteria.

Keywords **:** milk, conjugated linoleic acid, white cheese, nutrition

Variation of Milk Fatty Acid Composition Depending on the Alpine Vegetation Type Grazed by Dairy Cows

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First results of an experiment aimed at investigating variation of milk fatty acid composition depending on the Alpine vegetation type grazed by dairy cows are presented.
The experiment was carried out in a dairy farm located in the Italian South-Western Alpine region during Summer 2007. Two vegetation types interesting for dairy production were investigated: *Festuca nigrescens* type (*treatment* F) and *Trifolium alpinum* type (*treatment* T). Paddocks were located at similar altitudes (2230 - 2350 m a.s.l.), in similar lithologic, climatic and topographic conditions.
Two groups of 9 dairy cows exploited F and T pastures for 6 d for rumen adaptation + 6 days of experimental trial. Cows were milked twice a day (at 7.00 and 18.00) directly into the paddocks using the same milking machine type.
During the experiment, representative samples of both the pasture types were collected. In addition, at day 3, 4 and 5, three samples of "bulk" milk from each group were collected. A total of 3 vegetation samples and 18 milk samples for each treatment were analyzed to characterize their fatty acid composition.
Results showed many differences in milk fatty acid composition according to the Alpine pasture vegetation grazed by cows, in particular for alpha-linolenic acid (ALA) content, occurring in higher amount in milk from *treatment* F.
Such difference seemed related neither to vegetation fatty acid composition, nor to ALA estimated total assumption. Consequently, there should be other factors influencing ALA content in milk in different ways depending on the specific composition of pasture vegetation.
Milk concentration of the main isomer of conjugated linoleic acid *(c9t11*-CLA) didn't seem influenced by treatments, although its high values were comparable to literature data for milk from Alpine pasture-fed cows.

Keywords: fatty acids, milk, Alpine pastures, alpha-linolenic acid, *Trifolium alpinum*, *Festuca nigrescens*

Large Inter-Breed Variations in Coagulation Abilities of Cheese Milk from Danish Dairy Cows

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The coagulation properties of cheese milk directly influence the cheese yield. Hence, non- or poorly-coagulating milk is unsuited for processing or results in a low cheese yield. Observations on non-coagulating milk from dairy cows of different breeds have increased, and the urge to investigate the underlying causes, as well as to establish the extent of this phenomenon is obvious.
By use of a free oscillation rheometry based technique (ReoRox) we investigated the coagulation properties, i.e., rennet coagulation time, gel-strength by storage modulus G' and curd firming rate of fresh skimmed milk obtained from three Danish dairy breeds (Jersey, Danish red and white (RDM), and Danish black and white (SDM)). An initial screening of individual cows' milk (n = 151) showed significant differences in coagulation properties between the three breeds. Within the breeds, the samples were grouped according to the curd firming rate i.e. low, average, high, for further studies of the relations between coagulation properties and milk composition of individual cows.
Multivariate data analyses (Partiel Least Squares model) showed, for the two breeds RDM and SDM, that milk yield negatively influenced the curd firming rate. This indicates that breeding for high yield could have a negative impact on milk coagulation properties.

Keywords: Breed, Coagulation Ability, Cheese Milk, Danish Dairy Cows

Impact of a Pasture-Based Diet Supplemented with Sunflower Seeds on Fatty Acid Composition and Aroma Profile of Butter

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In our study we investigated the possibility to produce butter enriched in unsaturated fatty acids (UFA) and conjugated linoleic acid (CLA) by studying the feeding of cows, the fatty acid (FAs) profiles as well as the aroma composition of conventional and UFA/CLA enriched butter products.
Two groups consisting of 10 cows each were fed different diets to obtain milk with different CLA contents. The control cows grazed on a pasture and additionally received maize silage. The other group grazed on the same plot and received a sunflower seeds mixture. After 12 days the milk was collected during two days to produce the butter. The fatty acid contents of the two butter types were determined using high resolution gas chromatography. CLA isomers were analysed by Ag+ -HPLC. Headspace solid phase microextraction (HS-SPME) combined with gas chromatography/mass spectrometry/olfactometry (GC/MS/O) was employed to evaluate the aroma compounds of the two butter types.
The addition of sunflower seeds resulted in a lower proportion of saturated and a higher proportion of unsaturated fatty acids in the milk fat. In particular the UFA/CLA butter had a higher concentration of monounsaturated, polyunsaturated (PUFA), including CLA, C18:1 trans FA, C18:2 trans FA without CLA and omega-6 FA. Conventional butter revealed significantly more short and medium chain fatty acids (C:4 to C:16). The cis-9 trans-11 CLA isomer amounted to

about 88 % of the total CLAs, and the isomer trans-7 cis-9 was significantly higher in UFA/CLA enriched butter.
Olfactometric analyses revealed similar odour profiles of UFA/CLA and conventional butter, characterised by creamy, milky, fruity and soapy notes.
The results show that a diet rich in sunflower seeds was suitable to produce UFA/CLA enriched butter with an aroma profile similar to conventional butter.

Keywords: butter, feeding, fatty acids, CLA, olfactometry

Study on the Non-Volatile Hydrocarbon Fraction of Dairy Products

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Food traceability and knowledge of the relationship between production processing and food composition have become subjects of great interest during the last decade, due to the increasing demand for genuineness, high quality, and origin assurance of food. In the last few years several researches have approached the problem of food authenticity, mainly studying the traceability by examining volatile compounds. Besides this fraction, extensively considered in literature as biochemical markers of origin, even non-volatile hydrocarbon molecules could be of interest for the evaluation of cheese origin.
Linear, branched, saturated, unsaturated and terpenic hydrocarbons are minor components of the unsaponifiable fraction of milk fat. Actual knowledge of hydrocarbon compounds in milk fat is limited and dated. In literature there are studies about hydrocarbons in butter, goat milk, and milk powder but there are no researches about cheese.
In this research, the non-volatile hydrocarbon fraction of mountain dairy products, from animals feeding on pasture, have been compared with milks and cheeses of lowland origin.
Hydrocarbons were separated from the whole lipid matrix, previously extracted from milk and cheese without using solvents, by silica gel column chromatography. The fractions isolated were analyzed by gas chromatography-mass spectrometry and the identification of the compounds was made by comparing the mass spectra with both the ones reported in the library and those obtained from authentic standards.
The main compounds detected belonged to the following chemical classes: n-alkanes having odd and even carbon number (C14 to C29), aldehydes, isoprenoid hydrocarbons and esters. The latter two chemical classes and, in particular, 1-phytene, 2-phytene and neophytadiene, could be promising markers of the origin of the product.

Keywords: traceability, hydrocarbons, milk, cheese

Development of a Method of Analysis for Phenolic Acids in Milk

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Phenolic compounds are secondary metabolites of plants generally involved in defence against ultraviolet radiation or aggression by pathogens. Among the different classes of these compounds, defined according to their molecular structure, very important are the phenolic acids, constituted of

a phenolic ring bonded to a short acidic chain and one or more –OH and $–OCH_3$ groups. These compounds can be distinguished in two classes, the derivatives of benzoic acid and the derivatives of cinnamic acid. Their presence was highlighted generally in plants but also in some foods such as drinks, jam and bakery products.
The aim of this work was to develop and validate a method of analysis for these compounds in milk in which have never been determined before.
The analytical procedure includes four steps: 1) extraction of phenolic acids with acetone and acetate buffer (pH = 4,6); 2) hydrolysis of the conjugated phenolic acids using β-glucuronidase at 37°C; 3) clean up, with a solid-phase extraction to remove co-extracted materials with a DPA-6S cartridge and finally 4) analysis with reversed phase liquid chromatography and an UV diode array detector equipped with a 5 cm length cell, operating in full scan modality.
The recovery and the repeatability of the method has been evaluated according to European current guidelines for trace methods then L.O.D. and L.O.Q. of 19 phenolic acids have been determined.
Analysis of milk samples produced in highland and lowland pastures were performed with this new method and results showed significant differences in phenolic acid composition due to the different feeding of the cows.

Keywords: phenolic acids, milk, liquid chromatography, traceability

Effect of the Diet of Lactating Ewes on the Lipolysis of Ripened Cheese

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The aim of this study was to evaluate the effect of including residues from citrus and artichoke crops in the diet of lactating ewes on the lipolysis of ripened cheeses. For this purpose 48 ewes were distributed in four groups, fed with rations containing 0, 10, 20 and 30 % (dry matter basis) of entire citrus during the first experiment, and artichoke silage during a second trial. Bulk milk samples for cheese manufacture were collected three times during the lactation period from each experimental group. Manchego type cheeses (Control, Ch1, Ch2, Ch3) were evaluated for chemical composition and free fatty acids (FFA) after 15, 30 and 60 days of ripening.
The inclusion of citrus in the diet of lactating ewes reduced the fat and total solids content in cheeses only at the level of 30 % of citrus. Artichoke silage in the diet also reduced fat content in cheeses mainly in those corresponding to diets with 20 and 30 % of artichoke.
The use of entire citrus and artichoke silage in the ration did not change significantly ($P > 0.05$) the FFA profile of cheeses. The major FFA present in cheeses, despite the diet and ripening time were myristic, palmitic and oleic acid, while the butyric acid was the major short-chain FFA in cheeses. However, significant differences ($P < 0.05$) between cheeses were observed during the evaluation of citrus rations: content of medium-chain FFA (C10-C16) and total content of FFA (C4-C18:2) were significantly affected by diet, but it was not possible to describe a specific trend. In contrast, the inclusion of artichoke silage at 20 and 30 % (DM) of the ration led to a reduction of the content of short (C4-C8) and medium-chain FFA in these cheeses (Ch2 and Ch3) and therefore in the total amount of FFA at the end of ripening.

Keywords: citrus residues, artichoke silage, ewe milk cheese, lipolysis

Effects of Milk Type and Ripening Conditions on Carra Cheese

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Carra cheese, is manufactured in South Anatolia region provinced in Hatay. Carra means pottery. In the production of Carra cheese, both rennet curd from goat's milk and heated acid curd (coekelek) from cow's milk are prepared.
Carra cheeses were made from raw and pasteurized cow's and goat's milk in triplicate. They were ripened for 90 days in pottery by burying in the ground which is the traditional way and also in refrigerator. pH, titrable acidity, dry matter, fat, protein, water soluble nitrogen (WSN), ripening index (RI), trichloroacetic acid soluble nitrogen (TCA-SN), proteose-peptone nitrogen (PPN), sodium chloride and organoleptic properties of cheese samples were determined at 1, 30, 60 and 90 days of ripening.
Milk type and the storage conditions had significant effects on pH, titratable acidity, dry matter, fat, protein, WSN, RI, PPN and organoleptic properties of cheeses ($p < 0.01$). pH, dry matter, fat, WSN, RI, NPN and PPN content of Carra cheese made from goat's milk were higher than in cow's milk cheese. Titratable acidity, dry matter, fat, salt, WSN, RI, NPN and PPN content of Carra cheese ripened by burying in the ground were higher than ripened in refrigerator.
The results of the organoleptic evaluation indicated that the cheese produced from pasteurized goat's milk and ripened for 90 days in the refrigerator received higher scores than other cheeses. The average composition of experimental cheese was 25.1 g fat, 23.7 g protein, and 6.32 g salt per 100 g cheese with an average ripening index of 49.1 %.

Keywords: Cara cheese, milk type, storage, goat milk, cow milk

Influence of Coagulating Enzyme Types on the Chemical and Sensory Characteristics of White Pickled Cheese Made from Ewe's Milk

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The possibilities of using recombinant chymosin as an alternative coagulant to commercial calf and microbial rennets in the production of white pickled cheese made from ewe's milk was investigated. For this purpose, white pickled cheese produced by using commercial calf rennet, recombinant chymosin (*Aspergillus niger* var. *awamori*) and microbial rennet (*Rhizomucor miehei*) were compared in terms of their chemical and organoleptic properties. The cheese samples were stored in brine containing 12 % salt at 4 ± 1°C for 60 days. In the study, which was carried out in duplicate, pH, titratable acidity, dry matter, fat, fat-in-dry matter, protein, salt, nitrogene fractions (WSN, RI, NPN), electrophoretic and organoleptic properties of the cheese samples were determined at 1, 15, 30 and 60 days of storage.
According to the results, the effects of enzyme type on the titratable acidity, dry matter, salt, nitrogenen fractions and all sensory properties, except odour was significant ($p < 0.05$). At the end of storage, the titratable acidity, salt, WSN, RI, NPN values and sensory scores of the cheeses increased, while the pH, fat, TN, protein, β- and α_{s1}-casein contents of cheeses decreased compared to initial values.

Keywords: recombinant chymosin, ewe's milk, white pickled cheese

Production Method of Carra Cheese

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Carra cheese, is manufactured in South Anatolia region provinced in Hatay (Antakya). Carra means pottery. In the production of Carra cheese, both rennet curd from goat's milk and heated acid curd (coekelek) from cow's milk are prepared.
Firstly coekelek is produced. Secondly goat's milk cheese manufactured. Cheese was manufactured from non pasteurized milk. Rennet in such a quantity to clot the milk and give curd ready for cutting after 80-90 min. When milk coagulation is completed, the curd is cut into cubes with an edge of 1-3 cm. The curds are transferred into vats lined with cheesecloth to drain whey and pressed. Then, curd is cut into 8 cm^3 segments to shape. After cutting cheeses are sliced 1cm thickness, and salt is spread on it.
When cheese reached the desired moisture content it is coated with black cumin. Finally, a pottery is filled one layer rennet curd an done layer coekelek in alternating order until it is full. The pottery is turned up side down and stored in a cool place for a few days. Then, some salt and thyme are sprinkled on or a vine leaf is put to the surface of the cheese before pottery is closed down with a piece of cloth. Finally, the mouth of the pottery is closed with mud or a mixture (slurry) containing ash, salt, olive oil and water. When the mud or slurry dries, the pottery is buried in ground in upside position and left for ripening at least for 3 months.
The mean composition of Carra cheese are pH 5.63, titratable acidity 0.85 %(l.a), dry matter 53.4 %, fat in dry matter 46.6 %, protein 18.9 % and sodium chloride 8.84%.

Keywords: Cara cheese, production, composition

Protein Degradation in Feta Cheese: A New Approach

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Variability of the proteolysis pattern in industrial Feta cheese as an effect of the local conditions of production was investigated. Feta from four cheese plants (3 batches each) was examined. The factories were located at different regions of the geographic area defined for this PDO cheese and variations in the processing method were applied (commercial yoghurt culture vs. home made yoghurt vs. other commercial starter, level of salting, pre-ripening time in the warm room). Sampling was performed up to 360 days. Analyses included N fractionation and subsequent determination by the Kjeldahl method, free amino acid analysis by RP-HPLC, and free amino group estimation by TNBS and Cd-ninhydrin methods. All parameters measured were calculated as percentages of the total N of the cheese.
The level of proteolysis differed between cheese plants. In all cases, data throughout ripening for water soluble N (WSN), 12 % TCA-soluble N (TCA-SN), TNBS (TNBS-N) and Cd-ninhydrin methods (NIN-N), as well as total free amino acid content (FAA) fitted very well logarithmic equations but slopes and intercepts between factories differed as a result of the processing conditions.
Irrespective the age and the cheese plant, values for TCA-SN were highly correlated to those for WSN ($R^2 = 0.9390$) as the majority of peptides in the soluble N fraction of Feta was soluble in 12 % TCA. A high correlation was also found between FAA content and TNBS-N or NIN-N scores ($R^2 = 0.8692$ and 0.9080, respectively) but differences were observed between factories and cheese ages.

It seems that the rapid methods could be a good alternative for monitoring amino acid release in Feta cheese. However, a large database should be established before proposing a model for the accurate estimation of FAA because variations in processing technology affect proteolysis, especially in respect of the amino acid pattern.

Keywords: Feta cheese, proteolysis, N fractions, correlations

Cheese Authenticity Recognition with Chemometric Complex Approach

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Italy is among the countries producing the greater number of typical dairy products, many of which are known worldwide. The originality of a cheese depends on several factors such as milk and cheese making procedures, which are strictly dependent on geographic origin. Since food quality prediction involves numerous input and output variables, statistical analysis results in a group of complicated, difficult to understand, mathematical expressions.
The objective of the present work was to obtain a greater understanding about some typical Italian cheeses, important for the Piedmont economy, and to discriminate their different productive origin using the smallest number of parameters.
Principal Component Analysis (PCA) was used to estimate the usefulness of the various chemical analyses used to differentiate the Piedmont cheeses studied. When the dataset was made of a sufficient number of samples, Artificial Neural Networks (ANNs) and Genetic Algorithms (GAs) were applied in order to obtain a more accurate classification.
The results showed that PCA is a good tool for the classification of Piedmont PDO cheeses. It was even possible to perform a data reduction and to identify the most relevant variables for classification purposes. Coupling ANNs and GAs, it has been possibile to achieve equally good or better results than what obtained with PCA, using only approximately a half of the parameters.
The high accuracy of the results here reported, suggests that this chemometric approach could be extended to classify other dairy products, in order to verify and safeguard Protected designation of Origin products.

Keywords: cheese geographic origin, PCA, ANN, GA

Microbial Dynamics During Parmigiano Reggiano Cheese Production and Ripening

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The use of length heterogeneity PCR (LH-PCR) was exploited to monitor the evolution of Parmigiano Reggiano cheese microflora during production and ripening. Have twin wholes available, allowed us to have samples representative of the subsequent stages of the same Parmigiano Reggiano dairy process. With the aim to randomly isolate representative microflora of the cheese we used different nutritive media. Moreover, random amplification of polymorphic DNA PCR of the isolates was applied to choose biodiverse strains to compose a LH-PCR database. This database was developed employing the peak profile obtained for isolated, molecular typed and identified by 16S rRNA gene sequencing strains.

The use of a culture independent method as LH-PCR overcame traditional agar plate and culture dependent method limitations. Moreover, the modality of LH-PCR samples preparation allowed to evaluate both the entire and lysed cells evolution during cheesemaking and ripening
L. helveticus and *L. delbrueckii* subsp. *lactis* were the dominant species until the second month of ripening, even if an increasing number of them underwent to autolysis process. One month after brining, at least two new species were able to grow in the cheese: *L. rhamnosus* or *L. casei* or *L. plantarum*, and *P. acidilactici* or *L. parabuchneri*. After six months of ripening, the same species were found even if no one of them seems to be dominant. Interestingly, in this stage of ripening, also *L. rhamnosus* or *L. casei* or *L. plantarum* which seems to increase, undergo to autolysis process. From the sixth to the twentieth month of ripening any microbial evolutionary change were observed.
Monitoring both entire and lysed cells through LH-PCR allows to coming aware of the importance of this two fractions in PR cheese production and ripening. This approach opens perspectives for insight microbial evolution in fermented food environment.

Keywords: Parmigiano Reggiano, LH-PCR, microbial evolution

Characterisation of Proteolysis in Castelmagno PDO Cheese

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Castelmagno is a semi-hard cheese produced in the province of Cuneo (Piedmont-Italy), made from raw milk without the use of starter. After cutting, the curd is left 24 hours at room temperature and then putted under acidified whey for 3 days. Then, the curd is crumbled, salted, putted into cylindrical moulds and pressed strongly. Finally the cheese is ripened in natural caves for at least 2 months. The commercial product has a cylindrical shape (20 cm high, 20 - 23 cm in diameter) and a weight of 4 – 6kg.
Although Castelmagno PDO cheese plays an important role in Piedmont dairy economy, there is a lack of knowledge on its evolution during manufacture and ripening. Therefore the aim of this work was to define the proteolysis during production and ripening of 3 batches of Castelmagno PDO cheese. For each sample two layers were analysed: under the rind (3 cm thick, located 2 cm below the rind) and the core (4 cm thick, located in the centre of the cheese).
Proteolysis was assessed at 1, 2, 5 days of manufacture and after 1, 30, 60, 90 and 150 days of ripening by urea-PAGE of the pH 4.6-insoluble fractions from the cheeses, by RP-HPLC of the pH 4.6-soluble fractions and amino acid analysis therefrom.
All cheeses showed a higher degradation of α_{s1}-casein compared to β-casein and this was superior in the layer under the rind. The higher degradation of α_{s1}-casein started in the cheese at 1 day of ripening as a consequence of leaving the cheese under acidified whey for 3 days. The principal free amino acids detected in all the samples were Leu, Glu, Phe, Val and Asp, while the greatest level of all amino acids was found in the cheese at 150 days of ripening in the layer under the rind.

Keywords: Castelmagno PDO cheese, proteolysis, cheesemaking, ripening

Free Fatty Acid Characterization During Ripening of Two Traditional Goats' Milk Cheese Varieties from Canary Island

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Palmero and Majorero POD cheeses are two of the traditional goat cheeses produced in the Canary Island, in Spain. Their main characteristics are 1- the use of raw milk from local goat breed, Palmera or Majorera goats', respectively; 2- the use of autochthonous forage for feeding the breed; and 3- their particular cheese-making procedure.
In this work the lipolysis of Palmero and Majorero POD cheese was studied throughout 180 days of ripening. Changes in the free fatty acid (FFA) profile of cheeses were followed using a gas chromatography. The total FFA concentration increased ($P < 0.05$) considerably during ripening of Majorero and Palmero cheeses. Nevertheless, the total FFA content of Palmero cheeses, were higher ($P < 0.05$) than in Majorero cheeses, reaching at the end of ripening values of approximately 20 and 11 g kg^{-1} of cheese, respectively.
Although quantitative differences in the total FFA content of both cheeses were observed during ripening, the FFA profile of Majorero and Palmero cheeses was similar. Globally, the dominant FFA produced throughout ripening of Majorero and Palmero cheeses were palmitic, oleic, myristic, and capric acids, which represented approximately 70 – 80 % of the total FFA content. According to chain length, the main FFAs released in both cheeses were, butyric (short-chain, C4 to C8), myristic (medium-chain, C10 to C14), and palmitic and oleic acids (long-chain, C16 to C18:2).

Keywords: Palmero cheese, Majorero cheese, lipolysis, free fatty acid

Characterization of Two Types of Argentinian Hard Cheese: Reggianito and Goya

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Reggianito and Goya are two types of hard ripened cheese produced in Argentina, principally in the pampas region. Origin of Goya cheese is situated at the very beginning of the XIX century. It is related to a little village of the same name, 800 km from Buenos Aires to north, where this hard cheese were produced to ship in boats and feed people doing the travel by Paraná river between this capital and Asuncion. Origin of Reggianito cheese is situated at the middle of the XIX century. It is related with the arrival to our country of Italian immigrants who bring his own technologies and recipes. At present this cheeses are elaborated by 1100 cheesemaker of different sizes. Approximately 40'000 tons are produced yearly and 15 % are sold abroad.
Reggianito and Goya cheese (in this order) are elaborated with standardized milk (2.4 % fat) and whole cow milk. Both are hard ripened cheese with rind; its body has a close texture without eyeholes and it has a slightly salty taste. The consistency is hard. Fermented cheese whey used as starter contains *Streptococcus thermophilus* and *Lactobacillus bulgaricus.* Coagulation is obtained with liquid or rennet powder. Curd is heated to 53 or 49°C. Cheeses are salted in brine for 10 or 6 days. Then, it is ripened at 14 to 18°C for 180 or 90 days in a cheese room with 80 to 90% relative humidity.
Samples of Reggianito and Goya cheese from 10 and 5 very important cheese factories were characterized. The work also describes the main composition, proteolysis indicators, volatile free

fatty acids, texture and flavor sensory profiles and other characteristics. Although cheese making technologies are similar, results shows differences between this two hard argentine cheese very well accepted by consumers in our country or abroad.

Keywords: Reggianito cheese, Goya cheese, origin, characterization,

Characterization of Milk and Cheese from an Argentine Ewe Breed

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Pampinta breed was developed locally in 1990 for meat and milk purposes in extensive operation by backcrossing local Corriedale (25 %) ewes with East Friesian (75 %) ewes. The result was an animal well adapted to pampas region, huge, without any wool in faces and foots, without horns, high milk production and non fatty flesh.
Milk from lactating Pampinta ewes grown at INTA Anguil Station was studied during a period of 1 year taking monthly samples. Raw milk average composition was 6.72 % total proteins, 5.03 % casein, 7.44 % fat, 4.72 % lactose monohydrate and 19.18 % dry matter. At the beginning of the lactation period DM represented more than 25 %. Mineral composition, freezing point, pH, casein as well as free fatty acid, total fatty acids profiles and cholesterol content was determined.
Semi hard cheese was elaborated 4 times during the year. Three replicates of each batch were analyzed. 10 cm diameter and 6 cm high cylindrical cheeses weighing 400 g presented a natural color and resistant rind covered with paraffin and packaged in a vacuum plastic film. Average composition was 36.9 % of moisture, 51.2 % fat in DM, 108 mg of cholesterol 100 g^{-1}; 15.5 % of total proteins.
Sensorial evaluation of texture and flavor was determined on a 1-7 scale with a trained panel from INTI. Results are: Elasticity: 3.4 ± 1.0; firmness: 2.6 ± 0.4; deformability: 3.1 ± 0.6; friability: 3.1 ± 0.9; adhesivity: 3.4 ± 0.2; crystals: only in some samples; solubility 4.4 ± 0.5; moisture impression: 3.5 ± 0.8. Sensorial evaluation of flavor shows the following results: Intensity of odor: 3.9 ± 0.8; intensity of aroma: 2.6 ± 0.2; sweet: 0; salted: 3.6 ± 0.3; sour: 1.9 ± 0.2; bitter: 2.5 ± 1.2; pungent: 1.8 ± 0.8.
After 17 years of selection, nowadays there are more than 20000 animals distributed in about 40 dairies along Argentina, and the semi-hard cheese is well accepted among local consumers.

Keywords: Pampinta, milk, cheese, characterization

Influence of Genetic Polymorphism and Technological Parameters on Proteolytic and Aromatic Profile of Goat *Cacioricotta* Cheese

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In cheese-making of typical Italian cheeses, goat milk from different autochthonous Italian breed is mainly employed alone or in mixture to ovine or cow milk. It is known the renneting behaviour of goat milk is strictly related to casein amount, which is controlled by quantitative class of α_{s1} alleles present in bulk milk and associated with high, intermediate or reduced level of α_{s1} (strong, intermediate or weak alleles). These alleles in turn determine the technological "fate" of goat milk, transformed in pressed (strong alleles) or fresh cheeses (weak alleles) by lactic fermentation as well

as drinking milk (weak alleles). Moreover, the α_{s1} level expressed, associated to different genotypes, seems to be inversely relate to "goat odours" intensity in obtained cheese.
The main objective of this work is the valorisation of goat *Cilentana* breed, reared in Southern Italy, aimed at its biodiversity safeguard by derived dairy products characterisation. For this scope, two typical *Cacioricotta* cheese productions, obtained from two bulk milks containing strong or weak goat α_{s1}–CN variants, respectively, are carried out with the same technological process, except for the coagulant agent (*mucor pusillus* or kid rennet paste). Furthermore, the determination of proteolytic and aromatic profiles of final products is respectively carried out by means of immuno-electrophoresis techniques coupled to mass spectrometry and GC/MS analysis.
The results can be summarized as follows:
- the proteolytic cheese profile is depending both on α_{s1}–CN genotype and coagulant agent (microbic or paste rennet);
- the volatiles free fatty acids (FFA) composition of *Cacioricotta* cheese only seems to depend on coagulant type, while terpenes composition, on pasture composition, as expected.

Keywords: α_{s1}–CN genotype, goat cheese, 2D electrophoresis, mass spectrometry, GC/MS

Proteomic Approach to Define Authenticity of Typical *Pasta Filata* Cheeses

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Italian bovine *pasta filata* cheeses, such as *Caciocavallo* or *Provolone* and well known *Caciocavallo Silano*, are the most typical cheeses produced in Southern Italy; whereas, the production of *Provolone Valpadana* is spread in Northern Italy, namely in "*Pianura Padana"*. The main steps of cheese-making process are consisting of: i) use of goat or lamb paste rennet as coagulant agent; ii) long time of curd acidification under whey, so that it can gain the stretching aptitude. In 1996 the PDO mark was assigned to *Provolone Valpadana* and *Caciocavallo Silano* cheeses, whose productions were strictly linked to Lombardia, Veneto, Emilia Romagna, Trentino, in Northern Italy and Calabria, Campania, Molise, Basilicata and Puglia in Southern Italy. Apart from bovine breeds, the two products are differing both in form and weight of cheese, which in turn determine their minimal ripening time, 30 or 90 days, for lower or higher size, respectively.
The aim of this work is to identify molecular markers either of geographical origin or cheese-making process by using a proteomic approach. The insoluble nitrogen fractions at pH 4.6 of *Provolone Valpadana* and *Caciocavallo Silano* cheeses are analysed combining 2D electrophoresis with mass spectrometry.
The obtained results indicate that geographical origin of *Caciocavallo Silano* and *Provolone Padano* cheese can be discriminated on the basis of rare alleles B and C at β-CN *locus,* respectively. Actually, the rare β-CN B variant is more frequent in individual bovine milk of *Podolica* breed, reared in Southern Italy, than the rare β-CN C variant mainly occurred in French breed. Finally, a very similar peptide composition and the same α_{s1} fragments, derived by specific chymosin action, are obtained from the same technological procedure.

Keywords: 2D electrophoresis, mass spectrometry, proteome cheese

Genetic Biodiversity of Mesophilic Lactobacilli from Fiore Sardo PDO Cheese

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Fiore Sardo is a traditional PDO hard cheese manufactured in Sardinia at farmhouse level from ewe's raw milk, generally, without any addition of selected or natural starter culture.
Previous studies showed that mesophilic lactobacilli are dominant non-starter lactic acid bacteria colonizing Fiore Sardo PDO cheese. They may have entered adventitiously from the milk and the immediate surroundings during cheese manufacture, and play an important role during the ripening of cheeses.
One sample of cheese was collected at 3.5 months after the production (the minimal ripening period for marketing according to the product specification), from 21 farms (almost 50 % of the total producing farms) located in different dairy areas of Sardinia.
More than 200 isolates grown in FH agar medium were identified at species level, by species-specific PCR or DNA sequencing.
Six species of mesophilic lactobacilli were detected: *Lb. brevis, Lb. coryniformis, Lb. curvatus, Lb. paracasei, Lb. paraplantarum, Lb. plantarum* and *Lb. rhamnosus*. *Lb. plantarum* and *Lb. paracasei* were the most frequently detected species. Moreover, the distribution of the mesophilic lactobacilli species was different in the twenty-one batches of cheese analysed.
All the isolates were typed by repetitive-element PCR (rep-PCR) technique, using the primer set GTG_5. Rep GTG_5 profiles were analyzed by both eye and Bionumerics 4.5 software (Applied Maths, Belgium).
The aim of this investigation was to characterize the mesophilic lactobacilli isolates for different species and strains and to study their potential correlation with their origins.
The high species and strain-level biodiversity of mesophilic lactobacilli isolated seemed was linked to the individual farms.
These results, therefore, bear out the link with the geographical environment and emphasize the significance of protected designation of origin and the specificity of the Fiore Sardo PDO cheese.

Keywords: mesophilic lactobacilli, strain typing, Rep-PCR, PDO cheese.

Oligopeptides in Parmigiano-Reggiano Cheese: Molecular Markers for the Enzymatic Activity and for the Assessment of the Ripening Time

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Parmigiano-Reggiano is an italian extra-hard cheese produced in the northern part of Italy from raw cow's milk and aged for at least 12 months. During the ageing process, α_{S1}, α_{S2}, β and κ caseins undergo an extensive degradation, due to the action of the endoproteases and exoproteases produced by the lactic acid bacteria present in the natural whey starter, in rennet and in raw milk. The nitrogen fraction of the aged cheeses is composed by a mixture of native caseins, high, medium and low molecular mass peptides and free amino acids. By using originally developed extraction techniques and LC/MS methodologies, a detailed study of the oligopeptide (< 10 kDa) fraction during the ageing process of Parmigiano-Reggiano cheese has been performed. The most abundant oligopeptides were identified and semiquantified in samples regularly taken from the curd up to 24 months of ageing. About 80 peptides deriving from the proteolysis of α_{S1} and β casein have been identified, obtaining indications on the preferential cleavage sites of the enzymes present in cheese and on their activity at different stages of the ageing process. In particular, it has been found that in the first days of ageing the peptide fraction is mainly characterized by the peptides produced by the rennet enzymes (chymosin). These peptides are rapidly cleaved by the proteolytic enzymes of the lactic acid bacteria, generating new smaller peptides which are the most abundant up to 3-4 months

of ageing. After this time the peptide fraction becomes richer in longer peptides apparently generated by the combined action of milk endoproteases and bacterial exoproteases. Several derivatives of amino acids, newly formed during the ageing period by enzymatic reaction with glutamic, pyroglutamic or lactic acid, have also been identified. The evolution of the peptide pattern during the ageing process allows to precisely assess the age of the cheese.

Keywords: Parmigiano-Reggiano, oligopeptides, LC/MS, proteases

Rheological Tests for Predicting of Sensory Texture in Swiss Raclette Cheese

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In the present study the meltability of Swiss Raclette cheese was investigated by comparing rheolgical properties and sensory texture. The aim of the study was to predict sensory texture based on rheological tests. Two identical series of seven experimental cheeses with one control were made from 70 l of milk under different conditions in order to study the impact of various cheesemaking parameters on melting properties. Three rheological tests including, a small amplitude oscillatory shear experiment (SAOS), a compression test (CT) and a tensile test (TT, Fig. 1) were applied for the investigation of melting, resolidification and force properties. Sensory texture analysis of the molten cheese was carried out by a trained panel. and focussed on, the two attributes ”ropy” and ”gummy” that are associated with poor melting properties. The trial was analysed by ANOVA and the means of the different fabrication methods were compared pairwise by Fischer’s LSD test. Pearson’s correlation was used between the rheologically determined values such as the melting and resolidification point (SMP, ERP), the storage modulus G’, the loss modulus G’’, tan δ_{max} (G’’/ G’), compression force and tensile force and the scores for the attributes ”ropy” and ”gummy”. The most distinct impact of cheesemaking on sensory attributes ”ropy” and ”gummy” was obtained by adding 50 g of citric acid to the curd with the wash water. In comparison to the control cheeses, the addition of citric acid significantly lowered total calcium content ($p<0.05$) and improved the scores for the attributes ”ropy” and ”gummy” ($p<0.001$). In the SAOS experiment the same cheeses showed significantly lower values for both G’ and G’’ ($p<0.05$) at 80°C. Similarly, significantly lower force values were obtained in the CT experiment ($p<0.05$) whereas the results of the TT (Fig 1) experiment showed only a trend to lower force values. The best correlation coefficient was obtained between the force values of the CT experiment and the scores for the attributes ”ropy” ($r=0.757$) and ”gummy” ($r=0.606$).

Keywords: Swiss Raclette Cheese, rheological test, sensory texture, ropy, gummy, meltability

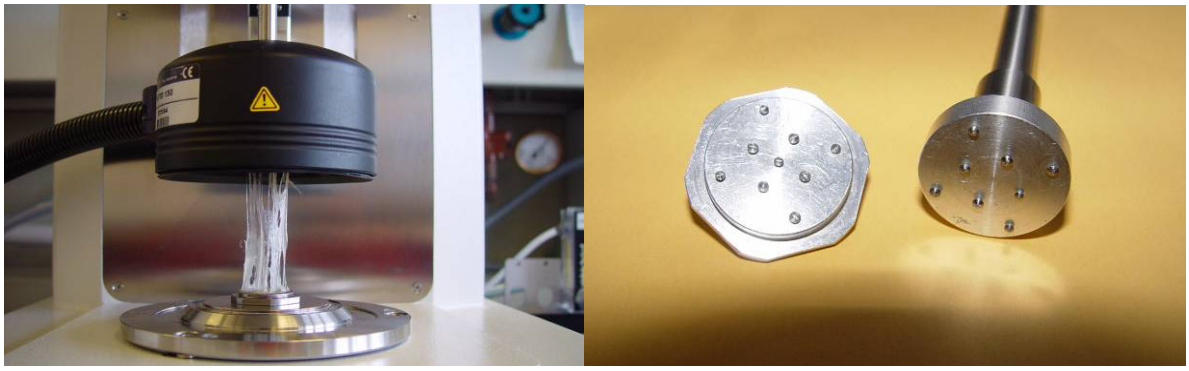

Figure 1: Tensile test for molten Raclette cheese with a commercially available rheometer (left) and two new designed plates (right).

Influence of Milk Pasteurization and Scalding Temperature on Proteolysis in Malatya Cheese

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Malatya cheese, a farmhouse Halloumi–type cheese, is characterized by scalding in hot whey during manufacture which produces an elastic and compact texture. Malatya cheeses were manufactured from raw or pasteurized milk and then the curds were scalded in hot whey at 60, 70, 80 or 90°C to understand the effect of heat treatment of cheesemilk or scalding temperature on the chemistry and biochemistry of the cheeses during 90 d of ripening. Differences in the levels of pH 4.6-soluble nitrogen between cheeses made from raw or pasteurized milk were significant ($P < 0.05$) after 30 d of ripening and these differences became greater as ripening advanced. Urea-polyacrylamide gel electrophoresis of the pH 4.6-insoluble fractions of the cheeses showed that both α_{s1}- and β-caseins were extensively degraded, especially after 30 d of ripening, but β-casein was less degraded than α_{s1}-casein. Peptide profiles by reversed-phase HPLC of the ethanol-soluble or ethanol-insoluble fractions of the cheeses showed that significant differences in the concentrations of some peptides between the cheeses made from raw or pasteurized milk; however, age-related changes in peptide concentrations were significantly different among the cheeses. Principal component analysis (PCA) was applied to simplify interpretation of the chromatographic data and distinguished between cheeses made from raw and pasteurized milk. The samples were also classified based on scalding temperature by PCA, but no regular distribution was observed. The results suggest that the pasteurization of cheese milk had a greater effect on peptide profiles of cheese than scalding temperature of the curd.

Keywords: Malatya cheese, proteolysis, scalding, raw milk, packaging

Chemical, Biochemical and Microstructural Characterization of Mihalic Cheese

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Mihalic is a hard, brined cheese, with a slightly acidic and very salty taste, regular openings (2 - 4 mm) and a 3 - 4 mm rind. It has been produced in Bursa and Balikesir cities of Turkey for more than 250 years. The chemistry, biochemistry and microstructure of Mihalic cheeses ripened in polyethylene bags or under brine were studied during 360 d. The gross composition of the cheeses ranged between 32.9-39.6 % moisture, 54.6-54.8 % fat-in-dry matter, 11.7-13.4 % salt-in-moisture and 20.4-23.2 % protein. The mean pH value of the cheeses was 5.0 at 1 d of ripening; however, it increased to 5.31 to 5.37 at 360 d. Proteolysis in Mihalic cheese was quite low due to its high salt-in-moisture and low moisture contents. The levels of soluble nitrogen fractions and urea-PAGE profiles of the pH 4.6-insoluble fractions were found to be significantly different at various stages of ripening. Urea-PAGE patterns of the pH 4.6-insoluble fractions of the cheeses showed that different degradation patterns of α_{s1}-casein were observed between the cheeses during 360 d of ripening. However, β-casein was more resistant to hydrolysis in the cheeses. Degradation of α_{s1}-casein was slower in comparison to other brined cheeses probably due to the higher salt-in-moisture content of Mihalic cheese. Packaging the cheese in polyethylene bags significantly increased the levels of pH 4.6-soluble nitrogen, 12 % trichloroacetic acid-soluble nitrogen and free amino acids compared to the brine salted cheeses. Total concentrations of free amino acids (AA) in cheeses

increased with age and Glu, Val, Leu, Lys and Phe were the most abundant AA in the cheeses. Higher levels of the corresponding AA were determined in the cheeses ripened in polyethylene bags. The peptide profiles (reverse phase-HPLC) of 70 % (vol/vol) ethanol-soluble and insoluble fractions of the pH 4.6-soluble fraction of the cheeses revealed some differences in the concentrations of some peptides among the cheeses. Images obtained by scanning electron microscopy of the cheeses ripened in polyethylene bags were clearly different (the protein matrix was more compact) from those of brine salted cheeses. In conclusion, ripening Mihalic cheese in polyethylene bags may be useful for easy storage while retaining quality.

Keywords: Mihalic cheese, proteolysis, microstructure, salt, packaging

Textural and Functional Improvement of Paneer Cheese Using Camel Milk with Exopolysaccharide-Producing Cultures

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Paneer, analogue to cottage cheese, is an indigenous coagulated milk product of Indo-Pak subcontinent, traditionally prepared by the addition of organic acid to milk (cow, buffalo or goat milk) at higher temperature. Cheese results in coarse and fibrous texture with typical nutty flavour (Masud et al. 2007). Paneer made by different varieties of milk gives characteristics flavour. Camel milk is traditionally used in many countries for preparation of different dairy products because it's noble properties of three times higher in Vitamin C than cow milk and lower in saturated fat, cholesterol, and lactose contents (Saima et al. 2003). These characteristics make camel milk suitable for preparation of a dietary cheese having low final saturated fat, cholesterol and lactose contents. But cheese making from camel milk is cumbersome process because of its low amount of κ-casein and a very limited ability of acidification, long cogulation time and weak coagulum (Mohammed, 1990). Paneer made by coagulating the camel milk at ambient temperature enhances the sensory characteristics but results in very soft texture which could not hold its shape during subsequent cutting/slicing. This needs to use some thing to improve the texture of cheese. Exopolysaccharide producing cultures could be an answer to improve the texture of camel milk Paneer.
We compared the texture and functional properties of Paneer made by using mixed starter of *Lactococcus lactis* AS1.18, *Lactobacillus delbrueckii subsp. Bulgaricus* AS1. 2132 with and without exopolysaccharide (EPS) producing strain *Lactobacillus kefiranofaciens* ZW3. Paneer made with starter culture having EPS producing strain reduced coagulation time, resulted strong coagulum with improved aroma, taste and mouth feeling as compared to cheese which lacked the EPS producing strain.

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Keywords: Paneer, cheese, exopolysaccharide

Proteolysis of Galotyri-Type Cheese Made Using Different Procedures

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The objective of this study was to determine the effect of different manufacturing procedures of Galotyri-type cheese, an acid/rennet-curd fresh cheese, on its proteolysis during storage. Three cheesemaking methods were evaluated, namely production of cheese using salted ewes' milk, starter culture and rennet (SM+R), and starter culture with or without rennet and salting the curd after draining (R+SC or SC, respectively). Gross proteolysis, as measured by the percentage of water soluble nitrogen (WSN), was found to be significantly affected by the manufacturing method. Omission of rennet during Galotyri-type cheese preparation resulted in reduced proteolysis. The SM+R cheese had higher levels of WSN than the other two cheeses throughout storage. There were no significant ($P > 0.05$) differences among the cheeses in the levels of total free amino acids, measured by the cadmium-ninhydrin method, at any sampling age. No differences in the urea-PAGE electrophoretograms were observed between the cheeses made with addition of rennet to cheese milk (R+SC and SM+R cheeses). From the PAGE electrophoretogram of SC cheese was evident that the two bands in front of the β-CN remained unchanged during storage while in the other two cheeses these bands were totally or partially hydrolysed. Also, some faint bands in front of α_{s1}-CN were additionally present in the electrophoretograms of the cheeses made with the addition of rennet. Primary proteolysis of all cheeses was very limited during storage, as indicated by the WSN and urea-PAGE.The way of milk coagulation had a relatively small effect on the RP-HPLC elution profiles of water-soluble extracts of cheeses. Apart from quantitative differences, the differences between the cheeses were limited to the absence of only two peaks in the peptide profile of SC cheese.

Keywords: Galotyri cheese, fresh cheese; acid/rennet-curd cheese; proteolysis

Free Fatty Acids and Volatile Compounds in Galotyri-Type Cheese Made by Different Cheesemaking Procedures

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The objective of this study was to determine the effects of different manufacturing processes of Galotyri-type cheese, a Greek acid/rennet-curd cheese, on the free fatty acids (FFA) composition and volatile compounds of the resultant cheeses, during storage for 30 days. Three cheesemaking methods were evaluated, namely production of cheese using salted ewes' milk, starter culture and rennet (SM+R), and starter culture with or without rennet and salting the curd after draining (R+SC or SC), respectively. No significant ($P > 0.05$) differences were observed for the total FFA content ($C_{2:0} - C_{20:0}$) at 1 and 15 days of storage among the three cheeses. However, at 30 days, the R+SC and SC cheeses had significantly ($P < 0.05$) higher content of total FFA than the SM+R cheese. The most abundant FFA in all cheeses, was lauric acid ($C_{12:0}$) and at 30 days of storage its content was significantly ($P < 0.05$) higher in the R+SC and SC cheeses than in the SM+R cheese. Almost the same volatile compounds were identified in the three cheeses, i.e. aldehydes, alcohols, ketones and acids. The SC cheese had more volatile compounds than the other two cheeses. Ethanol, acetone

and acetic acid were the most abundant volatile compounds in all cheeses. Moreover, the R+SC cheese had more ethanol and acetone than the other cheeses at 30 days of storage.

Keywords: Galotyri cheese, lipolysis, free fatty acids, volatile compounds

Effective Label Friendly Food Protection Against Yeasts in White Brined Cheese Applications

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Market samples of white brined cheese from Greece, Turkey and Bulgaria have been analysed for the presence of spoiling yeasts. The isolates were further analysed by Rep-PCR technology and identified as *Pichia fermentans*, *Yarrowia lipolytica*, *Saccharomyces cerevisiae*, *Kluyveromyces lactis* and *Debaryomyces hansenii*.
The sensitivity of these spoilage yeasts against Danisco's HOLDBAC™ YM protective cultures was tested on a screening agar with a "Feta"-like composition. A wide variability in the inhibitory effect of the protective cultures was found between yeast species but also within the same species.
Two protective cultures from Danisco's HOLDBAC™ YM range were applied to the vat milk together with the standard acidifying starters and white brined cheese was produced on industrial scale. These cheeses were microbiologically tested for presence of yeasts. During the first three months storage at 4 °C the natural yeast contamination in cheeses produced with protective cultures was below or just above the detection level of 100 cfu·(g cheese)$^{-1}$. The unprotected cheeses from the same industrial plant reached levels >10^4 cfu·(g cheese)$^{-1}$ within 3 months storage and >10^5 cfu·(g cheese)$^{-1}$ at end of shelf life, here 4 months. The yeast contamination in the samples where the two different protective cultures were applied reached levels of $3 \cdot 10^2$ cfu·(g cheese)$^{-1}$ respectively $2 \cdot 10^4$ cfu·(g cheese)$^{-1}$ after 4 months storage, and only one out of 6 assessors described in one of two replicate samples a yeasty off taste.
These results show that HOLDBAC™ YM range protective cultures can effectively inhibit the growth of spoilage yeasts in white brined cheese, without any adverse sensory effect.

Keywords: white cheese, yeast, mould, HOLDBAC™

A Model to Assess LAB Aminopeptidase Activities in Parmigiano Reggiano Cheese During Ripening

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The aim of this work was to investigate in which stages of ripening of Parmigiano Reggiano cheese, LAB aminopeptidases, liberated after cells autolysis and present in cheese extract, could be involved in secondary casein degradation.
In particular we evaluated six different substrates to reproduce PepN, PepC, PepA, PepL, PepI and PepX activities releasing different N-terminal amino acids. The effects of pH, NaCl concentration and temperature on the enzymes activities of amino acid βNa-substrates were determined by

modulating the variables in 19 different runs of an experimental design. It allowed us to obtain mathematical models able to assess the effect on aminopeptidases activities over a range of values covering different environmental conditions in different zones of the cheese wheel at different aging times.
The aminopeptidases tested in this work were present in cell free Parmigiano Reggiano cheese extract after 17 months ripening and were active in model system. The modelling approach explicit that to highlight the individual and interactive effects of chemical-physical variables on enzyme activities, is helpful to determine the true potential of an aminopeptidase in cheese. Generally, temperature and pH exerted the more relevant effects on the enzymatic activities and in many cases a relevant interactive effect of these variables was observed. Increasing salt concentration slowed down PepC, PepA, PepI and PepL. Interestingly, this variable did not affect PepN and positively affected PepX.
Our results evidenced that the six different LAB peptidases participate to cheese proteolysis and are induced or inhibited by the cheese production parameters which, in turns, depend on the cheese dimension.
The models elaborated varying pH, temperatures and salt concentration resulted to be a useful, low costly and time-consuming tool to understand the role of the main peptidases in the different phases of cheese ripening in relation to the major environmental factors influencing enzyme activity.

Keywords: aminopeptidase activity, lactic acid bacteria, Parmigiano Reggiano cheese, predictive model

Free Fatty Acids and Acidity Level in Romanian Traditional Cheese Burduf, Associated with Changes in *Listeria spp.* and *Lactobacillus spp*. Populations During Ripening

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In order to evaluate specific changes occurred in free fatty acids (FFA), acidity, Listeria and Lactobacillus levels, traditional Romanian cheese Burduf was tested monthly during 90 days of ripening. FFA level increased slightly during ripening, such tendency being common for most cheese types. The acidity of the cheese decreased slowly as shown by pH fluctuation (from 5.32 to 5.51 and 5.67 for the first, second and third monthly evaluation respectively). Neither the pH values, nor the FFA levels showed significant differences between the outer and inner cheese layers in any of the investigation assays. Natural contaminating *Listeria monocytogenes* was isolated from both inner and outer layers of the cheese chunk within the first two sets of investigation. After the third month, the qualitative test repeatedly failed to isolate *Listeria spp*. bacteria from both layers of the ripened cheese. The level of *Lactobacillus spp.* bacteria increased during the first two months and remained constant throughout the third month of ripening. The initial number of *Lactobacillus spp.* was higher in the outer layer (7.03 log cfu g^{-1}) than in the core (6.93 log cfu g^{-1}), whereas after ripening the inner layer (7.83 log cfu g^{-1}) was more populated with *Lactobacillus spp.* than the outer layer (7.67 log cfu g^{-1}). The present study suggests that a traditional ripening process featured by a regular biochemical pattern (as shown by the FFA evolution) is associated with the loss of *Listeria monocytogenes* which naturally contaminates raw milk cheese. The loss of this contaminant appears to be caused by the *Lactobacillus spp.* bacteria, as no processing or additives were used and the acidity decrease was favourable for *Listeria spp*. survival and even growth.

Keywords: *Listeria spp*., *Lactobacillus spp*., cheese, ripening, Burduf

Biofilms Present on Wooden Shelves Used for Cheese Ripening: Description and Inhibition of *Listeria monocytogenes*

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Wood has a long tradition as a natural material used in food production. Nowadays, its regulative water sorption properties and the cheese organoleptic qualities it provides make this support particularly well-adapted for cheese ripening. However, the question of its use during cheese ripening has frequently been asked for safety reason and little work has been carried out on the microbiological life in this environment. There is a clear need of scientific investigations on the microbiological ecosystems on dairy wooden shelves.
To answer this question, the description of the biofilm ecology was first made on wooden shelves used in the ripening of a raw milk smear cheese, at two different steps, the beginning and the end of the ripening cycle. In the two cases, the biofilm appeared to be mainly composed by technological cheese micro-flora and it remained stable over time. Secondly, the behaviour of *Listeria monocytogenes* after deposition on wooden shelves used for ripening revealed the protective effect against *Listeria* of the shelves. The inhibitory effect of the ripening shelves was observed for the two *L. monocytogenes* strains tested (serotype 1/2a and 1/2b). It appeared to be more important at the beginning than at the end of the ripening cycle. Thirdly, the protective effect against *Listeria* of consortia removed from shelves was investigated by direct inoculation of *Listeria* in removal suspensions. The consortia revealed also an anti-*Listeria* effect on the two strains. This analyse threw new light on the anti-*Listeria* effect mechanisms.

Keywords: biofilm, wood, *Listeria monocytogenes*, cheese

The Role of Autochthonous LAB in the White Brined Cheeses Ripening

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The effects of different autochthonous lactic acid bacteria, isolated from Serbian cheeses, were studied throughout 90 days of white brined cheeses ripening. The cheese A was produced with *Lactococcus lactis* ssp. *lactis*, *Lc. lactis* ssp. *lactis* bv. *diacetylactis*, *Lactobacillus paracasei* and cheese B was made with *Lactococcus lactis* spp. *lactis* i *Lc. lactis* ssp. *cremoris, Lc. lactis* ssp. *lactis* bv. *diacetylactis*, as starter cultures.
The number of starter culture cells maintained on the high level of 6-8 log cfu g^{-1}, during the monitored cheese ripening period. There was no significant influence of the different LAB on the gross chemical composition, but the course of proteolytic changes was slightly different. At each ripening time (30, 60, 90 days), proteolysis was assessed by the water-soluble fractions (WSN), soluble fractions in 5 % PTA (PTA-N) and SDS PAGE electrophoresis.
The cheese A exhibited the higher rate of proteolysis in the early ripening stages. At 30 day-old cheese A the content of WSN/TN and PTA-N/TN was 16.54 and 2.54 %, while residual α_s-casein and β-casein were 26.12 and 36.71 % respectively. Data for the content of WSN/TN and PTA-N/TN of cheese B was 14.36 and 2.41 %, while residual α_s-casein and β-casein were 52.37 and 46.11 % respectively. At the end of observed ripening period (90 days), the proteolytic changes of

cheese B were more intensive (residual α_s-casein and β-casein were 17.33 and 18.43 %, respectively) than of cheese A (22.58 and 33.63 %, respectively).
The sensory evaluation showed that white brined cheeses achieved high total scores at the each ripening time.
As conclusion, isolated and selected autochthonous LAB could be applied as starter culture in white brined cheese production. The using of autochthonous LAB provides white brined cheese with specific sensory characteristics, as well as high and standard product quality.

Keywords: white brined cheese, autochthonous LAB, electrophoresis, proteolysis

Evaluation of Sensory Characteristics of D.O. Zamorano Cheese by Consumers

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The use of trained panels is a common practice in the sensory evaluation of dairy products. However, the development of sensory profiles involves systematic and careful training of the judges in descriptive methodologies over long periods of time. For this reason, the aim of the present work was to determine whether the use of ranking tests by frequent consumers of hard ewe's cheeses might offer an effective method for the study of the sensory attributes of such products.
To accomplish this, consumers were asked to perform ranking tests of the most relevant cheese attributes previously determined by a trained panel. Indeed a rank preference test and a hedonic test were performed. Instrumental analyses of colour and hardness were also carried out to compare both methods. The cheeses were elaborated according with D.O Zamorano cheese Board with milk from three breeds of sheep (Castellana, Churra and Assaf) with different somatic cell counts (lower than 500,000 cells mL^{-1}; between 1,000,000 and 1,500,000 cells mL^{-1}, and more than 2,500,000 cells mL^{-1}).
From the results it may be concluded that it is possible to evaluate the sensory characteristics of cheeses using ranking tests carried out by frequent consumers, since it was observed that these latter were able to distinguish among the different cheeses. The sensory characteristics of hardness, intensity of taste and pungency reflected a significant influence of the SCCs of the original milk whereas breed significantly affected hardness and colour intensity. Finally, the SCC affected Warner-Bratzler Shear Force (WBSF) and the colour parameter L* and b*, although it did not follow a trend in accordance with the values of somatic cells. The instrumental parameters best related with the assessments of the frequent consumers were WBSF and the colour value of *a** for hardness and colour respectively. Finally, no statistically significant differences were found regarding preference.

Keywords: breed, SCC, consumer preference, ranking test

Method for Measuring Casein Particles Size in Artisanal Fresh Sheep Cheese by Dynamic Light Scattering

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Dynamic light scattering (DLS) has, up to recently, mostly been applied for scientific purpose. But, in recent years, it is gaining importance in quality control of different industrial products as well as for analysis of biological systems. The purpose of this preliminary study was to evaluate the possibility of using DLS for measuring the casein particles size in artisanal fresh sheep cheese. It is well known that casein size has great impact on the cheese structure. The samples of the artisanal fresh sheep cheeses (n = 10) were collected during the lactation season in 2007. The cheeses were made from Friesian sheep un-refrigerated raw milk and had the shelf life of three days. The samples were analyzed on the first day after production. Casein was isolated using modified Ridascreen Casein method: Enzyme immunoassay for the analysis of bovine casein (Art.No.:R5102, R-Biopharm AG, Darmstadt, Germany, 2006.). pH of prepared casein suspensions has been adjusted to the value of pH measured in cheeses. The casein particles sizes were determined by DLS using Zetasizer Nano ZS (Malvern, UK) instrument operated with green laser (532 nm). For each sample ten measurements were performed on 25°C. The measured mean size of casein particles in the analysed samples was 12.81 ± 0.36 nm. No significant differences in sample variability were observed according to Bartlett's test. These results indicate that DLS method might be useful and efficient tools for studying the structure of the artisanal cheese. Moreover, the publications dealing with cheese structure are scarce and data poorly understood. In addition, DLS method is non-invasive and can be applied for measuring the sizes of the isolated individual particles using conditions which are similar or identical to those in the original samples.

Keywords: cheese, casein, size, DLS

Characterization of Yeasts and Moulds in White Pickled Travnički Cheese and Brine

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This study was conducted to determine species of yeast and moulds that could occur as contamination of traditional Travnički cheese from Vlašić mountain in Bosnia and Herzegovina (B&H). 13 samples of cheese and the brine from different vats were analysed after 60 days of ripening. Besides, for each cheese and related brine sample, pH value and salt concentration were analysed.

Total amount of yeast and moulds on Sabouraud-Maltose agar plates counted from 10^2 to 10^4 in cheese (cfu g^{-1}) and the same in brine (cfu mL^{-1}). More divergent population of yeasts and moulds were present in brine then in related cheese samples. Thus, each cheese and brine sample represented special microbial niche.

Following yeast Genera were isolated from Travnički cheese and related brine: *Rhodotorula, Hanseniaspora, Debaromyces, Cryptococcus, Pichia* and *Saccharomyces.* Mold Genera isolated from Travnički cheese and related brine were as follows: *Penicillium, Cladosporium, Geotrichum, Asperigillus, Caphalosporium, Trichotecium, Fusarium* and *Mucor.*

Keywords: Yeasts, Moulds, White Pickled Travnički Cheese

Changes of Nitrogen Fractions During Ripening of Gorgonzola-Type Cheese Produced in Serbia

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In this study the chemical composition and changes of nitrogen matters in two Gorgonzola-type varieties were examined. Cheeses with commercial names "Blue sapphire" and "Blue birch" were produced using two types of *P. roqueforti* moulds, type "esportazione" and "dolce", respectively. The cheeses are cylindrical in shape and the small ones ca. 2 kg weight were made for the experimental purpose. Analyses were conducted on the core and under the rind, in order to define and compare the chemical composition and the degree of proteolysis in cheese layers of both varieties. The pH increase was initially higher in both cheese surface areas. The final pH value was ca. 6.1 under the rind of both "Blue sapphire" and "Blue birch" variety, while in the core area it increased to pH 6.56 and 6.81, respectively.
The water-soluble nitrogen fractions increased throughout the ripening process, especially after 30 days, concomitant with mould growth and sporulation. The proteolytic activity increased from the surface to the centre of the cheese mass. In 60 days old „Blue sapphire", the ripening index under the rind and in the core was ca. 19 and 34 %, respectively. In „Blue birch", this value under the rind was ca. 31 % and markedly higher in the inner area, ca. 50 %. The increases of trichloroacetic acid soluble nitrogen (TCA-SN) and phospho-tungstic-acid soluble nitrogen (PTA-SN) as well as primary and secondary nitrogen products (PNP and SNP), were also higher in „Blue birch" variety. The maximal values of these fractions were detected in the core area (ca. 29 % TCA-SN, 15 % PTA-SN, 30 % PNP and 20 % SNP of total nitrogen), which indicates extensive proteolysis in the core of „dolce" comparing to „esportazione" variety. Generally, the intensity of proteolytical activity of *P. roqueforti* varies considerably among strains, which is confirmed in this work.

Keywords: blue-veined cheese, chemical composition, proteolysis, *P. roqueforti*

The Market of Ripening (hard) Cheese in Poland Versus Main Competitors in the EU

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Cheese market in Poland shows a high growth rate, which is confirmed with growing production and turnover in foreign trade. Over the period of 2003-2007 cheese production increased by 35 % to 265 thousand tons while exports doubled and reached 104 thousand tons. The rise in went along with a widening market offer. Despite it the Dutch-type cheese still dominate the production structure. With 17 % of the sales value and 31 % of the export value are products of great importance for the branch. At the same time the market supply is poorly supplemented with imports.
Cheese production in Poland is very fragmented. There are very few companies producing circa 10 thousand tons. The market is dominated with dairy co-operatives. Most private companies are based on foreign investments.
Despite a dynamic development the production of ripening cheese in Poland still lags behind the main competitors from the EU: France and Germany, Italy and the Netherlands. Unlike Poland the EU countries are featured with high volume imports and exports, i.e. high intensity of intra-industry trade.
Per capita consumption of ripening cheese amounts to 4 kg per capita, which is significantly below the level reported in the EU-15. Low income and a lack of historical tradition are the main reasons for low level of consumption. Low level of consumption indicates a high capacity for the development of the Polish market. However the milk quota foxed for Poland is a serious obstacle for further development of the sector.

The EU enlargement eroded the competitiveness of the entire dairy sector. The increase in procurement (farm-gate prices) and producer prices of dairy products along with appreciation of Polish currency against euro. In the future the price conditions will be of declining importance on the competitiveness of the domestic cheese sector. Therefore it is necessary to improve the efficiency of production and trade as well as to intensify marketing activities.

Keywords: hard cheese, dairy sector, foreign trade, competitiveness, consumption, price

The Effect of Different Ripening Temperatures and Starter Types on Proteolysis and Bitterness of Kasar Cheese

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Kasar cheese, is one of the most popular cheese varieties manufactured in Turkey. It is a kashkaval like, scalded and kneaded cheese. It has semi-hard or hard texture, manufactured from cows' milk. It can be consumed fresh or after ripening. Bitterness is an important quality defect that limits the acceptability and marketability of these cheese types.
In this study, the influence of the storage temperature and two starter types on proteolysis and bitterness in Kasar cheese was investigated. As starter cultures; single culture of *Streptococcus salivarius* subsp. *Thermophilus* (cheese A) and the mixed culture of *St. salivarius* subsp. *thermophilus* + *Lactobacillus delbrueckii* subsp. *bulgaricus* + *Lb. helveticus* (cheese B) were used in Kasar cheese production. Cheese samples were ripened at 4 and 8°C to determine the effect of storage temperature on the cheese quality during ripening at 1, 30, 60, 90 and 120 days of. The level of proteolysis in the cheese samples was checked by applying chemical methods, UREA-PAGE electrophoresis, and RP-HPLC techniques. Gel filtration and RP-HPLC techniques were used to identify the molecular weight and the peptide composition of bitter fractions in cheese samples where bitterness was determined by sensory evaluation. Principal component analysis (PCA) was applied to the data in order to explore the effect of different ripening temperatures and starter types on proteolysis and bitterness of Kasar cheese.
When the results were evaluated, increasing and decreasing variations were observed in bitterness scores during the ripening period depending on proteolysis. Proteolysis level was limited, probably because of the high scalding temperature. However, the highest bitterness scores were obtained at the cheese B samples which were produced with proteolytic starter cultures and ripened at 8°C for 60 days. According to statistical evaluation storage temperature, ripening time and starter type have an important effect on Kasar cheese quality ($p<0.05$). The molecular weight of the bitter fractions was found approximately between 500 and 1000 Da. It was determined that bitter fraction having 500 Da contains high amount of tyrosine, phenylalanine and methionine.

Keywords: Kasar cheese, Bitterness, Proteolysis, Electrophoresis, RP-HPLC, PCA

The Effect of Different Ripening Temperatures and Starter Types on the Quality of Turkish White-Brined Cheese

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The objective of this study was to evaluate the influence of defined starter cultures and different ripening temperatures on the gross composition, proteolytic profile and bitterness of Turkish White brined (TWC) which limits the acceptability and marketability of these cheese types.
As starter cultures; lyophilized mixed culture of *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*, and *Leuconostoc mesenteroides* subsp. *cremoris* (Probat M3) and lyophilized mixed culture of *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. cremoris (R-704) were used in cheese production. The compositional, proteolytical and sensory analysis of cheese samples were carried out at the 1st, 30th, 60th and 90th day of ripening at 4 and 8°C storage.
The chemical compositions of the cheese samples showed changes over the ripening period. Interactions of ripening temperature, starter type, and ripening time had an important effect on cheese composition (except moisture, pH, and protein in total solid) ($P < 0.05$). Nitrogen fractions (WSN, TCA-SN, PTA-SN) of cheese samples showed that Probat M3 cultures have higher proteinase and peptidase activity and the influence of the starter type and ripening temperature and their interaction have a significant effect on WSN, TCA-SN, and PTA-SN of cheeses ($P < 0.05$). The results were supported by Urea-PAGE electrophoresis and RP-HPLC techniques.
Cheese samples which were produced with R-704 culture culture had lower sensory scores and had the highest scores for bitterness attribute after 90 days of ripening compared with cheese samples which were produced with Probat M3. This may be due to the accumulation of high level of bitter peptides, indicating low proteinase and peptidase activity of R-704 culture. The results clearly showed that bitterness in TWC could be controlled by starter type and ripening temperature combination effect. The use of Probat M3 culture may enhance cheese proteolysis through ripening and may reduce bitterness defect with combination effect of ripening temperature at 8°C in TWC.

Keywords: Turkish White brined cheese, Bitterness, Proteolysis, Electrophoresis, RP-HPLC

Utilization of Sodium Chloride Reduced Brine in the Production of White Cheese and the Effects on Quality

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In this study, potassium and magnesium chloride, were utilized in combination with sodium chloride for the production of white cheese with reduced sodium content. The salt combinations of brines used in the cheese production were: NaCl:KCl (1:1) and (3:1) and NaCl:($KCl+MgCl_2$) (1:1) and (3:1), while ripening of control cheese was achieved with brine containing only sodium chloride. The effect of utilization of alternative salt substitutes on white cheese quality was investigated by physical, chemical, microbiological and sensory analysis performed at 1, 30, 60 and 90 days of ripening.
The usage of alternative salts did not affect the dry matter and fat content of the different cheese samples. Also, there was no significant difference between the acidity, pH, ripening index and salt content as well as microbiological and sensory analysis of the control cheese and the sample ripened in the brine containing NaCl:KCl (3:1). However, in the case of NaCl:KCl (1:1) and NaCl:($KCl+MgCl_2$) combinations, bitterness was observed.
As a result, it was concluded that the use of NaCl:KCl (3:1) does not cause any technological disadvantage in the production of white cheese and does not affect the technological, microbiological and sensorial quality characteristics of the product adversely.

Keywords: white cheese, reduced sodium chloride, potassium chloride, magnesium chloride

Detection of LAB Bacteriocin Genes in Two Traditional Slovenian Raw Milk Cheeses and in their Microbial Consortia – Viable Part of Microbial Population

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In Europe there is an increasing interest to preserve the production of traditional cheeses. However, since such cheeses are typically produced from raw milk, the safety issue has to be of great concern. Bacteriocin(s)-producing lactic acid bacteria (LAB) naturally present in traditional cheeses present an inexhaustive pool of microbes with safeguarding potential. Two Slovenian traditional raw milk cheeses, Tolminc cow's and Kraški ewe's cheese were examined for the presence of LAB bacteriocin determinants by PCR. In addition to analysis of the total DNA extracted from cheeses, the DNA from cheese consortia grown on Rogosa, M17 and CATC agar media were examined in order to establish the viability of bacteriocin producers whose bacteriocin genes were detected in the cheese samples. Nine bacteriocin genes out of 19 tested for were detected in at least two cheeses. In two Kraški cheeses only plantaricin A genes were detected, while in the other cheeses 5 to 9 bacteriocin determinants were present, irrespective of the cheese type. Plantaricin A gene determinants were present in all cheese and consortia samples, while enterocins A, B, P, L50A, L50B, cytolysin and nisin genes were present in most of them. Lacticin 481 gene was found in three Tolminc cheeses only and/or in consortia. Helveticin J determinants were detected in consortia of isolates grown on Rogosa agar but not directly in cheese, indicating the low concentration of *Lb. helveticus* cells. The study results confirmed Tolminc and Kraški traditional cheeses to be a promising source of bacteriocinogenic lactic acid bacteria which could be good candidates for tailor-made cultures for these traditional cheeses. Whether or not the particular bacteriocin genes detected in cheese samples and/or in microbial consortia are also expressed in situ in cheeses and thus play a protection role against the development of potentially pathogenic bacteria needs to be elucidated.

Keywords: traditional cheeses, safety, LAB bacteriocins, total cheese DNA

Effect of Starters on Proteolysis During Storage of Galotyri-Type Cheese

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The effect of four commercially available starter cultures on the proteolysis during storage for 30 days of Galotyri-type cheese, a Greek acid/rennet-curd cheese, was investigated. Two mesophilic starter cultures MA011 (containing *Lactococcus lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris*) and Probat 222 (containing *Lc. lactis* subsp. *lactis, Lc. lactis* subsp. *cremoris, Lc. lactis* subsp. *lactis* biovar. d*iacetylactis* and *Leuconostoc mesenteroides* subsp. *cremoris*), a thermophilic yoghurt culture, CH-1 (containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus)* and a mixed mesophilic/thermophilic starter culture, CHOOZIT MT 1 (containing *Lc.lactis* subsp. *lactis, Lc. lactis* subsp. *cremoris, Str. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus)* were added in the cheese milk at the suppliers' recommended levels. Gross proteolysis, as measured by the percentage of total nitrogen soluble in water (WSN), was not affected by the different starter cultures at 1 and 15 days of storage. However, at 30 days the levels of WSN were significantly ($P < 0.05$) higher in the cheeses made with the mesophilic cultures MA011 and Probat 222 than in the cheeses made with the thermophilic and mixed cultures (CH-1 and CHOOZIT MT

1, respectively). The concentrations of total free amino acids, as measured by the cadmium-ninhydrin method, were similar in the cheeses made with the mesophilic and mixed cultures throughout storage but significantly ($P < 0.05$) lower than that in the cheese made with the thermophilic culture. No differences were observed between the urea-PAGE electrophoretograms of the cheeses made with different starters at any sampling day. The starter cultures MA011, Probat 222 and CHOOZIT MT 1 had no obvious effect on the RP-HPLC peptide profiles of the water-soluble extracts of the resultant cheeses at any sampling day. In comparison, the profiles of the cheese made with the thermophilic culture were not only quantitatively but also qualitatively different.

Keywords: Galotyri cheese; fresh cheese; acid/rennet-curd cheese, proteolysis

Consumer Behaviour and its Impact on Kosovar Dairies

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Kosovo is still rural and poor. Two thirds of the population of Kosovo live in rural areas and one third of the small household income is spent for food. Milk (Pasteurized milk, UHT milk) and milk products (ayran, yoghurt, kos, kos with cream, white cheese, Kashkaval, Sharri cheese, peppers with cream) have a long tradition in the region, but most of the existing dairy farms and dairies were destroyed in the war. Today, 257'500 tons of raw milk are produced per year in Kosovo, but only a small part of the milk produced is processed by the dairy industry (Kosovo 13 %) compared to the region (Romania and Bulgaria 15-20 %, Turkey 19 % and Serbia 49 %) or even Western Europe. Most of the milk is still consumed on the farm or sold on the "green markets" (=Bazaar) – a high risk from a public health view. Only the processed milk from the licenced dairies can guarantee a minimal level of food security for consumers.
The aim of this diploma thesis was to give an overview of the dairy market in the Balkan region in general and in Kosovo in detail. Appropriate surveys analyse consumer behaviour:
a consumer survey on yoghurt, kos and cheese sold in retail stores; consumer survey in the Bazaar; store check of dairy products; preference test of different salt content in cheese; sensory testing of the products participating in the Award for the Best Kosovar Dairy Products 2007.
Recommendations for the Kosovar dairies are as follows: improve the quality of the products to be competitive (QMS systems); reduce cost and do not increase prices; improve packaging to get a better reputation, especially with young consumers, who tend to buy imported products. There is a market demand for fruit yoghurts (children!) and less salty cheese.

Keywords: Kosovo, Consumer behaviour, Dairy products, Consumer survey

Oral and Intestinal Survival of Probiotics given in Different Food Matrices

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The aim of this study was to investigate the colonisation of specific probiotic strains in the oral cavity and GI-tract, and to compare colonisation when probiotics were given in yoghurt, cheese or capsule.

The study consisted of a 4 week run-in period, a 2 week probiotic intervention, and a 3 week follow-up period. During the intervention, 36 persons consumed the probiotic combination containing *L. rhamnosus* GG (LGG®), *L. rhamnosus* LC705 (LC705), *Propionibacterium freudenreichii* ssp. *shermanii* JS (PJS) and *Bifidobacterium lactis* Bb-12 (Bb-12®) in the forms of vegetable capsules (n=12; 1-2x10^{10} cfu day^{-1}), yoghurt (n=12; 3x10^{10} cfu day^{-1}) or cheese (n=12; 3x10^{9} cfu day^{-1}). The survival time of the probiotics was analysed from faecal and saliva samples by a strain- or subspecies-specific qPCR.
All the subjects carried all the four strains during the intervention. During the follow-up, the mean gastrointestinal survival time was 14.5 days for LGG, 10.4 days for Bb-12, 7.0 days for PJS, and 5.4 days for LC705. At the end of the follow-up, 29 % of the volunteers still carried LGG, 9 % Bb-12, 3 % PJS but none carried LC705. During the intervention, 5 % of subjects carried Bb-12 and 58 % LGG in saliva. During the follow-up, LGG was the only strain found in saliva.
The persistence of probiotics is strain-dependent. Product form did not affect the GI-survival of *Lactobacillus* strains, but the survival of *Bifidobacterium* and *Propionibacterium* was better when administered in yoghurt, in comparison to cheese.

Keywords: probiotic bacteria, gastrointestinal survival, qPCR

Survival of Probiotic Bacteria in Finnish Emmental-like Cheese

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Cheese matrix may protect bacteria from oxygen, offer a buffered surroundings, and the cheese fat might even protect the probiotic strain during the passage through the gastrointestinal tract. However, the manufacturing process, long ripening time and shelf life of the cheese may have an effect on the viability of the probiotic bacteria. The objective of this study was to follow the survival of four probiotic strains in an Emmental-like cheese.
Emmental-like cheese was produced in Valio Ltd cheese factory with starter cultures in combination with *Lactobacillus rhamnosus* GG (LGG®), *L.rhamnosus* LC705 (LC705), *Bifidobacterium animalis* subsp. *lactis* BB-12 (BB-12®) and *Propionibacterium freudenreichii* subsp. *shermanii* JS (PJS). Viability as well as the total amount of probiotic strains was analyzed on day 54, 99, 139 and 201 after the cheese manufacture. Viability was determined by cultivation and PCR, and the total amount of probiotic strains was quantified using qPCR.
All the probiotic strains survived the manufacturing process and maintained their viability of 10^{5}->10^{7} cfu g^{-1} cheese at the end of storage. LGG®, LC705 and PJS survived at 10^{7} cfu g^{-1} but BB-12® showed lower adaptability in cheese and survived at 5-7x10^{5} cfu g^{-1} at the end of storage, the survival rate varied from 33 to 72 % in the two batches tested. LGG® was the best survivor, and together with PJS they dominated the probiotic population by 39-47 %. qPCR reported higher prevalence of probiotic strains than viability test due to detection of DNA from non-cultivable and possibly decayed bacterial cells.

Keywords: Probiotic bacteria, survival rate, PCR, qPCR

Effects of Somatic Cell Counts on the Composition and Fatty Acid Profile of Milk from the Churra and Castellana Breed Sheep

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The Churra and Castellana sheep breeds are indigenous to North Western Spain and are the most important milk producers in Spain. Milk production is directed towards the manufacture of Zamorano cheese, a hard variety made in province of Zamora (Spain) from raw or pasteurised ewe's milk obtained only from these breeds. The somatic cell count (SCC) is often used to differentiate between healthy and infected mammary glands in ruminants, and is increasingly used in routine dairy sheep milk testing procedures as an indicator of individual and flock udder hygiene and health. Many discrepancies still exist in the literature with regards to "normal" or "acceptable" SCCs in sheep milk. In the present study, the effects of two different SCCs: < 500,000 (group I) and 1,000,000 to 1,500,000 (group II) on ovine (Churra and Castellana) milk composition were determined.
The concentrations of pH and lactose were higher in milks with the highest SCC levels: milk fat, true protein and casein (CN) behaved differently, depending on the breed studied. The most abundant free fatty acid (FFA), irrespective of SCC levels, was palmitic acid (representing 12- 18 % of total FFA). Short-chain FFAs (C4:0 - C8:0) comprised 11 - 14 % of total FFA, of which caprilic acid was the main short-chain fatty acid, accounting for 3 - 5 % of total FFA. Medium-chain FFAs (C10:0 - C14:0) comprised 31 to 43 % and long-chain FFAs comprised 39 to 57 % of all FFAs. SCCs have a significant effect on total FFAs, total FFA levels being higher in group II milks for both breeds.

Keywords: Ewe's milk, Fatty acids, SCC

Poster Session 2

Culture-Independent Analysis of Bacterial Ecosystems in Milk and Cheese Samples Using Denaturing High Performance Liquid Chromatography

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Denaturing high performance liquid chromatography (DHPLC) technique is an ion-pair reverse-phase chromatography. It associates the principles of HPLC with methods of DNA fragments separation in denaturing conditions (i.e. TTGE/DGGE). It uses specific cartridges that elute double stranded DNA molecules according to their melting temperature (Tm) and size. In non-denaturing conditions, it allows the detection of nucleotide polymorphisms.
DHPLC has been commonly used since the 1990s in medical research to detect DNA mutations and polymorphisms. More recently, DHPLC protocols were adapted for the separation of bacterial PCR-amplified fragments.
We tested the feasibility of applying DHPLC (WAVE system; Transgenomic Ltd) to the field of microbial ecology of dairy products for: i) Identification and separation of pure strains in mixed cultures (lactic acid bacteria, staphylococci), ii) analysis of bacterial biodiversity in dairy products, iii) diagnosis of mastitis (by analysis of milk samples), and iv) following the dynamics of bacterial populations from milk to ripened cheese.
We then compared the potential of DHPLC with TTGE/DGGE methods (Ogier *et al*. 2004). Results showed different advantages of the DHPLC compared to TTGE/DGGE methods. For example, we observed a better separation of the staphylococci species. Moreover, DHPLC offers a greater detection sensitivity using fluorescence, and an automatic collection of DNA fragments for further sequencing or other uses.
We conclude that the DHPLC technique is a promising novel tool that complements the current repertory of analytic methods at the molecular level, and is valuable for studies the complex ecosystem of dairy products.

Keywords: Bacterial ecosystem, dairy products, 16S rDNA, DHPLC

Head Space Gas Chromatography Screening of Dimethyl Disulfide Produced by Corynebacteria Strains

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To fully answer cheese makers needs in smear cheese ripening, texture, colouring, flavours, corynebacteria must express defined intrinsic properties according to the technological constraints. They have to grow quickly on the substrate under conditions of difficult pH and temperature managed by surface cheese ecology and ripening conditions. Flavour and especially sulphur compounds production capacity is known to be one of the main corynebacteria specificity.
The objective of this study was to evaluate the performance of 350 corynebacteria strains on the dimethyl disulfide (DMDS) production capacity, which is representative of sulphur compounds production by corynebacteria metabolism.
The development of a high throughput method was necessary to well answer to this project. The Headspace technique coupled with a Gas Chromatograph was the best compromise to reach this target.
It has enabled to classify corynebacteria strains according to DMDS production ability and also to notice differences between bacteria species. The implementation of this method was of major

interest to enrich a screening database for corynebacteria screening and then to better understand combination of strains on flavour impact.

Keywords: Corynebacteria, Sulphur compounds, DMDS, HS-GC

Influence of Antimicrobial Facultative Heterofermentative *Lactobacillus* on Cheese Ripening

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Facultative heterofermentative *Lactobacillus* (FHL) dominate the microflora of most cheeses after some time of ripening. The biodiversity of FHL is large and their impact on ripening is thereby strain dependent. In this work, the influence of FHL on cheese ripening was investigated using cocktails of antimicrobial strains isolated from semi-hard cheese.
Two cheese making experiments were made. In the first, a cocktail of four *Lb. paracasei* strains, were tested in semi-hard cheese produced at a dairy pilot plant. The second experiment was made at a dairy production plant and a cocktail with five anticlostridial *Lactobacillus* strains was tested in semi-hard cheese ripened both vacuum-packed and with smeared surfaces. The cheeses were analysed for viable counts on MRS pH 6.3 incubated at 30°C (lactic acid bacteria) and MRS pH 5.6 incubated at 37°C (*Lactobacillus*). Re-isolated strains were identified using ITS-PCR, pulsed field gel electrophoresis (1st experiment) and repetitive-PCR (2nd experiment). Amino acids and peptide composition were analysed by HPLC and casein composition by capillary electrophoresis.
The experimental cheeses were dominated by added FHL strains, while these were rarely found in control cheeses without addition. Successions of *Lb. paracasei* strains were shown. Gas formation measured as changes in the cheese density indicated pronounced activity of *Clostridium* in some of the control cheeses in the second experiment, but not in cheeses added the anticlostridial FHL cocktail. Evaluation of sensory properties indicated a more mature flavour in experimental cheeses as compared to the controls. Proteolysis developed similarly in control and experimental cheeses, except that the total amount of amino acids in experimental cheeses were only about 90 % of that in controls in both experiments. The FHL strains had significant input on the amino acid composition.
It could be concluded that cocktails of antimicrobial FHL strains could be used to control ripening of semi-hard cheese.

Keywords: cheese ripening, FHL cocktail, *Lactobacillus*, antimicrobial activity

***Lactobacillus delbrueckii* Growth During the Manufacture of Swiss-Type Cheese Largely Depends on the Other Thermophilic Lactic Acid Bacteria Present**

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This study investigates some sources of variability for the growth dynamics of *Lactobacillus delbrueckii,* a thermophilic lactic acid bacteria species used in the manufacture of numerous cheese varieties. The dynamics were studied from the tank milk to the unmoulding of Swiss-type cheeses manufactured from clean raw milk inoculated with different thermophilic starter species.
18 cheeses with 18 different combinations of starters were manufactured in similar and controlled conditions. The cheeses were inoculated with one strain of *Streptococcus thermophilus* – added at a low or a high level, *Lactobacillus helveticus* – strain 1, 2 or not, and *Lactobacillus delbrueckii* – strain 1, 2 or not. Strains 1 and 2 differed by their acidifying and proteolytic potentials evaluated in

milk (high for both strains 1). The results showed a large variability of *L. delbrueckii* growth dynamics during cheese-making. In milk already and then all along pressing, this dynamics significantly varied according to the *L. delbrueckii* strain, to the inoculation level of *S. thermophilus*, and to both the presence/absence and the strain of *L. helveticus*.
During the pressing of Swiss-type cheeses, the growth dynamics of *L. delbrueckii* were significantly influenced by the other thermophilic bacteria present, strongly suggesting that some interactions took place between *L. delbrueckii* and both *S. thermophilus* and *L. helveticus*. If one of them probably corresponds to the published interaction from *S. thermophilus* towards *L. delbrueckii* characterised in milk and yoghurt, the others are yet unknown.
These interactions and resulting *L. delbrueckii* growth dynamics have been found determinant for the characteristics of the ripened cheeses. Like *L. delbrueckii*, growth variability for *S. thermophilus* and *L. helveticus* may generate variability in ripened cheese. This variability may be used to create or maintain some diversity within Swiss-type cheeses.

Keywords: Swiss-type cheese, Lactobacillus delbrueckii, thermophilic lactic acid bacteria, growth dynamics

The Effect of Arginine Metabolism on the Survival Capacity of *Lactococcus lactis* During Cheese Ripening

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Lactic acid bacteria constitute the dominant population of undefined bulkstarters that are used for at least 95 % of Gouda cheese produced in The Netherlands. The starters generally contain around 70 % *Lactococcus lactis* subsp. *cremoris* strains, 25 % *L. lactis* subsp. *lactis* strains and 5 % *Leuconostoc* sp. strains. Starter culture activity results in acidification of the cheese milk and final cell counts up to 10^9 cfu g^{-1} are reached. Due to lactose depletion and other stress factors the cell count decreases to levels around 10^6 cfu g^{-1} after 4 weeks of ripening. Since lactose is depleted, survival of the cells may be enhanced by energy (ATP) generation during the metabolism of another substrate i.e. arginine. Notably, *L. lactis* subsp. *lactis* strains can metabolise arginine, whereas subsp. *cremoris* strains cannot. To investigate whether arginine metabolism contributes to survival during ripening of industrially produced Gouda cheese, the fractions of the *L. lactis* subsp. *lactis* and subsp. *cremoris* subpopulations have been assessed after 1, 2, 4 and 7 weeks of ripening. Total cell counts and subpopulation levels were determined by plating on non-selective and selective agarplates, respectively. Notably, the proportion of arginine metabolising *L. lactis* subsp. *lactis* strains increased within one week of ripening from 20 – 30 up to 80 %. This is in line with the hypothesis that arginine metabolism may contribute to the survival capacity of *L. lactis* subsp. *lactis*. Recently evidence has been obtained that metabolically active viable cells may also contribute to the specific flavour development during cheese ripening concomitant with flavour compounds produced by cellular enzymes released from lysed cells. Therefore our future work is directed at the characterisation of the impact of arginine metabolism on flavour development in Gouda type of cheese.

Keywords: Lactococcus, survival, arginine metabolism, cheese ripening

Phenotypic and Genotypic Identification, and Technological Characterization of *Lactococcus lactis* ss *lactis* Isolated from Traditional Italian Raw Milk Cheeses

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Artisan and traditional Italian cheeses produced in the Alps are manufactured using raw milk, and their fermentation is driven by adventitious microbiota. In many traditional cheeses *Lactococcus lactis* ss *lactis* is the most frequently isolated NSLAB species.
In the present study, 64 strains of *Lc. lactis* ss *lactis* were isolated from dairy products from different Lombardy Alpine areas and identified phenotypically and genotypically, and evaluated for technologically relevant biochemical activities: growth conditions, acid production, redox, lipolytic and proteolytic activities. Phenotype identification was performed on carbohydrate fermentation profile galleries (Api 20 Strep (bioMérieux, France) and Biolog AN Microplate (Biolog, USA)). All the isolates were subjected to PCR amplification specific to *Lc. lactis* ss *lactis* to confirm the phenotype identification.
Identification was performed by Api 20 Strep and Biolog AN and the results were in agreement in 61 of the considered 64 strains (Biolog AN identified 3 strains as *Lc. raffinolactis*).
PCR with species-specific primers gave different results for 6 strains identified as Enterococci.
The strains displayed wide heterogeneity in the biochemical characteristics: 23 strains were able to grow at 45°C, 6 did not grow at 15°C, 9 did not produce acid from maltose and 5 from ribose, while only 1 strain utilized melibiose. Only one isolate fermented raffinose and 20of the 64 produced acid from sucrose. Glucose, fructose and trehalose were fermented by all the isolates, and none utilised melezitose.
The technological properties differed within the species, in particular *Lc*. *lactis* ss *lactis* strains isolated from curd showed lower acidifying and higher reducing activity than strains isolated from cheese.
The results suggest that the peculiar sensory characteristics of these traditional cheeses are very closely related to the high biodiversity of the indigenous microflora.

Keywords: *Lactococcus lactis* ss *lactis*, Enterococci, acidifying, reducing activity

Impact on Proteolysis and Flavour Development in Low-Fat Semi-Hard Cheese Using Complex Blends of *Lactobacillus* subsp. and *Lactoccocus* subsp. Versus Use of a *Lactobacillus helveticus* as Adjunct Cultures

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Literature has over the past decades demonstrated the beneficial flavour effect of adding adjunct cultures (e.g. *Lb. helveticus*, *Lb. paracasei*, *Lb. plantarum*, *Lb. delbrueckii* and *Lactococcus* subsp.) into the cheese to accelerated/modify the cheese flavour. A range of potential adjunct cultures have been characterized in details in the laboratory according to their specific enzymatic systems (e.g. proteinases, peptidases, aminotransferases, glutamate dehydrogenase) especially related to the cheese proteolysis and derived flavour compounds; each system very important for the different steps during proteolysis. Most of these adjuncts have been tested in cheese applications as single strain cultures, but not always with expected high impact on desired flavour formation most likely due to accumulative limitations/bottlenecks during the proteolysis into low molecular and potent

flavour compounds. Experiences have indicated that complex cultures of different LAB species promote formation of desired flavours more efficient than use of a single strain adjunct culture.
The aim of this study was to evaluate the beneficial effect of using complex blends of ripening culture possessing different, but synergetic enzyme systems for the development of desired cheese flavours. A designed cheese trial including 12 cheese pilot plant productions were setup in order to study the impact on cheese ripening and flavour development of low-fat semi-hard cheese using two new commercial adjunct ripening blends and one commercial adjunct *Lactobacillus helveticus* compared to use of starter culture alone. An analysis program of cheeses include gross composition, sensory evaluation, analysis of protein degradation (total N, soluble N, free amino acids, chromatography) and aroma analysis were performed during the ripening period of 12 weeks.
This presentation will discuss results of the study focusing on the correlations discovered between adjunct cultures, sensory quality, degree of proteolysis and the aroma profile by use of multivariate analysis.

Keywords: Adjunct culture blends, cheese ripening, flavour development, low-fat cheese.

Pink Discolouration in Romano Style Cheese – Role of α-Dicarbonyls and Ammonia

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A rindless dry salted Romano style cheese manufactured in Australia using cheddar cheese processing equipment intermittently produces a pink/brown discolouration in the cheese when matured at 15°C. While there is little reported information on the cause of pink/brown discolouration it has generally been attributed to the presence of galactose and its involvement in the nonenzymic Maillard type reaction with proteins. However this reaction requires higher temperatures than normally encountered during maturation and therefore other reaction intermediates that are more reactive than a sugar are likely to be involved. In this investigation α-dicarbonyls found in maturing cheese (glyoxal, methylglyoxal and diacetyl) were added to UHT skim milk with an amino acid and heated at 55°C for 5 days. The addition of diacetyl resulted in a pink/brown colour although in the model milk system it was evident that the added amino acid was not necessary for colour formation. Further work demonstrated colour formation following incubation at 25°C for 3 days and at 15°C after 4 weeks incubation. Variations in simulated cooking regimes typical for Romano manufacture had no effect on colour intensity.
In a potassium hydrogen phthalate buffer system the role of various amino acids, urea and ammonia were assessed for their involvement in colour formation with diacetyl. Ammonia added either directly or generated from urea by urease or from glutamine by glutaminase was the most reactive compound. Further work is continuing examining the possible interaction of *Streptococcus thermophilus* (a urease positive organism) and diacetyl production by strains of *Lactobacillus helveticus* both of which are used as starters in the manufacture of this particular Romano style cheese.

Keywords: pink discolouration, Romano style cheese, α-dicarbonyl, ammonia

Influence of Properties and Manner of the Application of Starters on Quality of Ripened Emmental-Type Cheese

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The formation of typical regular round eyes in Emmental-type cheeses is an essential attribute of the high-quality ripened cheeses in the consumers market.
The use of the thermic cycles method simulating the production of Swiss type cheese with eyes seems to be the most suitable method for studying the properties of pure dairy cultures and further for the modification of the conditions of the technological process of cheesemaking.
Using thermic cycles, the properties of single strains or mixture of strains of *Str. salivarius subsp. thermophilus, Lb. helveticus* and *Lb. delbrueckii susp. lactis* were studied. They were used in two modifications of thermic cycles (highest applied temperature 50-51°C or 52-53°C) and the results of the cultivations were compared with the results of cultivations at a constant temperature of 37°C. On using the cultivation of microorganisms in simulation of the thermic cycles there were found to be significant differences between single strains as well as between mixtures of tested strains. Differences were mainly in the ability to grow and in the production of lactic acid during first 24 hours of cultivation.
Results of the study of the behaviour of strains *Str. salivarius subsp. thermophilus, Lb. helveticus* and *Lb. delbrueckii susp. lactis* by method of thermic cycles were verified in practice, and the modification of the conditions of the technological process of cheesemaking led to the formation of typical round regular eyes in cheeses.

Keywords : Emmental-type cheese, starters, eyes, cultivation in thermic cycle

***Streptococcus macedonicus*, a Multifunctional and Promising Species for Cheese Manufacture**

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The species *Streptococcus macedonicus* has been described recently and is among the *Streptococcus thermophilus*-like microorganisms that belong to the *Streptococcus bovis*/*Streptococcus equinus* complex. This thermophilic, homofermentative, dairy streptococcus, first isolated from naturally fermented Greek Kasseri cheese, possesses a food-grade and non-pathogenic status. Recently, it has been reclassified as *Streptococcus gallolyticus* subsp. *macedonicus*.
Strains of *S. macedonicus* are moderately acidifying and proteolytic. They participate in fat hydrolysis, citrate fermentation, and peptidolysis (PepX and PepN activity) during secondary hydrolysis of milk casein. No decarboxylation of amino acids occurs, except for a few strains that produce the biogenic amine tyramine from tyrosine. Haemolytic activity and sensitivity to antibiotics used in human clinical therapy underline the safety of *S. macedonicus* strains. Certain strains of *S. macedonicus* produce bacteriocins, for instance the anti-clostridial lantibiotic macedocin produced by *S. macedonicus* ACA-DC 198, and exopolysaccharides, for instance the high-molecular-mass and highly texturizing heteropolysaccharide produced by *S. macedonicus* Sc136. These physiological and technological properties make *S. macedonicus* a multi-functional, candidate adjunct culture for dairy manufacturing.
Growth of and macedocin production by *S. macedonicus* ACA-DC 198 have been assessed and modelled under conditions simulating Kasseri cheese technology. Macedocin production occurred during the first cooling step (16°C), which corresponded with the coagulum ripening until the desired acidity level of pH 5.1-5.2. This indicates that anticlostridial activity may be already present in the early stage of Kasseri cheese manufacture. The increase of the viable cell counts after heating at 75°C was probably due to resuscitation of heat-injured *S. macedonicus* cells or the re-growth of a stress-adapted subpopulation, as the culture conditions present (25°C, pH 5.0, 3 % NaCl) abolished

cell growth. Recovery of sublethally injured cells may be ascribed to stress responses (e.g., production of stress proteins) or the occurrence of a so-called viable but nonculturable state. The presence of *S. macedonicus* ACA-DC 198, used both as an adjunct and as a sole starter strain in Kasseri cheese production trials, has been confirmed until the end of cheese ripening. This was also observed for the bacteriocin produced by the strain. PepX activity has been detected in all experimental cheeses. Physicochemical and sensory properties of all mature cheeses corresponded with the ones characterizing traditional Kasseri cheese, indicating the usefulness of the macedocin producer, *S. macedonicus* ACA-DC 198, as adjunct culture. Moreover, macedocin and its food-grade producing strain are interesting alternatives to nisin and nisin-producing strains, respectively, to combat late loss of hard and semi-hard cheeses.

Keywords: *Streptococcus macedonicus*, milk fermentation, macedocin, Kasseri cheese

Evaluation of Lactic Acid Bacteria Diversity of Domiati Cheese by Using Culture Dependent and Culture-Independent Methods

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Culture-independent molecular techniques are now available to study microbial ecosystems. It is opening interesting perspectives to problems related to composition and population of microbial communities in foods.
In this investigation culture dependent and culture-independent methods were applied to focus on the dominant lactic acid bacteria (LAB) in Domiati cheese. The culture dependent methods used in this study were Apparatus and Procedure Identification system (API), sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and genotypic identification using rep-PCR fingerprinting technique. Temporal temperature gradient gel electrophoresis (TTGE) was the culture-independent method used for the detection of LAB
species present in 15 Domiati cheese samples collected from different regions in Egypt. The whole bacterial genome has been extracted directly from the sample; bacterial identification was facilitated by comparison with an extensive bacterial reference database that was established with DNA fragments of pure bacterial strains. All samples were also analyzed for moisture content, salt content and pH value.
Identification and differentiation of 50 isolates obtained from Domiati cheese samples was possible using the culture dependent methods. The isolates were shown to belong to the following species, *Enterococcus faecium*, *Lactobacillus delbrueckii subsp lactis*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum* and *Lactococcus lactis*.
Temporal temperature gradient gel electrophoresis results showed that *Enterococcus faecium* was the predominant specie, in addition *Lactococcus lactis*, *Lactococcus garvieae* and *Leuconostoc mesentroides* were also among the main microbial species in Domiati cheese. Many other bacterial species belonging to *Lactobacillus delbrueckii subsp lactis*, *Lactococcus raffinolactis*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Enterococcus faecalis* and *Pediococcus pentosaceus* were identified as subdominant or minor species.

Keywords: Culture dependent, Culture-independent, *Lactobacillus*, *Lactococcus*

MicroCheese: A high Throughput Cheese Manufacturing Model

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Cheese production has a long history and nowadays is of major economical importance. There is an increasing demand for both improving and enhancing of cheese production and ripening and for diversification of cheese flavour. However, effective and targeted research in these areas requires, in addition to *in vitro* experiments, extensive systematic screening of cultures and processing conditions during real cheese-making.
To reduce screening costs, models were developed to miniaturize the cheese manufacturing process, but an effective high throughput model until now was still lacking. Here, we describe a protocol which allows the simultaneous manufacturing of up to 600 cheeses in individual, miniaturized micro-titer format, cheese vats. Each cheese can be manufactured with a separate protocol. The manufactured cheese resembles the properties of conventionally produced cheese in terms of acidification profiles, moisture content, salt concentration, flavor profiles and micro structure. The protocol has been developed for Gouda- and Cheddar-type cheeses and can be adapted to other cheese varieties. Individual cheeses are made from as little as 1.7 ml of milk and can be analyzed for many parameters, including enzyme activities, bacterial survival, bacterial gene expression and flavor profiles.
This "MicroCheese" model offers new possibilities to study many aspects of cheese production e.g. the screening of microbial culture collections for desired fermentative capacities, screening of bacterial mutant libraries, variation of processing conditions and assessing of health aspects. This system will not only accelerate product development but will also allow a more systematic approach to better understand the complex process of cheese-making.

Keywords: miniaturized cheese, fast screening, product development

Modelling of Technology-Stress Effect on Physiological Characteristics of *Streptococcus* spp.

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The cheese starter cultures are stressed by many physiological effects during the cheese-technology operations. The aim of our study was to determine the impact of these conditions on growth characteristics (specific growth rate, final density and acid production) and proteolytic activity of selected strains of *Streptococcus salivarius subsp. thermophillus*. The collection of strains was tested for resistance to NaCl concentration under 42°C and 9 strains were selected for detailed analysis. The values of individual characteristics couldn't have been compared directly. We used the method of point correlation. Each of the 12 characteristic was rated with 1 point for positive characteristic and the final sequencing was based on the summarization of this points. The strains were tested for growth under reduced water activity (high NaCl concentration) with or without heat stress (simulated technology operation). The sequence of strains based on growth characteristics was compared with the proteolytic-based sequence. This characterization can be used when aiming for a precise selection of the optimal strains for particular technology.

Keywords: starters; LAB; physiological characteristics; Lactococcus; water activity; stress

Application Impedance Methods for Studying of Starters and Milk Contaminated Microorganisms

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Direct and indirect microbiological methods using Rabit apparatus were implemented for studying the resistance of 10 strains of *Str. salivarius subsp. thermophilus,* 10 strains of *Lactobacillus ssp*. against the temperature between 30-50°C, and for properties comparison of group strains *Pseudomonas fluorescens* (n=10), *Bacillus cereus* (n=10) and *Bacillus licheniformis* (n=10). The direct and indirect impedance methods were further tested for determination groups of coliform bacteria, thermoresistant (85°C, 10 min) anaerobic or aerobic bacteria, and psychrotrophic microorganisms in sample of raw milk (n=102).
The achieved and presented results confirm the suitability of using the Rabit apparatus and impedance microbiology method for studying properties of individual strains of microorganisms. The tested method for determination of thermoresistant aerobic bacteria in raw milk is unsuitable (R^2 = 0,0845), however the method for determination of psychrotrophic microorganisms is suitable (R^2 = 0,7248), and the method for determination of coliform bacteria is unsuitable for samples of raw milk containing < 100 cfu mL^{-1}. Impedance method with Whitley Anaerobe Broth recommended for determination thermoresistant anaerobic bacteria is unsuitable for determination strains of *Cl. butyricum a Cl. tyrobutyricum.*

Keywords : impedance microbiology, milk, starters, contaminated microorganisms

Identification and Cloning of a Cystathionine β/γ-lyase from *Lactobacillus casei* FAM18168

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The sulfur compounds hydrogen sulfide, methanethiol, methional, dimethyl disulfide and dimethyl trisulfide (DMTS) are key odorants of several cheese types and are presumably derived from the bacterial catabolism of methionine and cysteine. *Lactobacillus casei* is a dominant non-starter lactobacillus in many cheese types and may be used as an adjunct bacterium to enhance cheese flavor development and ripening. To identify mutants with selectively enhanced enzymatic activities it is prerequisite to understand sulfur metabolism and its regulation. However, the catabolic pathway(s) of methionine and cysteine in lactobacilli are not well characterized. Principally, there are two potential pathways: one is initiated by a transamination reaction whereas the other is initiated by an elimination reaction.
Several *L. casei* strains produced volatile sulfur compounds when incubated on methionine. The cell free extracts of these strains showed considerable cystathionine activity, indicating that this activity may contribute to the formation of volatile sulfur compounds. To characterize this activitiy, we applied proteomic tools and identified a protein with possible cystathionine lyase activity which is not encoded by the published genome of *L. casei* ATCC 334 in several *L. casei* strains. The gene was cloned from *L. casei* FAM18168 and expressed in *Escherichia coli* to study its enzymatic activities. The recombinant enzyme catalyzed α,β- and α,γ-elimination reactions and cleaved cystathionine, cysteine, homocysteine and methionine and thereby released volatile sulfur compounds. The findings indicate that cystathionine lyase may contribute to flavor development

and that *L. casei* strains overexpressing this enzyme may be used as flavor intensifying adjunct cultures.

Keywords: Lactobacillus casei, cystathionine lyase, volatile sulfur compounds

Tracing of a Probiotic *Lactobacillus reuteri* in Reduced-Fat Cheese

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The majority of fermented foods already on the market are fermented milk products and yoghurts. However, cheeses may offer certain advantages over freshly fermented milk products in delivering probiotic micro-organisms. On account of pH higher than that of the more traditional fermented foods, cheeses may provide a more stable milieu for the survival of health-promoting micro-organisms. Recently, reduced-fat cheeses have entered the market along with the traditional full-fat cheeses. In order to assess the potential of reduced-fat cheese to support the survival of a probiotic micro-organism, a reliable tracing method must be accessible.
In order to develop a tracing method for a probiotic *Lb. reuteri*, reduced-fat cheeses supplemented with *Lb. reuteri* were manufactured in pilot-scale. Samples taken after 9 weeks of ripening were homogenized and suspended into peptone-salt – medium. Dilutions of the suspensions were plated onto MRS-plates, which were incubated at 37°C for three days under microaerophilic conditions. Colonies with an appearance typical of *Lb. reuteri* could be counted on MRS-plates. Species-specific identification was verified by PCR using *Lb. reuteri*-specific primers. In addition to *Lb. reuteri*, numerous small bacterial colonies were growing on MRS-agar. These were determined to be mainly lactococci, which suggests that the starter culture strains used in cheese making were viable after the 9 weeks ripening period and showed retarded growth on MRS-agar after incubation at 37°C. Pulsed-field gel electrophoresis (PFGE) was used to generate a DNA fingerprint for the probiotic strain in order to distinguish it from other strains belonging to the species *Lb. reuteri*.
In addition to freshly fermented dairy foods, such as yogurts and fermented milks, traditional full-fat cheeses have been used as delivery systems for probiotic lactic acid bacteria. In response to the growing demand for health-promoting food, reduced-fat cheeses could be developed as carriers of probiotic micro-organisms.

Keywords: Probiotic, Lactobacillus reuteri, reduced fat cheese

Modelling of Technology-Stress Effect on Physiological Characteristics of LAB – *Lactobacillus* spp.

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The cheese starter cultures are stressed by many physiological effects during the cheese-technology operations. The aim of our study was to determine the impact of these conditions on growth characteristics (specific growth rate, final density and acid production) and proteolytic activity of selected strains of *Lactobacillus helveticus, Lactobacillus delbrüeckii subsp. lactis* and *Lactobacillus rhamnosus*.
Our results prove that the resistance to low water activity (a_w), simulated by NaCl, are strain-dependent. The heat stress (technology-dependent) has a minimal effect on the strains-growth characteristics. An interesting phenomenon was the increased acid production in *L. helveticus* under

the suppressed water activity. This effect is positive for the technology, because the lactic acid produced has positive effects on cheese quality. *L. delbrüeckii subsp. Lactis* are more sensitive to heat stress than *L helveticus*, but the difference is small. The physiological characteristics of *Lactobacillus rhamnosus* strains are significantly dependent on Aw. The differences are remarkable at $a_w = 0.95$. This value is critical for the development of contaminating *Clostridium* strains.

Keywords: starters; LAB; physiological characteristics; Lactobacillus; water activity; stress

Effect of Fat Content and Starter Composition on Casein Hydrolysis and Viscoelastic Parameters During Ripening of Semi-Hard Dutch-Type Cheeses

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The effect of varying fat content and secondary starters on proteolysis of caseins and viscoelastic properties were studied in semi-hard Dutch type cheeses. Caseins were analyzed using capillary electrophoresis (Beckman P/ACE MDQ), free amino acids were quantified with amino acid analyzer. Dynamic rheological measurements were performed with Physica MCR301. Starters were selectively counted on M17 and MRS media. Routine chemical analysis were also made according to IDF-standards.
Dutch-type cheese varieties are produced in Saaremaa Dairy (Estonia) using mesophilic LD-cultures and CHY-MAX™ (Chr. Hansen) rennet. Cheeses are made in open 5000 L vat from pasteurized and standardized milk. Cheeses are produced with three different fat contents, 26, 15 and 10 %. *Lactobacillus helveticus* is used as adjunct culture in one brand, Saaremaa Ekstra. Depending on the variety there are differences in cooking temperature (up to 4 degrees) and pressing time. Samples for analysis from commercial cheese batches were taken after 1, 30 and 45 days and further until at least 6 months.
Our preliminary investigations have shown quite extensive hydrolysis of α_{s1}-casein by chymosin and lactococcal enzymes and β-casein degradation to the lesser extend in Saare cheese (26 % fat). The degradation of α_{s1}-casein was confirmed by analysis of water soluble extract which contained mainly peptides from α_{s1}-casein N-terminal part. It is interesting to mention that much less α_{s1}-casein was degraded in Saaremaa Ekstra. The cause of this phenomena will be discussed and we will also investigate the relationship between fat content and starter counts in cheese during ripening.

Keywords: casein hydrolysis, fat content, viscoelastic parameters

The Biofilm Color Depends on Both the Yeasts and the Bacterium Strain Used

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The color of Munster cheeses is traditionally thought to be due to the bacterial flora, e.g. *Brevibacterium linens*. This study was carried out to evaluate effects of yeast blendings (2 *D. hansenii*, *Candida utilis*, 2 *K. lactis*) on the color of two bacteria isolated from cheese rind (*Micrococcus varians* (Mv) and *Brevibacterium linens* (Bl)). A 60 % Munster medium was sterilized before addition of agar-NaCl. Each mixture of yeasts (10^4 cfu cm^{-2}), differed by their yeast concentrations (M1 to M4), and a bacterium or a mixture of bacteria (50 % cfu each) were

inoculated on the medium surface and incubated at 12°C until day 28. The biofilm color was evaluated by L*C*h° (brightness, chroma, hue angle) spectrocolorimetry.
The color of all yeast mixtures was around the same: cream-colored/light yellow. With or without Bl, the color of M1Mv or M4Mv was lemon-yellow (C* = 35 – 40; h° = 85) while the one of M3Mv was dark orange (C* = 47 – 49; h° = 72). For M2 and Bl with or without M, the color was orange (C* = 44 – 47; h° = 75). For M1, M3 or M4 with Bl, it was dark cream-colored (C* = 37 – 40; h° = 79). For M2Mv, it was yellow-colored (C* = 42; h° = 88).
The color of each association (yeasts + bacterium or bacterium) didn't determine *a priori*. The color parameters depended on the initial concentrations of yeasts as well as the bacterium or the blending of ripening bacteria. Positive or negative interactions between yeasts and bacteria were underlined.

Keywords: color, Munster, yeast associations, bacteria

Livarot Color Depended on Deacidified Yeast, *G. candidum*, and Bacterium Associations

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The color of Livarot is traditionally due to the bacterial flora. This study was carried out to evaluate effects of deacidified yeast (*D. hansenii* DH or *K. lactis* KL), *G. candidum* (2 strains G1 and G2), on the color of four bacteria isolated from Livarot rind (two *B. linens* (Bl1, Bl2); *Microbacterium gubeenense* Mg, and *Arthrobacter arilaitensis* Aa). A 60 % Livarot medium was sterilized before addition of agar-NaCl. Yeast or a yeast + *G. candidum* (10^3 cfu cm^{-2}) with or without one bacterium were seeded on the medium surface and incubated at 12°C until day 28. The color on day 28 was evaluated by brightness, chroma, and hue angle spectrocolorimetry.
This study confirmed that the color of DH – bacterium biofilms was darker and more orange than the one of KL – bacterium biofilms. For each association with Bl2, the color was near the one of the reference (without seeding). For Mg biofilms, it was more yellow-colored than the one of reference. For Bl1 or Aa associations, the color of the biofilms depended on deacidified yeast and *G. candidum* used. *G. candidum* lightened the biofilm color by forming a light and white shade. This was stronger with G2 than with G1. Only five associations gave the dark orange color expected by the cheese makers: Bl1, DhBl1, KlBl1, L1G1Aa and L2G1Aa.
The color of each association wasn't determined *a priori*. The color parameters depended on the deacidified yeast, the *G. candidum* strain as well as the bacterium used. Positive or negative interactions between deacidified yeast, *G. candidum* and bacterium were underlined.

Keywords: color, Livarot, microorganisms, interactions

Water Activity Evolutions of Camembert-Type Cheeses During Ripening in Chamber

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Water activity [a_w] impact on microorganism growth and activities was highlighted by many authors. However, a_w dynamics have not been studied in detail during chamber ripening for a Camembert-type cheese. Under aseptic conditions, experimental cheeses were ripened under controlled conditions (temperature, gaseous atmosphere, and RH) during 15 days. A_w measurements

were done with a FA-st lab (GBX, Romans sur Isère, France) at 14°C. The salt (NaCl) concentration was determined using NF V 04-288 reference method.
No significant surface a_w differences between the two cheese faces were found. Surface a_w did not change when RH varied between 91 and 97 %. On day 6, a small a_w increase was observed and it could be due to *P. camemberti* mycelium development. For ground cheese samples, rind a_w raised from 0.94 (d1) to 0.97 (d3) when the salt diffuses from the rind (3.7 % NaCl on d1) to the core (1.6 % on d4). Core a_w did not varied significantly although the salt concentration increases from 0.0 to 1.6 %.

Keywords: water activity, cheese, ripening in chamber

Temperature and Relative Humidity Influence the Microbial and Physicochemical Characteristics of Camembert - Type Cheese Ripening

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To evaluate the effects of temperature and relative humidity (RH) on ripening kinetics, Camembert cheeses were prepared from pasteurized milk seeded with *Kluyveromyces lactis, Geotrichum candidum, Penicillium camemberti,* and *Brevibacterium aurantiacum.* Microorganism growths and physicochemical changes were studied under different ripening temperature (9, 12, 16°C) and RH (88, 94, 98 %).
K. Lactis and *G. candidum* growths increased with temperature but RH did not have any effect on them. Their specific growth rates were 3.3 times when temperature increased from 9 to 16°C, whatever the RH. Whatever the temperature, for 98 % RH, specific growth rate of *P. camemberti* spores was significantly higher (between 2.5 (16°C) and 3 times (9°C)), but the mycelium appeared damaged due to disruption of the equilibrium between *P. camemberti* and *G. candidum* in favor of this yeast. *B. aurantiacum* growth depended on both temperature and RH. For 88 % RH and 9°C, its growth was restricted ($8.6x10^7$ CFU g^{-1}) while for 98 % RH and 16°C, it reached $1.8x10^9$ cfu g^{-1} and the rind had an orange color after 20 days.
Temperature had a significant effect on lactose and lactate consumption rate in core and rind of cheeses. In the rind, for a temperature increase from 9 to 16°C, lactate consumption rate was 1.6 time higher under 98 % RH and 15 times higher under 88 % RH. Whatever the RH, temperature had significant positive effects on rind pH increase (from 4.6 to 7.5). At 9°C, the rind pH increase was observed between d6 and d9 while at 16°C between d3 and d4. Temperature and RH had an effect on underrind increase: at 16°C and half of the cheese thickness appeared ripened on d14. However, under 98 % RH, the underrind was runny.
The best ripening conditions to have an optimum between microorganism growths and biochemical kinetics were 12°C and 94 % RH.

Keywords: cheese ripening, temperature, relative humidity, microbiological and physicochemical kinetics

Metabolic Characteristics of Lactic Acid Bacteria from Camel Milk

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Most camel milk is consumed as fermented milk: the milk is usually allowed to ferment naturally at ambient temperature without any heat treatment. Information is lacking on the microbiology of camel milk and more research is needed. In order to define the natural microbial population of whole camel milk and to create active starters, the different metabolic characteristics of some lactic acid bacteria selected from camel milk were considered.
From samples of frozen camel milk 92 strains of Lactic Acid Bacteria were isolated: cocci (51) or rods (41). The study of the metabolic characteristics (gas production, sugar fermentation, growth temperatures, caseinolytic, reducing and acidifying activities) was carried out so as to compare these strains with those of cow milk. Growth at different temperature (15, 37 and 45°C) gave quite different results, as was expected: all the strains grew better at low temperatures. All the Cocci (51 strains) were homofermentative, while for the Rods 23 strains were homofermentative, 10 strains facultatively heterofermentative and 8 strains obligately heterofermentative. *Lactococcus lactis* ssp *lactis* predominated (Fig. 1). The pattern of fermented carbohydrates is sometimes unusual: 30 % of the strains were able to ferment tagatose, a sugar utilised by few species. Wide biodiversity was found for the fermentative profile within the same species and compared to the same species from other animals (cow, goat). Genotypic identification was necessary to achieve good identification for some of the 92 isolated strains.
The poor rennetability of camel milk can be improved by good fermentation using some lactic acid bacteria. Thus an appropriate selection of these bacteria, and the creation of a culture collection of strains isolated from camel milk, and identified and typed for their metabolic activities to use as starter cultures, would provide improved dairy product quality from the camel.

Keywords: camel milk, lactic acid bacteria, metabolic activities, starter culture

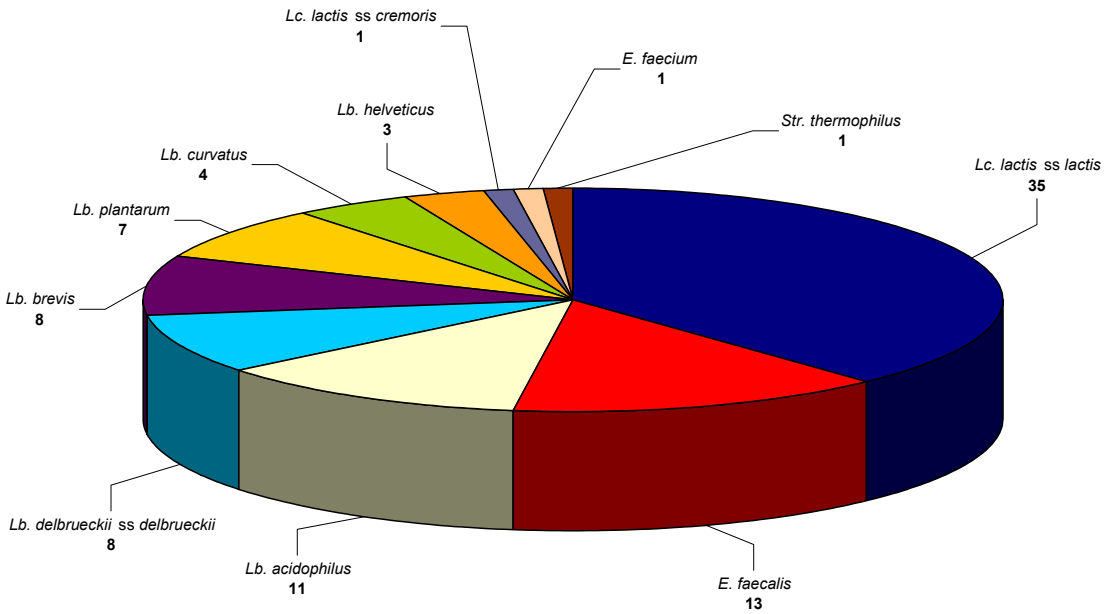

Figure 1: Distribution of lactic acid bacteria

Aroma Formation as a Criterion for Strain Selection in the Development of a Direct Vat Set Culture

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Aroma formation is an important criterion in addition to lactate formation from lactose when selecting strains for the development of a direct vat set culture.
In this paper two strains of *Lactococcus lactis* ssp. *lactis* (LL1 and LL2), 2 strains of *Lactococcus lactis* ssp *lactis* biovar *diacetylactis* (LLD1 and LLD2), one strain of *Lactococcus lactis* ssp. *cremoris* (LLC1) and one strain of *Leuconostoc mes.* ssp. *cremoris* (LN) were combined to form a trial culture.
Investigation of the acidification activity in the laboratory and the production of laboratory and model cheese showed that the mixture of strains resulted in a distinctive malty aroma which was described as having an unpleasant sensory effect. The unwanted aroma was attributed to the LLC1 strain by the electronic nose and was identified as 3-methyl butanal or 3–methyl butanol using GC/MS. A yeasty note could not be conclusively identified. Analysis of the aroma formation of strain LLC1 over 24 h showed the strongest unwanted aroma formation after 10 h. The electronic nose results showed that this malty note was less detectable when the proportion of LLC1 in the mixture was reduced, or when LN was present in the mixture. A significant reduction in the malty note was achieved by interaction with the Leuconostoc strain. This was confirmed in the cheese trials.
The LLC1 strain was replaced by a different LLC2 strain (likewise *Lc. lactis* ssp *cremoris*) which showed no such aroma formation. In the laboratory and model cheeses made with the new mixture there was less citrate breakdown, less acetic acid, and the yeasty malty note was no longer detectable. The aroma of the model raclette cheese made with LLC2 was judged to be significantly better.
The results show how important it is to check the aroma formation of individual strains and strain mixtures in culture formation and that it makes sense to use the electronic nose in combination with GC/MS and sensory evaluation.

Keywords: direct vat set culture, aromatic components, raclette cheese

Effect of Copper in Growth and Viability of Strains used as Starters and Adjunct Cultures in Emmental Cheese

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The aim of the study was to investigate the effect of supplemented copper (Cu^{2+}) on growing medium on growth and viability of strains used as starters and adjunct cultures for Emmental cheese manufacture, and to elucidate possible variability in copper resistance at species and strains level.
Thirteen strains belonging to *Lactobacillus delbrueckii, Lb. helveticus, Lb. rhamnosus, Streptococcus thermophilus* or *Propionibacterium freudenreichii* species were exposed to various copper concentrations in growth medium. The effects of copper on bacterial growth and cell viability were determined by optical density and pH measurements, and by platings. In addition, effect of oxygen present during copper exposure period was evaluated.
Among all strains, *Str. thermophilus* strain T101 was the most sensitive to copper, and anaerobic conditions increased this sensitivity significantly. There was a considerable variation in copper

resistance among strains of each *Lactobacillus* species included in this study. Copper resistance is both a species- and strain-dependent property. This variability may reflect the variability in copper binding capacities by cell wall components and/or excreted compounds to medium by the different species and strains. The increased sensitivity to copper under anaerobic conditions by the same strain could be a consequence of the quemical state of copper.
This study indicates that copper resistance is a highly variable property among starter and adjunct strains. However, very limited literature is available in this topic. The observed variability should be considered when strains will be selected for a particular type of Emmental cheese manufacture such as cheese milk handling in a traditional copper vat or in stainless-steel vat, with or without copper salt supplement.

Keywords: copper, Emmental cheese, starter, adjunct culture.

Impact of Milk Pretreatment on *Enterococcus* and *Staphylococcus* spp. and Antibiotic Resistances in Semi-Hard Cheese

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The impact of milk pretreatments on a traditional semi-hard Swiss cheese is an important issue driven by labelling issues with the brand protection AOC (Appellation d'Origine Contrôlée). Semi-hard cheeses can be heavily contaminated with *Enterococcus* spp. which often carry antibiotic resistance (ABR) genes. In this study, we tested the effects of different heat and physical milk pretreatments on enterococci in milk destined to cheese production and their survival during the cheesemaking process, ripening and storage. Five productions (~12500 liters milk each) of semi-hard cheese were made in a cheese factory (standard recipe) with differently treated milk: pasteurization (15 s at 73°C) and thermization (15 s at 62°C), with or without bactofugation, and raw milk (control). The microbiological quality of milk, cheese curd and cheese rind was assessed during 70-day ripening, with special focus on *Staphylococcus* spp. and *Enterococcus* spp. and ABR patterns. ABR were characterized phenotypically (disc diffusion testing of 18 antibiotics) and genotypically (microarray hybridisation with 90 antibiotic genes and confirmation by PCR).
Milk pasteurization eliminated efficiently enterococci and staphylococci in cheese curd. No secondary contamination was observed after milk treatment. Thermization only slightly decreased the number of enterococci (6×10^3 cfu g^{-1}) compared to raw milk cheese (5.2×10^4 cfu g^{-1}) after 70 day ripening. Bactofugation did not enhance the efficiency of thermization for decreasing the number of *Enterococcus* and *Staphylococcus* in cheese. The number of *S. aureus* in raw milk cheese did not exceed 10^3 cfu g^{-1} during ripening.
ABR incidence among enterococci isolated was high, including a high number of multi-ABR strains. Depending on the production, 13.3 - 66.7 % of all enterococci displayed resistance to erythromycin and 14.9 - 33 % exhibited resistance to tetracycline. The diversity of *Enterococcus* strains was assessed by rep-PCR revealing the presence of the same resistant strains in raw milk and in cheese curd and rind.
Our study showed that only pasteurization was efficient to remove enterococci from the cheese curd, and therefore avoid the spread of ABR bacteria. Pasteurization, or an equivalent treatment with respect to microbial reduction, should be recommended to reduce the number of ABR bacteria in traditional semi-hard cheeses.

Keywords: *Enterococcus*, milk pretreatment, antibiotic resistance, cheese microbiology

The Fluorescent Staining Method to Assess the Viability and Physiological State of *Lactococcus* and *Propionibacterium* Industrial Strains

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The aim of the present study was the development of method based on fluorescent staining to quantify the viability and physiological state of microbial populations of lactic and propionic acid bacteria during long-term cultivation in skim milk.
The method optimization was performed with pure cultures of industrial strains representing *Lactococcus* and *Propionibacterium*. The cultures with initial cell frequency of 10^6 cfu mL^{-1} in five repetitions were incubated at 10 and 30°C and 30°C with 3 % NaCl addition for 4 weeks. The sampling was performed within 24 hours from inoculation and next in regular intervals. Bacterial cells were vacuum-filtered onto polycarbonate filters and stained with 4',6-diamidino-2-phenylindole (DAPI) for the enumeration of total bacteria count. LIVE/DEAD® *Bac*Light™ Bacterial Viability Kit was further used for the viability studies and carboxyfluorescein diacetate (CFDA) to quantify the bacteria with intracellular esterase activity. The enumeration of bacterial cells was assessed using epifluorescent microscopy. Additionally the classical plate counting on nutritive media was performed.
The viable cell counts of both *Lactococcus* and *Propionibacterium* estimated by plating technique were lower than those obtained by direct microscopic counting. The DAPI bacterial cell frequency has not changed significantly during the examination. The temperatures of incubation strongly affected the cultivability and viability of the microbial populations. The viability of investigated strains decreased during the cultivation at 30°C, while it was stable at 10°C until the end of examination reaching the final level of nearly 70 % of viable cells in population. Moreover, *Propionibacterium* was more resistant to NaCl addition and maintained the cultivability longer than *Lactococcus*. The application of LIVE/DEAD and CFDA staining methods the best reflected the dynamic growth and changes in physiological state of both tested strains.
This study confirms the usefulness of fluorescent techniques for rapid and accurate evaluation of bacterial viability during the production processes in fermented dairy food.

Keywords: *Lactococcus, Propionibacterium*, epifluorescence microscopy, viability, fluorescent dyes

The Ability of NSLAB Cheese Isolates to Utilize Milk Fat Globule Membrane Carbohydrates

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The ability of four non-starter lactic acid bacteria (NSLAB) strains isolated from Norwegian semi-hard cheese and one *Lactococcus* sp. to utilize three carbohydrates associated with the milk fat globule membrane was studied. Growth of the LAB were studied in a carbohydrate-restricted media, supplied with one of four carbohydrates (glucose (Glc), N-acetyl-D-glucosamine (GlcNAc), N-acetyl-D-galactosamine (GalNAc) and N-acetyl-D-nauraminic acid (NANA)). Growth was measured by optical density (OD_{620}) and counting of colony forming units (cfu) on MRS- or M17 agar.
No growth was obtained for any of the NSLAB on NANA. *Lactobacillus plantarum* reached higher OD_{620} when grown on Glc compared to GlcNAc, however the number of colony forming units were

comparable. The same strain did not grow on GalNAc. The total number of viable cells and OD_{620} of the three *Lb. paracasei* strains decreased after 48 h of growth, when GlcNAc was the only supplied carbohydrate. After the slight decrease the OD_{620} stabilized, although the number of viable cells decreased continuously. Two of these *Lb. paracasei* strains grew faster and to higher numbers when grown on GlcNAc compared to Glc. The two *Lb. paracasei* strains that could utilize GalNAc for growth, had a faster increase and obtained a higher OD_{620} in CRM supplied with GalNAc, if the cells were pregrown in GluNAc compared to Glc. The *Lc. lactis* ssp. *lactis* strain reached higher OD_{620} when grown on GlcNAc compared to Glc, however the number of cfu were comparable. When grown on GalNAc the lag phase of the *Lc. lactis* ssp. *lactis* strain lasted for more than 24 hours, however after 48 hours, the cfu were comparable with the other substrates.

Keywords: NSLAB, cheese, milk fat globule membrane, carbohydrate

Inhibition of *Listeria monocytogenes* Growth at the Surface of Cheese

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The objective of this study was to identify complex microbial consortia able to inhibit *Listeria monocytogenes* at the surface of St Nectaire's cheeses.
The anti-listerial effect of 35 complex microbial consortia from St Nectaire's surface cheeses were tested on non cooked pressed type cheeses during ripening process (28 days). Five different microbial consortia were able to reduce *L. monocytogenes* growth (ΔLog (CFU cm^{-2}) $\geq$ -1.5) compared to a commercial ripening starter. One of them (TR15) can be stored at -20°C without losing its inhibitory properties.
The microbial composition of TR15 consortium was determined by Restriction Fragment Length Polymorphism, 16 or 23 S rDNA sequencing. Nineteen microbial species belonging to four groups were identified: 1) lactic acid bacteria (5species), 2)non lactic Gram+ bacteria (7 species), 3) Gram negative bacteria (3 species) and 4) yeasts (4 species). None of these strains were able to produce bacteriocins and inhibit *L. monocytogenes* by *in vitro* test. A consortium constituted by including one strain of each species was spread at the surface of one day cheese for testing their anti-listerial effect in comparison with the complex consortium. This reconstituted consortium was less inhibitory of *L. monocytogenes* than the complex one (TR15). Microbial analysis revealed that *Leuconosotoc meseneteroides* at 3 Log at day 1 on the surface of cheese spread with the reconstituted consortium was not detected after 8 days of ripening whereas it reached 7 Log in cheese spread with the complex consortium. Such difference may explain the less inhibitory ability of the reconstituted consortium. Further studies are in progress to understand the microbial interactions and the nature of inhibition by the complex consortium.

Keywords: microbial surface flora, *L. monocytogenes*, inhibition

What are the Interests of Microbial Biofilm on Chestnut-Wood Vat (Gerle) in PDO Salers Cheese?

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This study focuses on the formation of microbial biofilm at the surface of "gerle" and its role in the inoculation or contamination of milk and barrier against pathogen growth. In manufacturing PDO sales cheese, "gerle" name of the traditional wooden vat was questioned in the framework of hygiene regulation enforcement. Then to understand its role in cheese making, experimental cheeses were made.
After elimination of tannin and washing with whey during two weeks, under scanning microscopy and by microbial analysis, it has been shown that the wood vat was colonised by yeast and bacterial colonies. Among bacteria, Lactic acid bacteria, Gram negative, non lactic acid bacteria Gram positive were found but neither *Listeria monocytogenes* nor *Staphylococcus aureus* .
After cheese making it was demonstrated that washing daily the vat with whey or water maintain this microbial biofilm on wood vat. This microbial biofilm on wood vat was sufficient to inoculate in less than one minute pasteurized milk poured into the vat. Then the levels of lactic acid bacteria and yeasts in pasteurised milk were 4 log cfu mL^{-1}, that of Gram-positive non lactic acid ripening bacteria 3.4 log and that of Gram negative 2.8 log cfu mL^{-1}. Non-cooked cheeses were produced during several days in the "gerle"with pasteurised milk without any further inoculation of starter culture. This wood microbial biofilm may also prevent colonization of wood vat by *Listeria monocytogenes* and *Staphylococcus aureus* as even after voluntary inoculations several times in milk in contact with wood vat, these pathogens were never found on it.

Keywords: wood, PDO cheeses, safety, microbial biofilm

Structural and Functional Diversity of Microbiota in Istrian Cheese: Analysis of Indigenous *Enterococcus* Community

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Istrian ewe's cheese is one of the most important Croatian traditional products with a long history of manufacturing. The production of Istrian cheese includes many traditional techniques without selected starter cultures such resulting in a significant variation in cheese quality. However, to standardize the technology procedure and to produce the cheese with more defined composition it is necessary to characterize the indigenous microbial community during the ripening process. In order to collect the preliminary knowledge on indigenous microbiota, the present study aimed at investigating the structural and functional diversity of the cheese microbial community with respect to *Enterococcus* species. Fresh milk and cheese samples were collected from 6 dairy farms during the ripening on monthly base. Samples were subjected to microbiological analysis. In order to preliminary characterize the microbial communities present in respective samples, the total DNA was isolated by Maxwell 16 System procedure. The DNA was subjected to arbitrarily primed PCR (AP- PCR) fingerprinting assay. *Enterococcus* species were isolated on selective medium for viable count and 10-15 randomly selected colonies for each sample were Gram stained. A total of 97 Gram positive isolates from two farms were further identified by combining RAPD PCR using M13 and 10 mer primers, PCR with genus- and species-specific primers. According to RAPD grouping, representative isolates were further analyzed at genus and species level. All isolates were characterized as *Enterococcus* whereas RAPD analysis classified them into eight distinct groups. Furthermore, all obtained isolates were identified as *E. faecalis* or *E. faecium* such presenting the dominance of two respective species and low interspecies diversity. MPN number of isolates was between 7.3×10^3 and 9.5×10^7 showing the lowest abundance in milk samples. Comparison of the profiles generated by AP-PCR revealed that the profiles were distinct for each sample.
Keywords: Istrian cheese, microbial community, *Enterococcus*

Volatile Compounds in Cheeses made with Milk Cultures of High Enzymatic Activity Curds of *Micrococcus* sp. INIA 528

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The effect of milk inoculation with cultures of *Micrococcus* sp. INIA 528, and of the addition of high enzymatic activity (HEA) curds prepared with this strain to standard curds made with a lactic starter culture, on the volatile compounds of cheese was investigated. Esterase activity was significantly higher in all cheeses with added *Micrococcus* than in control cheese. Fifty-two volatile compounds were quantified in cheeses. Levels of acetaldehyde, two branched-chain aldehydes (2-methyl propanal, 3-methyl butanal) and some alcohols (ethanol, 2-methyl propanol, 3-metyl butanol) were higher, and those of diacetyl and acetoin lower, in cheese made from milk with added *Micrococcus*. Free fatty acids and esters (ethyl, propyl, isobutyl esters) were at higher levels in cheeses made with added HEA curds. Total esters increased 6.4-fold in cheese made from milk with added *Micrococcus* and up to 37.4-fold in cheeses made with added HEA curds.

Keywords: Volatile compounds in cheeses, *Micrococcus* sp. INIA 528

Characterization of the Microflora in a Norwegian Semi-Hard Cheese with Adjunct Culture of Propionic Acid Bacteria During Ripening

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Cheese ripening is a complex biochemical process that is influenced by enzymes from the milk, the coagulant, the starter lactic acid bacteria (LAB) and the non-starter lactic acid bacteria (NSLAB). The diversity of the cheese microflora contributes greatly to the complexity of the cheese ripening process, which is of crucial importance for development of the unique organoleptic characteristic of each cheese. Microbiological sampling of Norwegian commercial semi-hard, Dutch-type cheese made with adjunct culture of propionic acid bacteria was done during ripening. The objective of this study was to evaluate the diversity of the microflora in the cheeses according to different dairies and different ripening time.
Cheese from two Norwegian dairies situated in different geographic parts of Norway was analyzed. One of the dairies used microfiltered pasteurized milk and the other used pasteurized milk without microfiltration. The evolution of aerobic mesophilic bacteria, lactococci, presumptive lactobacilli and propionic acid bacteria was investigated after 1, 8, 24 and 40 weeks of ripening. Isolates (120) of non-starter lactic acid bacteria (NSLAB) from 12 cheeses after 8, 24 and 40 weeks of ripening were examined. The isolates were tested by traditional phenotypic and physiological tests, API and 16S rDNA sequencing.
Results showed that the development and the evolution of the microflora varied according to dairy and ripening time.

Keywords: Semi-hard cheese, microflora, propionic acid bacteria, ripening

Biochemical and Sensorial Changes in Parmesan Cheese Manufactured with Autochthonous Starters of *Lactobacillus helveticus*

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Autolytic *Lactobacillus helveticus* metabolism in Parmesan cheese tends to reduce processing costs, since autolytic cultures can accelerate cheese ripening.
This study aimed to study the *in situ* effect of autochthonous autolytic *Lactobacillus helveticus* cultures over biochemical and sensory attributes of Parmesan cheese, and also analyze the physicochemical characteristics and the sanitary quality of pasteurized milk employed in the manufacturing process.
Commercial *L. helveticus* "Cc_1" and autochthonous *L. helveticus* "E_5", "D_1" and "A" cultures were used. The first two cultures showed higher autolytic activity, and the others, intermediate autolytic profile and absence of autolysis, respectively. In the products, the following analysis were monitored during 180 days of ripening: water activity, ash, fat, dry matter, fat in dry matter, total protein, salt, tyrosine and tryptophan, and ripening extension and depth indices. In milk, bacteria belonging to coliform group, mesophilic bacteria, *Salmonella* sp. and coagulase-positive *Staphylococcus* were investigated, and in cheese, the same analyses were carried out, except for the mesophilic microorganisms and *Salmonella sp*. The Etana model was used for quantitative descriptive sensory analysis at day 90 of ripening.
Chemical and microbiological evaluation of milk and cheese showed results according to the current Brazilian legislation ($p > 0.05$). After 180 days, the highest average of titratable acidity was 2.06 % for "E_5", depth proteolysis index equal to 11.39 % for "D_1", proteolysis extension index equivalent to 16.45 % for "Cc_1", tyrosine was 412.6 mg $100g^{-1}$ for "D_1", and tryptophan equal to 138.8 mg $100g^{-1}$ for "Cc_1" ($p < 0.05$).
The evolution of both chemical compounds in the cheese matrix and sensory attributes were due to proteolytic and lipolytic activity that are often more expressive in the final period of ripening.

Keywords: Parmesan, autolytic *Lactobacillus helveticus*, ripening, sensory analysis.

Cheese Model to Assess the Production of Biogenic Amines by Coagulase Negative Staphylococci in Paste

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Coagulase Negative Staphylococci (CNS) may be naturally present in some foods. They are also used as starters in dairy fermentation processes where they are involved in the development of the flavour and typicity because of their aromatic, enzymatic and pigmentary capacities. Most species of CNS are generally regarded as safe but their inoccuousness in food processing need to be prooved at a species and infra-species level. Indeed starter producers and food manusfacturer are having to face to more and more severy sanitary criteria and rules. In particular the capacity to produce biogenic amines in food matrices need to be evaluate for the CNS strains which own the coding gene of decarboxylase and exhibit *in vitro* production.
A semi-hard pasteurised milk cheese model was developed to optimize biogenic amines production by CSN added. Two starters were combined in either low/high or high/low levels: (1) the commercial EzalMA400 (lactococci and *Streptococcus thermophilus*) and (2) a thermophilic proteolytic *Lb helveticus* + *Lb delbrueckii* mixture. In comparison to starters on their own, we tested

the addition either of a decarboxylase plus (*dc*+) lactobacillus as control or *dc*- or *dc*+ CNS strains (10^6). The cheeses were salted in brine to reach a sodium chloride content of 25 g kg^{-1} water, covered with wax and ripened for four months at 14°C. We adopted the low/high level starter combination because it produced more favorable environmental conditions for the survival of *dc*+ or *dc*- CNS which initial count was maintained until 4 months of ripening, a higher proteolysis and higher production of biogenic amines by dc+ lactobacilli. When *dc*+ CNS was added, biogenic amines were produced in higher amounts than with starters on their own or when *dc*- CNS was added. The nature and amount of amines was strain dependent and in relation to their *in vitro* capacity.

Keywords : biogenic amines, cheese model, Coagulase Negative Staphylococcus, inoccuousness

Autochthonous Lactic Acid Bacteria from Serbian White Brined Cheeses

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The white brined cheeses in Serbia are made using traditional techniques without the addition of starter cultures and fermentation occurs as a result of lactic acid bacteria naturally present in the milk. From 15 artisanal cheeses 65 autochthonous lactic acid bacteria were isolated and characterized with aim to identify strains, which could be used as starters in commercial dairy fermentations. All strains were tested for their Gram reaction, catalase test, acidification activity, growth ability at 20, 40, 60 g NaCl L^{-1}, proteolytic ability to hydrolyze β-casein by SDS PAGE electrophoresis and biochemical identification using API 50CHL and API RAPID ID 32 Strep.
Isolated microflora belonged to the genera *Lactococcus* (48 %), *Enterococcus* (11 %), *Lactobacillus* (38 %) and *Leuconostoc* (3 %). Dominant lactocci strain was *Lactococcus lactis* ssp. *lactis* (35 %), but presence of *Lactococcus lactis* ssp. *lactis* bv. *diacetylactis*, as aroma producer is very important. For lactobacilli dominant strain was *Lactobacillus para. paracasei* (21 %). Isolated strains varied significantly according to acid production, from very good (pH < 4.6) to poor acid producers (pH< 6.0) for 8 hours fermentation. All of isolated lactic acid bacteria grew in the presence of 20 and 40 g NaCl L^{-1}, while 25 isolates grew in the presence of 60 g NaCl L^{-1}. Isolates showed different proteolytic ability to hydrolyze β-casein. Some of them very extensively degraded β-casein while the others had been inferior in this activity.
According to these results, autochthonous lactic acid bacteria isolated from Serbian artisanal cheeses have some important attributes of starter bacteria, and several of them are potentially useful as new starters for industrial production of cheeses.

Keywords: autochthonous lactic acid bacteria, white brined cheese

Lacticin 3147 Produced by *Lactococcus lactis* IFPL 3593 Prevents Late Blowing in Cheese Caused by *Clostridium*

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Bacteriocins are valuable biological tools usable to ensure food safety and to reduce risk of food spoilage. Main accepted additives in cheesemaking (lysozyme and nitrate) are intended for the

prevention of late blowing caused by heat-resistant spore-forming organisms such as those belonging to the genus *Clostridium*. Lacticin 3147 is a two-peptide lantibiotic produced by *Lactococcus lactis* that possess a great bio-protective potential due to its inhibitory capacity against most Gram positive bacteria. Ability of Lacticin 3147 and the bacteriocin producing strain, *Lactococcus lactis* IFPL 3593, to inhibit growth of *Clostridium tyroburyricum* and *Clostridium sporogenes* was analysed by following their inhibition in co-cultures of pure strains in milk and curd and by performing cheesemaking trials. Growth of *C. tyroburyricum* CECT 4011 in co-culture with *L. lactis* IFPL 3593 caused a reduction of *Clostridium* counts of 3 - 4 log cfu mL^{-1}. A semi-hard cheese produced from pasteurized milk artificially contaminated with *C. tyrobutyricum* CECT 4011 and *C. sporogenes* A1 and B2 spores (1×10^4 mL^{-1}) was also used for studying the ability of bacteriocin-producing *L. lactis* IFPL 3593 to inhibit clostridia. Late blowing as a result of *Clostridium* outgrowth was only observable in control cheeses, where *C. tyrobutyricum* counts attained 7.5 log cfu mL^{-1}, while it was prevented in cheeses with added *L. lactis* IFPL 3593 that resulted in reduction of *Clostridium* counts to 2.5 log cfu mL^{-1}. Starter counts and the cheese pH did not differ between cheeses, indicating an effective contribution of Lacticin 3147 to total antagonistic activity against clostridia. The use of Lacticin 3147 to prevent late blowing in cheese seems a great alternative to the increasing concerns about potential allergenicity of lysozyme in egg allergic consumers.

Keywords: Lacticin 3147, cheese late-blowing, *Clostridium* spp., *Lactococcus lactis*

YtjE is a C-S lyase from *Lactococcus lactis* IL1403 Involved in the Formation of Methanethiol

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Volatile sulphur compounds (VSCs) derived from methionine such as methanethiol, dimethyl sulphide, and dimethyl trisulphide are regarded as essential components in many cheese varieties; therefore, enzymes involved in the metabolism of methionine are considered to play a major role in flavour formation during cheese ripening and the foci in biotechnological approaches to aroma improvement. The product of *ytjE* of *Lactococcus lactis* IL1403, suggested as a methionine-specific aminotransferase based on genome sequence analysis, was therefore investigated for its role in methionine catabolism.

The *ytjE* gene from *L. lactis* IL1403 was cloned in *Escherichia coli*, over-expressed and purified as a recombinant protein. When tested, the YtjE protein did not exhibit a specific methionine aminotranferase activity. Instead, YtjE exhibited C-S lyase activity, in agreement with its shared homology with the MalY/PatC family of enzymes involved in the degradation of L-cysteine, L-cystine and L-cystathionine. YtjE also exhibited α,γ-elimination activity towards L-methionine. Gas chromatographic-mass spectrometry analysis confirmed YtjE activity to result in the formation of H_2S from L-cysteine and methanethiol (and its oxidized derivatives dimethyl disulfide and dimethyl trisulfide) from L-methionine.

In summary, although genome sequence analysis is an invaluable tool for unravelling the function of a gene product, biochemical characterization of the encoded protein is essential for a correct identification of its function. The results shown here demonstrate that YtjE is a C-S lyase able to produce methanethiol. Given their significance in cheese flavour development, VSC production by YtjE could offer an additional approach for the development of cultures with optimized aromatic properties.

Keywords: Methionine, cheese flavour, volatile sulphur compounds, *Lactococcus lactis*

Microbial Community Structure and Dynamics of Bacterial Surface Flora of Smear Cheese Studied with Culture-Dependent and Culture-Independent Methods

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The microbial community structure of bacterial surface flora was investigated on Swiss Raclette cheese ripened with two surface cultures (defined and complex undefined), using both culture-dependent and culture-independent methods (TTGE for population profiles, and FISH and RT-PCR for quantitative population analyses). The impact of different protective cultures (*Lactococcus lactis* UL719 [nisin Z], *Pediococcus acidilactici* UL5 [pediocin], *Lactobacillus plantarum* SM71 [unknown bacteriocin] and two commercial cultures, HOLDBAC™ Listeria dairy [pediocin] and HOLDBAC™ YM-C) for Listeria inhibition on the development of the smear was also studied.
The surface microflora of control cheeses treated with a defined surface culture was analyzed during 6 week ripening. During the first two weeks, control cheeses showed a decrease of starter lactic acid bacteria and growth of three *Staphylococcus* species likely originating from the milk. Then the surface culture became predominant, with *Corynebacterium variabile* strains growing first, followed by *Arthrobacter protophormiae* and finally *Brevibacterium linens*. The development of the smear was not or little affected by the tested protective cultures. TTGE showed that *Lc. lactis* UL719 [nisin Z] inhibited two among three *Staphylococcus* species detected on control cheeses. On the other hand, counts of all protective cultures decreased concomitant with the starter lactic acid bacteria. *P. acidilactici* UL5, *Lb. plantarum* SM71 and HOLDBAC™ Listeria dairy showed strong protective effects with cheeses contaminated with Listeria (3 cfu cm^{-2}), whereas *Lc. lactis* UL719 and HOLDBAC™ YM-C had no effects. Cheeses treated with the undefined complex flora showed significantly different smear composition compared to control cheeses, with higher content of low GC-bacteria, and inhibited growth of Listeria (15 cfu cm^{-2}).
Our data showed that culture-independent methods are powerful tools to analyze qualitatively and quantitatively smear composition, complementary to culture-dependent methods, and can help in the development of high quality surface cultures with protective effects against Listeria contamination.

Keywords: semi-hard smear cheese, surface cultures, Listeria inhibition, molecular methods

A Microscope Chamber System to Study Microbial Interactions and Cell Death in Cheese

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The carbohydrate source of non-starter lactic acid bacteria in cheese is limited and mainly unknown. One possible source is cell material from starter bacteria cells. Introductory studies have indicated that some *Lactobacillus paracasei* strains isolated from Danish cheese may lyse cells of attenuated *Lactococcus lactis* in carbohydrate restricted medium (CRM).
The purpose of this investigation was to study interactions between selected strains of *Lb. paracasei* and *Lc. lactis* in a microscope chamber system that we have developed to monitor the growth of individual cells into micro-colonies. To investigate if nutrient material from dead *Lactococcus* cells may facilitate the growth of lactobacilli, we deliberately killed *L. lactis* with heat.

The fluorescent dye DAPI was investigated in order to follow the growth of microcolonies. The growth pattern with DAPI was similar to the growth without, but staining intensity still needs optimisation in the opaque medium. When bacteria are incubated with propidium iodide (PI) in the medium, the dye is only taken up by dead cells, which gives us the opportunity to actually monitor the progression of cell death over time in a population of bacterial cells.
In a mixture of the *Lb. paracasei* and *L. lactis* strains added to CRM, the *Lactobacillus* grew better than in a pure culture. Addition of heat treated *Lactococcus* cells, caused a stronger growth of *Lb. paracasei*, than the viable *Lactococcus*. We also showed that when live *Lactococcus* are added to CRM, the number of dead cells increases over time. With deliberately killed cells, all cells are red (e.*g*. dead) from the beginning. Interestingly, *Lb. paracasei* cells did not stain with PI, even after incubation for several weeks. This is consistent with the general observation that these *Lb. paracasei* are very robust.

Keywords: Microbial interactions, fluorescence microscopy, survival and cell lysis

Identification and Investigation of Genes from *Lactobacillus helveticus* DPC4571 for their Potential in Cheese Ripening

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The genome sequence of *Lactobacillus helveticus* DPC4571 has been determined. 2115 putative genes were identified and screened for metabolic functions intrinsic to cheese flavour; proteolysis, lipolysis, and cell lysis. Eight predicted lysis genes, three predicted lipolytic esterase genes and eight predicted peptidase genes were selected. The genes were cloned and protein expression evaluated in the cheese starter expression system and a commercial *Escherichia coli* over-expression system. The presence of cell lysis activity in DPC4571 was demonstrated in the DPC4571 cell extracts against DPC4571 cells. Eight lysin genes were cloned and levels of expression evaluated in the lactococcal and *E. coli* protein over-expression systems. Neither expressed proteins nor lytic activity was detected possibly due to their lethal nature. Reverse transcriptase PCR confirmed the expression of three lysin and two helveticin genes at a point in the DPC4571 growth curve where cell lysis occurred. Three putative esterase genes involved in fatty acid breakdown were cloned and two demonstrated expression in a *L. lactis* expression system. One of these, EstA was cloned into *L. lactis* and shown to be biologically active against the *ρ*-nitrophenol butyrate substrate. A model system to determine esterase activity using a pre-hydrolysed UHT cream substrate did not reveal an increase in esterase activity in any of the clones. Two peptidase genes *pepM* and *pcp* were selected based on their novelty, both were expressed and purified in *E. coli*. In conclusion, bioinformatic analysis of the genome of *L. helveticus* has revealed a plethora of genes with industrial potential including those responsible for proteolysis, lipolysis and cell lysis.

Keywords: *Lactobacillus helveticus* DPC4571, Potential, Cheese Ripening

Detection of Cell Lysis in *Lactococcus lactis*

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The lysis of starter bacteria can have a significant impact on the cheese ripening process, but the evaluation of the lytic phenomenon is complicated and influenced by the used method.
The work was aimed at the determination of lytic properties of five chosen *Lactococcus lactis* strains by different methods (determination of cell lysis in citrate buffer, determination of cell lysis in citrate buffer with nisin, determination of cell lysis in LM17 broth with mitomycin C, determination of cell lysis on GM17 agar with *Micrococcus lysodeikticus* lysate cells).
Four strains showed lytic properties in citrate buffer (pH 5) at the temperature 13°C after 12 days of cultivation; cells lyses ranged from 9 to 36 %. Four strains showed high lytic properties in citrate buffer (pH 5) with the highest tested nisin concentration (5000 IU ml^{-1}) at the temperature 13°C after 12 days of cultivation; cells lyses ranged from 31 to 52 %. It was found, that the lowest tested mitomycin C concentration (0.5 μg ml^{-1}) caused lyses in four strains during their cultivations in LM17 broth at the temperature 30°C for 8 h; cells lyses ranged from 3 to 71 %.
It was found, that the addition of *Micrococcus lysodeikticus* commercial lysate cells (0.2 % w/w) to GM17 agar caused formation of lytic zones with diameter from 1.5 to 3.0 mm around colonies of four strains cultivated at the temperature 30°C for 48 h.
Evaluation of lactococci lysis depended on the used method. Four out of five strains were assessed as lytic strains with different level of lytic abilities caused probably by induction of lysogenic phage and one strain was assessed as non-lytic strain by usage of all tested methods.

Keywords**:** cells lysis, detection, *Lactococcus lactis*

Identification and Quantification of NSLAB from Finnish Emmental Cheese

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The number of non-starter lactic acid bacteria (NSLAB) increases during cheese ripening and these bacteria may constitute a dominant population in the mature cheese. The NSLAB diversity, their metabolism and interactions with starter bacteria have an effect on the ripening process of the cheese. The diversity of NSLAB in a Finnish Emmental cheese was studied using species-specific PCR. Emmental cheeses were produced in Valio Ltd cheese factory. Cheeses were ripened for 12 months and the samples were taken at day 1 and 24, then at the age of 3, 6, 9 and 12 months. Genomic DNA was isolated from cheese and PCR-analyses were performed using species-specific primers. Cultivation and PCR were used to determine the NSLAB species present in cheese and the total amount of bacteria from each species was quantified by qPCR.
Three Lactobacillus species of non-starter origin were found in the Emmental cheeses studied, namely *Lactobacillus delbrüeckii*, *Lactobacillus fermentum* and *Lactobacillus casei*. The total number of lactobacilli in the cheeses ranged from 10^3 to 10^7 genome copies per gram of cheese. All of the species studied were found in the mature cheese.
Although the Emmental cheeses were produced in the same production plant the NSLAB varied in diversity and number.

Keywords: non-starter lactic acid bacteria (NSLAB), cheese ripening, PCR, qPCR

Lipolysis in Swiss Cheese: Identification of Lipolytic Esterases of *Propionibacterium freudenreichii*

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The lipolytic activity of *Propionibacterium freudenreichii,* a species used as starter for the manufacture and ripening of Swiss cheese, is a key factor in the formation of the flavour. The aim of this study was to characterise the lipolytic esterases of *P. freudenreichii*.
The genome data of *P. freudenreichii* CIP103027^T, recently obtained in our laboratory, was mined using AGMIAL, an open-source platform developed by INRA. To find sequences coding for putative esterases, automatic and manual searches were performed on the basis i) of homologies with putative esterases and biochemically characterised lipolytic enzymes; ii) of the presence of consensus motifs and domains. The coding sequences (CDS) identified were cloned in *Escherichia coli* and expressed using a strategy designed to increase the probability of obtaining a high level of soluble proteins. Thus, each CDS was introduced in five expression vectors containing each a hydrophilic HIS-tagged fusion protein, by a two-steps cloning method by recombination. The constructs obtained were tested for expression in four *E. coli* strains grown in three culture media at three temperatures, using an incomplete 4 x 3 x 3 factorial design. The proteins cloned were analysed by mass spectrometry to confirm their identity and tested for esterase and lipolytic activity.
Thirty-three CDS of putative esterases were found in *P. freudenreichii* genome. Only the 14 CDS that contained an exact esterase consensus motif were retained for cloning and expression. Predicted gene products had sizes ranging from 25 to 44 kDa, except one with 117 kDa. Three gene products contained putative signal peptide. The first protein purified using this strategy hydrolysed 1-naphtylacetate after native PAGE. However, this esterase seemed to have no lipolytic activity. The cloning and expression of the other selected CDS are under way.
The results obtained should help to identify key enzymes involved in the lipolysis of Swiss cheese.

Keywords: lipolysis, *Propionibacterium*, lipolytic esterase, Swiss cheese

Influence of Adjunct Cultures Isolated from Traditional Turkish Cheese on Organic Acid Contents of White Brined Cheese During Ripening

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The effect of the adjunct cultures (*Lactobacillus paracasei* subsp. *paracasei* and *Lactobacillus paracasei* subsp. *tolerans*) isolated from traditional Turkish cheese on microbiological, compositional and organic acid (OA) content of White brined cheese were studied. Ten organic acids (citric, orotic, pyruvic, succinic, lactic, uric, formic, acetic, propionic, butyric) were analyzed throughout ripening (1, 30, 60 or 90 days) by HPLC.
Lactobacilli in the experimental cheeses produced with addition of adjunct cultures reached maxima (10^8 cfu g^{-1}) after 30 days of ripening. Number of lactobacilli in control cheese was lower than in the experimental cheeses until 60 days of ripening. Thereafter, similar numbers of lactobacilli (~10^8 cfu g^{-1}) were present in control cheeses.
In comparison with the control cheese, the results indicated that the adjunct containing cheeses exhibited no significant differences in compositional (fat, protein, pH). However, the effect of

treatments on moisture and salt content were found significant ($P < 0.05$). The effect of ripening time was significant ($P < 0.05$) for all OA but treatments affected only some of them. The main OA of all cheeses throughout ripening were lactic, citric and acetic acids. The use of the adjunct culture could enhance the production of OA in cheeses with eventual positive effect on their sensory properties.
Lactic acid content showed an important increase because of the high activity of the lactic acid bacteria. Acetic acid content increased significantly ($P < 0.05$) during ripening and the effect of treatments were found significant ($P < 0.05$) on the acetic acid content. The decreasing rate of citric acid content during ripening was low, differences were significant ($P < 0.05$).

Keywords: White brined cheese, adjunct culture, organic acid

Study of the Origin of the Enterococci Found in an Idiazabal Type Cheese

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Enterococci are an ubiquitous group and are natural residents of humans, normal constituents of the intestinal flora of nearly all animals and grow to high levels in a variety of artisanal cheeses. The aim of this study was to determine the origin of the strains of enterococci, present in the different stages of the cheeses maturity, by typing enterococci isolated from the raw milk, the cheese and the cheesemaking environment and the faeces of the ewes and humans associated with the cheesemaking. Also, the presence of enterococci resistant to vancomicine (VRE) was studied throughout the whole process, together with their possible presence in the cheese. The study was performed twice, in a cheesemaking factory. Samples of ewes faeces, shepherd´s faeces, cheesemaker faeces, teatcups, brine vats, milk from the vats, milk from barrels, cheese after salting and cheese with 1, 15 and 60 days of ripening were taken. 97 % of the strains found were confirmed by PCR as enterococci. The species identified by PCR were: *E. faecalis* (57 %), *E. faecium* (20 %), *E. casseliflavus* (10 %), *E. gallinarum* (6 %) y *E. hirae* (3 %). *E. faecalis* was present in all the places sampled and was the main species in all the milk, cheese and ewes samples. In the cheesemaker´s faeces, the main species was *E. faecium*,. *E. gallinarum* and *E. casseliflavus* were the main ones found in brine samples.
With regards to the traceability studied using Rep-PCR in the strains of *E. faecalis* that were present in all the sampled places, it appears that the origin of the enterococci found in the cheeses could be the cheese maker and the machinery used in the cheese making facilities and not the ewes faeces. However other possible non faecal origins are still to be determined.
Strains of enterococci vanC1 y vanC2/C3 were found in samples of ewes faeces, milking machines, brine and vats. No strains of VRE were found in samples of milk or cheese.

Keywords: VRE, enterococcus, raw milk, cheese

Protective Lactobacilli: Inhibition of *Clostridium* sp. in Cheese Model System

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It was found, that non-starter lactobacilli constitute the majority of the NSLAB population occurring naturally in semi-hard type cheese and they posses many interesting properties useful in

the future to improve the quality of cheese. Some of these strains are able to inhibit food spoilage bacteria, moulds, pathogens, due to the production of antimicrobial compounds (lactic acid, carbon dioxide, bacteriocin, diacetyl, hydrogen peroxide, ethanol, organic acid, etc.). The application of protective lactobacilli as adjunct cultures in semi-hard cheese production is often limited by its narrow activity spectrum and its inactivation due to the interaction with cheese ingredients. The testing in cheese-like condition is necessary for successful application of protective cultures.
The anticlostridial activity of 23 selected NSLAB strains isolated from Czech and Danish semi hard cheese was tested. The lactobacilli possessed different inhibition effect against *Cl. tyrobutyricum*, *Cl. butyricum* and *Cl. sporogenes*.
The inhibition effect of selected strains *Lb. paracasei* ST 68 (hydrogen peroxide producing) and *Lb. paracasei* 171R2 (bacteriocin and hydrogen peroxide producing) was tested at the different cheese-like conditions and different results for the production of inhibitory substances were observed.
The effect of both strains was similar in reducing the numbers of gas-producing *Cl. butyricum* by > 3 log cycles in RCM medium, > 1 log cycles in milk and by 2 log cycles in cheese slurry, compared to control during the test period.
The anticlostridial active lactobacilli strains were characterized phenotypically, by 16S rDNA and REP-PCR as *Lb. paracasei*, *Lb. fermentum* and *Lb. rhamnosus*. All tested *Cl. tyrobutyricum* strains were identified by species-specific PCR for their further confirmation in cheese.

Keywords: NSLAB, Clostridium, cheese, PCR

Biodiversity of Propionibacteria During Ripening of Emmental PDO Cheese

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Emmental PDO cheese is made from raw milk containing low concentrations of wild propionibacteria (PAB). Today, the addition of selected strains of *Propionibacteria freudenreichii* enhances regular eye formation and storage quality. However, in spite of using established cultures, cases of late fermentation still rarely occur. In order to get a better insight in the diversity and dynamics of PAB in Swiss Emmental, two cheeses were made of the same raw milk and inoculated with two different PAB cultures A and B containing strains with low and high aspartase activity, respectively. The composition of PAB flora was investigated in the curd before and after scalding and at intervals of two months during ripening. Eighty randomly selected PAB isolates per sample were typed and the relative distribution of wild strains and culture strains was determined. Genotyping was performed by Insertion sequences restriction fragment length polymorphism (IS-RFLP) and Minisatellites. After warm room storage at 24°C the proportion of wild strains ranged in both cheeses between 22 % (culture A) and 32 % (culture B). During further ripening the proportion of wild strains increased up to 80 % in the cheese containing culture A (Figure 1), indicating that wild strains of raw milk can affect cheese quality despite the addition of PAB cultures. Contrarily, the proportion of wild strains decreased during cold ripening in the cheese containing culture B. The results imply that growth temperature and aspartase activity of PAB strains present in cultures or raw milk may be important factors affecting the diversity of PAB in Emmental cheese.

Keywords: Emmental cheese; ripening; propionibacteria; IS-RFLP; Minisatellites

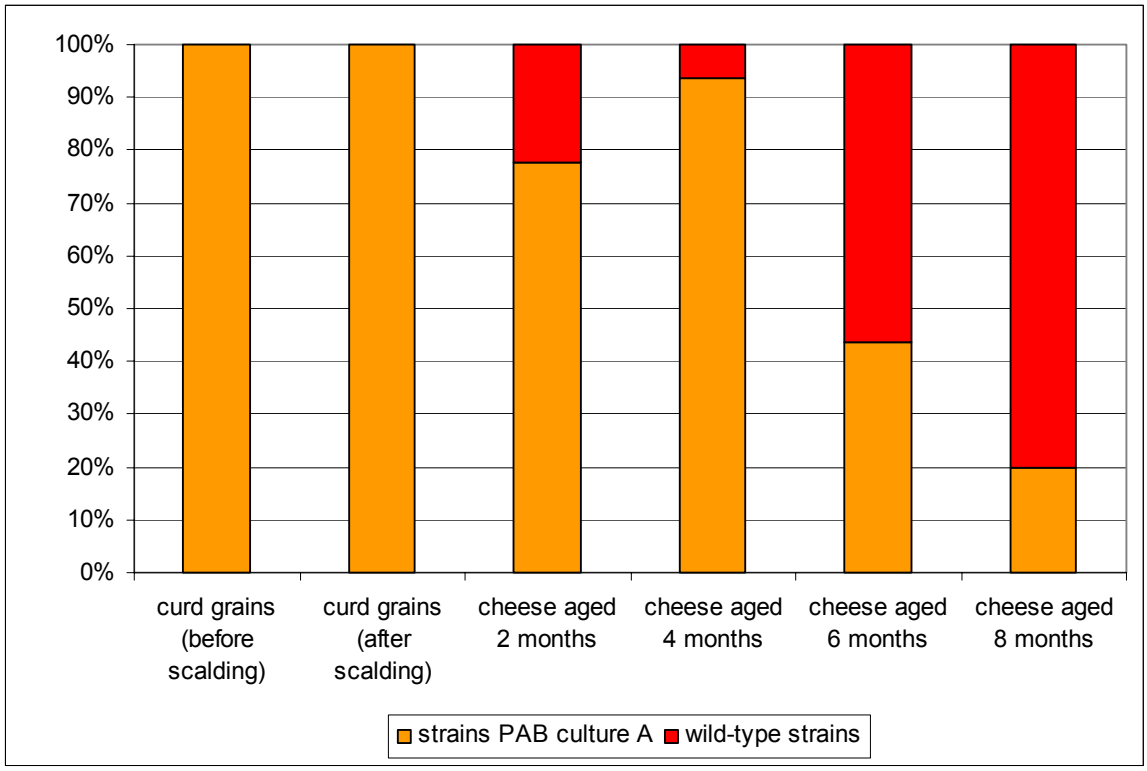

Figure 1: Composition of PAB flora obtained in Emmental cheese made with culture A containing strains with low aspartase activity.

Quantification of the Competition Between Lactic Acid Bacteria and *Staphylococcus aureus* in Milk and Lump Cheese

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In connection with ewes' lump cheese production, the inhibitive potential of two mesophilic cultures of lactic acid bacteria was described. The initial amounts of lactic acid bacteria necessary for keeping the growth of *Staphylococcus aureus* under control were estimated in ultra-pasteurized milk.

The growth inhibition of *S. aureus* 2064 with mesophilic culture Fresco was the more effective the higher addition of competitive microflora and the lower incubation temperature were used. At temperature of 25°C, *Staphylococcus aureus* 2064 in stationary phase reached counts higher than 10^6 cfu mL^{-1} by the Fresco culture addition $N_{0\_Fr} = 2.95$ $\log_{10}$ counts and by the addition of Fresco $N_{0\_Fr} = 6.6$ $\log_{10}$ in the beginning there was an increase about 1 log in staphylococci counts. At 18 °C and comparable addition of Fresco culture, $N_{0\_Fr} = 6.7$ $\log_{10}$ counts, the increase in *S. aureus* counts was only about 0.53 log. Increasing of *S. aureus* 2064 in stationary phase against its initial numbers in co-culture with Fresco culture at 25°C was determined using the equation N_{max_Sa}-N_{0_Sa}= - $0.7258N_{0\_Fr}$ + 5.6748 ($R^2_{Nmax\_Sa - N0\_SA}$ = 0.9281). The behaviour of *Staphylococcus aureus* 2064 and mesophilic culture A at temperature 25°C was also studied. The culture A showed similar, even higher inhibitory effect on *S. aureus* growth at 25°C. In this case the growth of *S. aureus* 2064 in co-culture with culture A at 25°C was described by the equation N_{max_Sa} - N_{0_Sa} = -2.1814 N_{0_A} + 12.605 ($R^2_{Nmax\_Sa - N0\_Sa}$ = 0.9564).

The duration of pH lag-phase, hence the time during which no changes of pH values were observed was determined by initial inoculum of Fresco culture at each incubation temperature. These

dependencies were described by statistically high significant linear relations in this work. The maximal increase of *S. aureus* counts in stationary phase by 1 log against its initial numbers was controlled by the addition of the Fresco culture or the mesophilic A culture higher than 10^6 or 10^5 cfu mL^{-1}, respectively.

Keywords: lactic acid bacteria, Staphylococcus aureus, growth quantification

Concerted Acceleration of Cheese Flavor and Texture

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Flavor and texture are crucial attributes of cheese for consumer perception. Each cheese has its own particular characteristics which are formed during the ripening period varying from weeks to years. Ripening is performed under carefully controlled conditions and is the result of a well balanced, complex series of flavor forming processes. Acceleration of ripening is interesting from an economic point of view, but it is difficult to maintain the flavor balance while ripening. We have identified a carboxypeptidase that is capable to induce accelerated, balanced flavor development. The enzyme itself liberates amino acid as flavor precursors. Using head-space GC-MS, we have surprisingly found that not only the development of amino acid derived flavor compounds is accelerated, but also of compounds derived from lactose and fatty acids. Bitterness is reduced in presence of the carboxypeptidase. In addition we observed a softening of cheese texture upon addition of the carboxypeptidase. We will present a model, based on experimental data, to explain this unexpected phenomenon.

Keywords: Accelerated cheese ripeing, texture, flavour

Technological Properties of Lactic Acid Bacteria Isolated from Silter

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Silter is a traditional Italian cheese made from raw cow's milk in the Sebino Bresciano and Valcamonica areas. It is frequently the product of marginal farmers who adopt traditional technology in their small-size milk production and processing. These farmers have long been endeavouring to maintain an old tradition by handing it down to the present time, generation by generation. Raw milk cheeses are characterised by the presence of indigenous microflora that is associated with a more intense, and qualitative, difference in flavour.
The aim of this study was to characterize, and evaluate, important technological properties of the autoctonous lactic acid bacteria (LAB) isolated during the manufacture of Silter produced on the mountain and on the plain.
The microbiological analyses show high levels (10^7cfu mL^{-1} in the milk and 10^8 cfu g^{-1} in the curd) of LAB species in the samples from both mountain and plain. 484 strains of LAB were isolated: 67 strains, selected after sensory analyses of cheeses, were evaluated for phenotypic characteristics, and for technologically relevant biochemical activities such as acid production and redox activity in milk, proteolytic activities. The strains were identified by Api 20 Strep, Api 50 CHL (Api System BioMérieux France), Biolog AN and GP Microplate (Biolog System USA) and were subjected to Specie-specific PCR in order to confirm phenotypic identification. A high level of biodiversity in the indigenous microflora: *Lactococcus lactis* ss *lactis* predominated (31/67) (Fig.1).

The strains showed an Eh value between –217.0 mV and +151.4 mV: in 39 strains the reduction activity was high (Eh < -102.0 mV). The titratable acidity after 24 h ranged from 5 to 10 g lactic acid L^{-1} for 38 strains. Regarding acidifying activity, that of *Streptococcus thermophilus* was the fastest, reducing the pH of skim milk to 4.30 after 6 hours and 3.84 after 24 hours of incubation.
The results suggest that the strains isolated from the raw milk and curd are a potential source of new starters with particular properties.

Keywords: Silter, characterization, lactic acid bacteria

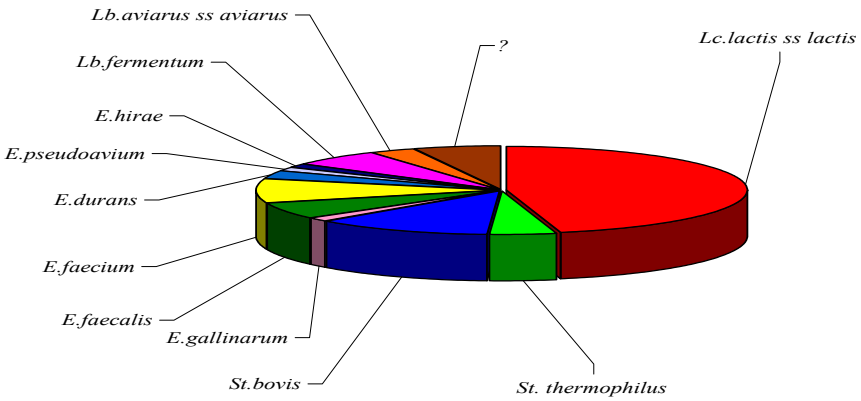

Figure 1: Distribution of LAB

Tyrosine Decarboxylase Activity in *Enterococcus* Strains

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Histamine and tyramine are the most studied biogenic amines (BA) due to the toxicological effects derived from their vasoactive and psychoactive properties. The concentration of these BA is especially high in cheese and other fermented foods.
Bacteria belonging to genera *Lactobacillus, Entorococcus or Carnobacterium* have been described as possessing tyrosine decarboxylase (TDC) activity which converts tyrosine into tyramine. The ability of microorganisms to decarboxylate tyrosine is variable even between strains of the same specie. Molecular methods based on the *tdc* gene detection have proved to be fast and sensitive but differentiating between high and low tyramine producers is not possible.
he present work describes the development of a quantitative method for determining the capacity of *Enterococcus* strains to decarboxylate tyrosine. *Enterococcus* genus is chosen as it is the predominant non-starter microflora in raw milk cheeses. The method is based on the quantification of tyramine production by cell free extracts, after strain incubation in a tyrosine rich medium. The activity measured was linear with the reaction time and with the protein concentration.
A qualitative screening method was used to classify as tyramine producers or non-producers thirty four *Enterococcus* strains, isolated from ewe's raw milk and cheese and genotipically identified as *E. faecalis* (14 strains), *E. durans* (13 starins) and *E. faecium* (8). Then the quantitative method was applied to analyze their TDC activity.

Strains were classified into three groups according to their TDC activity: a) low activity strains possessing TDC activity producing less than 0.01 μmol tyramine. min^{-1}.prot mg^{-1}. To this group belonged all strains classified as non-producers by the qualitative method (7); b) medium activity strains had activities between 0.01 and 0.1 μmol tyramine. min^{-1}.prot mg^{-1}; c) high activity strains produced more than 0.1 μmol tyramine. min^{-1}.prot mg^{-1}. Seven of the fourteen *E. faecalis* strains had high activity whereas none of the *E. durans* strain had low activity.

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Keywords: *Enterococcus*, tyrosine decarboxilase activity, tyramine production

The Nomenclature of *Lactobacillus casei,* an Archaeology Approach

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The nomenclature of the species in the so-called *Lactobacillus casei* complex has not been clearly solved, and this has let to confusion in the literature. The species names at present used within this complex are *Lactobacillus casei, Lactobacillus zeae, Lactobacillus paracasei,* and *Lactobacillus rhamnosus*. A literature review shows that only 3 species, not 4, should be recognized in the complex of which *L. rhamnosus* is one of them. It is also clear that the present type strains for *L. casei* (ATCC 393^T) and *L. zeae* (ATCC 15820^T) belong to the same species, while the proposed neotype strain for *L. casei* for (ATCC 334^T) and the type strain for *L. paracasei* (NCIMB 700151^T (formerly NCDO 151^T)) belong to a second species. However the discussion about which name is the correct is still running. According to the ATCC homepage the type strain of *L. casei* 393^T is the "*Streptobacterium casei* strain #7" (later renamed to *Lactobacillus casei*) and *Lactobacillus casei* would therefore have priority over the name *Lactobacillus zeae*, as it was published first. However, Acedo-Félix & Pérez-Martínez (1) showed that strains named ATCC 393 from different laboratories and culture collections based on polyphasic taxonomy belonged to two different species, namely a *L. zeae* like strain from all public culture collections, and a *L. paracasei* like strain from several research laboratories.

The original *"Streptobacterium casei* strain #7" has been stored in freeze-dried form in the original Orla-Jensen collection at BioCentrum-DTU since 1948. However, the strain is no longer viable. Therefore, we isolated DNA from the freeze-dried material. We amplified the 16S ribosomal gene by PCR and DNA sequenced the gene. The obtained DNA sequence of strain #7 was closely related to the proposed neotype strain for *L. casei* ATCC 334^T, and the type strain *L. paracasei* NCDO 151^T, and more distantly related to ATCC 393^T. The ATCC 393^T strain in the public culture collections is therefore not the same strain as #7, and therefore do not represent the original Orla-Jensen strain as quoted. This means that the name *L. zeae* has priority for the strains resembling *L. zeae* ATCC 15829^T, and therefore the ATCC 393^T present in the public culture collections should be renamed as *L. zeae* ATCC 393, while the name *L. casei* should be reserved for those resembling *L. casei* ATCC 334^T.

We are presently investigating if the ATCC 393 laboratory strains, that were similar to *L. paracasei* (Acedo et al., 2003), are identical to strain #7 using a Multi Locus Sequence Typing approach (Cai et al., 2007)

E. Acedo-Félix, G. Pérez-Martínez (2003). *Int. J Syst Evol. Microbiol.,* **53,** 67-75
H. Cai, B.T. Rodríguez, W. Zhang, J.R. Broadbent, J.L. Steele (2007). *Microbiol.,* **153,** 2655-2665

Keywords: nomenclature, *Lactobacillus casei*

Evaluation of the Effects of Attenuation Methods on Accessibility of Intracellular Enzymes from *Lactococcus lactis* Strains

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During cheese ripening starter cells become non-viable, increase in permeability and can completely autolyse thereby allowing their intracellular enzymes to contact with substrates. A widely used method to increase the LAB enzyme content in cheese and accelerate ripening involves the inclusion of LAB strains as starter adjuncts during cheese manufacture. These adjunct LAB strains have been previously subjected to attenuation treatments to retard acid production and enhance intracellular enzyme release. However, to date, little published information exists on the effects of various attenuation methods on the permeability and release of intracellular enzymes such as Pep X or Pep N. In this study, three *Lactococcus lactis* strains used as Cheddar cheese starter cultures were subjected to the following attenuation treatments: sonication, chemical permeabilization, heat and freeze shock. In general, as duration of sonication increased the levels of intracellular enzymes released into the cell free supernatant increased for all strains. The effects on cell permeability and accessibility of intracellular enzymes were subsequently monitored in cell pellets of strains treated with isopropyl alcohol, sodium dodecyl sulphate, hexadecyltrimethlammonium bromide (CTAB), heat (45 to 65 º C) or freeze shocking. Chemical treatments, in particular CTAB, had a strongly positive effect on accessible intracellular enzyme activity of Pep X or Pep N. Heat treatments did not result in increases in accessible enzyme activity. Permeability of cells following treatments was monitored using flow cytometry and sub-populations corresponding to intact and permeabilized cells were identified and quantified. CTAB treatment appeared to generate a uniquely permeabilized sub-population which corresponded to enhanced intracellular enzyme accessibility.

Keywords: Enzymes, Permeability, Autolysis, Attenuation

Characteristics of Cheddar Cheese Made with Adjunct *Lactobacillus* sp. that Exhibited Differing Activities of Amino Acid-Releasing and Catabolising Enzymes

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Cheddar cheese was made in duplicated 45 litre pilot plant scale trials using either *Lactobacillus plantarum* 12028 or *Lactobacillus paracasei* I7 adjunct cultures that were added at 10^5-10^6 cfu ml^{-1} vat milk. The effect of α-ketoglutaric acid (KGA) addition (20g kg^{-1}) to the adjunct-containing curd was also evaluated. The specific activities of several aminopeptidases, dipeptidyl peptidase, aromatic and aspartate aminotransferase were up to 10-fold higher in the glutamate dehydrogenase (gdh) forming *Lac. plantarum*. Cheeses were monitored after 8, 16 and 24 weeks of ripening to confirm adjunct establishment, to monitor amino acid levels and volatile components, and for sensory analysis by a trained taste panel.
The presence of both adjuncts resulted in higher amino acid levels developing than in the control cheese during ripening with the accumulation being greater for several amino acids with the more peptidolytic *Lac. plantarum*. The inclusion of KGA together with the adjunct resulted in a pronounced reduction in amino acid levels in the *Lac. plantarum* cheese and a concomitant increase in some typical amino acid catabolites. Although this adjunct was able to recycle KGA enzymically, KGA supplementation of the curd during manufacture enhanced aminotransferase-mediated amino

acid turnover. Amino acid levels were not similarly affected by KGA inclusion and increased in the cheese containing *Lac. paracasei* that exhibited lower aminotransferase activities. Beneficial effects of both adjuncts were only evident in the initial stages of maturation and the acceleration of some ripening attributes was more pronounced with the more enzymically active *Lac. plantarum* adjunct; despite the marked differences in amino acid and volatiles profiles following KGA inclusion there was not a corresponding improvement in the sensory perception of cheese flavour and maturity. It can be concluded that the use of adjunct cultures pre-selected for key enzymic activities can have beneficial effects on the extent and rate of cheese character development during ripening and the use of such high activity gdh-producing strains obviates the need for KGA inclusion in the curd during manufacture.

Keywords: Cheddar Cheese, adjunct *Lactobacillus* sp.,amino acid, enzymes

A Toolbox to Quantify Enterotoxin Gene Expression in a Complex Microbial Ecosystem

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Cheese is a complex and dynamic microbial ecosystem characterized by the presence of a large variety of bacteria, yeasts and moulds. Some micro-organisms, including species of lactobacilli or lactococci, are known to contribute to the organoleptic quality of cheeses, whereas the presence of others may lead to spoilage or constitute a health risk. *Staphylococcus aureus* is recognized worldwide as an important foodborne pathogen, owing to the production of enterotoxins in food matrices.
In order to study enterotoxin gene expression during cheese manufacture, we developed an efficient method to recover total RNA from cheese and a robust method to study gene expression by quantitative RT-PCR. RNA is isolated from cheese in two steps: A cheese sample is first homogenized in citrated water with a mechanical blender and cells are isolated by centrifugation. Cells are then disrupted and total RNA is purified using a classical protocol. This method yields pure preparations of undegraded RNA accessible for RT-PCR.
It is important that quantitative RT-PCR data be normalized with a proper internal control to obtain reliable results with biological significance. We thus investigated the expression stability of ten housekeeping genes potentially useful as references in studies of *S. aureus* grown in milk or cheese, using the geNorm Visual Basic Application for Microsoft Excel. Enterotoxin gene expression can therefore be normalized using the geometric mean of the three most stably expressed reference genes. And gene expression is correlated with enterotoxin quantity.

Keywords: *Staphylococcus aureus*, gene expression, normalization, enterotoxin

Construction of a Reporter Vector for the Analysis of *Propionibacterium* Promoters

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A ß-galactosidase reporter system for analysis of promoter elements in *Propionibacterium* was designed. The pTD210 *in vivo* reporter vector was constructed using a promoterless *lacZ* gene from *Bifidobacterium longum* cloned in the pAMT1 plasmid. The utility of the pTD210 reporter vector was demonstrated by analysis of nine potential promoter fragments in *Propionibacterium freudenreichii*. The system produced accurate and reproducible measurements that facilitated both promoter identification and quantification of their activities ranging from 50 to 7000 Miller units.

Keywords: reporter vector, *Propionibacterium*

Development of Microarrays for the Detection of Proteolytic Cheese-Ripening

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Trace analysis and analyses of micro amounts belong to the standard important tasks of analytical chemistry. High throughput methods are important from a technological and economical point of view to meet the increasing demands of society for quality assurance and analytics in general. Nowadays demands increase on analytical chemistry - not only from an economical point of view but also due to ecological reasons - with respect to minimizing consumption of solvents and chemical reagents during the analytical process.
A way to achieve this goal should be the adaptation of microarray techniques developed in the fields of molecular biology and genetics for food technology based analytical tasks. From the point of view microarray techniques have been shown both, massive parallel throughput and small consumption of reagents leading to excellent sample to expenditure ratios.
In case of process control during cheese ripening microarrays would show a possibility to detect in time of process some different but essential parameter probes like amino acids and afford a change in production at once if necessary.
Some problems are given on the way: Where biological structures meet with technical systems there usually arises the biotechnological "interface problem", for example in biosensors and bioelectronics, in biocompatibility and biomolecular affinity chromatography and many more. The solution often lies in coating the technical surface, for instance by means of chemical coupling. We provide coupling protocols and thus offer specific solutions also including coupling with lateral structuring, e.g. for array technology.
Here, we implement a micrometer scale structured surface modification for adsorptive and covalent coupling of antibodies for the detection of amino acids generated from the water soluble extract, which are important for screening the cheese ripening process. Produced structures are detected by fluorescence marking or photometric.

Keywords: Microarray, proteolysis, cheese ripening

Quantitative Proteomic of Bacterial Enzymes Released During Swiss Type Cheese Ripening

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Due to increasingly available bacterial genomes in databases, proteomic tools have recently been used to screen proteins expressed by micro-organisms in various fermented food products. Numerous bacterial proteins were shown to be released into Swiss-type cheese through lysis of the

lactic starters *S. thermophilus* and *Lb. helveticus* (Gagnaire *et al.*, 2004). Proteins including various peptidases, glycolytic enzymes and stress proteins were identified on 2D-gel electrophoresis and tandem mass spectrometry. The new challenge consists in the quantification of these proteins in the cheese matrix at different stages of ripening. In order to circumvent the difficulty of quantifying proteins between gels, a new mass spectrometry-based approach was used. This technique used isobaric tagging reagent for quantitative proteomic analysis (iTRAQ), which are amine specific and yield labelled peptides identical in mass. After fractionation of the labelled peptides by reverse phase nano-LC coupled on line with ESI-Q-TOF, specific signature ions are observed in MS/MS mode permitting relative quantification of four different samples simultaneously. Experimental Swiss–type cheeses were performed using microfiltered milk and as starter lactic acid bacteria *S. thermophilus* ST20, *Lb. helveticus* LH1 and dairy propionibacteria *P. freudenreichi* P23. At four ripening times, cheese aqueous phases were extracted and fractionated to separate bacterial and milk proteins. Each sample, standardised in protein amount by amino acid analysis prior to proteomic analyses, were: i) analysed by 2D-electrophoresis for qualitative analysis and ii) submitted to trypsinolysis, and labelled with specific iTRAQ tag, one per ripening time. The four labelled samples were mixed together and analysed by nano-LC coupled on-line with ESI-QTOF mass spectrometer. This technique provided the identification of the bacterial proteins released through lysis as well as their respective abundance at different stages of Swiss-type cheese ripening. Such a dynamic investigation could be enlarged to a variety of fermented food products.

V. Gagnaire, M. Piot, B. Camier, J.P.C Vissers, G. Jan, J. Léonil (2004). *Int. J Food Microbiol*. **94**(2), 185-201

Keywords: proteomic, Swiss-type cheese, mass spectrometry, protein quantification, lysis

HPLC-MS-Based Methods for the Study of Sulfur Metabolism in *Lactobacillus casei*

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Microbial metabolomics is considered as an emerging new tool for functional proteomics. Metabolomics is a technology that involves the non-targeted, holistic analysis of the changes in the complete set of metabolites under defined conditions. Combined with proteome studies it can reveal insights over a wide range of microbial research areas from growth medium optimization, identification of bioactives in complex mixtures, the characterization of mutant strains, the exploration of the production potential of strains to many more issues.

In contrast to GC-MS- or NMR-based analysis, the application of HPLC-MS for metabolomic studies is relatively new and compared with NMR offers a higher sensitivity. In our studies, we examined metabolites of *Lactobacillus casei* which had been incubated in a buffer in the presence and absence of methionine. Metabolites were extracted by solid phase extraction and analysed by hydrophilic interaction chromatography coupled to electrospray ionisation mass spectrometry. Differential analysis of the HPLC-MS data was performed with the SIEVE software (Thermo Fisher Scientific), which enables semi quantitative comparisons between samples and an automated label-free differential expression analysis of metabolomic data.

We found that several metabolites were up- and down regulated in the presence of methionine. A major metabolite derived from methionine was identified as 2-hydroxy-4-methyl thiobutyrate. In a second step exploratory data analysis using principal component analysis and discriminant partial least squares was performed with the SIEVE data. The results show that the combination of LC-MS and multivariate data analysis is a powerful tool for non-targeted metabolomics studies.

Keywords: metabolomics, mass spectrometry, differential data analysis

Changes in Proteomic Profiles of Sweet Whey and Rennet Casein Fractions in Response to Pasteurization

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Proteomic methods have been increasingly employed for the profiling and detailed characterisation of proteins and peptides in milk and derived products. In the present study the protein profiles of sweet whey and rennet casein fractions prepared from raw or pasteurized (HTST) skimmed milk were studied by two-dimensional gel based proteome analysis coupled with MALDI TOF mass spectrometry. As some of the changes induced in the milk proteins in response to pasteurization involved changes in disulphides, the proteins were analysed by a modification of the traditionally used method for two-dimensional gel electrophoresis involving non-reducing running conditions in an attempt to visualize eventual formation of disulphide-linked complexes of moderate size in response to pasteurization.
The relative spot volumes obtained after separation of fractions obtained from raw or pasteurized milk, respectively, were compared using multivariate data analysis and statistical t-test. It was not possible by visual inspection alone to identify major differences in the proteomic profiles of sweet whey and rennet casein fractions prepared from raw or pasteurized milk, respectively.
By principal component analysis of relative spot values no clear grouping of samples according to heat treatment was obtained. By t-test, a number of different spots in the rennet casein and sweet whey fraction were found to significantly vary in response to pasteurization. Some of these represented higher molecular mass complexes and were identified by mass spectrometry to contain α_{S1}-casein, and turned out to increase by pasteurization. Furthermore, fragments of α_{S1}-casein, probably corresponding to α_{S1}-I casein fragment as a result of chymosin cleavage, increased in the whey after pasteurization whereas the content of PP3 in whey decreased after pasteurization. This shows that gel-based proteome analysis is a valuable tool for the characterisation of subtle variations in protein composition that occur as a consequence of pasteurization.

Keywords: proteomic profile, sweet whey, rennet casein

Study of the Metabolism of the Cheese-Ripening Yeast *Yarrowia lipolytica*: Focus on Lactate and Amino Acids Catabolism

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The role of the microorganisms within the cheese ecosystem has already been investigated. Nevertheless, the methods used essentially remained descriptive due to the lack of molecular tools. The sequencing of several microbial genomes from the cheese ecosystem opens up the route for new approaches to study the metabolism of the cheese-ripening microorganisms.
The substrates consumption such as lactate and amino acids is of major importance for the microbial development during cheese-ripening. Our study essentially focuses on the catabolism of lactate and amino acids (AA) by the cheese-ripening yeast *Y. lipolytica*.
A strain of *Yarrowia lipolytica* isolated from cheese was grown in a liquid medium containing lactate, in the presence of a low (0.1X) or a high (2X) concentration of AA. Our results show a

drastic rise in the growth of *Yarrowia lipolytica* in the medium containing a high AA concentration, but limited lactate consumption. Conversely, lactate was extensively consumed in a medium containing a low concentration of AA. These data suggest that AA are used by *Y. lipolytica* as a main energy sources, lactate being consumed after AA depletion.
The effect of AA addition in a culture of *Y. lipolytica* containing only lactate was investigated. Real-Time quantitative PCR analyses were performed with specific primers of four aminotransferases (Aro8, Aro9, Bat1, Bat2) and two lactate dehydrogenases (Ldh1, Ldh2). Our data show that Bat2, Bat1 and Aro8 were maximally expressed after 15 - 30 min following AA supplementation, while maximum expression levels for Ldh1 and Ldh2 were further delayed ($\geq$ 60 min).
Our data demonstrates that the catabolism of lactate and amino acids complement each other, although *Yarrowia lipolytica* primarily assimilates AA. The results of this study may constitute a first step in the investigation of the possible involvement of *Yarrowia lipolytica* in the ripening process.

Keywords: Lactate and AA catabolism, Real-Time quantitative PCR, *Yarrowia lipolytica*

A Step Towards the Investigation of Microbial Gene Expression in Cheese: Development of Improved RNA Extraction Methods

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Gene expression measurements based on real-time reverse transcription PCR or DNA arrays have many potential applications for the study of growth and activity of cheese microorganisms. However, extraction of RNA from cheese constitutes so far a limiting step.
We set up and compared two different methods for RNA extraction from the same model cheese. The first one is based on direct extraction, and the second on prior separation of the bacterial cells from cheese. With the direct method, limited amounts of RNA are recovered from cheese samples, due to the difficulty to treat quantities of cheese higher than 1 g. However, nearly 30 µg of RNA can be recovered per g of cheese containing approximately $2x10^9$ cfu g^{-1} of *Lactococcus lactis*. Using bacterial separation, significant amounts of RNA can be recovered, even at a concentration as low as $2x10^7$ cfu g^{-1}, due to the possibility to purify the RNA from cells isolated from 20 g of cheese. For the two methods, the integrity of RNA prepared from model cheeses manufactured with *L. lactis* LD61 was excellent, even for two weeks-old cheeses. Good quality RNA could also be recovered from commercial cheeses, except those in which autolysis of lactococci had occurred. When *L. lactis* gene expression was measured by real-time reverse transcription PCR, identical results were obtained in most cases for the two RNA preparation methods.
The RNA extraction methods that we set up will be useful for the study of microbial gene expression in cheeses. In the near future, this type of analyses will provide new insights into the microbial activities involved in cheese production.

Keywords: RNA extraction, cheese, real-time PCR, gene expression, *Lactococcus lactis*

A Genomic Analysis of Amino Acid and Sugar Metabolism in *Lactobacillus helveticus*

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Lactobacillus helveticus DPC4571 is a Swiss cheese isolate and is the most promising flavour development adjunct culture identified to date at Moorepark Food Research Centre. DPC4571 has a number of traits that are extremely desirable in cheese flavour development including autolysis, reduced bitterness and enhanced flavour development. To fully exploit the potential of this culture in cheese ripening, the complete genome sequence of DPC4571 was determined and will serve as the basis for an investigation of the desirable cheesemaking traits of this strain.
The overall trend observed in the metabolic pathways of DPC4571 as compared to other lactobacilli is the loss of biosynthetic capacity that reflects adaptation to the nutritionally rich dairy environment. *In silico* analysis of amino acid requirements reveals that DPC4571 can synthesis 4 amino acids *de novo* (aspartate, asparagine, cysteine and serine). The extreme level of auxotrophy for amino acids is similar to that reported for other dairy lactobacilli. There were substantial differences in the sugar utilisation capability of DPC4571 as compared to the closely related *Lactobacillus acidophilus* NCFM, 9 phosphoenolpyruvate dependent phosphotransferase systems were identified in the DPC4571 genome compared to 20 in the genome of *L. acidophilus* NCFM. In addition the number of glucosidase genes present in DPC4571 was substantially less than in *L. acidophilus*, the general trend observed for sugar metabolism was a reduced capability to metabolise complex oligosaccharides of the type that would be present in the intestinal and plant associated niches from which *L. helveticus* is absent.
The data presented will compare the differences in nutrient biosynthesis and sugar utilisation genes from the genomes of *Lactobacillus helveticus* and other *Lactobacillus species* that occupy different environmental niches.

Keywords: Lactobacillus helveticus, Genomics

Growth of *Lactobacillus casei* ATCC 334 in a Cheese Model System: A Genomic Approach

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Cheese ripening is a dynamic, poorly understood process that is essential for development of cheese flavor and requires non-starter lactic acid bacteria (NSLAB). The energy sources utilized for growth of NSLAB are unknown, however potential energy sources include simple carbohydrates, citrate, nucleic acids, glycopeptides and phosphopeptides. *Lactobacillus casei* is a typically dominant species of NSLAB present in ripening cheddar cheese. Our research group has developed cheddar cheese extract (CCE) as a model system medium that allowed us to examine the growth substrates present in ripening Cheddar cheese. The recently completed genome of *Lb. casei* ATCC 334, a cheese isolate, has enabled us to observe global expression patterns during the same growth curve. Triplicate fermentations were carried out in CCE at 37°C and pH of 5.2. *Lb. casei* ATCC 334 grew from an initial cell density of 10^3 to 10^9 cfu mL^{-1} during 65 h fermentation. Samples for microarray analysis were taken at early log, (12 h), mid-log (27 h), late-log (35 h) and stationary phase (50 h). *Lb. casei* ATCC 334 was able to reduce and maintain the CCE media at -340 mV after mid logarithmic phase a value similar to that found in ripening cheese. Differential gene

expression (DE) was determined using ODP approach (optimal discovery procedure), that identified 296 genes out of 2963 annotated at a false positive rate cutoff 10 %. The main category of DE were "poorly characterized or with unknown function" (36 %); "information storage and processing" (33%) and "metabolism" (22%). A detailed analysis of metabolism related genes indicated the possibility of a novel metabolic pathway to derive energy from the degradation of nucleic acids present in Cheddar cheese. Overall, the use of CCE is shown to be a good model system for the study of microbial growth of NSLAB and its metabolism in ripening cheddar cheese.

Keywords: *Lactobacillus casei* ATCC 334, cheese model system, global gene expression

Poster Session 3

Influence of Fat Reduction on Sensory and Microbiological Properties of Cheddar

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Production of high quality lowfat (< 6 % fat) Cheddar cheese is a current industry goal in the U.S., but critical information of how fat reduction, and corresponding compositional changes in lowfat cheese such as lower salt-in-moisture content, influences sensory and microbiological properties is lacking. Specific knowledge of how fat reduction influences these properties could lead to identification of methods to enhance or minimize formation of specific flavors. Our objective was to characterize sensory and microbiological differences in Cheddar cheeses containing 32 (full), 16 (reduced), or 5 % (lowfat) fat (wet wt). Cheeses were manufactured in duplicate with a single-strain *Lactococcus lactis* starter culture and ripened at 8°C. After 2 wks, 3 mo, 6 mo and 9 mo, the cheeses were evaluated by a trained sensory panel using an established flavor language and sampled for starter and nonstarter lactic acid bacteria (NSLAB). Sensory results were evaluated by univariate and multivariate analyses to document the influence of fat reduction and ripening time on flavor. Cheeses with 5 % fat were characterized by a lack of milkfat flavor across ripening. Additionally, these cheeses lacked sulfur and brothy flavor development after 6 and 9 mo ripening compared to reduced and full fat cheeses and were instead distinguished by whey flavor and burnt/animal/brothy flavors. Microbiological data also showed some interesting trends. First, starter populations remained stable out to 3 mo in lowfat and reduced fat cheeses before showing any decline, but in full fat cheese generally declined by at least 2 orders of magnitude by 3 mo. Additionally, NSLAB levels in low fat cheese exceeded 10^6 by 6 wks, but populations in reduced or full fat cheese did not attain that level even after 6 mo. These results provide greater insight into the problems, and potential solutions, related to manufacture of high-quality lowfat Cheddar cheese.

Keywords: lowfat cheese, cheese flavor, sensory analysis, cheese microbiology

Thermophilic Starters Influence the Sensory Characteristics of Swiss-Type Cheeses

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The final sensory characteristics of cheeses are known to develop during ripening, due to biochemical changes in the different constituents. However, the cheesemaking step is decisive for the final cheese characteristics. It influences the dynamics and activities of microorganisms during ripening because it determines at unmoulding 1/ the chemical composition and structure of cheese matrix, 2/ the balance between the microbial populations. The activity of acidifying starters during the cheesemaking step may largely contribute to this influence. An experiment was designed to determine the effect of different thermophilic starter combinations on the sensory characteristics of Swiss-type cheeses.
Eighteen Swiss-type cheeses were made using 18 thermophilic starter combinations, from 2 levels of one *Streptococcus thermophilus* (ST) strain, 2 strains of *Lactobacillus helveticus* (LH) and 2 strains of *Lactobacillus delbrueckii* (LD). Otherwise all the processing conditions were similar. A sensory evaluation of the cheeses was performed after 7 months of ripening by a panel of 11 trained judges. Thirty-seven attributes were used: 9 for texture, 5 for taste and trigeminal sensations and 23 for aroma.

LD had a bigger effect on cheese sensory characteristics than LH or ST. Cheeses without LD had aroma characteristics of less mature cheeses (more propionic, butter, less intense, toasted, animal). One of the LD strains showed characteristics of more mature cheeses (higher flavour and aroma intensities, more fat sensation, solubility, crystals, salty, acid, pungent, sour milk, toasted, roasted, animal, garlic, but lower firmness, elasticity and granular microstructure). ST level influenced only texture attributes (ST high level gave more granular microstructure and dryness, less fat sensation and solubility). Depending on LH, 6 attributes were found to be different (2 texture, 2 taste, 2 aroma). Interactions were found between LH and LD, between ST and LD, and between ST and LH (respectively 10, 6 and one attributes).

Keywords: swiss-type cheese, thermophilic starters, sensory characteristics

Mapping Regional Differences in Consumer Perception of Sharp Cheddar Cheese in the United States

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There is no legal age definition for U.S. Cheddar cheeses labeled as “sharp” or “aged”. Since there is no minimum age and due to the large number of commercial facilities, there is tremendous variability in flavor profiles of aged Cheddar cheese. Understanding what aged Cheddar cheese flavors are desirable to consumers and if these desirable flavor profiles are defined by regional location of consumers would be useful information to cheese manufacturers. The objective of this study was to evaluate consumer acceptability of aged Cheddar cheeses and to 1) determine the key drivers of consumer liking and to 2) determine if these preferences were distinct between U.S. consumers from different U.S. regions. Descriptive sensory profiles of a representative array of sharp Cheddar cheeses (n = 30) were determined using a trained sensory panel and an established cheese flavor sensory language. Nine representative Cheddar cheeses were evaluated by consumers in three regional locations: East coast (North Carolina, n = 150), Midwest (Illinois, n = 75), and West coast (Washington, n = 100). Consumers assessed the cheeses for overall liking and other consumer liking attributes. Five distinct consumer clusters were identified. The cluster membership distribution between East coast and Midwest consumers was similar while the West coast distribution was distinct ($p < 0.05$). A larger proportion of West coast consumers were present in cluster 3 which consisted of consumers that generally extremely liked the flavor profiles of all sharp Cheddar cheeses with specific likes for cheeses with intense brothy and nutty flavors. Consumers in other clusters were more discerning and key drivers of liking included milkfat, cooked, and sulfur flavors and sour taste. Sharp Cheddar cheese acceptance varies widely among consumers throughout the U.S. and is related to consumer preferences for distinct cheese flavor profiles. These data can help manufacturers understand consumer association of different sharp cheese in different geographical locations.

Keywords: Cheddar cheese, consumer acceptance, regional differences, preference mapping

Terpenoids and Benzenoids in La Serena Cheese Made at Different Seasons of the Year with a *Cynara cardunculus E*xtract as Coagulant

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La Serena cheese, protected by a designation of origin, is a soft variety made in Extremadura (Spain), from Merino raw ewes' milk, by using an extract of *Cynara cardunculus* flowers as coagulant. Terpenes are secondary plant metabolites and possibly good markers for the origin of dairy products. Benzenoids could come from plants or from the metabolism of aromatic amino acids.
The objective was to determine the influence of the season of manufacture and the ripening time on the volatile terpenoids and benzenoids of La Serena cheese. Ewes were fed with formulated feed (FF) in summer, and grazed on natural pastures in winter and spring. Extracts from cheeses, FF and thistle flowers were analysed for volatile compounds by dynamic headspace extraction coupled to a GC-MS system equipped with a chiral capillary column.
Most terpenoids and benzenoids present in cheese were observed in the FF (mainly terpenes), and in the *C. cardunculus* extract (mainly benzenoids). The enantiomeric α(+)/α(−)pinene ratio changed during ripening. γ-Curcumene, α-humulene, and all xylene, ethyl benzene and propyl benzene isomers decreased, while α-terpineol, verbenone, benzyl alcohol, 2-phenyl-ethanol, benzoic acid methyl ester, and the phenolic compounds increased significantly during ripening.
Safranal, geranyl acetone and two sesquiterpenes, γ-curcumene and α-curcumene, were more abundant in spring than in winter cheeses. Alkyl benzenes and other benzenoids were more abundant in summer cheeses. The presence of two sesquiterpenes (likely originating from the natural pasture) solely in winter and spring cheeses, was sufficient for the seasonal discrimination of the cheeses. Elevated abundances for alkyl phenols in winter and spring cheeses can be related to the higher protein and fibre content of the natural pastures. The high levels of alkyl benzenes in summer cheeses could possibly be ascribed to contamination with environmental pollutants during frozen storage, but a plant or microbial origin can not be discarded.

Keywords: La Serena cheese, terpenoids, benzenoids, plant coagulant, seasonal changes

Gas Chromatography Mass Spectrometry Olfactometry (GC-MS-O) Profiling of Cheese

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It is well-known that flavour in cheese is strongly linked to volatile compounds production. The objective of our study was to develop a methodology enabling the identification of the compounds that mostly participate to the cheese flavour. The selected key compounds will then be of interest when screening specific strains activities that could enhance particular flavours.
The olfactometry process consisted of high intensity training with a particular laboratory prepared solution to selection a jury of experts. The selected experts were then used to analyse the product in a 40 minutes GC-MS-O session.
The key point for the GC-MS tool was to best extract volatile compounds from the cheese. For that purpose, a method was developed using Tekmar Purge and Trap system.
Results obtained consisted of an olfactory signal and a mass spectrum chromatogram. Data were then treated by first integrating the olfactory peaks and then using the mass spectrum to identify the

molecules detected by the nose. The judge's descriptions corresponding to the molecules were added. Individual result tables were amalgamated to show the molecules detected, their frequency of detection by judges and the frequency of citation of the most common descriptions.
The profiling of cheese by GC-MS coupled with olfactometry detection has enabled the identification of the key flavour compounds by a pre-selectioned jury of experts. The aim of this work was the creation of a methodology which could be used to analyse by olfactometry and thus identify by mass spectroscopy the key compounds present in various cheeses.
These analyses will then provide useful information to screen strains on flavour properties, allowing a clever development of new starter ranges.

Keywords: GC-MS, Olfactometry, Cheese

RP-HPLC Evaporative Light Scattering Detection Method for Determination of Free Amino Acids in Cheese

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Ample literature proceedings describing methods for determination of free amino acids and peptides in variety of cheeses have been published. The methods are mostly based on chemical modifications of free amino acids and small peptides and their separation by RP-HPLC using UV/VIS or fluorescence detectors. Amino acids and small peptides soluble in trichloroacetic acid were treated with phenylisothiocyanate, *o*-phthaldialdehyde, 9-fluorenylmethylchloroformate, or 1,4,5-trinitrobenzenesulfonic acid to obtain derivatives suitable for HPLC analysis. In order to simplify these procedures we have tried to omit chemical modification step and to analyse free amino acids from cheese by HPLC combined with Evaporative Light Scattering Detection (ELSD).
Separations were performed on the Prevail 5μm, 250 × 4.6 mm, C18 reversed phase column using water containing trifluoroacetic and heptafluorobutyric acids - acetonitrile gradient. Good separation of a standard mixture of 17 amino acids with norleucine, added as the internal standard, was achieved in 25 minutes run. Developed method was applied for the analysis of a semi-hard full-fat ewe's milk cheese, that possesses a specific flavor and smell, collected at the farms located on the island Krk which are breeding Croatian autochthonous sheep.
It was found that glutamic acid, leucine, valine, phenylalanine and proline dominate in the trichloroacetic acid soluble fraction of the mature cheese. The results are in accordance with the data reported in literature for the similar type of cheeses obtained by other methods. Developed RP-HPLC – ELSD method is a simple and reliable, and could be adopted for determination of free amino acids in cheeses during their production.

Keywords: RP-HPLC-ELSD, ewe's milk cheese, cheese ripening, amino acids, peptides

Characterization of Flavour Development in Raw and Pasteurized Milk Cheddar Cheese

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Raw and pasteurized milk Cheddar cheeses were manufactured from a single milk source in three different weeks and ripened at 6°C. There was no significant difference in moisture, fat, protein,

salt, or pH between the cheeses made from raw and pasteurized milks initially or during aging. Acetate buffer (pH 4.6) and 12 % TCA soluble nitrogen (SN) increased during aging in all cheeses with the raw milk cheeses having a higher proportion of their total nitrogen present as TCA SN from 90 to 450 d of aging. Raw milk cheese had a greater depth of proteolysis. Descriptive sensory analysis with a trained panel was performed at 3 month intervals for 15 months. Flavour compounds were extracted at each timepoint by both Solid Phase Micro-Extraction (SPME) and Solvent Assisted Flavour Evaporation (SAFE) and characterized using Gas Chromatography Olfactometry and Mass Selective Detection (GC-O and GC-MSD). Cheeses were also tested for coliforms and *Listeria*, as well as starter and non-starter lactic acid bacteria (NSLAB). Raw milk cheeses were distinguished from pasteurized milk cheeses by low but distinct intensities of fruity and cowy flavours while pasteurized milk cheeses exhibited higher levels of cooked flavour. Nutty and brothy flavours developed more quickly in raw milk cheeses than in pasteurized milk cheeses. Methyl and ethyl esters of c-4 through c-10 fatty acids were found at higher levels in the raw milk cheese than in the pasteurized milk cheeses. Compounds exhibiting cowy/barnyard aromas were detected in the raw milk cheeses by GC-O. As expected, raw milk cheeses contained coliforms while these microorganisms were absent after pasteurization. Higher standard plate counts were also observed for raw milk cheeses. Higher starter and NSLAB counts were observed at the 12 month time point in the raw milk cheeses when compared to the pasteurized milk cheeses.

Keywords: Raw Milk, Flavour, Cheddar cheese, proteolysis, flavour chemistry

Yeasts as Enzyme Source for Accelerated Ripening of Cheddar Cheese

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Ripening of cheese involves the biochemical events glycolysis, lipolysis and proteolysis, which are caused by the enzymes and starter cultures used in the manufacture, and which determine the ripening time and also the flavour of the various types of cheeses. A great deal of research in cheese technology is devoted towards the manipulation of these processes in order to accelerate ripening times or to improve flavour. Cheddar cheese is constantly a subject for such research.
There is increasing evidence that certain yeast species contribute to flavour and texture development during ripening of certain cheeses, including Cheddar cheese. The addition of yeast cultures as adjunct cultures may accelerate ripening, or lead to a faster development of flavour and taste. This is brought about by an increase in primary proteolysis of the caseins as well as the break down of amino acids into flavour compounds. The respective enzymes involved seem to be proteases with plasmin or plasmin activation activity, and glutamate dehydrogenase.
This research reports on the search of yeasts of dairy origin that display plasmin or plasmin activation activity, and/or glutamate dehydrogenase, and their inclusion as adjuncts in the processing of Cheddar cheese. Yeasts that expressed both enzyme activities were added as single adjuncts, while combinations of yeasts with single enzyme activity were also employed. Changes during ripening were monitored by sensory and biochemical analyses. Primary proteolysis was followed as nitrogen released and gel electrophoresis, while secondary proteolysis was followed by means of HPLC of water-soluble peptides, which are responsible for flavour.
The yeasts were found to contribute to the biochemical events, with the most promising results being obtained with a combination of yeasts which express separate plasmin and glutamate dehydrogenase activity and proteolytic and lypolitic organisms as adjuncts. This was confirmed by sensory evaluation, and supported by proteolysis, where six month old yeast inoculated cheeses were evaluated as comparable with an eight month ripened one.
Keywords: Yeast, Cheddar cheese, proteolysis, plasmin, glutamate dehydrogenase

Preliminary Study on the Use of GAS FT-IR for the Evaluation of Cheese Volatile Fraction: Comparison with SPME/GC/MS Technique

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The FT-IR analysis, on condensed phase, is currently used for the determination of both major (i.e. total fat, proteins, lactose) and some minor (i.e. citric acid, free fatty acids) constituents of dairy products. As far as the volatile fraction analysis is concerned, among the different techniques, those based on the gas chromatographic separation coupled with mass spectrometry are the most used for the dairy products characterization.
In our research the possibility of the application of the FT-IR analysis to the cheese volatile compounds was investigated. Thirty nine samples of PDO Bitto cheeses were analysed by both GAS FT-IR and SPME/$GC/MS techniques.
A GAS FT-IR prototype, developed to detect some volatile molecules released from bottle grade PET, was adopted. It consisted of a FT-IR spectrometer, a multiple-pass long path absorption cell and a desorption unit. Dynamic headspace under nitrogen flow, followed by concentration in a Tenax trap, was used for the extraction of the volatile compounds.
The preliminary step of the work was dedicated to the set up of the best analytical conditions and instrumental parameters for cheese analysis. Spectra of authentic standards were acquired for band assignment. The bands present in the spectrum of Bitto belonged to the chemical classes of ketones, alcohols, free fatty acids and esters.
Since the GAS FT-IR device used in this research is not a hyphenated instrument and thus it does not perform the separation of the analytes, the comparison with SPME/GC/MS results was made by using chemometric techniques. Correlations were observed between the FT-IR spectra and the most abundant compounds detected by SPME/GC/MS.
Even though some aspects of the analytical conditions need to be improved, and particularly sample presentation and detector sensitivity, the results obtained are promising for a possible application of GAS FT-IR in the evaluation of volatile fraction of foods.

Keywords: GAS FT-IR, SPME/GC/MS, volatile fraction

"Aree Cru" classification by Volatile Compounds for Pecorino Siciliano Cheese

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Pecorino Siciliano is a PDO ewes' milk cheese produced in Sicily. The aim of this study was to investigate aroma profile differences for traditional Pecorino Siciliano cheese produced in different Sicilian parts. Two, four and eight months ripened cheeses were obtained from 21 farms, from throughout Sicily, that were classed into seven areas cru by geography: Iblean (A), Etnea (B), Southern-center (C), Northern-center (D), Western (E), Western-center (F), Peloritana (G). A MS-based Electronic Nose (SMartNose) was used to detect organic volatiles components in the mass-to-charge (m/z) range of 10 to 160 amu. Results were statistically elaborated by Principal Components Analysis (PCA). Comparison of the cheeses at 2, 4 and 8 months of ripening, generally showed differences in volatile compounds among the different areas. Cheese samples at 2 - 4- 8 months of ripening, as expected, showed less marked differences respectively, likely due to the more advanced

lipolytic and proteolytic processes releasing the aroma compounds that characterize the cheese. Looking at the 8 months cheeses, showing the best separation with PC1 (60 %) and PC2 (22 %), and comparing two areas each time we can conclude that all the areas showed a good separation but no difference between Iblea area (A) *versus* Southern-center area (C) and Iblea area (A) *versus* Northern-center (D) were detected. In conclusion Electronic Nose technology is simple and fast to use, especially if there are a lot of samples, and represents a very useful tool in discriminating the geographic origin of a product.

Keywords: Pecorino Siciliano, SMart Nose, Areas Cru, volatile compounds

Flavor of Goat Milk Cheese Characterized by a Rapid and Representative Tool

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Flavor of soft surface-ripened goat milk cheeses is linked to milk composition (e.g. fat) but mainly to the activity of ripening strains. To evaluate rapidly, effectively and costless, the aromatic potentiality of ripening strains, an eatable experimental lactic cheese was set up. Lactic curd, prepared with standardized pasteurized goat milk, was freeze dried (F) and stored at -20°C. For each trial, a control (C) was realized with fresh curd. F was reconstituted to obtain 43 % dry matter (50 % fat in dry matter) and 1.3 % salt, as for C. The two bases, F and C, were ripened with pure strains (*Penicillium* and *Geotrichum*) or mixed cultures including yeasts, in Petri dishes (140 mm diameter) at 12°C. The behavior of the strains in Petri dishes was also compared to this observed on classic lactic buchette type cheeses (B). Biochemical, microbiological and sensorial analyses were realized. During ripening in Petri dishes, proteolysis rates were similar on F and C but higher lipolysis levels were observed on F, for *Penicillium* only. This might be linked to a 4 log decrease in thermophilic bacteria count during storage process. Nevertheless, similar sensorial profiles were observed on F and C. Comparing with cheese making, Petri dishes enabled a ripening twice as fast as this observed with cheese. Therefore, a 14 days F base had the same biochemical characteristics (proteolysis and lipolysis) as a 26 days B cheese and also similar odor and flavor profiles (figure 1). Sensorial analysis realized on the F base enabled to distinguish genus, species and strains specificities, especially concerning goaty descriptors (figure 2) but also covering aspect. A 9 month storage had no significant effect on biochemical characteristics of F. This base enabled a further screening of more strains and mixes and was considered as a useful tool by cheese makers.

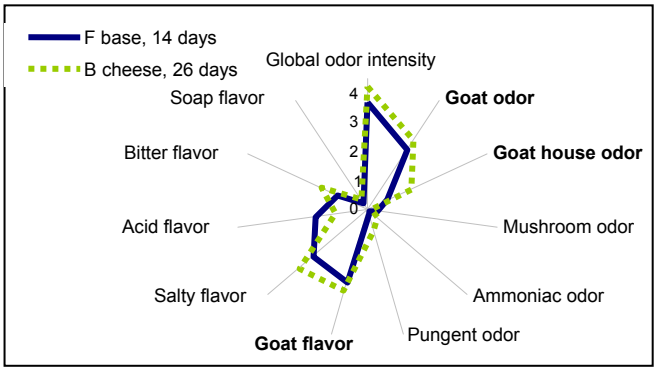

Figure 1: Sensorial profiles obtained on F base (model cheese) and B cheeses (lactic buchette type cheese) for mixed ripening strains (Geotrichum candidum + yeast)

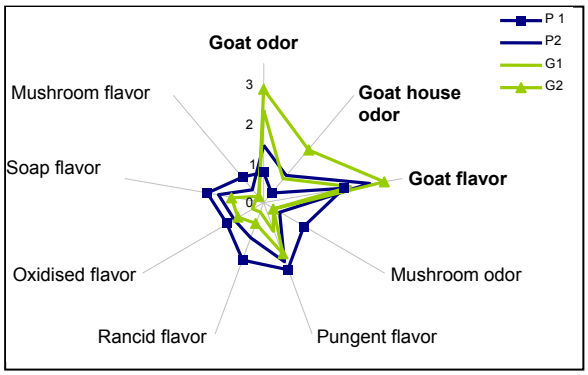

Figure 2: Sensorial profiles of strains of Penicillium camemberti (P1 and P2) or Geotrichum candidum (G1 and G2) on F base (Petri dish)

Keywords: model cheese, goat milk fat, flavor, ripening

New Insights on the Impact of Microbial Diversity on Flavours in Washed-Rind Soft Cheese

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Livarot cheese microbial ecosystem has been used as a model. The most common yeasts in this model were *Geotrichum candidum* and *Debaryomyces hansenii*, these and others occasionally present such as *S. cerevisiae*, *Y. lipolytica*, are able to produce volatile compounds (e.g. sulphur compounds) that are particularly important in these cheese varieties. They are able to produce 10 different sulphur compounds from cysteine and methionine. The production of some of these compounds by yeasts, such as 2 methyl-tetrahydrothiophen-3-one or 1,3 oxathiane, was reported for the first time on Potato Dextrose Broth enriched with cysteine and methionine.
Moreover, many newly isolated bacterial strains were unknown in relation to their contribution to cheese flavour. Twelve bacterial strains including *Proteus vulgaris*, *Psychrobacter sp.* and *Microbacterium foliorum* that are quite common on washed rind cheeses, produce compounds such as thioesters and 2,4 dithiapentane in a casamino acid medium enriched with methionine. A strain of *Staphylococcus cohnii* was also found to produce pyrazines in the same medium. These findings are new knowledge on bacteria common on cheeses, which have been rarely mentioned for their flavouring capabilities.
The use of a selective extraction of thiols, adapted from wine extraction, allowed the identification of new thiols in soft cheeses. In Munster cheese, a thiol already described in wines has been identified several times. This method is giving its first results and no doubt that, as in wine, it will permit to find new powerful thiols in cheeses.
Assays of formulations using a Livarot microbial sub-ecosystem already described, confirmed the importance of most of the yeasts on the flavour of a model ripened curd but also the one of specific bacteria such as *P. vulgaris*.

Keywords : flavour, sulphur compounds, washed rind, microbial ecosystem

New Sulphur Flavours in Washed Rind Soft Cheese

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The large surface area:volume ratio, the low buffering capacity and the aerobic growth conditions are as many factors which favours the microbial diversity on the surface of soft cheeses. For example, the diversity of yeasts and bacteria on the surface of Livarot cheese has been described and has shown a large diversity of yeasts and bacteria. The most common yeasts on these cheeses such as *Debaryomyces hansenii* and others occasionally present (*S. cerevisiae, Y. lipolytica*) are able to produce volatile compounds, i.e., sulphur compounds particularly important in these cheeses. They are able to produce 10 different sulphur compounds from cysteine and methionine (Lopez del Castillo-Lozano *et al*., 2007). The production of some of these compounds by yeasts, such as 2 Methyl-tetrahydrothiophen-3-one or 1,3 oxathiane, was reported for the first time on Potato Dextrose Broth enriched with cysteine and methionine. Moreover, the availability of a diversity of flavours obtained is increased by newly isolated bacterial strains. Among these bacteria, many were unknown in relation to their contribution to cheese flavour. Twelve bacterial strains

including *Proteus vulgaris*, *Psychrobacter sp.* and *Microbacterium folarium* which are quite common on washed rind cheeses, are producing compounds such as thioesters and 2,4 dithiapentane in TSYE medium enriched with methionine. A strain of *Staphylococcus cohnii* was also found to produce pyrazines in the same medium. These findings are new knowledge on bacteria common on cheeses which have been rarely mentioned for their flavouring capabilities (Deetae et al, 2007 et 2008, submitted). Moreover, the use of a selective extraction of thiols adapted as described in wine by Tominaga et al. (2006) allowed to identify new thiols in soft cheeses. In Munster cheese, ethyl-3-mercaptopropionate was identified on many occasions. It is described as "fruity" at low concentration or "fox" at higher concentrations in wines and particularly in old champagnes. This method is giving its first results and no doubt that, as in wine, it will permit to find new powerful thiols in cheeses (Sourabié et al, 2008, submitted).

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Keywords: Sulphur Flavour, cheese

Ultrasonically Aided Process Control in Emmental Cheese Manufacturing

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Improved quality control (QC) in Swiss cheese manufacturing would bring significant savings to the dairy industry. This could be achieved by developing process monitoring during cheese ripening. Non-destructive evaluation methods, such as ultrasound (US), have potential for on-line monitoring purposes. The focus areas in the Swiss cheese ripening process are early structural defect detection and the determination of the propionic acid fermentation stage.

The project aim is to assess the US method capability to image Emmental type cheese macro structure, defect detection potential and capability to measure proteolysis level during the propionic acid fermentation. The imaging potential was studied with multi-element phased array system. An US image was compared to an image of a sliced cheese sample. The methods feasibility to produce similar features as in optical reference image will be presented. However, the problems arising from high attenuation and structure inhomogeneity of cheese need still to be resolved. Simulations have been carried out to enhance signal analysis based defect detection. Structural defect detection was studied by analysing reflection signals for different cheese structural elements e.g. cheese eyes, cracks and whey-nest structures. In the preliminary measurements, reflection signals from Emmental cheese scannings were compared to the library of simulated signals. The method potential of monitoring the cheese ripening was studied with US velocity measurements. US velocity, which is related to Young's modulus, show correlation with α_{s1}-casein breakdown level during the warm room period. This could be used, in co-operation with US imaging, to determine the end point of the propionic acid fermentation and to predict potential crack formation.

Next steps in the project will be the validation of the method performance to fulfil on-line requirements for defect detection. Also the method's applicability to follow the eye and crack development will be evaluated.

Keywords: ultrasound, Swiss cheese, quality control, cheese structure

Investigation of Cheese Melting by Infrared Spectroscopy: Correlation with Rheology

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Texture properties play a key role in consumer acceptance of cheese. The rheological characterization of ripened cheeses is important as a mean of determining body and texture for quality and identity as a function of composition, processing techniques and storage conditions. Most of the methods available to study the phenomenon of cheese meltability are empirical and have a low repeatability. The purpose of this study was to evaluate the meltability and the viscoelastic behaviour of 2 cheeses using two methods - dynamic testing rheology and infrared spectroscopy.
Trends in the structure and texture as a function of temperature were determined for 1 hard cheese (Comté) and 1 semi-hard cheese (Raclette) using infrared spectroscopy and dynamic testing rheology, respectively. The storage modulus (G'), the loss modulus (G") and the complex viscosity (η*) decreased while strain and tan δ increased as the temperature increased from 25 to 80°C. Mid-infrared spectra in the 3000-2800 cm^{-1} region were recorded on cheese samples at 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 and 80°C. For each cheese, the data sets containing infrared spectra and rheology data were evaluated using principal component analysis and common components and specific weights analysis. It was shown that the maps defined by principal components 1 and 2 discriminated cheese samples as a function of temperature whatever the data (dynamic testing rheology data or infrared spectra). In addition, the melting temperature of fats for the two cheeses determined from the dynamic rheology data and the synchronous fluorescence spectra gave similar results.

Keywords: cheese, structure, texture, melting, mid-infrared spectroscopy, rheology

Quality Prediction in Mature Emmental Cheeses Using Proteolysis

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Proteolysis is an important factor influencing the final quality of mature cheeses. For the prediction of the relation between the total evaluation of the quality of mature Emmental cheeses and the proteolysis there were used samples from the 30 pilot productions sampled in the course of their maturation, namely after their salting (1st day of maturation), before fermentation in the fermentation cellar, after fermentation and at the age of 75 and 120 days, respectively.
The course of proteolysis was described by peptide mapping and by the determination of the content of free amino acids. The evaluation of the total cheese quality at the age of 120 days included sensory evaluation, formation of holes and fracture parameters as well as the evaluation of curd texture.
The relation between the content of individual peptides and amino acids determined in cheeses in the stages of cheese maturation in view and the final evaluation of mature cheeses at the age of 120 days was evaluated by means of Pearson correlation coefficients for individual peptides and amino acids.
For the prediction of the final quality of mature cheeses on the basis of the content of individual peptide fractions, there were selected three possible criteria – the total content of hydrophilic peptides and the contents of the peptide Nr. 2 or Nr. 6. The most suitable criterion is the content of

the peptide Nr. 6. Among the amino acids in view, the most suitable ones appear to be proline or glutamic acid.

Keywords: cheeses of Emmentaler type, proteolysis, peptides, amino acids

Risk of the Occurrence of Butyric Fermentation in Emmental Cheese

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The changes in cheese composition, butyric acid content and number of clostridia were studied in the course of the maturation of Emmental cheeses produced under operational fermentation conditions. Numbers of *Clostridium tyrobutyricum* in cheeses before fermentation in the warm cellar were at the limit of detectability (ca 10^1 spores g^{-1}). In spring and winter the numbers of *Clostridium sp*. increase up to 10^3 – 10^4 spores g^{-1} in mature cheeses. In summer and autumn the numbers were in order of 10^2 spores g^{-1}. The average content of butyric acid in cheese (before warm cellar) was 100 mg kg^{-1}. After fermentation in the warm cellar its amount was lowest in summer (ca 200 mg kg^{-1}) and highest in winter (ca 900 mg kg^{-1}).
The knowledge of the changes of active acidity, water activity, butyric acid content, and the types and numbers of *Clostridium sp*. in the set of Emmental cheeses in view enabled the proposal of criteria describing the risk of the occurrence of intensive butyric acid fermentation.
As long as the a_w of cheeses before fermentation in the warm cellar is higher than 0.95, it is necessary to regard the numbers of *Clostridium sp.* in order of 10^2 spores g^{-1} cheese as a risk factor, depending on the current pH value.
The risk of the occurrence of the intensive butyric fermentation in the warm cellar increases if the number of *Clostridium sp.* exceeds the limit of 10^3 spores g^{-1} cheese or if the content of butyric acid increases more than by 300 mg kg^{-1}.

Keywords: cheeses of Emmentaler type, butyric fermentation, clostridia,

Chemometric Evaluation of Swiss-Type Cheese Ripening

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Swiss-type cheese represents a large sale segment in the cheese market and has a high consumer acceptance. However the possibility of marketing can be reduced, if apart from classical eye formation incorrect eye, even split and crack formation may occur in the cheese matrix. For this reason it is important to find the cause relation between eye and split formation, measured chemical ripening indices, technological and ripening cultures factors. Therefore several industrial productions of Swiss-type cheeses were examined by means of different chromatographic methods and subsequently image evaluation of eyes and splits.
To the final statistical evaluation of all received results and data multivariate statistical methods were used like the Principal Component Analysis (PCA) and Partial Least Square Regression (PLSR) with the software "The Unscrambler 7.5 (Camo ASP).
During the analytical and the statistical evaluation correlations of instrumental analytic determined chemical ripening indices of the primary and secondary proteolysis (RP-HPLC) and of the

carbohydrate metabolism (HPLC) to sensory characteristics (stage of ripening, eye and split formation) as well as to technological parameters were observed.
As significant chemical ripening indices especially the organic acids of the carbohydrate metabolism are pointed out beside the products of the primary and secondary proteolysis. From the results of the organic acids analysis conclusions on the metabolic procedures of the cultures could be demonstrated, whereby besides the classical propionic acid fermentation also the metabolic Wood Werkman pathway took place. In addition, the metabolic procedure of propionic acid bacteria with aspartase activity was recognized, during that carbon dioxide could be formed additional from the degradation of aspartate arised from proteolysis. Thereby the split formation is favoured.
Concerning the evaluation of the ripening stage and the age of Swiss-type cheese, different ripening indices of the primary and secondary proteolysis could be determined as significant and the age of the cheese could be determined in different samples.
In summary chemometric evaluation procedures in this project represented an innovative and established tool for controlling, evaluating the ripening and detection of ripening defects of the cheese.

Keywords: Swiss-type cheese, eye, split, organic acids, chemometric evaluation

The Application of Near Infrared Spectroscopy Technology and a Remote Reflectance Fibre-Optic Probe for the Determination of Peptides in Cheeses (Cow's, Ewe's and Goat's) with Different Ripening Times

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It is well accepted that the amount of total, hydrophobic and hydrophilic peptides and the hydrophobic:hydrophilic ratio is a suitable way to study the proteolysis process and the changes of these parameters are significantly influenced by cheese age. Although chromatography and electrophoretic techniques provide valuable information about peptides produced in cheese during ripening, the use of such techniques involves considerable analysis time.
For this reason, the use of Near Infrared Spectroscopy (NIRS) technology employing a remote reflectance fibre-optic probe (with a 5cm x 5cm quartz window) for the analysis of the hydrophilic (HI) and hydrophobic (HO) peptides and the ratio HO/HI was assayed. To do so, cheeses with known and varying percentages of cow's, ewe's, and goat's milk were elaborated (112 samples). Ripening controls were performed over 6 months and at each ripening time NIRS spectra were recorded and the peptide composition obtained by reversed-phase high performance liquid chromatography were used as reference.
The calibration process was implemented with the spectra of the samples and their chemical data. The regression method employed to was modified partial least squares (MPLS). The multiple correlation coefficients (RSQ) and prediction corrected standard errors (SEP(C) obtained were respectively 0.879 and 1.83 % for hydrophilic (HI) peptides; 0.879 and 1.83 % for hydrophobic peptides (HO); 0.890 and 0.03% for the ratio HO/HI peptides. Results show that the method allows immediate control of the peptide cheese composition without prior sample treatment or destruction by direct application of the fibre-optic probe to the cheese.

Keywords: Peptides, NIRS, Determination, Cheese

Detection of Seasonal Origin of Milk in Cheeses (Cow's, Ewe's and Goat's) Using NIRS Technology and a Discriminant Analysis

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In the present work we study the use of NIRS technology together with the use of a remote reflectance fibre-optic probe (with a 5cm x 5cm quartz window) for the discrimination of the season (winter or summer) of milk collection in the elaboration of cheeses(cow's, ewe's and goat's) with different ripening times over 6 months. To do so, cheeses with known and varying percentages of cow's, ewe's, and goat's milk were elaborated (112 samples with milk collected in winter and 112 samples with milk collected in summer) and served as reference material. NIRS spectra of the samples in each ripening time were recorded.
The classification method applied to this procedure was DPLS, and the algorithm was PLS 2. With 100 samples in each group (winter or summer), and with no treatment of the NIR spectra and 15 PLS (partial least squares) factors, we obtained RSQ values (multiple correlation coefficients) of 0.814 and SECV values (standard error of cross-validation) of 0.257. Of the 200 samples used in the training procedure (internal validation) correct classification was made of 96 % of the winter milk and 97 % of the summer milk. The method was applied to 24 samples for external validation, with a prediction rate of 92 % in both cases.
This indicates that the model can be used to know the seasonal origin of the milk used in the elaboration of any type of the milks studied up to a ripening time of 6 months.

Keywords: Cheese, Seasonality, NIRS, Classification

Temperature and pH Effects on the Physical Properties of Milk Gels from Animal Rennet and Microbial Coagulants

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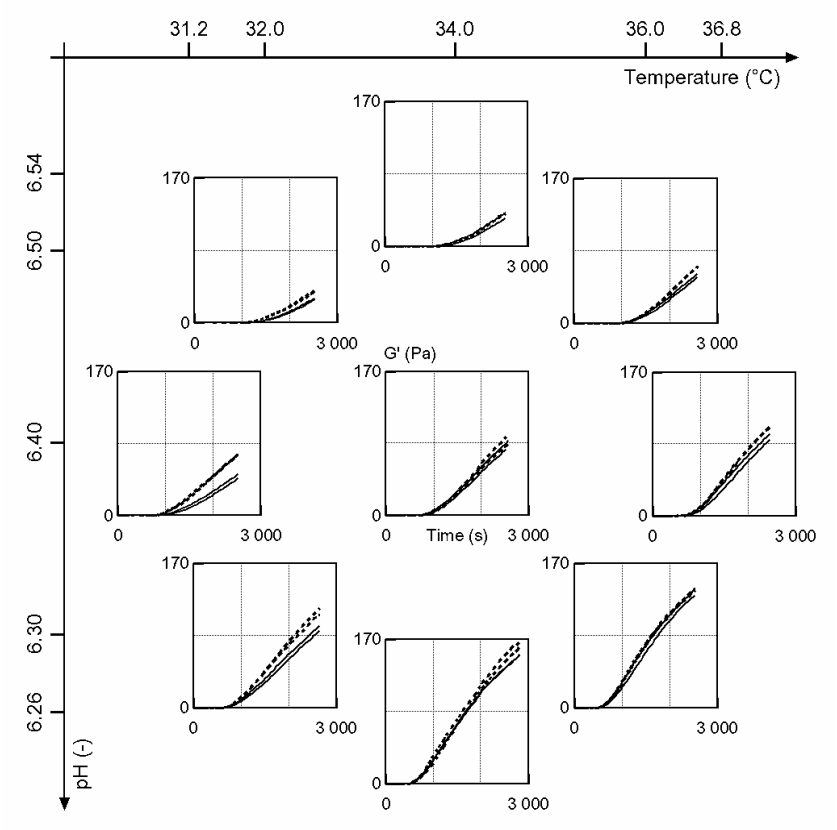
The enzymatic coagulation, the first important step in cheese-making, has a tremendous impact on the quality of the resulting cheeses. Because of an increasing demand for coagulating enzymes a number of substitutes, mainly of microbial origin, are available on the market. As compared to traditional rennet from the abomasum of ruminants these enzymes often show a higher proteolytic activity, which eventually causes the development of bitter peptides and lower cheese yield. Aim of the study was to establish a methodical basis for further work on different enzymes within the pH and temperature range relevant for hard and semi-hard cheeses.
Two animal rennets and two microbial coagulants from Rhizomucor were diluted to the same soxhlet strength, and gel firmness was monitored within a strain-controlled rheometer immediately after their addition to reconstituted skim milk. A central composite design was established to determine temperature and pH effects on gelation within a range of $31.2 < T > 36.8$ and $6.26 < pH > 6.54$.



Apart from a general trend that, for a particular temperature, gelation time decreases and firmness increases with decreasing pH, gels made by addition of animal rennets (dotted lines) show higher firmness as compared to microbial substitutes, especially at conditions typical for the manufacture of swiss-type cheeses. ANOVA and multiple t-tests of the central experiments revealed significantly higher gel firmness when applying animal rennets. The practice of adding higher amounts of microbial enzymes to achieve the same level of gel firmness within the same time-scale enhances unspecific proteolysis and may lead to a reduction of cheese yield and negative effects on the sensory properties of the end product. Based on the results of this study the determination of the cheese yield in laboratory scale experiments is under progress.

Keywords: milk coagulation, rennet, microbial coagulants, rheological properties

Characterisation of Ripening Stages of Emmental Cheeses by Mid-Infrared Spectroscopy

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Experimental Emmental cheeses were analyzed in order to evaluate the potential of mid-infrared spectroscopy to follow the ripening process. Nine cheeses were sampled at days 20, 27, 34, corresponding to a ripening at 13°C, and at days 51, 58, and 65 (ripening at 20°C). The infrared spectra were obtained by placing cheese slices directly over the attenuated total reflectance crystal. Analysis of variance showed significant absorbance changes on spectra sets in the regions assigned to the vibrations of alcohol, amide and hydrocarbon groups. The principal component analysis made it possible to distinguish four homogeneous groups of samples: the first with samples collected at day 20 and 51, the second with samples of day 58, the third with samples of day 65, and the fourth containing the ripened samples. A combination of PC1 and PC2 made it possible to describe a first step in the evolution from day 20 to 58. In a second step, the PC1 made it possible describe the evolution from day 58 up to the end of ripening. These evolutions can be linked to the two phases of Emmental ripening technology, in cold and warm temperature. The eigenvectors corresponding to PC1 and PC2 showed two important regions, around 1750 cm^{-1} assigned to esters and organic acids, and the amide 1700-1490 cm^{-1} region assigned to proteins respectively. Discriminant analysis was performed by dividing the samples spectra in three groups, a calibration set (n = 34), a cross

validation set (n = 14) and a prediction set (n = 14). By this analysis, three different groups were separated, young, intermediate and ripened cheeses.

Keywords: Cheese ripening, Mid-Infrared spectroscopy, Emmental, Discriminant analysis

Characterisation of Proteolysis of Camembert-Type Cheeses by Middle Infrared Spectroscopy and Protein Secondary Structure

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Previous results showed the potential of Fourier Transform Infrared spectroscopy (FTIR) to evaluate the cheese ripening notably by taking into account the Amide region of the spectra, characteristic of the proteolysis. In these works, FTIR was used to investigate the secondary structure of protein and theirs modifications during the ripening of Camembert - type cheeses. The parameters of the deconvolution curve program were fitted from the spectra of pure proteins (Concanavaline, Trypsin) and the comparison of the calculated percentages of α-helix, β-sheet, β-turns and undefined conformation with the published data. Then, the same algorithm was used to estimate the percentages of these four fractions of protein secondary structure at several times during two ripening trials. The proportions didn't change a lot during the 27 days of ripening. The mean values in amide I region were 55 % (SD = 4.7) for β-sheet, 22 % (SD = 3.5) for α-helix, 14 % (SD = 3.6) for β-turns and 9 % for random conformation. The principal component analysis factorial map didn't allow distinguishing an evolution depending of the ripening time.

Keywords: Mid-Infrared spectroscopy, deconvolution, protein structure, cheese proteolysis

Quantification of Cow's Milk Percentage in Czech Bryndza Sheep Cheese Using Isoelectric Focusing of γ-Caseins and RT-PCR

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In Europe, cheese varieties made from ewes' and goats' milk are of considerable economic importance as a result of widespread acceptance of traditional cheeses. However, the substitution of cows' milk for ewes', goats' and buffaloes' milk is a fraudulent practice in the dairy industry.
In some countries (e.g., Austria, Czech Republic), mixed cheese varieties are produced from cows' and ewes' milk; the maximum percentage of cows' milk allowed is 49 %. Therefore, food analysts are challenged not only by milk species identification, but also by the need for quantitative determination of cows', ewes' and goats' milk in mixtures.
The objective of this study was the quantitative determination of the cow's milk percentage in Bryndza sheep cheese. Winter and summer reference samples were analyzed according to the EU reference method. Cheese proteins were separated by isoelectric focusing and calibration curves were established after densitometric evaluation of γ-caseins. In addition, species identification was performed by conventional polymerase chain reaction (PCR) as well as quantitative real-time PCR using species-specific primers.

Usually, the EU reference method is performed as a qualitative technique using reference samples, which are certified with 0 and 1 % of cow's milk, respectively. However, after densitometric evaluation of γ-caseins, a quantitative estimation of cow's milk percentage was obtained in mixed-milk Bryndza cheeses, where an inaccuracy up to ± 4% of cow's milk was found. Conventional PCR was shown to be a qualitative method, although a certain semi-quantitative estimation could be achieved in some cases. Real-time PCR proved to be a high-sophisticated technique, which enables the quantitative determination of the cow's milk percentage in mixed Bryndza cheese samples, but turned out to have an unexpected high error probability. This was probably due to the fact that DNA-based methods are to be applied for quantitative adulteration control of mixed cheeses with extreme care, only!

Keywords: Cow's milk, Bryndza cheese, isoelectric focusing, real-time PCR

Characterization of Important Microbiological and Chemical Attributes in Full Fat, Reduced Fat and Low Fat Cheddar Cheeses Made with a Single, Defined *Lactococcus lactis* Starter Culture During 9 Month Ripening Period

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There is a lack on information and scientific understanding related to the flavor defects that are prominent in low fat cheese. A key to understanding why flavor development in low fat cheese is so different to that which occurs in full fat cheese is to determine the differences in microbial population and microbial activities between cheeses with different fat levels. Changes in chemical attributes during ripening such as simple and modified carbohydrates, organic acids, nucleic acids, serine-phosphate (free and bound), and glycoproteins; as well as starter culture enzyme activities: general aminopeptidase activity (AP), X-prolyl dipeptidyl aminopeptidase (PAP) and post-prolyl endopeptidase (PEP) were studied in cheeses made at 3 different dairy plants. Cheddar cheeses made with full, reduced and low fat content as well as the inclusion of a washing step in the making of the full fat cheeses were chemically analyzed. An HPLC method for the extraction and analysis of trace level carbohydrates was developed. Carbohydrate profile shows that as fat levels decreased, the levels of lactose and galactosamine and D-Lactate increased, however, only a slight decrease of enzyme activities (PAP and PEP) was observed. Soluble nitrogen had no apparent change as fat content was decreased. The effect of wash step decreased the levels of lactose, galactosamine and glucosamine from the available energy sources in cheese, yet only a slight increase in heterofermentative products was observed, suggesting that the washing step did not remove all the energy sources available for the starter and non-starter bacteria present in these cheeses. Overall, this research shows for the first time, a different carbohydrate preference by the microbial population in low fat cheddar cheeses. This change in metabolic preference may be a key component for the understanding and controlling the flavor chemistry and flavor sensory properties of such cheeses.

Keywords: Low fat cheddar cheese, carbohydrates, energy sources

Fast Estimation of Water Activity on Cheese Surface using a New Non-Destructive Method

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At the cheese surface level, water activity plays an important role in the mass loss and microbial growth. Several measurement systems exit to evaluate water activity but most of them are invasive, destructive and based on time-consuming methods leading to mean values on the thickness of the samples analysed.
Accordingly, a fast and accurate methodology has been set up to predict water activity on surface of solid biological products, such as cheeses. This methodology consists in performing experiments in a micro-reactor to obtain specific data and then, in processing these data to estimate the surface water activity, together with the very low water flux generated, without weighing the cheese.
A micro-reactor was therefore designed to generate around the cheese an airflow as homogeneous and steady as possible, with a velocity ranging from 3 to 12 cm s^{-1}, a temperature, from 6 to 20°C and a relative humidity, from 60 to 99 %. Obtaining high relative humidity values was possible only by recycling a part of the air flowing around the cheese by means of an automated 3-ways valve.
The micro-reactor was also used for determining water transfer coefficients for a plaster cast of a cylinder 4 cm height and 13 cm in diameter (i.e. the size of a small Saint-Nectaire-type cheese) submitted to air velocities lower than 0.1 m s^{-1}. The results highlighted a linear evolution of the water transfer coefficient with the air velocity, in the range 3 - 10 cm s^{-1}.
Once the water flux and the water transfer coefficient are known, the water activity on cheese surface can be quickly and accurately estimated in a non-invasive and non-destructive way. The table below shows the results obtained for two saline solutions (Exp.1 and Exp.2) placed inside the micro-reactor at an air velocity of 3.5 cm.s^{-1}. The experiments were repeated three times (a, b, c).

Experiments	a_w measured using NovaSina (Reference)	Relative air humidity (%)	Water Flux (kg.s^{-1})	Water transfer coeff. (kg.Pa^{-1}.m^{-2}.s^{-1})	a_w predicted
Exp.1a	0.95	73.9	$1.90.10^{-7}$	$7.82.10^{-8}$	**0.95**
Exp.1b	0.95	79.2	$1.41.10^{-7}$	$7.82.10^{-8}$	**0.95**
Exp.1c	0.95	84.9	$6.69.10^{-8}$	$7.82.10^{-8}$	**0.95**
Exp.2a	0.87	72.3	$1.60.10^{-7}$	$7.82.10^{-8}$	**0.87**
Exp.2b	0.87	75.9	$1.29.10^{-7}$	$7.82.10^{-8}$	**0.88**
Exp.2c	0.87	79.5	$4.54.10^{-8}$	$7.82.10^{-8}$	**0.85**

Keywords: water activity, cheese, non-invasive and non-destructive method, water transfer coefficient

RP-HPLC-Analysis and Validation of Amino Acids in Cheese by OPA-Pre-Column Derivatization

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The degradation of caseins to fragments, peptides and amino acids and their metabolites is one of the major biochemical process during cheese ripening. The proteolytical development is characteristic for every type of cheese and influences the flavour and texture generally. Therefore

the identification of products of proteolysis in different steps of ripening may enable to control the ripening process and leads to an early detection of ripening defects.
Beside the established HPLC-chromatographic monitoring of proteins and peptides it is also necessary to determine the individual amino acids quantitatively and to detect their development in the process of ripening. The aim of this work was to generate a new method for quantitative determination of cheese ripening relevant amino acids by a modified OPA-pre-column derivatization using mercaptoethanesulfonic acid as thiol component. For these investigation the water-soluble extract of cheese, which is already available by the monitoring of peptides, was directly used. The isoindole products, formed by the reaction of the free α- and ε-terminal amino groups in amino acids with o-phthaldialdehyde (OPA) and the new thiol component, were separated by reverse phase chromatography and detected fluorometrically.
Due to this method it was possible to detect the OPA-sensitive amino acids in the water-soluble cheese extract separately and calibrate them with available standards. The calibration, with 36 - 50 calibration measurements, leads to correlation coefficients of 0.966 – 0.998. The matrix dependent validation of the new method was carried out by measurement of precision data in three different times of ripening. The repeatability was determined for each of the amino acids (10-fold analysis in series). The coefficient of variation of the mean value ranges from 1.4 – 71.2 % depending on stage of ripening (low or high concentration of amino acid) and type of amino acid. Also the recovery of the OPA-sensitive amino acids on water-soluble cheese extract, which ranges from 70 - 100 %, depending on concentration and type of amino acid, was determined. In addition, to the monitoring of proteins and peptides, the amino acids profile and concentration in Swiss-type cheese during ripening could be determined with this new method.

Keywords: Swiss-type, amino acids, OPA-derivatization, ripening

Improvement of Cheese Ripening Monitoring Based on Airflow Pattern and Atmospheric Composition Control

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The aim of this work (European IP Truefood) was to developed new instrumentation and monitoring strategies favouring the control of cheeses ripening.
In a first step, a prototype able to control the cheese ripening rooms was designed. The prototype is including: (i) sensors (temperature, relative humidity, oxygen and carbon dioxide concentrations, air velocity, and cheese mass weight), (ii) advanced software (CRIC) in charge of data acquisition, on-line kinetic calculation and ripening process control, (iii) new control laws. It was tested and validated during Saint Nectaire-type cheeses ripening trials. Consequently, key ripening characteristics such as cheese mass loss rate and cheese respiratory activity were calculated in real time. In parallel, experimental results showed low air velocities inside the rooms, from 0 to 0.07 m s^{-1} and seldom higher than 0.03 m s^{-1}. Further, numerical calculations were performed by means of Computational Fluid Dynamics (CFD) techniques (Fluent code) to improve the ventilation level and the homogeneity. The air velocities were increased to an average of 0.20 m s^{-1} by changing the location of the fans and by adding a wall in front of the cheese stack.
New monitoring strategies involving changes in air circulation and CO_2 concentration control were defined. Their effects on the process efficiency and on cheese quality were evaluated.

Sequential ventilation allows a reduction of energy consumption evaluated at 14 %, in average, in our configuration process, with a cut of air circulation during 10 min every 15 min. The rind dry matter of cheeses was higher than those ripened in continuous air circulation
When the CO_2 concentration was controlled at 3 %, the mycelium density, evaluated by the cheese makers, increased more quickly and the rind pH reached higher value, 6.5 against 6 for the reference trial. The final firmness was also lower for the cheese ripened under CO_2. These changes were linked with the lactate consumption and proteolysis.

Keywords: Cheese ripening, ripening kinetic, air flow pattern, CO_2 concentration

Evaluation of Ewes' Cheese Texture by NIRS Technology Employing a Fibre-Optic Probe

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Characterisation of cheese texture is traditionally carried out in two ways by sensory and instrumental methods. Both methods are time-consuming and costly then a great interest exists in developing instrumental techniques to enable faster and less expensive assessment of texture such as NIRS technique. However a limited number of works on the prediction of cheese texture have been carried out. In the present work we studied the use of NIRS (Near infrared spectroscopy) technology together with a remote-reflectance fibre-optic probe for the analysis of ewe's cheese texture. The cheeses were elaborated with milk from three breeds of sheep (Castellana, Churra and Assaf) with different somatic cell counts (SCC) (lower than 500,000 cells mL^{-1}; between 1,000,000 and 1,500,000 cells mL^{-1}, and more than 2,500,000 cells mL^{-1}) and ripened for 12 months, thus 96 samples were obtained. Previous works have shown that breed and SCC affected casein composition and SCC also increase proteolysis during ripening, reducing the strength of the gel thus affecting the texture of the cheese. At each ripening time NIRS spectra were recorded and instrumental texture were determined as Warner-Bratzler shear force using a TX-T2i*plus*.
The regression method employed was modified partial least squares (MPLS). The calibration, with the multiple correlation coefficients (RSQ), 0.961 and prediction corrected standard errors (SEP(C)) of 2.1 Newton, allows determining texture in the range 0.0 - 49.0 Newton. The ratio performance deviation (RPD) value obtained, 5.4, indicate that the NIRS equation obtained was applicable to unknown samples. Taking into account the results, it can be concluded that results obtained from NIRS method are comparable with those obtained from instrumental texture analysis, indeed it can provide rapid, non-destructive and cheep measurements without prior sample treatment or preparation.

Keywords: Ripening time, breed, SCC, calibration

Determination of Ripening Time, Breed and SCC Level in Zamorano Cheeses Using NIRS Technology and Chemometric Studies

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Somatic Cell Counts (SCC) is an indicator of milk quality that affects fat content, total solids, and the amounts of casein and lactose of milk which are usually negatively correlated with the SCC, while in general SCCs are positively correlated with total nitrogen, non-protein nitrogen and pH. Ewes' milk is mainly used for the production of Zamorano cheese. This is a PDO Spanish traditional cheese made from ewe's milk obtained only from Churra and Castellana breeds. In the recent years, the Assaf breed is becoming important despite the lower quality of their milk and the higher incidence of mastitis. The determination of cheese quality parameters implies the use of several costly and time-consuming methods. The use of NIRS technique that provides rapid, non-destructive and multi-parametric measurements may be very interesting.
Bulk tank ewes' milks of three breeds (Churra, Castellana and Assaf) with low (<500,000 mL^{-1}), medium (1,000,000-1,500,000 mL^{-1}) and high (> 2,500,000 mL^{-1}) SCC were used to manufacture ewes' cheese. Cheeses that had been ripened for 0, 1, 2, 3, 9 and 12 months were used to obtain NIRS spectra. The classification method applied to this procedure was stepwise linear discriminant analysis (LDA). A discriminant analysis was carried out based on the differences among ripening months (seven variables, 93.7 % of samples correctly classified) and among SCC levels in each month. At the first month of ripening with one variable PDO cheeses, those made from Churra or Castellana milk, were discriminated (81.3 % of samples correctly classified) from Assaf milk cheeses. Using two variables the 87.5 % of samples were correctly classified according to the SCC group. Similar results were obtained for classification of PDO against no PDO cheeses at the third month of ripening (68.8 % correctly classified), and for SCC group at the ninth month (81.3 % correctly classified). All models were tested in cross validation leave-one-out.

Keywords: Ripening time, NIRS, PDO, classification

Determination of Total Solids and Protein in Cheese Curd Using Near Infrared Spectroscopy

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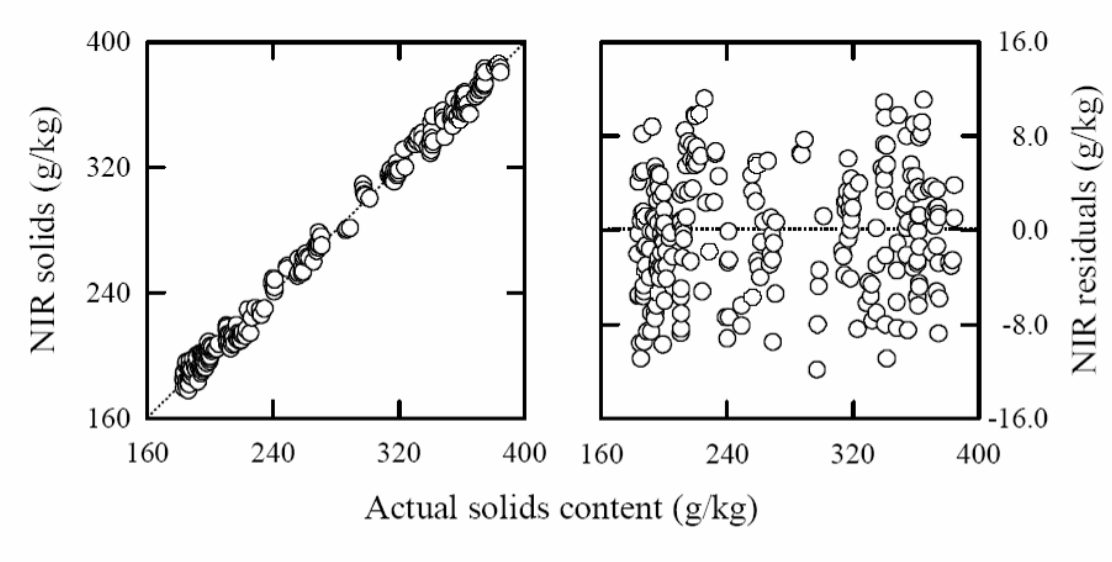
Due to automation, there is an increasing interest in methods with a high potential for in-line/on-line analysis of composition during processing. Despite of the changes occurring during pressing, salting and maturation, curd formation followed by curd cutting are considered as being essential in cheese manufacture. Hence, continuous information on the composition of cheese curd may help to control the cheese-making process, thus presumably contributing to the improvement of cheese quality. Aim of the present study was to evaluate whether Near Infrared Spectroscopy (NIR) can be successfully applied to analyze compositional properties of curd.
Cheese curd samples were made from UHT milk. After cutting the gel, the curd-whey mixture was warmed to a particular temperature for a specified period of time, and the curd grains were then separated from the whey by using sieves. Intensity and duration of cutting were varied, as was scalding temperature and scalding time. NIR spectra of the homogenized curd were recorded in diffuse reflection, and reference moisture and protein content was analyzed by standard methods.
Total solids and protein content of the curds varied in the range of 173 - 384 g kg^{-1} and 66.8 - 121.3 g kg^{-1}, respectively. Based on preliminary experiments, the second derivatives of the spectra in the 7,200 - 4,100 cm^{-1} range were subjected to PLS regression. The calibration models were developed and evaluated by full cross-validation. The accuracy of the predictive models was assessed using RMSECV and R^2 for predicted versus measured data. For solids and protein content, RMSECV was 0.502 and 0.548 %, and R^2 was 0.994 and 0.985, respectively. Curd homogenization prior to NIR measurement resulted in a slight improvement of RMSECV and R^2. Estimates for 15 independent samples do closely resemble the reference values, and NIR estimation was characterized by

variation coefficients of 1.28 % (total solids) and 2.42 % (protein). These data serve as a sufficient basis for the further implementation of NIR reflectance sensors in industrial scale.



Keywords: Cheese curd, composition, infrared, NIR

Formation of Furosine and α-N-(2-furoylmethyl) Amino Acids During the Ripening of Chester Cheese

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During cheese ripening, numerous biochemical reactions occur impacting the taste, texture and flavour of the final product. Protein degradation to peptides and amino acids is of particular importance. Proceeding proteolysis in cheese gives rise to peptide bound N-terminal α-amino groups, which, in addition to protein-bound lysine ε-amino groups, may react with reducing carbohydrates during processing and storage to yield Amadori products (APs) in the course of the Maillard reaction. During acid hydrolysis, N-terminal Amadori products are degraded to α-*N*-(2-furoylmethyl)-amino acids (α-FMAAs), while furosine (ε-FMLys) is formed from lysine bound APs. Furosine serves as an indicator of severity of heat treatment and storage conditions of dairy products and was investigated by Corzo et al. (2000) as a marker of ripening in Spanish Manchego cheese. The aim of the present study was to investigate the extent of α-FMAA and furosine formation during cheese ripening, in order to obtain an information about the extent of N-terminal glycation and to elucidate whether the ratio between furosine and α-FMAAs changes during maturation of cheese. A Chester (Cheshire) cheese served as a model and was stored over a period of 12 weeks. During this time, FMAAs were analysed by means of RP-HPLC/UV and LC-ESI-TOF-MS, respectively. Throughout the maturation period protein degradation was characterized by SDS-electrophoresis and quantification of trichloroacetic acid solube nitrogen (TCA-SN) and water-soluble-nitrogen (WSN) by Kjeldahl and the spectroscopic *o*-phthaldialdehyde (OPA) assay.
N. Corzo, M. Villamiel, M. Arias, S. Riménez-Pérez, F.J. Morales. (2000). *Food Chem.*, ***71***, 255-8

Keywords: furosine, α-*N*-(2-furoylmethyl) amino acids, cheese ripening, protein degradation

Knowledge Integration for Cheese Ripening Control

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Although cheese ripening has been widely studied under several aspects, it still remains difficult to manage. Complex interactions take place between microorganisms, bio- and physicochemical reactions. The link between them and the kinetics of the sensory changes during ripening is almost unknown. So, data and knowledge driven models have been implemented to improve the process control. Nevertheless, these approaches are both not satisfactory in terms of generalization due to a lack of data and uncertainty on the phenomenon. To go further, we propose an approach combining cognitive science, sensory "at line" analysis and experimental databases applied on camembert type cheese ripening.
Operators in charge of processes have a key function in the control of the product quality. Their know-how has already been used successfully to develop the "sensory indicators" method to follow-up food processes. Thanks to this method, six sensory indicators were defined with the experts to characterize the cheese evolutions throughout ripening. Pilot trials were achieved in regulated room under different temperatures and relative humidities to validate this approach. The cheese ripening was monitored by physico-chemical, biochemical, microbial measurements and sensory assessments.
The different relative humidities and temperatures applied on the ripening trials impacted microorganism growth, physico and biochemical kinetics as well as sensory indicators evolution. Finally, a map based on Principal Component Analysis (PCA) was carried out on six variables: pH level, three micro-organisms concentration, lactose and lactate concentration. Then we projected the sensory data on this map. The results highlighted the concordance between the sensory kinetics and the physicochemical and microbiological kinetics. So, instrumental data and sensory assessments could be integrated to predict efficiently cheese ripening evolution.

Keywords: soft mould cheese, "at-line" process control, knowledge integration

Influence of Ripening to Texture Properties of Edam Cheese

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In the study were evaluated the Edam cheeses with fat content 30 and 45 % w/w produced by two dairies (A, B) using two different starter cultures YY-88 and LL-50 during 6 months ripening. The effect of ripening was related to the texture of Edam, so as to determine the optimum ripening time. Sampling times were 26, 54, 89, 117, 146 and 180 days after production. Cheese texture was analysed by compression test which were done by using a Tira-test.
Ripening time had significant effect on rheological characteristics of the cheeses. It was found that the most decrease of the force needed for compression of the sample was after three months of ripening in both cheeses with 30 and 45 % w/w fat from producer A. Both cheeses from producer B were less firm after 6 months of ripening than after 3 months. In every Edam sample after 3 months of aging there was no dependence on the type of starter culture.

Keywords: texture, edam cheese, ripening

Investigation of Fat Replacers in the Use of White Pickled Cheese Produced from Sheep Milk

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Health concerns have led consumers worldwide to reduce consumption of food with a high fat content. There is a growing market of foods with natural ingredients considered as healthy, with a good mouthfeeling and pleasant flavour. The production of fat reduced cheeses is of special interest. Cheeses with reduced fat content may have less flavour and poor texture properties. For this reason, fat replacer compounds are used to fully or partially replace fat, they have been used to stimulate the functional and organoleptic properties of cheese with a substantial reduction in calorific value.
In this experiment, white pickled cheeses were made with low fat (0.9 %) and full fat (6.1 %) ewe's milk. The low fat milk was also used for the cheese making with the addition of Simplesse® 100, Maltrin 040 and Simplesse® 100 + Maltrin 040 as fat replacers. The cheese samples were stored in 12 % brine at 4°C for 60 days. In this study, which was carrried out in duplicate, pH, titrable acidity, dry matter, fat, fat in dry matter, protein, salt, salt in moisture, nitrogen fractions, electrophoretic pattern and organoleptic properties of cheese samples were determined at 1, 15, 30 and 60 days of storage.
Overall, quality evaluation showed that it was possible to produce acceptable low-fat cheese (with fat replacer) by the conventional production techniques. Low-fat cheeses, produced by adding Simplesse®100 and Maltrin 040 were highly acceptable compared to the low-fat cheese without fat replacers.

Keywords**:** White Cheese, Fat replacer, Simplesse® 100, Maltrin 040

The Influence of Ripening Temperature on Structure and Consistency of Cheese

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The aim of work was to determine the influence of ripening temperature on structure, consistency and eye formation process of Swiss – Dutch type cheeses manufactured on automated cheese production line in industrial scale.
Three variants (3 temperature combinations) of 6 weeks cheese ripening period were examined, namely: (I) 8ºC/2 weeks; 18ºC/2 weeks and 8°C/2 weeks, (II) 10ºC/2 weeks; 20ºC/2 weeks and 10°C/2 weeks and (III) 14ºC/2 weeks; 22ºC/2 weeks and 14 °C/2 weeks. Analyzed cheeses during ripening process were turned upside down once per week. The texture (structure and consistency) and eye formation in cheese were evaluated before and after 2, 4 and 6 weeks of cheese ripening. The rheological properties of cheeses were determined by texture analyzer TA-XT Plus of Stable Micro Systems company by performing squeezing and cutting tests. Additionally the structure was specified on the basis of quality and amounts of eyes visible in the cheese intersection.
The obtained results and observations showed that ripening temperature had significant influence on texture of examined cheeses. Especially higher ripening temperatures (14, 22, 14°C) had more favourable influence on structure and consistency of the Swiss – Dutch type cheese in comparison to those which ripened at lower temperature. The texture analysis showed that cheeses which ripened at higher temperatures were characterized by less concise consistency (more elastic and less hard of cheese body with the uniform structure already after 4 weeks of ripening) in comparison with cheeses which ripened at lower temperatures (it was confirmed by considerable drop of both squeezing and cutting forces applied during the tests). The cutting test showed that cheese ripened

at higher temperature was more dynamically that those ripened at lower temperatures. Moreover higher temperature especially after 2 weeks of ripening (rise from 14 to 22°C) had more favourable influence on proper beginning of eye formation in Swiss – Dutch type cheeses.

Keywords: cheese, structure, consistency, ripening temperature

Texture Development During Storage in Starter Free Cheese Model Based on Fully Concentrated Microfiltered Milk

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A starter-free cast cheese model system with constant gross chemical composition based on microfiltered skim milk retentate, depleted of whey protein (< 0.2 %) with 19.0 % casein was developed. The effect of pH, holding time at coagulation temperature, demineralisation, chymosin content and storage time at 12°C on texture was studied. The coagulation kinetics was investigated by low-amplitude oscillation, texture by uniaxial compression.
The rheological properties at 1 day were found to depend on pH, demineralisation, chymosin concentration and holding time at the coagulation temperature. A minimum in Young's modulus was found at a pH of 5.15-5.20 indicating a shift between "acid" and "rennet" gels. During storage, pH and chymosin concentration influenced the texture development. At a low level of rennet the cheeses tended to stiffen during storage and more so with decreasing pH. With increasing rennet concentration, the cheeses turned pasty with a maximum in softening at pH 5.2-5.4.

Keywords: cheese model system; microfiltration retentate; demineralisation; rheology

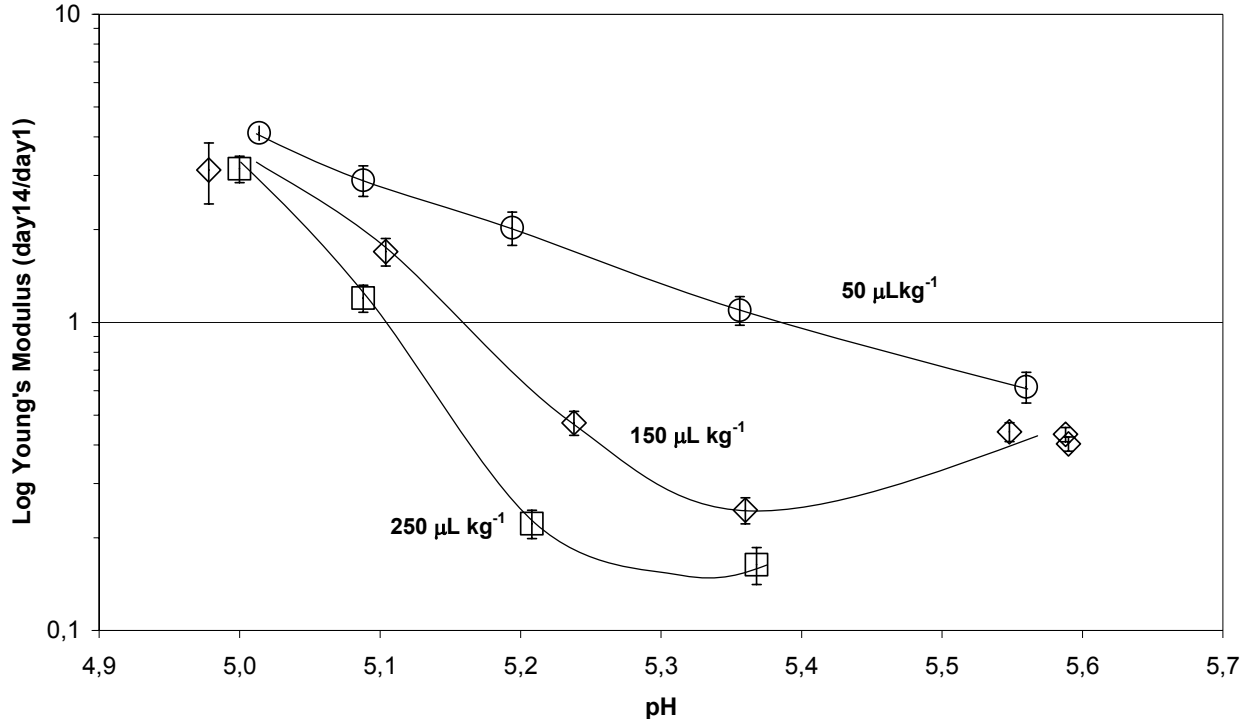

Figure 1: Changes in texture during storage as function of pH at varying chymosin content

The Influence of Transglutaminase Treatment on Renneting Course and Textural Properties of Rennet Gel

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The influence of enzymatic cross-linking on rennet coagulation of milk was investigated using different applications of Tgase. Protein cross-linking was carried out prior to, simultaneously and after the rennet addition. When the milk incubated with Tgase for 90 min at 32°C prior to the rennet addition, no gel was formed. The results showed that the milk proteins cross-linking prior to the rennet addition inhibited the primary enzymatic phase of rennet coagulation. In the Tgase induced sample, inhibition of CMP release can be related to form intra-molecular cross-links between κ-casein molecules. It was observed that enzymatic cross-linking affected the primary and secondary phase of renneting. But the effects occurred at lower and or higher degrees depending on the application method of Tgase. Among these methods, it was determined that the most suitable application was the addition of rennet prior to the Tgase treatment. In this case, the effect of Tgase on the primary phase diminished due to the increased CMP that was released and the decreased rennet floculation time compared to the simultaneous reaction of Tgase and rennet. Furthermore, the water holding capacity and hardness of the gel network was enchanced by inter-molecular cross-links during the progressive renneting times.

Keywords: Transglutaminase, caseins, cross-linking, renneting, textural properties

Effect of High Pressure Homogenization on Rennet-Induced Milk Gelation

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High pressure processing has the potential to alter the structure of renneted milk gels and thereby impact upon cheese structure and ripening characteristics. To investigate this further, low-heat and medium-heat skim milk powders (SMP) were reconstituted in deionized water to a casein level of either 0.8 or 5.4 %. The suspensions hydrated with constant stirring overnight, then adjusted to pH 6.40. Reconstituted SMP suspensions were passed four times through a Microfluidizer high pressure homogenizer one day after preparation at 0 (control), 23 or 116 MPa, under ice to minimize temperature increases. The suspensions were kept at 25°C for a further 4 days, and pH, calcium supernatant concentration, and particle size determined daily (for the 0.8 % casein samples). To estimate ionic calcium, samples were ultra-centrifuged (117,000 *g*) and total calcium determined by EDTA-colorimetric titration on the supernatant. Particle size was measured by diluting the SMP suspensions into simulated milk ultrafiltrate at pH 6.40. The point of gelation of 5.4 % calf-renneted casein suspensions were determined immediately after homogenization at 116 MPa by dynamic oscillatory rheometry at pH 6.40.
Higher pressures reduced the micelle diameter (~200 nm) in low-heat SMP by 5 nm at 23 MPa and 15 nm at 116 MPa, as measured by non-invasive laser light backscattering. The corresponding reduction in size was 3-7 nm for medium-heat SMP. Higher pressure increased pH by 0.02-0.03 units for both SMPs, likely caused by solubilization of calcium phosphate, as quantified by an observed increase in supernatant calcium of 7-14 mg kg^{-1} after homogenization at 116 MPa. The time for milk gelation to occur increased for SMP suspensions homogenized at 116 MPa, possibly due to disruption of the casein micelle surface and partial exposure of the more hydrophobic micelle

interior. These results are consistent with the removal of surface layers from the casein micelle after homogenization, and this occurs independently of calcium phosphate solubilization.

Keywords: Microfluidizer, calcium, pH, casein micelle

Use of Combined Protease & Peptidase Systems to Accelerate Ripening in Cheddar

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By adding commercial protease enzymes to the Cheddar cheese make it was possible to stimulate increased casein breakdown and also show that concurrent peptidase addition could hydrolyse some of the additional peptides created by the protease thus liberating corresponding additional free amino acids capable of both direct flavour effects and further potential catabolism into more complex flavour compounds. The biochemical (proteolytic) processes in the cheeses were monitored via HPLC over 4 months of ripening and then the acceleration of the ripening process assessed using sensory analysis techniques.

Keywords: accelerated cheese ripening, protease

Influence of Exopolysaccharide Produced by Isogenic Strains of *Lactococcus Lactis o*n Half-Fat Cheddar Cheese

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The effect of an exopolysaccharide on cheese manufacture, yield, composition and microbiology were examined in half-fat Cheddar cheeses manufactured using an exopolysacharide producing (EPS+) starter (*Lactococcus lactis* subsp. *cremoris* DPC6532) and its non-exopolysaccharide producing (EPS-) genetic variant (*Lactococcus lactis* subsp. *cremoris* DPC6533). The EPS- isogenic variant was obtained by inducing the loss of a ~60 kb EPS-encoding plasmid. Strain isogenicity was confirmed by comparing pulsed-field gel electrophoresis (PFGE) profiles and the absence of the exopolysaccharide plasmid was confirmed by PCR and Southern Blot.
Two cheeses were manufactured, in triplicate, using a 1.5 % inoculum of each of the starter strains. Rennet coagulation properties, evaluated by low amplitude oscillation rheometry, were not affected by the presence of the expolysaccharide (no significant differences were observed between the $GT_{0.5}$ and STC_{35Pa} between the two cheeses).
Actual yield per 100 kg of cheese milk (Ya) increased by 8.17 % in the EPS+ cheese relative to the EPS- cheese, while moisture increased by 9.49 % at 1 month of ripening. Similarly, moisture in the non fat substances (MNFS) increased while salt in moisture (S/M) decreased in the EPS+ cheese relative to the EPS- cheese by 6.8 and 15.8 % respectively. Fat in dry matter (FDM) was not affected by the presence of the exopolysaccharide. pH was lower in the EPS+ cheese (5.14) compared to the EPS- cheese (5.36).
Starter counts decreased from 10^9 to 10^6 cfu g^{-1} cheese by 6 months of ripening in both cheeses with similar levels of lysis detected. Although water activity was found to be higher in the EPS+ cheese, similar levels (10^6 cfu g^{-1} cheese) of non-starter lactic acid bacteria (NSLAB) were detected in both cheeses by 6 months.

These data show that the use of an exopolysaccharide-producing strain increases cheese yield and moisture without affecting coagulation, survival of starter and growth of NSLAB.

Keywords: exopolysaccharide, isogenic strains, cheese yield, cheese composition

Impact of Expolysaccharides on the Texture and Functional Properties of Half-Fat Cheddar Cheese

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Exopolysaccharides (EPS) produced by starter strains have been proposed as a mechanism to improve the texture and cooking properties of reduced-fat cheeses. The influence of EPS on the rheology, texture and melting properties of half-fat Cheddar cheese, manufactured using two isogenic strains (*Lactococcus lactis* subsp. *cremoris* DPC6532 (EPS+) and DPC6533(EPS-)) which differ only in their ability to produce EPS, was assessed over a 6 month ripening period.
Hardness and gumminess were lower in the EPS+ cheese compared to the EPS- cheese throughout ripening. For both cheeses, a similar reduction in the level of these parameters was observed over a 6 month ripening period. The level of hardness detected in the EPS+ cheese is similar to a full-fat Cheddar cheese. Similar levels of cohesiveness were detected in both cheeses throughout ripening. Fracture stress and fracture strain were found to be lower in the EPS+ cheese and decreased over ripening for both cheeses.
Cheese flowability, as measured by the modified Schreiber and Olson/Price methods, was higher in the EPS+ cheeses throughout ripening but increased for both cheeses over ripening. For example, using the modified Schreiber method, the flowability was 303 % higher at day 1 and 67 % higher by 6 months in the EPS+ cheese compared to the EPS- cheese. Levels of flow detected in the EPS+ cheese are similar to those reported for full fat Cheddar cheese.
No significant differences were observed in the apparent viscosity or elastic shear modulus (G'_{20} and G'_{90}) at 6 months between the two cheeses.
These data demonstrate that the presence of EPS in half-fat Cheddar cheese improves the texture and flowability to a level similar to a full-fat cheese, making the EPS-producing starter an attractive tool to overcome the undesirable texture proprieties of reduced fat cheeses.

Keywords: exopolysaccharide, cheese texture, rheology, melting proprieties

Effect of Different Whey Protein/Casein Ratio on the Ripening of UF White Cheese

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For studying the effect of whey proteins to proteolysis during the ripening of ultrafiltered (UF) white cheese, experimental UF cheese were produced by adding whey protein isolate with high purity (> 90 %) to retentate of pasteurized milk in different ratios of whey proteins to caseins (20/80, 30/70, 40/60 and 50/50 %). The addition of whey proteins did not have a significant effect on the development of the pH 4.6 soluble nitrogen fraction during cheese ripening, but caused differences in protein profiles determined by urea-polyacrylamide gel electrophoresis. The rate of

α_{s1}-casein degradation was decreased by increasing whey protein/casein ratio, while hydrolysis of β-casein was negligible in all samples. The cheeses contained high whey protein ratio had lower free amino acid concentrations as assayed by RP-HPLC. Finally it was revealed the higher whey protein/casein ratio, the softer texture and less acceptability of UF white cheese.

Keywords: UF white cheese, whey protein, ripening

Effect of Cream Homogenization on Textural and Sensory Characteristics of Reduced Fat Turkish White Cheese

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The effects of cream homogenization of cheese making milk on biochemical and textural (hardness, adhesiveness, gumminess, cohesiveness etc) characteristics, microstructure and sensory properties of reduced fat Turkish White cheese were studied during the storage of 90 days. Homogenization was applied at 20 MPa to cream containing 38 % milk fat. Reduced fat Turkish White cheeses were manufactured from 0.75 and 1.5 % fat in milk that were standardized with unhomogenized or homogenized cream. Thus, four treatments were carried out using 2000 liter standardized milk for each treatment.
The contents of total solids, milk fat and free fatty acid increased in reduced fat Turkish White cheeses produced by homogenized cream when compared to control samples. Homogenization of cream resulted in lower contents of total nitrogen and water-soluble nitrogen in reduced fat Turkish white cheeses ($p < 0.05$). Hardness was significantly lower in cheeses obtained from homogenized treatment and gradually decreased in all samples during storage ($p < 0.05$). Conversely, adhesiveness increased in cheese samples with homogenized cream in comparison to unhomogenized treatment ($p < 0.05$). Gumminess and cohesiveness were not affected by homogenization of cream in manufacturing process ($p > 0.05$).
Cream homogenization in cheese manufacture had also a significant influence on the microstructure of the product. The protein matrix in unhomogenized treatment was compact, occupied with small number of unevenly dispersed fat globules. Fat particles were embedded in the spongy appearance of protein matrix. In the micrographs of cheeses from homogenized treatment, large number of fat particles was dispersed in the casein matrix. The texture of this cheese was creamier.
With regard to sensory characteristics, homogenization of cream in the manufacture had a significant effect on the appearance, flavor and texture of reduced fat Turkish White cheeses. Higher mean scores of flavor were obtained in cheeses produced by homogenized cream in comparison to unhomogenized treatment during storage ($p < 0.05$).

Keywords: Reduced fat Turkish White cheese, homogenization, cream, texture

Primary Proteolysis in Concentrated Casein Systems with Added Whey Protein

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Ultrafiltration (UF) of cheese milk reduce rate of proteolysis in semi-hard and hard cheeses. Consequently, UF has not been successful for ripened cheeses in spite of considerably increased cheese yield because also the whey proteins and not only the casein will end up in the cheese.

Direct inhibition by whey proteins of plasmin and chymosin activities on casein has been suggested as an explanation. However, the mechanism has not been revealed.
To elucidate factors of importance to the reduced proteolysis in cheese made from concentrated milk, primary proteolysis of β-casein by plasmin in UF and microfiltrated (MF, excluding the main part of the whey proteins) skim milk concentrate (both pH 5.8) was investigated using capillary electrophoresis (CE). Furthermore, proteolysis by chymosin and plasmin in rennet-induced casein gels (pH 5.8) made from microfiltrated concentrate with varying whey protein concentrations and total solid (TS) contents were investigated by analysing casein components with CE and peptide composition with HPLC.
Interestingly, lower plasmin activity was obtained in MF concentrate (0.1 % w/w whey protein, TS 28.6 % w/w) than in UF concentrate (4.1 %, w/w, whey protein, TS 30.1 % w/w), which clearly showed that the inhibition of plasmin by whey proteins was not the explanation. Another explanation to less plasmin activity could be a harder mechanical treatment during milk filtration for the MF milk. Also, addition of whey protein to MF concentrate did not change the plasmin activity. In gels of MF milk, 10 to 25 % (w/w) of α_{S1}-caseins and β-caseins had been broken down after 61 days of storage (13°C). Proteolysis of α_{S1}-casein and β-casein by chymosin in gels did not decrease when the whey protein concentration increased as long as TS was constant. However, increase in TS decreased activity of chymosin. A minor decrease in plasmin activity in gels was detected when the whey protein concentration increased. After storage (61 days), the elasticity of gels had decreased due to primary proteolysis of caseins but differences between gels were small.

Keywords: cheese, milk filtration, whey protein, proteolysis

The Use of Microfiltration for the Manufacture of Mozzarella Cheese

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Pilot plant 151 module of Carbocep was used with a 0.14 μm cut off membrane (6.8m^2). The concentration experiment was carried out with 600 L of skim milk (dry matter 87.5 g kg^{-1}, pH 6.65) in two stages. In the first step, the milk was concentrated by a factor of three and in the second step diafiltrated with 200 L of water and concentrated to 90 L.
32 kg heavy cream with a fat content of 480 g kg^{-1} was added to the diafiltrated concentrate (= 15 g kg^{-1} fat in the initial partially skim milk used in the experiment). The final concentration factor was 5.1. The pH of the second concentrate was 6.2 and dropped to 5.9 by adding phosphoric acid and starter. The decrease in pH takes about one hour at temperature of 32 to 34°C. Rennet (microbial, Hansen) is added and coagulation is completed within 10 min (pH 5.4). The curd is cutted and cooked, moulded, and finally cold brined. 70.5 kg cheese with a pH of 5.15 to 5.2 was obtained with a dry matter and fat content of 495 and 220 g kg^{-1}, respectively.

Keywords: mozzarella, microfiltration, diafiltration

Evaluation of Commercial Accelerated Ripening Systems in Cheddar Cheese

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Acceleration of Cheddar cheese ripening is an ongoing research objective and involves attempts to reduce ripening costs without adversely impacting on product quality. Most commercially successful methods utilize exogenous enzyme preparations. There is a lack of published data on the effectiveness of such commercial ripening systems. This study was undertaken to independently evaluate the effects of three separate enzyme systems on Cheddar cheese ripening and quality. Two preparations (Accelase AM317, Danisco; Accelerzyme CPG, DSM) are added to cheesemilk and one (Accelase AHC50, Danisco) with the salt. These enzyme systems were compared to a control without enzyme addition. All cheeses were manufactured using LL50A (DSM) under identical conditions, ripened at 8°C for 112 days. The bulk whey from the AM317 cheeses contained significantly more free amino acids than the other cheeses, but no additional proteolytic activity was evident in the bulk whey of the experimental cheeses over the control. Levels of live, premeabilised and dead cells were similar in the curd at whey drainage, in the salted curd, in the pressed curd, in bulk and salted whey, except for the AHC50 cheeses, where significantly less live and permeabilised cells and more dead cells were evident in the salted curd and pressed whey. There was no apparent difference in starter and non-starter levels or water activity over ripening. Significantly higher levels of moisture and moisture in non fat substances were found in AM317 cheeses compared to the control and AHC50 cheeses. Some significant differences in pH were also evident. Levels of proteolysis were significantly higher in AM317 and AHC50 cheeses and no significant difference in levels of lipolysis over ripening. Significant textural differences were apparent. Significant differences in sensory attributes were noted between the cheeses at the end of ripening, but no off-flavours or bitterness were evident.

Keywords: Accelerated, Cheddar, Cheese, Ripening

Continuous Cheese Production by Implementation of Membrane Filtration Technology

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A milk concentrate produced by microfiltration (MF) and ultrafiltration (UF) offers a simple method of manufacturing soft, fresh or semi-hard cheese. This technology is state-of-the-art in the range of continuous cheese production. HF-Finnatec has enhanced the technology and enables further rationalisation of workflow with its prototype *HF ConFrom™*. Labour-intensive steps of production like curd processing, whey draining and pressing can be saved. With this technology the rate of cheese yield can be increased up to 10 %.
HF ConFrom™ consists of two units: filtration unit for processing concentrates with high dry matter and cheese unit for continuous dosage of ingredients, coagulating and forming compartment. The system is very flexible, multifunctional and innovative. The kind of cheese made with *HF ConFrom™* has got similar characteristics than cheese produced in the traditional way. Products manufactured in this way provide a variety of options in terms of quality, flavour and forming. In the future continuous production of semi-hard cheese is also possible with this technology.

Keywords: microfiltration, ultrafiltration, continuous cheese processing, yield, cheese innovations

Heat-Induced Changes on the Casein Micelle under UHT Conditions and their Influence on the Cheese Making Process

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The aim was to obtain insights regarding thermal reactivity of the casein micelle which so far has been considered very heat-stable. Milk was fractionated in its main protein fractions by means of microfiltration / diafiltration with a combination of ultrafiltration so that pure, whey-protein-free native casein could be made available. With pure casein solutions different structural and molecular changes in the casein micelle were assessed after UHT-heating. By means of measurements regarding dissociation and/or polymerization degree, hydrophobicity and voluminosity the actual thermally caused variability of the casein micelle was determined. These effects were then correlated with the gel formation characteristics of casein during cheese production. It was concluded that correlation between the renneting properties (regarding relative coagulation time and relative gel firmness) of heated casein dispersions and the heat-induced changes exists. Heat-induced changes can be responsible alone or in a combination with other changes for the impairment of renneting properties. Thus, it could be stated that the thermal changes of the casein fraction are substantially larger than so far assumed, because in presence of the whey protein the effects of both fractions were always overlaid.
From the side of cheese making properties of the microfiltration-retentate it was concluded that the higher the whey protein contents and heating temperature, the more strongly the renneting properties of the retentate were impaired after heating under UHT-conditions. Additionally, it was found that the casein / whey protein concentration ratios can be used to optimise the renneting behaviour of these modified milks.

Keywords: casein micelle, UHT-heating, renneting properties, physical-chemical changes

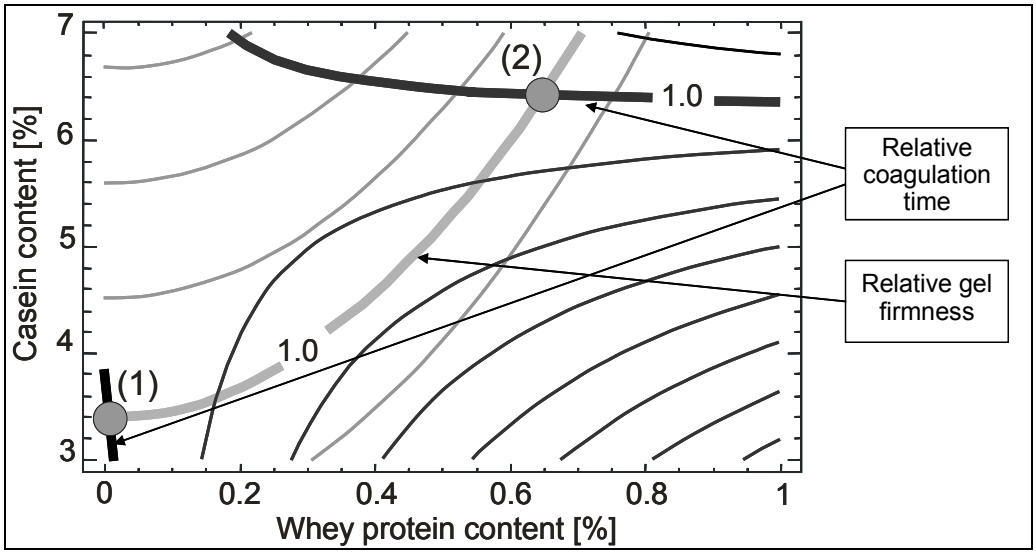

Figure 1: Optimisation of the protein composition of MF milk concentrates prior to UHT treatment using the relative rennet coagulation time and gel firmness as criteria

Inhibition of Rennet Activity in Cheese Using Equine Blood Serum

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Equine blood serum was added (0.1 to 2 %, v/v) to cheesemilk at 15°C after completion of the first phase of renneting and before aggregation of the rennet-altered casein micelles, to inhibit the residual coagulant in Cheddar-type cheeses. After 42 d ripening, there were no significant differences ($p > 0.05$) in the protein content and pH between the cheeses; the moisture and salt

content of the cheeses varied slightly both with the level of blood serum added and between trials. Throughout ripening the level of pH 4.6-soluble nitrogen as a percentage of total nitrogen (SN/TN) was significantly higher in the control cheeses than in experimental cheeses and was about twice that of the experimental after ripening for 180 d. During ripening, there was almost no hydrolysis of α_{s1}-casein in the cheeses made from milk containing 0.25 to 2% blood serum. Throughout ripening there were large quantitative differences between the peptide profiles of control and experimental cheeses. The results of this study suggest that the addition of equine blood serum to cheesemilk at levels from 0.25 to 2% was very effective in inhibiting the residual chymosin activity in Cheddar-type cheeses during ripening; the activity of plasmin remained unaffected by the added blood serum.

Keywords: Equine blood serum, Cheddar cheese, Cheese ripening, Coagulant

Suitability of Fermentation-produced Camel (*Camelus dromedarius*) Chymosin as a Coagulant for Cheddar Cheese

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Cheddar-type cheeses (10 kg) were manufactured using fermentation-produced coagulant CHY-MAX™ M (camel chymosin) or CHY-MAX™ (calf chymosin). There were no significant differences in the composition, pH and cheese yield between the cheeses made with either coagulant, but there was indication of higher cheese yield when using camel chymosin. The level of pH 4.6-soluble nitrogen as a percentage of total nitrogen and the level of primary proteolysis, as detected by urea-polyacrylamide gel electrophoresis, was significantly lower in cheeses made with camel chymosin than in cheeses made with calf chymosin. There were large quantitative differences between the peptide profiles, determined by reverse-phase high-performance liquid chromatography, of cheeses made with calf or camel chymosin as ripening progressed; however, there were no significant differences in the levels of free amino acids. The results of descriptive sensory analysis suggested that cheeses made with camel chymosin were characterized by lower intensities of sulfur and brothy flavours and had less bitter taste than the cheeses made with calf chymosin, however, the cheeses made with calf chymosin had greater breakdown, higher smoothness and mouthcoating and were more cohesive and adhesive. The results of this study suggest that camel chymosin can be used successfully to make Cheddar cheese with lower levels of proteolysis but with good flavour, which may be of significance in cases where there is a propensity to bitterness.

Keywords: Camel chymosin, Cheddar cheese, Cheese ripening, Coagulant

Model Studies on the Stability of Transglutaminase Crosslinked Casein Micelles Toward Enzymatic Proteolysis

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The enzyme microbial transglutaminase (mTG) [E.C 2.3.2.13] catalyzes an acyl transfer reaction between the gamma-carboxyamide group of protein-bound glutamine and the epsilon-amino group

of lysine residues. The reaction leads to a formation of intra- and/or intermolecular crosslinks (isopeptide) resulting in the polymerization of proteins, leading to improved functional properties (Yokayama et al., 2004).With regard to the application of mTG during cheese making, the incorporation of whey proteins into the curd and consequently the curd yield is enhanced (Han et al., 2003). In spite of using mTG in food industry, the relation between mTG induced structural modification and the associated functional consequences is not clarified in detail.
Caseins, which are aggregated in micelles in bovine milk, are a good substrate for mTG. Preliminary studies have shown that mTG treatment of casein micelles increases the micellar stability toward disintegration by addition of EDTA, ethanol as well as high hydrostatic pressure (Partschefeld et al., 2007). The objective of this work, therefore, was to examine the effect of mTG treatment on the enzymatic proteolysis of casein micelles. For this, casein micelles were isolated from pasteurized milk by centrifugation and resuspended in synthetic milk ultrafiltrate. The incubation with mTG (8U g^{-1} casein) was carried out at 40°C for 60 minutes followed by a thermal inactivation of the enzyme. Afterwards the stability of the micelle structure toward proteolytic degradation by pepsin and chymotrypsin was examined. The collapse of micelle structure was analyzed using DLS (dynamic light scattering) as well as turbidity measurements at 633nm and the protein degradation was examined by SDS-polyacrylamide gel electrophoresis.
The results showed an improved stability of crosslinked casein micelles toward treatment with proteolytic enzymes compared to untreated casein micelles. On the one hand, the protein degradation by pepsin as well as chymotrypsin was reduced. On the other hand, no collapse of the micelle structure, as it was observed for the untreated casein micelles, occurred. The observed stabilized effect could be traced back to the intramicellar formed crosslinks. It is suggested that the isopeptides were primarily formed net-like within the external region of the micelles, by what the attack of pepsin or chymotrypsin is decreased and the basic micelle structure is maintained. This result may be of importance with respect to proteolytic changes occurring during ripening of cheese prepared from mTG-treated milk.
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C. Partschefeld, U. Schwarzenbolz, S. Richter, T. Henle. (2007). *Biotechnol. J*., **2**, 456-461

Keywords: transglutaminase, crosslinked casein Micelle, proteolysis

Production of Fat-Reduced Ovine Ricotta Cheese from Whey Concentrated by Ultrafiltration

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Ultrafiltration (UF) is a membrane separation process largely used in cow dairy industry, but until now, less used in sheep dairies. Ricotta cheese, particularly the ovine type, is an Italian typical fresh dairy product obtained by heat-coagulation of whey proteins.
The aim of this work was to produce a fat-reduced ovine Ricotta cheese using whey concentrated by UF. Ovine Ricotta cheeses were obtained from natural whey (RCO) and from whey concentrated by UF (RUF). RCO was obtained by mixing skim whey and 68 g $100g^{-1}$ fat cream, while RUF by mixing UF skimmed whey retentate (VCR = 5.6) and 68 g $100g^{-1}$ fat cream. Fat/protein ratio in the whey was kept constant in all replicates (1.4 for RCO and 1.3 for RUF).
The results show significant differences ($P < 0.001$) in moisture and in fat/dry matter ratio which resulted 73.2 and 44.4 g $100g^{-1}$ *vs* 69.9 and 67.8 g $100g^{-1}$ for RUF and RCO, respectively. True protein/dry matter ratio, as expected, was significantly higher in RUF than RCO ($P < 0.001$). Accordingly, the fat/true protein ratio was lowest in RUF. Despite of the differences found in

chemical composition both RUF and RCO resulted of good quality in a sensory test. The use of UF concentrated whey in Ricotta cheese manufacturing allowed us to increase significantly the Ricotta cheese yield (+ 85 %) and the fat (+ 10 %) and protein (+ 154 %) recovery.
RUF, accordingly to its lower fat content, can satisfy the new consumer trends that prefer a diet with a reduced fat content products. In addition, the pre-concentration of ovine whey by UF improves the Ricotta cheese yield, increasing the fat and protein recovery in the product.

Keywords: ovine cheese, ricotta, ultrafiltration

Evaluation of the Impact of Microfiltration, Acidification with Carbon Dioxide and Prolongation of the Storage Period of Milk on the Diversity and Dynamics of the Bacterial Community During Ripening of Cheddar Cheese

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Until now, the impact of milk's treatments on the dynamics of cheese microbiota was difficult to investigate. To assess two treatments (microfiltration (MF) and CO_2 acidification) effects as well as storage period of nine days on the dynamics of the bacterial community during ripening in experimental Cheddar cheeses; three cheese-making trials were carried out at two different storage periods (2 and 9 days): control LT, CO_2 and MF cheeses which were analysed at four different stages: on day 1, day 14 and after 3 and 6 months of ripening. Total DNA was PCR amplified using universal and lactic acid bacteria (LAB)-specific primers targeting the rRNA gene. The amplicons were analyzed by terminal-restriction fragment length polymorphism (T-RFLP) method and by 16S rDNA clone library. Comparison of T-RFLP profiles showed few variations between LT, CO_2 and MF cheeses for two independent replicates. The two treatments as well as milk storage up to nine days have no effect on the diversity and dynamics of the bacterial community throughout ripening. The presumptive identification of the predominant peaks on the T-RFLP profiles was validated by sequencing 16S rDNA clone library. At all the sampled levels of ripening, cheeses were characterized by persistence (relative abundance ~ 95%) of the starter LAB. The dominant non-starter LAB (≤ 25 %) identified were *Lactobacillus* species (*Lactobacillus delbrueckii*, *Lactobacillus pentosus*, etc.). Principal component analysis and hierarchical clustering analysis revealed four distinct groups corresponding to the four levels of ripening without difference between LT, CO_2 and MF cheeses for both replicates fabrications. The results demonstrated that cheeses produced with milks treated by CO_2 acidification and microfiltration even after nine days of storage have comparable quality with those produced with two-day-old milk. T-RFLP in combination with multivariate analysis and 16S rRNA gene sequencing proved to be an effective strategy to study cheese microbial dynamics.

Keywords: Cheddar cheese, lactic acid bacteria, T-RFLP, 16S rDNA clone library.

Development of an Innovative Process: Production of Mozzarella and Ricotta from Ewes' Milk

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Fermented mozzarella from ewes' milk was successfully produced in the pilot scale cheese dairy at ALP Research Station from raw, thermised (batchwise, 62°C) and gently pasteurised milk (batchwise pasteurisation, 62°C/30 min.), but not from milk pasteurised at 72°C (batchwise). With 60°C the optimum *pasta filata* water temperature in the trials was significantly lower than in cow or buffalo mozzarella production. The resultant curd temperature after two water renewals was 54 - 55°C. Curd mass plasticizability was good at pH values of 5.15 - 4.85. One key factor in particular proved to be the thermal lability of ewes' milk.
A comparison of the important sensory properties such as hardness and flavour of the best ewes' milk mozzarella experiments with those of market products showed that ewes' milk mozzarella can compete very well with commercial products of cow or buffalo milk.
Ricotta was successfully manufactured from both sweet whey (pH > 6.4) taken at the beginning of the process and the fermented total whey occurring at the end of the process of ewes' milk mozzarella. For satisfactory results the latter first had to be adjusted to pH 7.5 with sodium hydroxide solution. Precipitation took place at 80°C without the addition of an acidifying agent. The thermally labile whey proteins of the ewes' milk began to precipitate spontaneously.
An evaluation of the economic feasability of the processes developed showed that the cost-effective production of ewes' milk mozzarella is possible similarly to cow and buffalo mozzarella. Furthermore this production can represent a higher added-value method than the production of semi-hard ewes' milk cheese.

Keywords**:** ewes' milk, ewes' milk cheese, mozzarella, ricotta

The Effect of Homogenization and Microfiltration on Functional Properties of Soft and Semi-Hard Cheese

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The objective of the study was to characterize the impact of homogenization and microfiltration (MF) on functionality of soft cheese and semi-hard cheese. Soft cheeses and semi-hard cheeses were manufactured from four batches of pasteurized and standardized (fat/protein ratio: 0.84 and 0.9, respectively) milk: untreated cheese milk (concentration degree i = 1; not homogenized 0 MPa), homogenized milk (i = 1; 8 MPa), MF concentrated milk (i = 2; 0 MPa) and homogenized and MF concentrated milk (i = 2; 8 MPa). Homogenization was performed at 65 °C and for microfiltration a ceramic membrane (cut off 0.1 µm) was used. Curd treatment times were adjusted by modelling to achieve comparable dry matter in the differently produced raw and ripened cheeses. The functional properties were assessed after 4 weeks of ripening. Meltability was evaluated using a modified Schreiber test. Cheese hardness was measured by applying a puncture test within the whole soft cheese and by uniaxial compression test for semi-hard cheese, respectively. Yellow Index was determined by means of L*a*b*-values with a Chromameter.
The composition was comparable among each cheese type made from differently pre-treated milk. In general, the colour of the cheese was mainly altered by homogenization due to increased number of small fat globules resulting in an increased light scattering. Additional MF concentration (i = 2; 8 MPa) had only little effect on colour but caused an increase in cheese hardness. Cheeses made of MF concentrated milk showed a slightly improvement in melting compared to untreated samples whereas homogenization hindered melting with lowest levels for solely homogenized (semi-hard cheese) and combined treatment (soft cheese). In addition, melted cheese samples made from homogenized milk revealed no or little browning.

The results reflect the impact of mechanical pre-treatment of cheese milk on functional properties for both soft and semi-hard cheese and it offers potential to design cheese products for special applications in food industry.

Keywords: homogenization, microfiltration, semi-hard cheese, functionality

Semi-Hard Cheese from Milk Retentate

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Concentration of milk by membrane filtration for continuous production of cheese has become a state-of-the-art technology in the cheese industry. The object of the present work was, to produce a semi-hard cheese from a highly concentrated milk retentate obtained with a new type of cassette filter modules at filtration temperatures of 50-55°C. Process milk was maximally concentrated by microfiltration (MF) and the fat content of the resulting milk retentate was increased to the final fat content by the addition of cream (70 % fat) before cheese making. The effects of calcium chloride, the prematuration process, pretreatment of the process milk and diafiltration on the quality of the final product were examined. The obtained results show that minimal curd separation is needed to achieve the dry matter of semi-hard cheese. The casein-bound calcium was also concentrated during microfiltration and impaired the melting properties of the semi-hard cheese. The addition of $CaCl_2$ is not advisable as, in addition to the hardness/rubberiness, it also slightly increased the bitterness of the ripened cheese. The use of microfiltration to reduce the count of bacteria of the industrial milk instead of pasteurisation and vacuum treatment of the cheese prior to smear-ripening had a positive effect on the texture and flavour.

Keywords: semi-hard cheese, microfiltration, protein concentration, cassette module

A Preliminary Study of Cheeses Manufactured from Varying Ratios of Bovine and Caprine Milks

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Consumer demand for goats milk and for cheeses made therefrom has grown steadily. An insufficient supply of caprine milk has resulted in increased opportunities in certain markets for cheeses made from caprine mixed with ovine or bovine milks. Research reports on cheeses made from mixed caprine and bovine milks are scant.
Semi-hard cheeses were manufactured in three replicate trials from milks mixed in ratios 100:0; 75:25; 50:50; 25:75; 0:100 (caprine:bovine) and ripened for 150 d. All cheeses had similar levels of moisture-in-non-fat-substances (~ 61 %) and salt (1.5 %). Cheeses made with ≥ 25% caprine milk had similar mean pH levels during ripening to cheeses made with 100 % caprine milk and had similar and significantly higher levels of primary proteolysis after 150 d ripening than cheeses made from 100 % bovine milk ($P<0.05$).
Descriptive sensory analysis showed that cheeses made from 100 % bovine milk had significantly higher levels of cooked and whey flavours and significantly lower levels of fruity, free fatty acid, cowy, waxy/goaty and bitter flavours than cheeses made from 100 % caprine milk ($P<0.05$).

However, with the exception of less intense waxy/goaty flavour cheeses manufactured from a 50:50 mix of milks had similar levels of these flavours to cheeses made from 100 % caprine milk.
These results indicate that cheeses made from ≥ 50% caprine milk display chemical and sensorial characteristics similar to those made from 100% caprine milk and may provide an appealing and available alternative to consumers desiring caprine cheeses.

Keywords: cheese, bovine and caprine milks, ripening, sensory properties

Manufacture of Cheddar Cheese from High-Pressure-Treated Milk

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High-pressure (HP) processing is a non-thermal technology which preserves food, usually without degradation of nutrients, flavour or the colour of the product. In 2007, the first HP-treated dairy product, a cheese spread, was commercialised in Spain. In addition to microbiological effects, HP has significant and often unique effects on proteins, such as caseins and whey proteins, which play an important role in cheese making. β-Lactoglobulin, for instance, is denatured at pressures over 100 MPa and then incorporated into the cheese, which leads to a higher yield, while changes in the casein micelle structure influence the rennet coagulation properties of milk. In this study, laboratory-scale Cheddar cheese was made from pasteurised milk and milk high-pressure treated at 400 MPa or 600 MPa. Compositional analysis showed a significant increase in moisture and salt content in cheese made from HP treated milk (600 MPa); protein and fat levels decreased slightly. The growth of starter and non-starter lactic acid bacteria was followed over a ripening time of three months and a change in the growth patterns was evident. Furthermore, proteolysis during ripening was studied using electrophoresis and effects of HP on this process elucidated. Results show, that α_{S1}-CN is more rapidly hydrolysed after treatment at higher pressures. Overall, HP treatment of cheese milk increased the yield but also changed microbial ecology and proteolysis during Cheddar cheese ripening.

Keywords: Cheedar cheese, high pressure treatment

The Effect of High Pressures on the Ripening of Irish Blue-Veined Cheese

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In recent years, there has been much research on the effect of high-pressure on milk. It is known that gram (+) bacteria are more resistant than gram (-) and that moulds are some of the most baro-sensitive microorganisms, and can be inactivated at pressures up to 300 MPa. Further studies have shown that HP could be used to increase the refrigerated shelf-life of cheese due to high bacterial inactivation. In this study, mature (i.e., ripened) Irish farmhouse blue-veined cheese was subjected to high-pressure at 400 MPa or 600 MPa and kept at 4ºC for one, 14 and 28 days. The effect of high-pressure on appearance, composition, microbiology and proteolysis was studied to establish if HP-induced inactivation of microbes and enzymes could arrest the ripening of such cheese and thus extend shelf-life at optimal quality. Results show that HP at 400 MPa increased the pH of cheese but the reverse occurred at 600 MPa; no significant effect was found for moisture, salt, fat and protein. Starter, NSLAB, mould, enterococci, total aerobic bacteria and yeasts were followed over

the storage time and the effect of HP was clear; in particular, the counts of moulds showed significant differences between the control and the HP-treated cheese. In general, all counts were lower in the cheese treated at 600 MPa. Primary proteolysis, measured by the pH 4.6-soluble nitrogen fraction, was higher in the high pressure-treated samples and electrophoresis indicated a change in breakdown pattern. Secondary proteolysis, studied by analysing the PTA- and TCA - soluble N fractions indicated increases in the high pressure-treated samples. Furthermore, the expressible serum increased with storage time and pressure. Overall, it could be shown that HP decelerated the ripening of Irish-blue veined cheese at 600 MPa.

Keywords: high-pressure, blue-veined cheese, storage, microbiology

Improvement of Milk Powder for Chocolate Manufacturing

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Milk powder is a main ingredient for the manufacture of milk chocolate which influences substantially the process behaviour and flavour, texture and rheological properties of the final product. The influence of the degree of denaturation (DN) and of the protein concentration on the rheological properties during the chocolate manufacturing process and on the quality of the chocolate are not known yet and therefore were investigated in this study. Six specific milk powders, either roller dried whole milk or spray dried skim milk with defined DN or reduced protein content were produced and chocolate was manufactured in small industrial scale. Rheological measures and sensory analysis by trained panels were carried out.
During chocolate manufacturing it was observed that for spray dried milk powder a higher DN resulted in a higher operational capacity of the refiner. With medium high heat powder (DN 84 %) it was 57 kg h^{-1} μm^{-1}(1800 mm refiner)$^{-1}$, 18 % higher than with low heat powder (DN < 1 %). Between these chocolates no significant sensory difference was found. A lower protein content of the milk powders had no influence on the operational capacity but reduced the viscosity during conching and in the final product (figure 1) and the hardness in the tasting.
Between the chocolate made with spray dried skim milk powder (34.1 % protein) and a chocolate made with the same type of spray dried skim milk powder but reduced protein content (either 30.6 or 23.7 % protein) no significant differences were found in the sensory analysis neither by descriptive methods nor by a triangle test. A tendency towards faster melting and reduced hardness (bite) with lower protein content could be observed though.
To confirm the present results further experiments have to be carried out. The recipes for chocolate manufacturing have to be adapted according to the type of milk powder used.

Keywords: chocolate, milk powder, denaturation, protein content

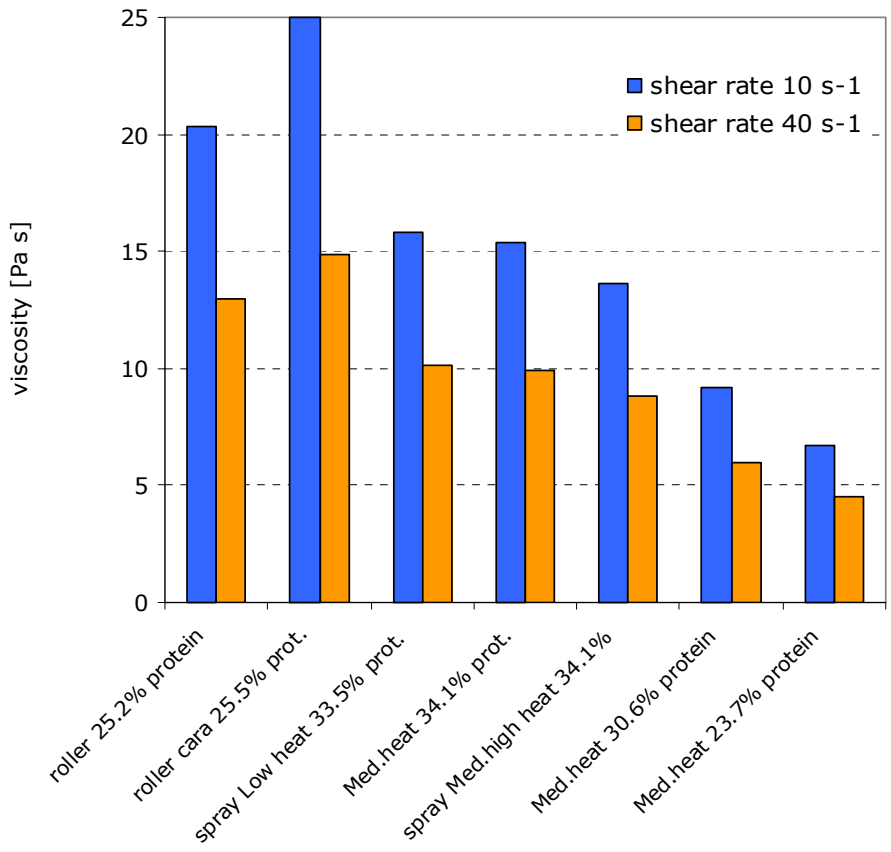

Figure 1: Viscosity of the different melted chocolates measured at 10 and 40 s^{-1}

Effect of Partial Replacement of Sweet Cream with Whey Cream on the Textural and Rheological Properties of Cream Cheese

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This study investigated the rheological and textural properties of cream cheeses (CC) when whey cream was used to replace various levels of sweet cream. Three different CC were manufactured: control cheeses (0 % whey cream), CC containing 25 % whey cream (25WC) (75 % sweet cream) and CC with 75 % whey cream (75WC) (25 % sweet cream). Dynamic small amplitude rheological properties of CC were measured during heating from 5 to 80°C at 1°C min^{-1}. The parameters measured were storage modulus and loss tangent. Hardness of CC was determined by texture profile analysis (TPA). Hardness of cheese cakes baked with CC was determined by a penetration test. A trained sensory panel used spectrum descriptive analysis to determine firmness and difficulty to spread. Storage modulus values at 8°C of CC made with 75WC were significantly ($p < 0.01$) lower than the storage modulus values of the control CC. Hardness determined by TPA and firmness determined by sensory indicated that CC with 75WC had significantly ($p < 0.0001$) lower values compared to control CC and to CC with 25WC. Cheese cakes manufactured from CC made with 75WC were significantly ($p < 0.05$) softer than those baked with the control CC or CC with 25WC. A similar trend was observed for the difficulty to spread attribute determined by sensory analyses. This study indicated that the use of 25WC resulted in CC with similar or slightly firmer characteristics than control CC, while CC with 75WC gave softer products. This could be due to the

presence in whey cream, of milk fat globule membrane fragments or higher levels of denatured whey proteins which may interfere with structure development.

Keywords: cream cheese, texture, whey cream

Characterization of Brazilian Type Cream Cheese with Addition of Isolated Soy Protein and Inulin

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Brazilian cream cheese is a typical Brazilian dairy product obtained by the fusion of the curdled mass, cooked or not, de-wheyed and washed, obtained by acid and/or enzymatic coagulation of milk, added with milk cream and/or butter and/or anhydrous fat or "butter oil". Aiming to obtain healthy products, companies manufacturing Brazilian cream cheeses have been using bioactive ingredients to create new products, which has a flavor similar to that of the cream cheese, and with a functional appeal.
The effect of ingredients (milk cream, hydrogenated vegetal fat, isolated soy protein and inulin) was studied on physicochemical characteristics of twenty different formula of cream cheese.
The curdled mass was obtained by direct acidifying the skimmed milk using lactic acid, de-wheying and washing the curd until pH 5.2. Hydrogenated vegetal fat (6.0 to 10.0 %), isolated soy protein (0.5 to 1.5 %), according to the trial, sodium chloride, melting salt, nisin and water were added to this mass. These ingredients were heated to 75ºC and mixed continuously in a dairy blender during 2 minutes. Following this, the milk cream (2.0 to 6.0 %) and inulin (3.8 to 6.3 %), according to the trial, whey protein concentrate, tapioca starch, xanthan gum and water were added. The product was mixed for an additional 2 minutes and heated to 90-95ºC and mixed continuously during 2 minutes. The product was cooled down and stored at 8 ºC for 12 h, when analytical evaluations were done.
The product presented of pH value ranging from 6.26 to 6.38, 0.24 to 0.39 % acidity, 72.9 to 100.4 g kg^{-1} protein, 16.2 to 17.7 g kg^{-1} ash, 80 to 120 g kg^{-1} fat and 24.26 to 39.76 % fat in dry matter. Among the samples of Brazilian cream cheese with addition of functional ingredients, some formulas had resulted in products with similar quality compared to the commercial counterparts, showing suitable viscous texture, spreadability, aroma, color and brightness.

Keywords: bioactive ingredients, functional cheese, cream cheese

Aroma Changes During Processing of Cheese Powders

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Cheese powders are used as flavouring ingredients in a wide variety of foods. In cheese powder production, cheese is disintegrated and melted with water, and sometimes with emulsifying salt. The blend is pasteurised and subsequently most of the water is removed by spray drying. These processes will inevitable change the aroma profile of the product. The flavour profile and intensity of the final cheese powder will of course also depend on the type and age of the cheese(s) used. To what extend aroma changes takes place during cheese powder processing has not previously been published.
The aim of this study was to analyse how the different steps in cheese powder production affect the aroma profile. Samples were collected from an industrial cheese powder plant: 1) three cheeses, a

young and a mature Danbo (Danish semi-hard) cheese as well as an Emmentaler cheese, 2) the corresponding processed cheese blends, each pasteurised at three different time/temperature schemes, and 3) the final cheese powders. Aroma compounds were isolated from the samples by dynamic headspace collection and analysed by GC-MS.
A total of 98 aroma compounds were identified in the samples. Expectedly, there were large differences in the aroma profiles of the three cheeses.
Pasteurisation of the cheese blends lead to some loss of aroma compounds, probably due to evaporation and thermal degradation. Increased pasteurisation temperature resulted in increased loss of many aroma compounds.
The most substantial loss of aroma compounds, however, occurred during spray drying as considerable amounts of aroma compounds evaporate together with the large amount of water removed. Nevertheless, higher concentrations of most of the aldehydes and some pyrazines were found in the cheese powders than in the pasteurised blends. These compounds may have been formed by chemical reactions such as lipid oxidation or Strecker degradation of amino acids. Between cheese powders, smaller differences in aroma composition were observed than within the original cheeses and within the pasteurised cheese blends, respectively.

Keywords: cheese powder production, processing steps, aroma changes

Zein Coating of Italian Gorgonzola PDO Cheese has Prophylactic Antimicrobial Properties

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Zein, a natural corn protein, is applied as a coating film to provide a moisture or gas barrier for nuts, meats or fruits. Gorgonzola PDO cheese is a blue-veined cheese produced in North Italy and consumed worldwide. Because the application of high pressures or detergents is not allowed by the PDO disciplinary law, the process of Gorgonzola PDO cheese ripening is constrained by efforts to reduce pathogenic bacterial contamination. In this work, we used a zein coating as a prophylactic method to prevent the presence of undesirable microorganisms on Gorgonzola surface. Gorgonzola PDO rinds were coated with both: zein alone, and with zein plus lactic acid. We evaluated the main microbiological parameters and some parameters of composition to investigate the influence of these films on cheese characteristics. Microbiological analyses revealed that both coatings (with and without lactic acid) significantly reduced the bacterial counts on cheese surface. Likewise, neither coating affected the internal microflora counts of beneficial cheese-ripening bacteria. Electrophoresis experiments showed a decreased protein hydrolysis on the samples treated with zein plus lactic acid compared to the control (non-coated). This result was confirmed by the analysis of free fatty methyl esters, which showed reduced lipolysis. Because the zein plus lactic acid coating induced modifications on the end product, it was discarded from the study. Conversely, zein film alone showed a good potential as coating agent for typical cheeses, reducing the external counts without affecting the cheese ripening time and characteristics. Further studies will focus on the ability of this film to inhibit the growth of specific pathogenic bacteria.

Keywords: edible films, cheese, zein

Protein Oxidation of Cheeses with Different Content of Fat

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The aim of the study was to investigate which mechanisms are involved in the formation of oxidation products in model cheese with different fat content (6 and 26 %) after exposure to fluorescent light. Cheeses packed in vacuum and in atmospheric air, respectively, were exposed to light for a period of 14 days. Riboflavin is believed to be the primary photo sensitizer in the cheeses, and complete loss of this compound was observed within the first two to three days of photo oxidation. The formation of dityrosine was negatively correlated with loss of riboflavin, and a plateau was reached within the first two to three days of photo oxidation. The formation of dityrosine and lipid peroxides was very much dependent on the content of fat in the cheese and on the concentration of oxygen in the packaging, while the formation of dimethyl disulfide (DMDS) was less dependent on the concentration of oxygen. Hence a high content of DMDS was also seen in the vacuum-packed cheeses. Reduced oxygen content during storage may therefore not by itself be able to avoid the formation of off-flavours from the oxidation of proteins.

Keywords: protein oxidation, fat, cheese, packaging

Evaluation of Two Packaging Systems on the Ripening Characteristics of Turkish White Cheese

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Turkish White cheese is the most popular cheese made and consumed in Turkey. A common feature of Turkish White cheese technology is that ripening occurs in brine and lasts from a few weeks up to three months. It has been manufactured and packaged in polystyrene cups with brine or cryovac containers in vacuum on a large scale in well-organized dairy plants.
In the study, the effect of two packaging systems on biochemical and textural characteristics, sensory properties and color of Turkish White cheese was investigated throughout 90 days of ripening. Full-fat Turkish White cheeses were manufactured in a modern dairy plant (Pinar Dairy Products) from cows' milk containing 3.3 % milk fat and packaged in polystyrene cup with brine containing 12 % salt or cryovac plastic material under vacuum. Turkish White cheese packaged into cryovac container had higher contents of total solids, milk fat, total nitrogen and water-soluble nitrogen than the cheese packaged in polystyrene cup during ripening ($p < 0.05$). The rate of proteolysis in terms of water-soluble nitrogen was also affected by the packaging system. Higher ripening index was obtained in cheeses with cryovac container ($p < 0.05$).
Naturally, significant differences in the level of salt were obtained between cheeses, higher content of salt was found in cheese packaged in polystyrene cup than cheese with croyovac container depending on the presence of brine ($p < 0.05$).
With regard to textural characteristics, packaging in cryovac container resulted in cheeses with significantly higher hardness. In the ripening period until 60th day, hardness decreased in both cheese samples packaged in cryovac or polystyrene container ($p < 0.05$). Whereas, Turkish white cheeses with polystyrene cup received higher sensory scores especially in terms of flavor. Both flavor and appearance scores decreased during the ripening period of cheeses. Packaging system had also a significant influence on the color values of the product.

Keywords: Turkish White cheese, packaging, brine, ripening.

Preparation of a Homogeneous, Standardised, Cheese-Like Matrix Usable as Reference Material

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Laboratories have to demonstrate their ability to generate reproducible and comparable testing results. This requires traceable methods with know measurement uncertainties. The use of appropriate reference material or the evaluation of the method in a ring trial or proficiency testing is needed to realize the requirements. In both cases a sufficient homogenous and stable reference material is necessary which moreover should have properties quiet close to those of the routine samples; this in order to include the often difficult sampling steps. For cheese loafs no proofed procedure exists.
Tests dealt with the preparation of a standardised cheese-like matrix usable as reference material with or without addition of substances or bacteria for chemical, enzymatic and microbiological testing as well as for ring trials or proficiency testing. The homogenization procedure proofed to be appropriate. For the evaluation of the homogenization procedure different parameters can be used but sodium proofed to be the best. Parameters already being homogeneously distributed in the cheese loaf or partly being below detection limits are not suitable.

Keywords: cheese, reference material, homogeneity, sodium