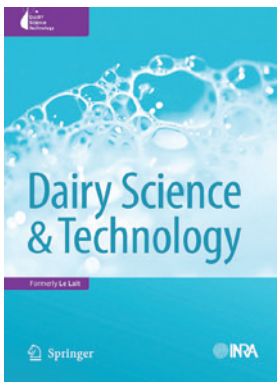

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65 rue de Saint Brieuc
F-35042 Rennes, France.
dst@rennes.inra.fr

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This international journal, first issued in 1921 (*Le Lait*), publishes peer-reviewed original articles, notes, and review papers on all aspects of dairy science and technology, i.e. microbiology, biochemistry, physico-chemistry, transformation procedures and nutritional qualities of milk and dairy products, including milk from bovine or non-bovine species and human milk. Papers related to milk production are not in the scope of the journal, unless there is a clear relationship to dairy technology, human health or final product quality. Special issues are also dedicated to scientific meetings in the fields. All papers are in English with abstracts in Chinese.

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A Special Issue of the journal will be dedicated to the **8th Cheese Symposium 2011**, held on 28-29 September at Cork. All submissions will go through the normal peer review process of the journal.

Magalie Weber, Managing Editor of *Dairy Science & Technology*, will be attending the symposium and will be happy to answer any queries related to the journal.

The papers selected for publication will then be offered a free access on the journal website at http://www.springer.com/13594 and on SpringerLink.

Contents

Dear Delegates,

On behalf of the scientific and organising committees I am delighted to welcome you to the 8th Cheese Symposium jointly hosted by Teagasc, University College Cork and INRA, the French National Institute for Agricultural Research.

The aim of this Symposium is to cover the most recent developments in fundamental and applied research in cheese in the following thematic areas: Flavour Development, Diversification, Health and Nutrition and Quality.

The Symposium provides a unique forum for both academia and industry to share experiences, knowledge and concepts on the latest developments and applications of cheese research and will hopefully serve as a catalyst to for new collaborations and friendships.

I hope that you enjoy your time in Cork and get to experience its unique culture and traditions.

Yours Sincerely,

Kieran Kilcawley, Chair of organising and scientific committees

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Valérie Gagnaire, INRA (FR)

Mary McCarthy Buckley, UCC (IE)

Scientific Programme

DAY 1 WEDNESDAY 28/09/2011

08.00–09.20	Registration and Coffee
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09.30–09.50	**John Jordan (Irish Dairy Board)** ***New Cheese Markets: UK/Europe/US Perspectives***

Session 1: Flavour Development

Chairperson: Martin Wilkinson, University of Limerick

09.50 – 10.25	**BART WEIMER (UNIVERSITY OF CALIFORNIA, USA)** ***Physiological role of Starter Bacteria in Cheese Flavour Development***
10.25 – 10.45	Use of microfluidization as mechanism to create attenuated LAB with increased metabolic/enzymatic activity to enhance cheese flavour development **Kieran Kilcawley, TFRC, Moorepark, Ireland.**
10.45 – 11.05	Application of enzymes to cheese foods – opportunities and hurdles **Tim Coolbear, Fonterra Research Centre, New Zealand.**
11.05 – 11.30	Coffee and Poster Viewing
11.30 – 12.00	**ANNE THIERRY (INRA, FRANCE) & JOHN HANNON (TEAGASC, IRELAND)** ***Cheese Flavour: Formation and Analysis***
12.00 – 12.20	Hydroxy acid dehydrogenase activities of cheese relevant *Lactobacillus helveticus* strains. **Soila Kananen, University of Copenhagen, Denmark.**
12.20 – 12.40	Typicality and geographical origin Markers of protected origin cheese from The Netherlands revealed by PTR-MS. **Martin Alewijn, Institute of Food Safety, Wageningen, Netherlands.**
12.40 - 12.45	A Matter of Taste, Agilent Presentation **Ken Brady**
12.45 – 14.00	**Lunch and Poster viewing**

Session 2: Cheese Diversification

Chairperson: Phil Kelly, TFRC, Moorepark

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14.35 – 14.55	Factors relating to the development of a pink discolouration defect in commercial cheese – A review **David Daly, TFRC, Moorepark, Ireland.**
14.55 – 15.15	*Lactobacillus helveticus*: in situ proteolytic activity and stretchability of Swiss-Type Cheese **Valerie Gagnaire, INRA, Rennes, France.**
15.15 – 15.45	Coffee and Poster Viewing
15.45 – 16.15	**JEAN-RENÉ KERJEAN (ACTILAIT, FRANCE)** ***Recent Developments in Hard and Semi-Hard Cheese Technology: Control of Quality by Mastering Interactions Between Curd and Ecosystems.***
16.15 – 16.35	A protocol for the construction of non-bitter Direct Vat Inoculation blends of *Lactococcus lactis* for use in semi-hard Gouda type cheese manufacture. **Jonathan Goodwins, Danisco, France.**
16.35 – 16.55	Unraveling fungal diversity in milk and determining the contribution of fungi to cheese ripening **Steve Labrie, Université Laval, Quebec, Canada.**
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Scientific Programme

DAY 2 THURSDAY 29/09/2011

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Chairperson: Tom Beresford, TFRC, Moorepark

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16.20 – 16.35	Migration of triclabendazole residues from raw milk to cheese **Clare Power, TFRC, Moorepark**
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	Symposium Close

SESSION 1:
Flavour Development

Chairperson:
Martin Wilkinson, University of Limerick

Abstracts for Oral Presentations

Notes

Keynote 1

WEDNESDAY 28/09/2011, 09.50 – 10.25

Physiological role of Starter Bacteria in Cheese Flavour Development.

Bart Weimer (Utah State University, USA)

Fermenting milk into cheese is a complex event that intertwines microbial metabolism of nutrients (sugars, protein and fat) to produce a complicated mixture of flavor compounds (small molecules, peptides, amino acids, and fatty acids) the summation of which produce impact flavors. Limited to no flavor production occurs without microbial metabolism, which led to active and persistent searches for starter cultures and flavor adjunct bacteria that produce accelerated and balanced flavor profiles during aging. While lactococci are a phylogenetically similar group of organisms the phenotypic traits are very different between strains, allowing a large selection of culture combinations to modulate cheese flavor production.

Genome analysis of various lactic acid bacteria reveals that flavor-production pathway regulation is complex and varied among genera and strains. Ultimately, flavor formation is controlled by gene expression regulation of existing pathways changes. Organisms respond to abiotic stress (i.e. salt, temperature, pH) via gene expression changes to modify flavor compound pathways. Nutrients (i.e. sugars, peptides, fatty acids) also impact expression of specific metabolic pathways to release simpler nutrients used for survival and production of small metabolites (i.e. flavor compounds) that impact the microbial community and overall flavor of cheese. Advanced molecular biology tools now enable examination of the entire bacterial community of cheese, as well as the metabolic pathways that are being expressed during aging. Addition of single strains alters the microbial community of cheese, which in turn changes the metabolite production of the entire community.

While the starter culture is a dominant group of organisms, it is only a fraction of the entire community that is also producing flavor compounds during aging. Identification of the non-culturable state in lactococci as part of the cheese community led to the discovery that some flavor molecules are only produced during this metabolic state, which explains production of branched chain fatty acids in hard cheeses. A significant portion of the lactic acid bacteria populations in hard cheeses is non-culturable lactococci that are producing these compounds only during sugar starvation, which is common after the initial aging period. In total, cheese flavor compound production is a diverse mixture of microbes producing small molecules in response to the stress of cheese production, the nutrient pool, the community structure, and their physiological state – all of which changes the metabolic productivity of the bacterial community.

Notes

Lecture 1

WEDNESDAY 28/09/2011, 10.25 – 10.45

Microfluidization as a mechanism to create attenuated LAB with enhanced metabolic and enzymatic activity.

C.M. Hayes[1], A.B. Yarlagadda[1], I.A. Doolan[2], M.G. Wilkinson[2], P.L.H. McSweeney[3] and K.N. Kilcawley[1].

[1] *Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland;*

[2] *Department of Life Sciences, University of Limerick, Castletroy, Limerick, Ireland;*

[3] *School of Food and Nutritional Sciences, University College, Cork, Cork, Ireland.*

kieran.kilcawley@teagasc.ie

Both starter and adjunct cultures are widely used to control and accelerate cheese ripening. The primarily role of adjunct cultures is to release intracellular peptidases that contribute both directly and indirectly to flavour development. It is thought that after cell lysis their direct contribution to cheese ripening ends. In addition the peptidases released on lysis appear to be unstable in the cheese environment and thus are only active for a short period.

However, recent research using advanced flow cytometry techniques has shown that most starter bacteria in Cheddar cheese enter a partially permeabilized or "dormant" state within the cheese over ripening rather than cell death. These cells cannot be enumerated by plate counting techniques as they are not viable growing cells, but it is postulated that they remain metabolically active and thus continue to significantly contribute to cheese flavour, as they remain the dominant species within the cheese. Thus, this study was undertaken to determine if "dormant" cells could be produced from lactococcal strains by attenuation using microfluidization.

Microfluidization is a high pressure technique where a cell suspension is pumped into an interaction chamber at high velocity which disrupts cells. The extent of disruption can be controlled by the type of chamber, pressure/flow rate and number of passes through the chamber. Disrupted cells were evaluated for viability, cell morphology using flow cell cytometry, enzymatic and metabolic activity using enzyme assays and subsequently assessed for flavour potential in a model system using sensory analysis and SPME GCMS. The study has highlighted the usefulness of microfluidization to create permeabilized/dormant LAB with enhanced metabolic and enzymatic activity, with potential for use as adjunct cultures in for flavour development in dairy systems.

Lecture 2

WEDNESDAY 28/09/2011, 10.45 – 11.05

Application of enzymes to cheese foods – opportunities and hurdles.

T. Coolbear.

Fonterra research Centre, Private Bag 11029, Dairy farm Road, Palmerston North, New Zealand.

tim.coolbear@fonterra.com

Enzymes have a long history of use in dairy. All traditional yoghurt-type and cheese-type products rely on enzymes in their manufacture, whether delivered through the activity of microorganisms or added as purified or semi-purified preparations. Over the last few decades a considerable amount of scientific understanding has developed with respect to how the various enzymes alter milk components to give structural, functional and flavour features. The science behind the art of cheese manufacture in particular has matured to the point where, for instance, knowledge of the enzyme activities involved in flavour formation is being applied to move flavour profiles to new and more commercially valuable paradigms – and move them more quickly, more consistently and more predictably.

However, enzymes can also cause problems in dairy – when they are present in the wrong place at the wrong time product integrity and quality can be quickly compromised. Thus the control of enzymes, from whatever source, in dairy processing is paramount to their use in the production of the high quality, value-added, nutritionally enhanced and more convenient foods that consumers are increasingly demanding. As the understanding of the potential of enzymes in dairy increases and hence the opportunities to create new dairy foods diversify, the complexity of the regulatory environment impacts increasingly strongly.

This paper provides some insights into both the opportunities and the hurdles, both scientific and non-scientific (including most commercially important "omics" approach: economics), that dairy processors are faced with in the application of enzymes to the delivery and development of cheese foods.

Notes

Keynote 2

WEDNESDAY 28/09/2011, 11.30 – 12.00

Cheese Flavour: Formation and Analysis

Anne Thierry[1] (INRA, France) & John A. Hannon[2] (Teagasc, Ireland)

[1] INRA, AGROCAMPUS OUEST, UMR1253 STLO, 65 rue de Saint-Brieuc, 35042 Rennes cedex, France;

[2] Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland.

Considerable knowledge has been accumulated on the biochemical processes occurring during the ripening of cheese, which have in turn major consequences on flavour development. The flavour profiles of cheeses are complex and are variety specific. According to the 'component balance' theory, the flavour of cheese is the result of the correct balance of a wide variety of volatile flavour compounds originating from the degradation of milk constituents during ripening.

Primary degradation of milk constituents leads to a whole range of flavour precursor compounds which are followed by a concerted series of secondary catabolic reactions that result in the formation of a variety of volatile compounds. Only some of the compounds formed directly contribute to cheese flavour. The formation of flavour compounds is mainly dependent on the enzymatic potentials of the microflora, their autolytic abilities and physiological states as well as on the manufacturing process, coagulant and indigenous milk enzymes. Some factors limiting the formation of flavour compounds have been recently identified.

The availability of improved sensory and volatile instrumentation methodologies for the analysis of volatiles have aided our understanding and characterisation of cheese flavour. The analysis of the volatile profile of cheeses relies heavily on the extraction technique used (DHS, SHS, SPME, Thermal desorption, ITEX, HS-trap) as well as the sensitivity of the detectors. The use of GC-olfactometry to identify the odour activity of separated compounds has also served to improve our understanding of the contribution of individual compounds to the perception of flavour. However, to fully elucidate cheese flavour, descriptive sensory analysis should be conducted in tandem.

New perspectives offered by analytical developments in -omic approaches can lead to a better knowledge of the potentials of micro-organisms (genome sequence), their global activity and physiology in cheese under starvation or at cold temperature (transcriptomics, flow cell cytometry, release of stress proteins observed by proteomics).

Notes

Lecture 3

WEDNESDAY 28/09/2011, 12.00 – 12.20

Hydroxy acid dehydrogenase activities of cheese relevant *Lactobacillus helveticus* strains.

S. Kananen and Y. Ardö.

Department of Food Science, Faculty of Life Sciences, University of Copenhagen, Denmark.

soka@life.ku.dk

Lactobacillus helveticus contributes to flavour formation in cheese by amino acid catabolism that begins with a transamination to form α-keto acids, which are degraded further to volatile flavour compounds or to less flavour active hydroxy acids. High activity of the hydroxy acid dehydrogenase (HADH) may therefore decrease the amount of volatile flavour compounds formed from α-keto acids. *Lb. helveticus* have typically aminotransferase activity on aromatic and branched-chain amino acids with the highest activity against the aromatic amino acids.

The objective of this work is to investigate HADH activities in the catabolism of various α-keto acids in different *Lb. helveticus* strains. The activities were analysed in crude cell free extracts by measuring enzymatic reactions using a microtitre plate scanner spectrophotometer. The results show that the HADH activities on β-phenylpyruvic acid, the α-keto acid from Phe, may be 100 times higher than the activity on α-ketoisocaproic acid, from transamination of Leu. These results will be confirmed by further analysis of eight cheese related *Lb. helveticus* strains, and the catabolism of various α-keto acids will be included.

Results are discussed in relation to other enzymes involved in amino acid catabolism, because properties of HADHs have an influence on flavour formation balance. It could be concluded that a much higher amount of volatile compounds from Leu than from Phe should be expected to be formed by *Lb. helveticus* in cheese.

Lecture 4

WEDNESDAY 28/09/2011, 12.20 – 12.40

Typicality and geographical origin markers of protected origin Cheese from The Netherlands revealed by PTR-MS.

M. Alewijn[1], S. Galle[1], A. Koot[1], C. Soukoulis[2], L. Cappellin[2], F. Biasioli[2], S. van Ruth[1].

[1]RIKILT, Institute of Food Safety, Wageningen University and Research Centre, Akkermaalsbos 2, 6708 WB, Wageningen, the Netherlands;

[2]IASMA Research and Innovation Centre. Fondazione Edmund Mach, Food Quality and Nutrition Area, Via E. Mach 1, 38010 San Michele all'Adige (TN), Italy.

martin.alewijn@wur.nl

Volatile fingerprints of 30 cumin cheese samples of artisanal farmers' cheese of Leiden with EU Protected Designation of Origin (PDO) and 29 (similar) cumin cheese samples of varying commercial Dutch brands without PDO protection were used to develop authentication models. The headspace concentrations of the volatiles, as measured with high sensitivity proton-transfer mass spectrometry, were subsequently subjected to partial least-squares discriminant analysis (PLS-DA).

Farmers' cheese of Leiden showed a distinct volatile profile with 27 and 9 out of the 60 predominant ions showing respectively significantly higher and lower concentrations in the headspace of the cheese in comparison to the other cumin cheeses. The PLS-DA prediction models developed classified in cross-validation 96% of the samples of PDO protected, artisanal farmers' cheese of Leiden correctly, against 100% of commercial cumin cheese samples. The characteristic volatile compounds were tentatively identified by PTR-time-of-flight-MS. A consumer test indicated differences in appreciation, overall flavour intensity, creaminess, and firmness between the two cheese groups.

The consumers' appreciation of the cumin cheese tested was not influenced by the presence of a name label or PDO trademark.

Notes

WEDNESDAY 28/09/2011, 12.40 – 12.45

Agilent Presentation

A Matter of Taste

Ken Brady

Agilent Technologies UK Ltd.

Particular groups of compounds have a huge effect on the flavour of foods.

Sulfur containing compounds are of particular interest due to their extremely low sensory threshold, but do however exist at very low levels in most foodstuffs.

The Agilent 7200 GC QToF can be used to identify unknown compounds and quantify their relative abundances in a heavy matrix. The High Resolution capacity and speed of the 7200 allows for a rapid, non targeted screening approach, while maintaining the necessary pg on column sensitivity with a wide quantitative dynamic range.

Notes

SESSION 2:
Cheese Diversification

Chairperson:
Chairperson: Phil Kelly, TFRC, Moorepark

Abstracts for Oral Presentations

Notes

Keynote 3

WEDNESDAY 28/09/2011, 14.00 – 14.35

Successful Examples of Cheese Diversification (Dubliner cheese and Specialty cheese in the US)

Professor John A. Lucey: (Director of Wisconsin Centre of Dairy Research)

Director of the Wisconsin Centre for Dairy Research,
University of Wisconsin-Madison, 1605 Linden Drive, Madison, WI 53706-1565, USA.

The cheese industry worldwide continues to grow as production facilities get larger and more automated. At the same time there is growing interest in producing differentiated products that could appeal to some consumer segment, e.g. with distinctive flavours/appearances, produced organically, or from grass-based production systems. The cheese industry in Ireland produces a relatively narrow range of cheese varieties, at least in its larger production facilities. Many other varieties are produced at the farmstead level.

This talk will discuss some successful examples of cheese diversification that has added value to the farmers as well as to the cheesemakers. Dubliner cheese development will be discussed as to why this was successful while many other attempts have not. The author will share some personal insights/ perspectives of the Dubliner story.

In Wisconsin specialty cheese has shown phenomenal growth in the past 20 years and Wisconsin now produces about 50% of all specialty cheese in the US and this segment is around 20% of total cheese production in the state. The University of Wisconsin-Madison/Wisconsin Centre for Dairy Research played a key role in this revolution primarily by providing specialized training/short courses/support to the cheese industry on these new artisan cheeses. Experts were brought in from around the world to provide hands-on insights into cheese varieties not previously made in the state. More recently the creation of the Dairy Business Innovation Centre helped to provide assistance to new cheesemaking ventures especially on the business planning, marketing and labelling aspects.

Notes

Lecture 5

WEDNESDAY 28/09/2011, 14.35 – 14.55

Factors relating to the development of a pink discolouration defect in commercial cheese – A review.

D.F.M. Daly[1,2], P.L.H. McSweeney[2] and J.J. Sheehan[1].

[1]*Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland;*

[2]*School of Food and Nutritional Sciences, University College, Cork, Ireland.*

diarmuid.sheehan@teagasc.ie

Development of a pink colour defect is an intermittent but persistent defect in a wide range of ripened cheese varieties (Swiss, Cheddar, Grana and Italian-types) with or without the colourant annatto. This defect results in down-grading or rejection of product and consequential economic loss to producers. Pink discolouration can manifest in a number of ways e.g. patches at the surface or within the cheese block, or as a uniform pink border occurring below the surface of cheese blocks.

A number of previous studies have investigated pink discolouration in cheese, however little consensus exists as to what the discolouration is and what the underlying causes are. This review seeks to provide a comprehensive overview and interpretation of the underlying factors associated with the defect to date for both research and commercial applications. In cheeses without added colourant, pink discolouration has been associated with certain strains of thermophilic lactobacilli, and particularly on their influence on cheese redox potential. Development of the pink defect has also been attributed to the Maillard reaction occurring in the presence of galactose, low molecular weight proteolysis products and the presence of a critical oxygen concentration. It has also been proposed that phenolic compounds may be responsible for development of pink-brown or dark brown discoloration in a number of cheese varieties.

In cheeses with added colourant (annatto) the development of pink discolouration has been associated with; bleaching of annatto due to factors such as varying pH levels within the cheese matrix (particularly > pH 5.4); oxidation of bixin in storage under high intensity fluorescent lights in display cabinets; the presence of oxygen; variations in redox potential; interactions between nitrates and annatto in plastic surface coating and also due to interactions between colourants and high heat treatment during processed cheese manufacture.

Lecture 6

WEDNESDAY 28/09/2011, 14.55 – 15.15

Lactobacillus helveticus: *in situ* proteolytic activity and stretchability of Swiss-Type Cheese.

L. Sadat-Mekmene[1,2], R. Richoux[3], L. Aubert-Frogerais[1,2], M.-N. Madec[1,2], C. Corre[1,2], M. Piot[1,2], J. Jardin[1,2], S. Lortal[1,2] and V. Gagnaire[1].

[1]*INRA, UMR1253, Science et Technologie du Lait et de l'OEuf (STLO), F-35042 Rennes, France;*

[2]*Agrocampus Ouest, UMR1253, STLO, F-35042 Rennes, France;*

[3]*Actilait, BP 50915, F-35009 Rennes, France.*

valerie.gagnaire@rennes.inra.fr

In contrast to other lactic acid bacteria, *Lactobacillus helveticus* can possess two cell-envelope proteinases (CEPs) called PrtH2 and PrtH. These CEPs exhibit different cleavage specificity on pure alpha s1-casein, and questions remain on their action in cheese and their potential contribution in stretchability. Indeed, *L. helveticus* is known to enhance stretching properties in contrast to other thermophilic lactobacilli, such as *L. delbrueckii* subsp. *lactis*.

The aim of this work was to investigate the proteolytic activity of *L. helveticus* strains in cheese matrix according to the number of their CEPs and the cheese stretchability. Two strains were selected, ITGLH77 and ITGLH1 which possess one CEP, PrtH2, and two CEPs, PrtH and PrtH2, respectively. Proteolysis was monitored during ripening: i) casein hydrolysis by urea-PAGE; ii) peptide pattern of the cheese aqueous extracts first by SDS-PAGE and second by RP-HPLC. Three chromatographic fractions were collected and peptides identified by RP-HPLC coupled on-line with tandem mass spectrometry. In parallel, the dynamic of stretchability of Emmental cheese was measured. The microstructure of the strands formed was observed by confocal laser scanning microscopy.

The stretchability of Emmental was significantly higher in cheese manufactured with strain ITGLH77. By using Principal Component Analyses, the stretching properties were found to be correlated with the presence of peculiar peptides derived from primary proteolysis and mainly from alpha s1-casein, hydrophobic peptides, (> 20 amino acid residues), which were accumulated in cheese manufactured using strain ITGLH77. These peptides seemed to be less hydrolysed by this strain, as previously observed *in vitro*, on pure alpha s1-casein. A lesser extent of proteolysis in cheese enhanced stretching capabilities. Further studies will use modified strains having only PrtH or PrtH2 on the cell surface of lactobacilli strains to understand how to orientate such cheese functionalities.

Notes

Keynote 4

WEDNESDAY 28/09/2011, 15.45 – 16.15

Recent developments in Hard and Semi-Hard Cheese Technology: Control of Quality by Mastering Interactions between Curd and Ecosystems.

Jean-René Kerjean (Actilait, France)

Actilait, BP 50915, F-35009 Rennes, France.

Milk protein standardisation is a critical step in the manufacture and quality of cheese. It is critical that the levels of milk casein and denatured whey protein, and their interaction are controlled to ensure consistency in the composition and structure of the matrix. This standardisation helps to maintain the regularity of all the draining parameters and to simplify the standard operation procedures in vats, in pressing and in cheese maturation. Many process technologies (UF, diafiltration, microfiltration) and milk protein powders are now available for this purpose.

Microfiltration is now a real alternative to pasteurisation in the preparation of cheese. While MF removes all bacterial flora, it is particularly effective in eliminating C *tyrobutyricum*, and minimising the risk of spoilage through secondary fermentation in pressed cheeses. Moreover, MF protects the indigenous milk enzymatic activity of milk, enabling it to make a contribution to flavour development.

The role of the natural ecosystems in traditional raw milk hard and semi-hard cheese is widely confirmed, their technological control is deeply improved and their place is no longer questioned in the cheese technological landscape.

A better understanding on the role of fat structure in hard and semi-hard cheese (due to the new microscopy techniques), its contribution to texture and cooking properties is now known. Microscopy has also contributed to a deeper understanding of the relative structure of the fat/protein network in different cheese-types, and how it affects the distribution of bacterial colonies, their physiology, and possible effects on quality.

It is particularly important to optimise the interface between research and industry, so as to embed the science underpinning the key mechanisms controlling quality, and consistency of key performance indicators, and to minimise quality defects. Advances in new mathematical models have the potential to reproduce the reasoning of defects by cheese experts, it will be an opportunity to preserve and enrich the technological know-how, worldwide.

Notes

Lecture 7

WEDNESDAY 28/09/2011, 16.15 – 16.35

A protocol for the construction of non-bitter Direct Vat Inoculation blends of *Lactococcus lactis* for use in semi-hard Gouda type cheese manufacture.

J.D. Goodwins, L. Pellerin, K. Jourdon, E. Manoury and A. Mornet.

Danisco SAS, BP10, 86220, Dangé St. Romain, France.

jonathan.goodwins@danisco.com

A system has been developed, optimising Lactococcal strain combinations for the purpose of avoiding the accumulation of hydrophobic peptides during cheese ripening. This work involved a combination of analytical and applicative approaches along with conventional strain development.

A novel laboratory scale cheese making process with 12 cheese vats in parallel (VIP) was employed with subsequent peptide HPLC chromatography and sensory analysis on the resultant cheeses after ripening. A means of identifying the "bitterness potential" of any particular acidifying strain was established and confirmed with the VIP. The effect of combining strains with different bitterness potentials was then explored and the resultant combinational effects observed were then exploited to improve the effectiveness of the Direct Vat Inoculation approach in European style semi-hard cheese.

Lecture 8

WEDNESDAY 28/09/2011, 16.35 – 16.55

Unraveling fungal diversity in milk and determining the contribution of fungi to cheese ripening.

S. Labrie.

Department of Food Sciences and Nutrition, Nutraceuticals and Functional Foods Institute (INAF),

STELA Dairy Research Centre, Department of Food Sciences and Nutrition, Université Laval, Quebec City, QC, Canada

Steve.Labrie@fsaa.ulaval.ca

The microbiota of milk influences milk quality and the characteristics of the resulting cheeses. Many studies have investigated deleterious bacteria in milk as well as positive non-starter lactic acid bacteria (NSLAB). However, little attention has been paid to milk non-starter fungal microflora.

This conference will review approaches that can be used to characterize fungal diversity in milk and specialty cheeses. Recent studies have used new molecular techniques to monitor the development of ripening yeasts and molds in specialty cheese, including real-time PCR and T-RFLP genetic profiling. The species diversity of the native fungal microflora of raw milk has also been investigated.

Milk from 19 Quebec dairy farms that produce their own specialty cheeses was analyzed. One hundred and eleven raw milk samples were analyzed, and 505 fungal isolates were identified. Yeasts made up 67% of the isolates and were assigned to 37 species. Mold isolates (33%) were assigned to 33 species. A semi-quantitative analysis of milk from four dairy farms over a 5-month period revealed that the fungal microflora of the milk was relatively stable, although it varied from farm to farm. Interestingly, the fungal ecosystem of the milk from one farm was dominated by yeast species often use as ripening agents while the milk from another was dominated by undesirable molds. An analysis of one-week-old raw milk cheeses identified non-starter yeasts and molds (NSYM) that could potentially influence the cheese ripening process. A multi-locus sequence typing (MLST) approach was used to show that the non-starter yeast *Issatchekia orientalis* found in the milk from one dairy farm persisted into one-week-old raw milk cheese.

Altogether, these results suggested that the native fungal community in milk is a source of non-starter yeasts and molds that are detectable in specialty cheeses and may contribute to cheese ripening.

Notes

Lecture 9

WEDNESDAY 28/09/2011, 16.55 – 17.15

Effect of altering curd washing on the biochemistry, quality and yield of Cheddar cheese.

J. Hou[1], J.A. Hannon[1], T.P. Beresford[1], P.L.H. McSweeney[2] and T.P. Guinee[1].

[1]Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland;

[2]Department of Food and Nutritional Sciences, University College, Cork, Ireland.

tim.guinee@teagasc.ie

Relatively little information is available on the effects of seasonal variations in milk lactose, which can range from ~ 4 to 4.8% (w/w) over lactation, on the quality of Cheddar cheese. The aim of the current study was to investigate the effects of curd washing, as a means of varying the concentration of lactose plus lactic acid in cheese moisture (LLAMC), on the quality of Cheddar cheese.

The level of curd washing in the cheese vat was varied to give target levels (%, w/w) of LLAMC in the final cheese of 5.3 (control), 4.5, 4.3 and 3.9; these values correspond to expected LLAMC levels in non-washed cheeses made from milks with lactose levels of 4.8 (control), 4.6, 4.3 and 3.8% (w/w), respectively. The cheeses were manufactured in triplicate from mid-lactation milk and analysed over a 270-day ripening period.

Increasing curd washing significantly reduced mean levels of lactose, total lactate and LLAMC over the ripening period and the reduction in LLAMC coincided with a significant increase in pH by ~0.3-0.4 at times greater than or equal to, at 90 days. For all cheeses, the LLAMC was ~ 1.0-1.6 units lower than predicted based on concentration of lactose in the cheese milk and the degree of lactose removal by curd washing. Otherwise, alteration of curd washing generally did not affect gross composition and the counts of starter or non-starter lactic acid bacteria.

Curd washing resulted in a non-significant numerical decrease in cheese yield, approximating 0.08kg cheese (per 100kg milk) when washing at a rate of 0.1kg per kg of cheese milk. Grading of the cheese indicated that curd washing resulted in cheeses that were less acid, sweeter and creamier tan non-washed cheese.

Notes

SESSION 3:
Health and Nutrition/Fat & Salt reduction

Chairperson:
Tom Beresford, TFRC, Moorepark

Abstracts for Oral Presentations

Notes

Keynote 5

THURSDAY 29/09/2011, 09.50 – 10.25

Health and Nutritional Aspects of Cheese with a Focus on Bioactive Peptides

Isidira Recio: (CSIC, Spain)

Institute of Food Science Research (CIAL) CSIC-UAM., Nicolás Cabrera 9, 28049 Madrid, Spain

i.recio@csic.es.

Cheese has an undoubtedly nutritional value as source of good quality proteins, fat, vitamins and minerals. For instance, cheese provides all essential amino acids (except methionine and cysteine) and it is an excellent source of bioavailable calcium, phosphorous, zinc and magnesium. In addition, it is practically free of lactose which is an advantage for lactose intolerant population.

During cheese ripening, milk proteins are hydrolysed into a large variety of peptides and some of these can exhibit different biological activities. Although, some of the health benefits derived from cheese consumption are related to its mineral content or to specific fat components, we will review the presence of bioactive peptides in cheese and their potential role in health. Among others, the presence of casein phosphopeptides, that can improve mineral absorption and exert anticariogenic properties, has been reported in different cheese types. Several short peptides with antihypertensive properties can be released during cheese manufacture and maturation and their contents can be assessed by using advanced analytical approaches.

The presence of these peptides with angiotensin converting enzyme-inhibitory activity has also been related with a potential anti-obese effect or with a possible role in building up mineral density. Several opioid peptides or longer fragments containing these sequences have been also detected in cheese. Food digestion is now receiving a special interest and cheese can be seen as an important source of precursor longer peptides where the active form will be released after gastrointestinal digestion.

Further studies are needed to confirm these potential activities, substantiate health claims with sufficient evidence, study mechanisms of action or identify novel peptide sequences with different physiological effects.

Notes

Lecture 10

THURSDAY 29/09/2011, 10.25 – 10.45

Effect of an exopolysaccharide produced by a strain of *Lactococcus lactis* on the manufacture and ripening of half-fat Cheddar cheese.

N.E. Costa[1], D.J. O'Callaghan[1], J.A. Hannon[1], T.P. Guinee[1], P.L.H. McSweeney[2] and T.P. Beresford[1].

[1]*Moorepark Food Research Centre, Teagasc, Fermoy, Co. Cork, Ireland;*

[2]*Department of Food and Nutritional Sciences, University College, Cork, Ireland.*

john.hannon@teagasc.ie

The influence of starter-produced exopolysaccharide (EPS) on the manufacture, yield, composition, proteolysis, flavour, microstructure and functionality of half-fat Cheddar cheese was assessed. Two half-fat cheeses were manufactured using single strains of an EPS-producing starter (*Lactococcus lactis* subsp. *cremoris* DPC6532, EPS^+) and its non-EPS-producing genetic variant (*Lactococcus lactis* subsp. *cremoris* DPC6533, EPS^-), which differ only in their ability to produce EPS.

Results indicated that the EPS did not interfere with milk coagulation but had a significant effect on reducing syneresis. A significant increase in cheese yield was detected in the EPS^+ cheese, attributed to an increase in moisture content and MNFS, which consequently caused an increase in water activity. However, the water desorption rate at relative humidities ≤ 90% was also significantly higher in the EPS^+ cheese, indicating that this extra water was not strongly bound. TPA hardness and gumminess were successfully reduced while adhesiveness increased in the EPS^+ cheese, which resulted in very similar texture parameters to a full-fat cheese. Cooking properties were also significantly improved in the EPS^+ cheese.

Although primary proteolysis was significantly higher in the EPS^+ cheese throughout ripening, no significant differences were detected in the levels of volatiles or free fatty acids between the two cheeses. The microstructure of the cheeses, as observed by CRYO-SEM and CLSM, revealed a more open structure for the EPS^+ cheese, with the EPS located around the pores. The EPS, produced in milk at a rate of 322 g/L, had an average molecular weight of 280 KDa, was un-charged and composed of glucose and galactose at a ratio of 1.29:1.

These data suggest that as the EPS was un-charged, it did not interfere with coagulation but remained in the serum phase, binding extra water, thus increasing the moisture content and hence yield without adversely affecting cheese flavour.

Lecture 11

THURSDAY 29/09/2011, 10.45 – 11.05

Soft goat cheese enriched with polyunsaturated fatty acids: Manufacture, physico-chemical and sensorial characterizations.

J.-Y. Gassi[1,2], M. Thève[3], E. Beaucher[1,2], B. Camier[1,2], F. Rousseau[1,2], F. Gaucheron[1,2], L. Leboeuf-Schneider[3] and E. Lepage[3].

[1]*INRA, UMR1253, Science et Technologie du Lait et de l'Œuf (STLO), F-35042 Rennes, France;*

[2]*Agrocampus Ouest, UMR1253, STLO, F-35042 Rennes, France;*

[3]*CCPA, ZA Nord- Est du Bois de Teillay, F-35150 Janzé.*

jean-yves.gassi@rennes.inra.fr

The consumer is particularly attentive to nutritional factors which can affect their health. Particular attention is paid to the quantity and quality of lipids. Milk and dairy products are considered rich in saturated fatty acids (SFA). One way to decrease SFA and increase polyunsaturated fatty acids (PUFA) in dairy products is to modify the composition of milk by modifying the diet of animals. The aim of this work was to manufacture and characterize soft goats cheese made with milk enriched with PUFA.

Two groups of 30 Alpine dairy goats were fed with a traditional ration (control) or a ration enriched with PUFA (supplemented). The milks were characterized for total solids, contents in protein, fat and mineral, fatty acids profile and fat globule size distribution. The cheese making parameters were supervised and the cheeses were made in triplicate. Levels of proteolysis and lipolysis were monitored in the cheeses during ripening. Sensory evaluation was also performed.

Milk acidification and cheese drainage were similar. The biochemical modifications (proteolysis and lipolysis) during ripening were also equivalent. On the contrary, an increase in cheese yield of 5.7 % was observed with the enriched milk due to its higher fat and protein contents. At the same time, the cheese composition after 30 days of ripening was different. Thus, it was determined an increase in the concentration of PUFA (for example, the concentration of C18:3 (n-3) increased from 0.8 to 1.74 %) and a decrease in SFA (66.8 to 60.4 %). The sensory analyses results were similar for both cheeses.

This work showed that it was possible to make soft goats cheese with milk enriched with PUFA. The parameters of the cheese making and the final quality of the cheese were positively affected. The commercial development of this type of cheese is in progress.

Notes

Keynote 6

THURSDAY 29/09/2011, 11.30 – 12.00

Reduction of Fat and Salt in Cheese.
Reduced-fat/Reduced-salt cheese: approaches to quality improvement

Tim Guinee & Kieran Kilcawley (Teagasc, Ireland).

Moorepark Food Research Centre, Teagasc, Fermoy, Co. Cork, Ireland;

Manufacture of hard cheese involves a concentration of casein from ~ 2.7 % in the aqueous phase of milk to a concentration level in the range 35–50%. At this casein concentration, the aqueous system is "jammed", in the form of a structural continuum (matrix) of fused para-casein aggregates linked primarily through calcium/calcium-phosphate mediated crosslinks. The system is characterised by the occlusion of the serum phase within the casein aggregates, with little or none free to confer 'liquidity' to the cheese. Increasing structural jamming results in a more translucent, firm, chewy and rubbery cheese. The quality of reduced-fat hard cheese is frequently less acceptable than the full-fat equivalent because of the higher protein in moisture and higher pH, which leads to a hard/rubbery texture, lack of opacity, and in some cases an unbalanced flavour.

In contrast to fat, reducing salt content has only a relatively minor direct effect on the level of matrix, but affects the quality of the solvent (serum) permeating the matrix and its interactivity with the matrix and indirectly effects the levels and physiology of the microflora and the enzymatic activities in the serum. Salt reduction reduces pH, lowers casein hydration, increases moisture, increases water activity and enhances proteolytic activity; this results in a softer cheese and sometimes in bitter cheese.

Reducing both fat and salt in cheese adversely affects both the development and release of key compounds associated with cheese flavour, and thus flavour perception. Hence, key strategies in the manufacture of improved quality reduced-fat, reduced-salt cheeses require studying their interactive effects; optimization of the degree of structural jamming, lactate-to-protein ratio, pH (by alteration of the manufacturing process), the choice of starter cultures (acid profile, enzymatic/autolytic/salt sensitivity) and rennet (type and level).

Process parameters of relevance include: dilution of matrix by inclusion of non-interactive fillers; adjusting casein aggregation by factors such as milk heat treatment and pH; regulating degree of curd dehydration via control of cheesemaking process.

Notes

Lecture 12

THURSDAY 29/09/2011, 12.00 – 12.20

Impact of NaCl on ripening of Cheddar cheese as studied by moisture equalisation.

K.K. Moeller[1,2], F. Rattray[2], E. Hoeier[2] and Y. Ardö[1].

[1]*Faculty of Life Sciences, University of Copenhagen, Rolighedsvej 30, 1958 Frederiksberg C, Denmark;*

[2]*Chr. Hansen A/S, Boege Allé 10-12, 2970 Hoersholm, Denmark;*

kikm@life.ku.dk

Increasing demands for low/reduced-salt food challenges the cheese industry. The objective of this study was to investigate the impact of NaCl on flavour and texture development during Cheddar cheese ripening, thus establishing an approach for salt compensation. Unlike previous studies, the final moisture content was kept constant as a function of decreasing NaCl content.

Duplicate cheeses with four NaCl levels (0.85, 1.32, 1.74, and 2.36% (w/w), designated low-, reduced-, normal- and high-salt cheeses, respectively) were produced. The moisture content was equalised (37.7±0.1% (w/w)) by iterative parallel adjustments of the dimensions of curd grains and milled curds, scalding time/temperature and level of NaCl addition. Cheeses were analysed physico-chemically, microbiologically and sensorically (trained panel) over the course of nine months ripening.

Salt reduction resulted in faster acid production by the starter, as measured by pH and lactose/lactate levels at day one. Highest plasmin and highest chymosin activities were encountered in low-salt and high-salt cheeses, respectively. During early ripening, the former was correlated to higher γ-casein/proteose peptone levels as analysed by CE/LC-MS. Low/reduced-salt cheeses showed lower levels of intact β-caseins, αS1-caseins and higher amounts of hydrophobic peptides. Direct correlations were evident between %NaCl and intracellular aminopeptidase activities in cheese serum, which manifested in the free amino acid levels.

Due to high starter viability, non-starter counts were significant only in nine-month-old cheeses and predominantly in low-/reduced-salt cheeses. Water activity (aw=0.945-0.973), calcium and magnesium levels were inversely correlated, while the level of potassium was directly correlated to %NaCl. Uniaxial compression revealed similar textural properties, except for high-salt cheeses. PCA of sensory data significantly distinguished low-salt, reduced/normal-salt and high-salt cheeses. Low-salt cheeses received higher 'bitter' and lower 'umami', 'salt', 'cheddary' and hedonic meta-descriptors scores.

Adjustment of the cheese-make technology largely recovered high-quality textural properties of salt-reduced cheeses. Still, additional actions to re-establish a balanced proteolysis/peptidolysis and flavour formation are required.

Lecture 13

THURSDAY 29/09/2011, 12.20 – 12.40

Influence of proteolysis and amino acid release on quality of reduced-fat Cheddar cheese.

M.W. Børsting[1,2], K.B. Qvist[1], F.K. Vogensen[2], N.B. Jensen[1], J. Vindeløv[1] and Y. Ardö[2].

[1]*Chr. Hansen A/S, DK-2970 Hørsholm, Denmark;*

[2]*Department of Food Science, Faculty of Life Sciences, University of Copenhagen, DK-1958 Frederi.*

dkmew@chr-hansen.com

In spite of a considerable amount of research, it is still difficult to produce high quality, reduced-fat Cheddar. Here, the objective was to study how reduced-fat Cheddar (30 % fat in dry matter) structure may be influenced by choice of coagulant (bovine chymosin (BC) vs. camel chymosin (CC)) and adjunct culture (AB) (*Lactobacillus delbrückii*) selected to accelerated the amino acid release. CC is more specific and efficient in coagulating milk than BC, which makes it possible to reduce the dosage in cheese production. However, the proteolytic activity is lower, leading to longer ripening time, harder structure and lower bitterness.

Four Cheddar cheeses were produced repeated at four different days in a Latin square design: BC-AB, CC-AB, BC+AB, CC+AB. Peptide development, amino acid release and mechanical texture measurement were analysed six times during 28 weeks of ripening. Gross composition was analysed after eight weeks and sensory characteristics after 28 weeks.

BC±AB compared to CC±AB had a higher level of both αS1-casein (f1-23) and peptides derived from it. During ripening the differences in derived peptides were reduced in cheeses –AB and vanished in cheeses +AB. A significant higher amount of the bitter peptide β-casein (f193-209) was seen in cheeses with BC±AB compared to CC±AB during the whole ripening period. Stress at fracture was significant lower (softer structure) after 28 weeks in cheeses with BC±AB compared to CC±AB. This was confirmed by the sensory panel for cheeses –AB only. Strain at fracture was significantly lower (shorter texture) in +AB cheeses compared to –AB cheeses, which correlated with a significantly higher (4 x increase) amount of free amino acids.

CC reduced problems about bitterness in reduced-fat cheddar but did not soften the cheese. The selected AB vanished differences in αS1-casein (f1-23) degradation during ripening but due the high amino acids release the structure became shorter.

Notes

SESSION 4:
Cheese: From Quality to Concepts

Chairperson:
Paul McSweeney, University College Cork

Abstracts for Oral Presentations

Notes

Keynote 7

THURSDAY 29/09/2011, 14.00 – 14.35

Quality and safety of artisan cheese production system and consumer perception.

Guiseppe Licitra, (Corfilac, Sicily)

Giuseppe Licitra, CoRFiLaC Regione Siciliana, DISPA, Catania University.

Word Wide Traditional Cheeses have strong linkage to the territory of origin and are unique expression of the symbiotic interaction of human resource, culture of the communities and the nature. Every traditional cheese originates from complex systems tied to several "bio-diversity factors": the environment, the climate, the natural pasture, the breed of the animals, the use of raw milk and its natural micro-flora, the cheese-making technology with the unique role of humans, the historical tools, and the natural aging conditions. Artisan (traditional) products are almost banned under the "false" reason of "food safety". Researchers have reviewed published outbreaks associated with dairy products, and results indicate that raw milk cheeses are not riskier than cheeses made from pasteurized milk. Improper pasteurization, post-process recontamination, storage, and cross contamination are often reported as responsible for outbreaks.

Artisan cheeses cannot be identified with just the use of "raw milk"; a "multiplicity of practices" have the potential to make safe products.

The real meaning of the linkage with the territory is related to farming systems: grazing animals, non intensive production system, and strong connection with the environment. The results are higher product quality in terms of flavour and aroma profile, antioxidant and other health properties (α-tocopherol, β-carotene, retinol, CLA, PUFA, bioactive tripeptides, eicosapentaenoic acid, docosahexaenoic acid, ...). The defense of artisan cheeses has been encouraged also by the consumer purchasing behavior. Consumer interviews suggest that the first seven criteria on cheese purchase intention are: food safety, use of natural ingredients, health properties of products, local products, Protected Denomination of Origin, artisanal production and typical flavour. In agreement with other studies there is evidence that European consumers may trade-off some degree of inconvenience in the purchase, expensiveness and preparations of traditional food products in order to enjoy the specific taste, quality, appearance, nutritional value, healthiness and safety.

Notes

Lecture 14

THURSDAY 29/09/2011, 14.35 – 14.50

Effect of sodium chloride on the properties of a model cheese system.

I. Piska and T.P. Guinee.

Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland.

ivo.piska@teagasc.ie

Most rennet-curd cheeses are salted post coagulation, close to the end of manufacture, either by adding salt directly to, and mixing with, curd pieces that are subsequently moulded/pressed, or by placing the moulded cheeses in brine. The aim of the current study was to investigate the effect of adding salt at different levels in the range 0 to 2.3% prior to rennet-acid-induced gelation on the properties of model cheeses.

Model cheeses (~ 47 % dry matter, protein-to-fat ratio ~ 1) were prepared in triplicate by dispersing milk protein in an aqueous-based solvent comprised of water, fat, lactose and sodium chloride. The dispersion, which was a liquid pre-cheese with the dry matter content of the final cheese, was subjected to gelation by the addition of rennet and glucono-delta-lactone and incubating at defined temperature of 32°C. Following gelation and storage at 4 °C for 1-4 d, the model cheeses were analysed for: composition; textural properties using large-strain deformation compression; composition of expressible cheese serum using HPLC; microstructure by confocal scanning laser microscopy and/or cryo-SEM; and flowability on heating (change in dimensions on melting at 280°C for 4 min).

Increasing NaCl from 0 to 2.3% slightly increased firmness and fracture stress, but significantly reduced chewiness (from ~ 12 to 6 N) and meltability. The latter changes, on increasing NaCl, coincided with increases in levels of non-expressible serum and serum concentrations of total protein and whole alpha-s and beta caseins. The protein matrix became more continuous and less punctuated by free-water inclusions, as salt level was increased.

The results suggest that addition of NaCl promotes water uptake by, and swelling of, the matrix polymer structural units (para-casein aggregates). However, a simultaneous decrease in the effective concentration of dispersed polymer in the moisture phase of the structural units may explain the decrease in chewiness.

Lecture 15

THURSDAY 29/09/2011, 14.50 – 15.05

Strategies for lowering salt in cheese.

W.J.M. Engels, K. Burseg and E.-M. Düsterhöft.

NIZO food research, P.O Box 20, 6710 BA, The Netherlands.

wim.engels@nizo.nl

Sodium chloride is one of the most widely used additives in the food industry because of its low cost and varied properties. It has a preservative and antimicrobial effect, it acts as flavour enhancer and the salt has effects on microbial and biochemical processes. Alteration of flavour in fermented foods may occur because of reducing or enhancing the activity of enzymes responsible for the formation of flavour compounds. Sodium chloride is present in significant amounts in e.g. bread, soup, cheese and sausages. As a consequence, sodium intake exceeds the nutritional recommendations in many industrialized countries. Excess dietary sodium intake from salt overconsumption has been linked to the development of hypertension, cardiovascular disease and other health problems.

The aim of this talk is to discuss the lowering of the salt content in food products, such as cheese, without changing consumer acceptability. NIZO food research studies various aspects of lowering salt in cheese. In addition to gradual stepwise reduction over time of the salt concentration, possibilities to apply salt replacers and natural taste boosters are studied. Research towards aroma induced taste enhancement, using gustometer and olfactometer, is an example of the latter aspect. A feasible approach to counteract loss or alteration of flavour due to lowering salt levels in cheese is by adapting and tailoring the formation of compounds to such an extent that restoration and/or compensation of flavour is reached.

Dedicated, real cheese, screening systems, such as MicroCheese and ScreenCheese, are now available for this tailoring of flavour formation. These models also offer tremendous possibilities to study growth of undesired micro-organisms in cheese upon lowering of salt

Notes

Lecture 16

THURSDAY 29/09/2011, 15.05 – 15.20

Food choice attitudes and motives of cheese consumers.

S. McCarthy[1], M. McCarthy[2], L. McKeown[1,2], J. Walton[3] and A. Flynn[3].

[1]*Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland;*

[2]*Department of Food Business and Development, University College, Cork, Ireland;*

[3]*School of Food and Nutritional Sciences, University College, Cork, Ireland.*

sinead.mccarthy@teagasc.ie

Understanding food choices is problematic due to the complex interplay between the individual, the environment and food products. Establishing the extent to which various motives frame specific food choices is of value. In the case of cheese, insights into the impacts of food choice attitudes and motives on consumption patterns could aid in the development of existing and new markets.

The recently completed representative National Adult Nutrition Survey (n=1500; 18-90 years) collected data on Irish consumers food choice motives and attitudes as well as food consumption patterns (4 day food diary). Pearson correlations were used to measure the linear association between consumers' cheese consumption levels and their food choice attitudes and motives. Cheese consumers accounted for 65% of the population with a mean daily intake of 21g (std. dev =18). There was no significant difference in cheese consumption across age groups. Mean daily intake of cheese was correlated with 20 different attitudinal and motive constructs.

Significant correlations were identified for five constructs. Mood ($P=0.013$) was negatively correlated with cheese consumption. This suggests that cheese may not be viewed as a mood enhancing product. Furthermore, when greater importance was placed on sensory motives lower consumption levels were observed ($P=0.046$). This may be as a result of 'sensory driven' consumers seeking greater variety across products and thus consuming less in any particular food domain. Good cooking skills ($P=0.013$) good intention to eat a healthy diet ($P<0.001$) as well as choosing smaller portion sizes ($P<0.001$) were also significantly negatively correlated with cheese consumption, indicating that cheese may not be an obvious healthy choice for consumers.

Although these analyses indicate that food choice motives do not distinguish well for the cheese product category, certain recommendations can be made. Marketing opportunities exist to promote the sensory, feel good and health aspects of cheese.

Lecture 17

THURSDAY 29/09/2011, 15.50 – 16.05

Influence of oxidation-reduction potential on cheese quality.

V. Caldeo and P.L.H. McSweeney.

Department of Food and Nutritional Sciences, University College, Cork, Ireland.

p.mcsweeney@ucc.ie

Oxidation-reduction (redox) potential is a fundamental physicochemical parameter that affects the growth of microoganisms in dairy products and contributes to a balanced flavour development in Cheese. Even though redox potential has an important impact on the quality of dairy products, it is not usually monitored during cheese manufacture. Since bacterial growth drives the reduction in redox potential during cheese manufacture and ripening, the ability of *Lactococcus lactis* strains to affect redox potential was studied.

Redox potential of the medium was altered by bacterial growth and there were strain-specific differences in the nature of the redox potential/time curves obtained. Methods were also developed for monitoring redox potential during cheesemaking and early in ripening. Changes in redox potential during laboratory scale manufacture of Cheddar, Gouda, Emmental and Camembert cheeses were determined. Distinctive kinetics of reduction in redox potential during cheesemakings were observed, and depended on the cheese technology and starter culture utilised.

Redox potential was also measured early in ripening by embedding electrodes into Cheddar cheese at moulding together with the salted curd pieces. Using this approach it was possible to monitor redox potential during the pressing stage. Redox potential was also controlled during the ripening of Cheddar cheese by adding oxidizing or reducing agents to the salted curd before pressing. By adding an oxidising agent (potassium iodate), it was possible to maintain a positive redox potential during cheese ripening while reducing agents further reduced the redox potential of Cheddar.

Our studies give an evidence of the importance of the redox potential on cheese quality.

Notes

Lecture 18

THURSDAY 29/09/2011, 16.05 – 16.20

Classical enterotoxin production by coagulase-positive *Staphylococcus aureus* isolated from raw milk used for raw milk cheese production.

K. Hunt[1,3], J. Schelin[2] P. Rådström[2], F. Butler[3] and K. Jordan[1].

[1]*Teagasc, Moorepark Food Research Centre, Moorepark, Fermoy, Co. Cork. Ireland;*

[2]*Applied Microbiology, Lund Institute of Technology, Lund University, Lund, Sweden;*

[3]*Biosystems Engineering, UCD School of Agriculture, Food Science and Veterinary Medicine, Belfield, Dublin 4.*

Kieran.jordan@teagasc.ie

Coagulase positive *Staphylococcus aureus* can produce *Staphylococcus* enterotoxins (SE's) that can be present in raw milk and raw milk cheeses. SE's are considered as food poisoning toxins which can induce an emetic response following consumption. The objective of this study was to survey Irish raw milk suppliers for raw milk cheese producers, for the prevalence of classical SE producing *S. aureus*.

From 5 of the Irish raw milk suppliers, 151 isolates of coagulase positive *S. aureus* were selected. Of the isolates investigated, 83% were found not to produce any classical SE, nor did they harbour classical *staphylococcus* enterotoxins (se) genes. The remaining 17% of isolates, which were detected from one of the raw milk suppliers, were found to harbour the c type se gene (sec), and only produced Enterotoxin C (SEC). These SEC producing isolates were further characterised into two distinct DNA types, both of which were detected in both the raw milk and raw milk cheeses. The growth and production of these 2 SEC strains was further investigated in sterile 10% reconstituted skim milk over a range of low temperatures (8-16°C) and inoculation levels (10 to 10^4 cfu/mL).

The results showed that in milk stored below 14°C and in the absence of competing bacteria a limited production of SEC was detected over 4 days even when the level of coagulase positive *S. aureus* were high. SEC was also found to only be produced after *S. aureus* had reached its stationary phase of growth.

Lecture 19

THURSDAY 29/09/2011, 16.20 – 16.35

Migration of triclabendazole residues from raw milk to cheese.

C. Power[1,2], B. O'Brien[3], M. Danaher[4], A. Furey[2], M. Whelan[4], R. Sayers[3] and K. Jordan[1].

[1]*Teagasc, Moorepark Food Research Centre, Moorepark, Fermoy, Co. Cork. Ireland;*

[2]*Cork Institute of Technology, Bishopstown, Cork, Ireland;*

3Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork. Ireland;

[4]*Teagasc, Food Research Centre, Ashtown, Dublin, Ireland.*

Kieran.jordan@teagasc.ie

Triclabendazole is used in treating parasitic infections in food producing animals. Until recently, triclabendazole was licensed for use as a flukicide in lactating animals. However due to the lack of information on excretion of residues into milk no maximum residue limit (MRL) has been established and its use on farms in Ireland is now prohibited. This study was undertaken to investigate the migration of triclabendazole residues from raw milk into cheese and their stability in cheese. Six lactating cows were orally dosed with Fasinex 10% Oral suspension at 12ml Fasinex per 50kg body weight, which contained triclabendazole as the active ingredient. Samples of milk were taken from each cow, morning and evening for 29 days post administration as the residue was no longer detected in the milk in all animals. Twenty four hours after administration of Fasinex, the complete days milk production form each cow was collected and pooled into two aliquots, each aliquot containing the milk from three cows.

Half of each pooled aliquots was pasteurised and a laboratory scale, semi soft cheese was manufactured from each of the four aliquots obtained. Triclabendazole residues were measured by mass spectrometry, in both the raw and pasteurised milk, curds and whey, fresh and ripened cheese. The results showed that the residue concentration within raw and pasteurised milk were similar.

During cheese manufacture, only 10% of the triclabendazole residues were lost in the whey; the remaining 90% were retained in the curd and therefore the cheese. Triclabendazole residues were unstable during the 5 week ripening period of the cheese. If triclabendazole residues are present in milk they can migrate to cheese, although they appear to be unstable during cheese ripening.

Notes

Lecture 20

THURSDAY 29/09/2011, 16.35 – 16.50

Effects of variation in cheese composition and maturation on water activity in Cheddar cheese during ripening.

D.K. Hickey[1], T.P. Guinee[2], J. Hou[2] and M.G. Wilkinson[1].

[1]*Department of Life Sciences, University of Limerick, Castletroy, Limerick, Ireland;*

[2]*Teagasc Food Research Centre, Moorepark, Fermoy, Cork, Ireland.*

Martin.Wilkinson@ul.ie

Water activity (aw) is a measure of the availability of water for enzymatic reactions, microbial growth and metabolism. However, the extent to which variation in cheesemaking conditions and compositional parameters influence water activity during ripening is unclear. A total of 32 Cheddar cheeses from three different experimental studies undertaken at pilot scale (500L) were investigated for their effects on water activity in Cheddar cheese over 270 d ripening at 8ºC.

Within two studies, the concentration of lactose or lactic acid in the cheese moisture phase (3.9-5.3%) was varied by curd washing and in the third study the cheesemilk protein composition was also varied (from 3.3 to 4.0%). Experimental cheeses had mean ranges of compositional parameters typical of those found in commercial Cheddar cheese; moisture (35.6–38.6%), fat (29.9–33.4%), salt (1.4–2.0%), salt-in-moisture (3.8–5.2%), moisture-in-non-fat substance (52.4–56.3) and pH (5.0–5.7).

Overall, water activity decreased significantly from ~0.965 to ~0.956 over ripening in all cheeses. Linear regression analysis indicated that water activity within individual cheese treatments was generally not significantly influenced by variations in the magnitude of individual compositional parameters. However, within each cheese trial, water activity was significantly correlated with both primary and secondary proteolysis over the 270d ripening period.

In conclusion, the preponderant factor controlling water activity in Cheddar cheeses made using standard procedures appeared to be proteolysis, with typical variations in compositional parameters having a much lesser effect.

Notes

THEME A:
Flavour Development

Abstracts for Poster Presentations

Notes

Poster A1

Analysis of the odour profile of food products using a micro chamber thermal extraction system and thermal desorption (TD) GC-TOF (MS) detection.

G. Roberts and D. Cooper.

Markes International Ltd, Gwaun Elai Medical Science Campus, Llantrisant, RCT, CF72 8XL, UK.

garethroberts@markes.com

The ability to identify the odour profile from food products is commercially important for several reasons. These include product quality/consistency, consumer attraction and off-odour analysis as an indicator of decay/contamination. To study this profile, a micro chamber thermal extraction system (μ-CT) is described which enables the volatile and semi volatile organic chemicals (VOC/SVOC's) to be monitored from a variety of food products including cheese. The work will show the efficiency of a multi hyphenated technique (μ-CTE-thermal desorption-GC-TOFMS) to analyse the odour profile of cheese for the determination of both primary and trace components.

Bulk samples are placed into a series of chambers, heated (optional) and purged with an inert gas. The effluent from each chamber obtained from dynamic headspace extraction is subsequently trapped by a thermal desorption sample tube containing selective sorbents allowing the whole VOC/SVOC profile to be monitored, including Sulphur compounds. Analysis is performed using a thermal desorption system connected to a high performance GC time-of-flight mass spectrometer.

The ability of the TD system to quantitatively recollect a (split) proportion of the samples after desorption from the TD tube and/or cold trap is described which enables re-analysis of the same sample. This provides a technique to look at both the high concentration components (high split), followed by a low split method for trace level analysis. The inherent sensitivity and classical EI spectra derived from the TOF-MS enables trace level compounds to be detected and identified using the NIST library. In this example, analysis of the GC-TOF MS data will employ novel chemometric based software incorporating spectral deconvolution and multivariate analysis (PCA) for the identification of target compounds. The data presented in this poster will show the VOC/SVOC profile of a cheese sample can be derived using a multi hyphenated TD-GCMS technique.

Poster A2

Origin and control of the typical goat flavour: example of French products.

P. Gaborit and K. Raynal-Ljutovac.

Actilait, BP 49, F-17700 Surgères, France.

p.gaborit@actilait.com

Goat milk cheeses are characterized by their unique sensorial properties and especially their typical goat flavour, whose definition depends on the country of origin. In the Netherlands and Italy they are perceived as rather "pungent and lipolysed" while they are perceived as more "animal" in France. This presentation gathers all the results obtained concerning this topic. First the compounds responsible for this flavour are presented. Then the study focuses on 4 ethyl octanoic acid, its threshold detection levels depending on the matrices and its concentration in raw milk at different stages of lactation. The impact of technological steps during cheese making on goat flavour and the release of 4 ethyl octanoic acid is described (cold storage, homogenization, pasteurization, acidification and the use of exogenous lipases) showing the incidence of the specificity and the "quality" of lipolysis. Indeed, as fat is the main fraction of goats milk involved in cheese flavour, the level and quality of lipolysis are of the highest importance The results concerning the relationship between lipolysis of milk and lipolysis of French cheeses and more specifically the strong impact of the surface ripening strains (*Penicillium camemberti*, *Geotrichum candidum*, yeasts...) in relation to the technological parameters and biochemical composition of cheeses are discussed. Emphasis is placed on the optimum ratio between 4 ethyl octanoic and other free fatty acids to induce a favourable typical goat flavour. The scarce results obtained concerning the incidence of goat diets on goats cheese flavour and on the release of 4 ethyloctanoic acid are also presented. Finally, the most recent studies performed on an edible lactic cheese model for use as a rapid screening model to assess the potential of ripening strains to producer aromatic compounds are also presented.

Notes

Poster A3

Development of a new edible goats milk model cheese to assess the potential of ripening strains.

P. Gaborit and K. Raynal-Ljutovac.

Actilait, BP 49, F-17700 Surgères, France.

p.gaborit@actilait.com

The flavour of soft surface-ripened goats milk cheeses is linked to milk composition but more importantly to the activity of the ripening strains. In order to rapidly evaluate, in a comprehensive and cost-effective manner,, the potential of ripening strains to produce aromatic compounds, taking into account the specificities of goats cheese (i.e. goats milk fat and lactic technology), an edible experimental lactic cheese model was developed. Lactic curd, prepared from standardized pasteurized goats milk, was frozen or freeze dried and stored at -20°C. For each trial, a control was performed with fresh curd. The model cheese was made (in a Petri dish) from frozen curd or from reconstituted freeze dried curd to give the same characteristics as the control (43% dry matter). Salt was added to all cheeses at a level of 1.3 %. The 3 cheese model bases (fresh, frozen and reconstituted freeze dried curd) were ripened with pure strains of Penicillium and Geotrichum or using mixed cultures including yeasts, in Petri dishes (140 mm diameter) at 12°C. The behaviour of the strains in the Petri dishes was also compared to their behaviour observed in classic lactic buchette type cheeses. Biochemical, microbiological and sensory analyses were performed. Similar sensory profiles were obtained from the three cheese model bases. The ripening of the model cheeses in Petri dishes was twice as fast as that observed during classical lactic buchette type cheese making: a 14 day old model cheese made from reconstituted freeze dried curd had the same biochemical characteristics (proteolysis and lipolysis) as a 26 day old buchette lactic cheese and also had a similar odour and flavour profiles. Sensory analysis performed on the freeze dried curd base enabled genus, species and strains specificities to be distinguished, especially concerning goaty descriptors but also yeast or mould morphology on ripened cheese. This base enabled a further screening of more strains and mixtures of strains.

Poster A4

Accelerzyme® CPG; a carboxypeptidase to accelerate cheese and EMC ripening.

P. Dekker, B. Folkertsma, K. Muijlwijk and G. van den Hoven.

DSM Food-specialties, P.O. Box 1, 2600 MA Delft, the Netherlands.

peter.dekker@dsm.com

Enzymes are often used in cheese production for coagulation, but have not been widely applied for enhancing cheese ripening. The main reason for this is that the enzymes that are currently available for this purpose can cause severe processing, flavour and structural side-effects in both cheese and whey.

For the acceleration of ripening small peptides and amino acids need to be released and converted to flavour components by the cheese cultures. Endo-acting proteases, that have sometimes been used to enhance cheese ripening, need to be added in high amounts to get sufficient release of small peptides and free amino acids. Consequently, this results in disturbing and softening of the cheese matrix structure. In theory, aminopeptidases are more suitable for the release of free amino acids without disturbing the cheese matrix structure, but these enzymes have the disadvantage that their pH optimum is too high. Therefore, aminopeptidases will have a high activity in milk and whey but a low activity in the acidic cheese matrix.

We have found that the addition of a serine carboxypeptidase (Accelerzyme® CPG) to the cheese milk results in an accelerated, balanced flavour formation in a number of different cheese types. Accelerzyme® CPG releases free amino acids from the carboxy-terminus of caseins at an acidic pH. The enzyme has no activity at neutral pH in the cheese milk, preventing side reactions during cheese making and whey processing. The availability of free, cheese flavour related, amino acids in the cheese matrix was increased when Accelerzyme® CPG was used. Cheese flavour was more mature, and bitterness was reduced in Accelerzyme® CPG treated cheeses. The effect of Accelerzyme® CPG on the ripening of several different cheese types will be discussed at the meeting.

Notes

Poster A5

Ability of thermophilic lactic acid bacteria to produce aldehydes and alcohols.

S. Buchin[1], I. Ben Slima[2], M.O. Coquillard[1] and J.C. Salmon[1].

[1]*INRA, UR342, Technologie et Analyses Laitières, BP 20089, F-39801 Poligny, France;*

[2]*ESIAT, 58 av. Alain Savary, cité El Khadhra, 1003 Tunis, Tunisia.*

solange.buchin@poligny.inra.fr

Aldehydes, alcohols and esters are volatile compounds potentially involved in the formation of cheese flavour. In Swiss-type cheeses, variations of such compounds have been linked to the presence of different species: propionibacteria, lactobacilli or streptococci.

To identify bacteria responsible for the production of aldehydes, alcohols and esters in cheeses, strains previously isolated from cheeses were screened for their ability to produce such compounds in a milk-based medium. The identification of precursors was also attempted. Preliminary results obtained on strains of thermophilic lactic acid bacteria, *Streptococcus thermophilus* (ST) and *Lactobacillus delbrueckii* (LD) are presented.

One strain of ST and one strain of LD were inoculated separately at 5 x 10^6 cfu/mL in sterile skimmed milk containing 1% yeast extract, and added with nothing or either 10mM of pyruvate (Pyr), threonine (Thr), isoleucine (Ile), valine (Val), methionine (Met) or anhydrous milk fat (3%). Inoculated milks with and without respective additions were incubated at 42°C for 72 hours. Non inoculated milk incubated under the same conditions was used as control. ST and LD were enumerated up to 24 hours. Volatile compounds were analyzed by Purge and Trap / GC / MS at 0, 24 and 72 hours.

Maximum counts of ST (3 x 10^8 cfu/mL) and LD (3 x 10^7 -10^8 cfu/mL) depending on the medium was reached in 5 to 8 hours. Aldehydes, acetaldehyde and propanal accumulated from 0 to 72 hours for both strains in each medium except for ST with the addition of Thr, Ile, Val, and LD with the addition of Pyr and Thr. Butanal and branched aldehydes decreased for both strains in all media. With respect to the alcohols, the quantities of methanol and 1-propanol decreased in all media, especially for LD. Conversely, the levels of 2- and 3-methyl-butanol were enhanced for both strains, and 1-butanol and 2-methyl-propanol for LD. No esters were detected in any media for either strain. No major effect of possible precursors was observed.

The ability to produce aldehydes and alcohols needs confirmation with other thermophilic strains.

Poster A7

Coryneform bacteria as adjuncts to accelerate the ripening of Cheddar cheese.

D. Ristagno and P.L.H. McSweeney.

School of Food and Nutritional Sciences, University College, Cork, Ireland.

p.mcsweeney@ucc.ie

Coryneform bacteria are generally found on the surface of smear-ripened cheeses and contribute to cheese ripening by the production of proteolytic, lypolitic enzymes and pigments which are responsible for the formation of a glistening, viscous and red-orange-yellow layer at the surface. In this study, *Microbacterium casei* DPC 5281, *Corynebacterium casei* DPC 5293 and *Corynebacterium variabile* DPC 5305 were used as adjunct culture in Cheddar cheese and their role during ripening was evaluated. The strains were grown to a final concentration of 10^9 cfu/mL and the cultivated broth was added to the cheese milk at level of 1%. Control cheese (C1) was made with 1% of sterile broth, cheese 2 (C2) contained 1% of *Microbacterium casei* DPC 5281, cheese 3 (C3) contained 1% of *Corynebacterium casei* DPC 5281, cheese 4 (C4) contained 1% of *Corynebacterium variabile* DPC 5305. *Corynebacteria* numbers in experimental cheeses at day 1 were around ~10^8 cfu/g, decreased by ~2 log units during the first week and remained almost constant during ripening. The composition of each cheese at 14 days was typical for Cheddar cheese indicating that the use of coryneform bacteria as adjunct culture did not have a significant effect on composition. No differences were observed between the electrophoretograms of experimental and control cheeses. After 60 and 180 d of ripening, levels of pH 4.6-soluble N as a % of total N in experimental cheeses C2 and C3 were significantly higher than the control cheese whereas no differences were observed in C4. All the experimental cheeses showed significantly higher levels of free amino acids than the control cheese. These results show that *Microbacterium casei* DPC 5281, *Corynebacterium casei* DPC 5293 and *Corynebacterium variabile* DPC 5305 accelerated the ripening process of Cheddar cheese through their proteolytic enzymes system.

Notes

Poster A8

Investigation of the acidic and basic proteome of *Lb. helveticus*.

S.A. Harvey[1], T. Coolbear[1], C. Chambers[1], J.R. Reid[1] and T. William (Bill) Jordan[2].

[1]*Fonterra Research Centre, Private Bag 11029, Palmerston North, New Zealand;*

[2]*Victoria University of Wellington, P.O. Box 600, Wellington, New Zealand*

tim.coolbear@fonterra.com

Flavour in cheese develops as a result of the concerted action of an array of enzymes, including proteases, lipases, glycolytic enzymes and those involved with amino acid catabolism. Products of these biochemical processes are also converted to additional flavour compounds via chemical conversions. Whilst much is known about the development of some flavour compounds, it is not known which metabolic pathways are the most influential, or whether other processes, perhaps some previously uncharacterised, are involved in cheese flavour development. Proteomic technologies provide an opportunity to investigate a broad range of metabolic pathways without pre-supposing which pathways are influential.

Lactobacillus helveticus strains can reduce bitterness and enhance fruity, sweet and mature flavour notes in cheese (Kiernan et al., 2000). The acidic and basic proteomes of a *Lb. helveticus* strain were characterised as part of an overall strategy to identify the metabolic pathway(s) responsible for such flavours. A range of proteins were identified, including those involved in carbohydrate metabolism, protein, RNA and amino acid biosynthesis and transport. Post translational modifications of some proteins were suspected, as they were detected as protein "trains" on 2-DE gels. In addition, a cluster of low molecular mass, high pI proteins in the basic gels were identified as ribosomal structural proteins.

Poster A10

Cold adaptation of the cheese ripening bacterium *Propionibacterium freudenreichii*.

M. Dalmasso[1,2], H. Falentin[1,2], D.-M. Deutsch[1,2], G. Jan[1,2], S. Parayre[1,2] and A. Thierry[1,2].

[1]*INRA, UMR1253 Science et Technologie du Lait et de l'Œuf, 65 rue de Saint-Brieuc, 35042 Rennes cedex, France.*

[2]*AGROCAMPUS OUEST, UMR1253 STLO, 65 rue de Saint-Brieuc, 35042 Rennes cedex, France.*

marion.dalmasso@rennes.inra.fr

Propionibacterium freudenreichii is known to play a key role in the formation of cheese flavour during the ripening of Swiss-type cheese and especially during the cold storage stage. At every stage of cheese manufacture, *P. freudenreichii* has to face several stress-generating conditions and especially a cold-induced stress when Swiss cheeses are transferred from a warm ripening room (24°C) to a cold room (4°C). The aim of this study was to investigate the adaptation and survival of *P. freudenreichii* at cold temperature by means of the first global gene expression profile for this species. The temporal transcriptomic response of *P. freudenreichii* was analyzed during its growth phase at 30°C and then during further incubation at 4°C for 9 days, always preventing any exhaustion of the main carbon source (lactates). As the cells moved from the warm to the cold storage, most of the down-expressed genes were involved in cell division, protein turnover, translation, transcription and DNA replication. During incubation at cold temperature, *P. freudenreichii* adopted multiple strategies for maintaining its viability. It used polyphosphate supplies by activating genes coding for Nudix hydrolases and pyrophosphatases. It accumulated carbon supplies by up-regulating genes of lactate, alanine and serine conversion to pyruvate, of aspartate conversion to fumarate, of gluconeogenesis and of glycogen synthesis. Thus, even if the metabolic activity is slowed down at cold temperature, *P. freudenreichii* remains active, which could explain its ability to continue the production of aroma compounds in cheese during their ripening at low temperature.

Notes

Poster A11

Effect of *Enterococcus faecium* ET031 on the microbiological and physicochemical characteristics of Cheddar cheese.

V. Caldeo[1], A. Topcu[2], A.A. Hayaloglu[3] and P.L.H. McSweeney[1].

[1]*School of Food and Nutritional Sciences, University College, Cork, Ireland;*

[2]*Department of Food Engineering, Hacettepe University, Ankara, Turkey;*

[3]*Department of Food Engineering, Inonu University, Malatya, Turkey.*

p.mcsweeney@ucc.ie

Enterococcus is a lactic acid bacterium found widely in nature and has different technological applications including cheese production. The aim of this work was to study the influence of *Enterococcus faecium* EF031 as adjunct culture on the quality of Cheddar cheese. Four batches of Cheddar cheeses were manufactured in triplicate. The control batch contained starter culture only, while the experimental batches contained starter culture and different levels (10^6, 10^7 or 10^8 cfu/mL) of *E. faecium* EF031 (Bioprox, Levallois, France). During aging the number of enterococci decreased by one log cycle in all three experimental cheeses while the control cheese contained a low level of enterococci. No differences in the levels of starter and non-starter lactic acid bacteria between the control and experimental cheeses were evident and typical trends were found for all the cheeses. Composition was not influenced by the addition of *E. faecium*. Redox potential was measured during cheesemaking and at four months of ripening; redox potential of the cheese made with the highest level of *E. faecium* had a lower value compared to the control cheese. Assessment of proteolysis was investigated by principal component analysis (PCA) of the RP-HPLC peptide profiles of pH4.6-SN fractions of cheese samples at 15 days, 2 months and 6 months of ripening. Cheeses were separated by ripening period and cheeses made with different levels of adjunct cultures had different peptide profiles. Moreover, volatile compounds were assessed by SPME-GC-MS and data analysed by PCA. Quantitative differences were found between the control cheese and the cheeses made with *E. faecium*. These findings support the technological utility of *E. faecium* as an adjunct culture for Cheddar cheese.

Poster A12

The right interaction between lactic acid bacteria and flavouring cultures: The solution for differentiated flavours in cheeses.

J. Ligot[1], M. Saubusse[1], M. R. A. Mueller[1], P. Bonnarme[2], F. Irlinger[2] and S. Mansour[1].

[1]*Cargill SAS, 18/20 rue des Gaudines - CS 182215 – FR 78 105 Saint Germain En Laye;*

[2]*Agro Paris Tech-INRA, UMR 782 GMPA2, FR 78850 Thiverval Grignon.*

Soulaf_Mansour@cargill.com

Adjunct cultures such as non starter lactic acid bacteria, ripening bacteria and yeast are widely dispersed naturally in the dairy environment. Some adjunct cultures are desirable in dairy products, and their beneficial effects are recognized in ripened cheeses due to their lipolytic and/or proteolytic activities as well as their positive interaction with lactic starters.

The aim of this study is to select a well defined cocktail of adjunct cultures together with lactic acid bacteria (LAB) as a solution to create and or improve the flavour of semi-hard cheeses while shortening the ripening times.

A model cheese that mimics semi-hard cheese conditions was developed. Different associations of LAB and adjunct cultures were tested. Microbiological, flavour and sensory analyses were performed. Flavouring associations selected from the model cheeses were then tested in semi-hard cheeses.

The model cheese was validated as a good tool to assess the flavour production in semi-hard cheeses. The production of volatile compounds was measured in model cheeses produced with more or less complex associations. The model cheeses made with the combination of LAB and adjunct culture was, by far, the most efficient at producing volatile compounds of interest such as ethyl acetate, phenyl ethyl alcohol and dimethyl disulfide. Sensory analysis of semi-hard cheeses/ model cheeses confirmed the positive impact of the adjunct culture addition in the fruity, ripened and sharp notes production.

The addition of a defined association of adjunct culture and LAB in semi-hard cheese efficiently improves the overall cheese flavour and brings ripened and sharp characters to young semi-hard cheeses.

Notes

Poster A14

Characterisation of the non-coagulating-enzyme part of different milk-clotting preparations.

O. Rolet-Répécaud[1], F. Berthier[1], E. Beuvier[1], S. Gavoye[2], E. Notz[2], S. Roustel[3], V. Gagnaire[4,5], J. Jardin[4,5] and C. Achilleos[1]

[1]*INRA UR 342, Technologie et Analyses Laitières, F-39801 Poligny, France;*

[2]*Actilait, Pole Technologie Fromagerie et Laitiere, BP 19, F-25620 Mamirolle, France;*

[3]*ENILBio, Rue de Versailles, BP 49, F-39801 Poligny cedex, France;*

[4]*INRA, UMR1253, Science et Technologie du Lait et de l'Œuf (STLO), F-35042 Rennes, France;*

[5]*Agrocampus Ouest, UMR1253, STLO, F-35042 Rennes, France*

Christine.Achilleos@poligny.inra.fr

The first step in cheese manufacture is the formation of a gel from milk. This step can be achieved by adding milk-clotting preparations prepared from various sources and different methods. Each preparation contains not only coagulating enzymes but also an associated support. Our objective was to characterise this support and to identify differences between preparations according to their manufacturing process and sources. Twenty-four preparations representative of the diversity available for cheese manufacture were sampled 3 times over a period of 6 months: 10 commercial rennets, 9 artisanally-produced calf rennets, 2 recombinant chymosins and 3 microbial preparations. Preparations were analysed for sodium, chloride, phosphorus, ammonia, free amino groups and ash contents, pH value and the phosphotungstic-acid-soluble nitrogen fraction. The protein diversity was determined by SDS–polyacrylamide gel electrophoresis and particular bands identified by reversed-phase nano liquid chromatography coupled on line to tandem mass spectrometry. The preparations were clearly discriminated according to the composition of their support. Preparations from *Cryphonectria parasitica* had the highest content in ammonia and nitrogen fractions. Commercial rennets and preparations from *Rhizomucor miehei* had the highest values for mineral composition while traditional rennets had the lowest. Commercial rennets presented the highest variability in composition. This variability was also confirmed by electrophoresis performed at the same milk-clotting activity, in which numerous protein bands ranging from 10 kDa to 150 kDa were shown in contrast to other preparations. Among the identified proteins, bovine serum albumin was mainly present in commercial rennets as well as minor bovine proteins and as expected not in recombinant chymosins. The observed variability in the support between the investigated preparations was linked to the source of coagulating enzymes and within the same source was inherent to the method used to obtain preparations. Work is under progress to determine if support impacts the cheese manufacturing process.

Poster A15

A field study on Sicilian dairy farms: pasture and cattle breed effect on milk volatile odour active compounds.

T. Rapisarda[1*], I. Schadt[1], G. Belvedere[1], C. Pasta[1], C. Guardiano[1], G. Licitra[1,2] and S. Carpino[1].

[1]*CoRFiLaC, Regione Siciliana, s.p. 25 km 5 Ragusa Mare, 97100 Ragusa, Italy;*

[2]*DISPA, Catania University, via Valdisavoia 5, 97100 Catania, Italy.*

t.rapisarda@corfilac.it

The aim of the present study was to evaluate the influence of pasture feeding and cow's breed on the volatile odour active compounds of milk. Four Sicilian dairy farms were selected, two with both Holsteins and Brown Swiss cows and two with only local breed Modicana cows. Bulk milk of each breed per farm was sampled two times per experimental period (P1 = spring and P0 = summer). Samplings within period occurred with weekly intervals. Pasture was available in P1 but not in P0. During P0 cows were grazing stubble. Additional hay and concentrate was supplemented during all periods. The volatile compounds were extracted by Steam Distillation (SD) and analyzed by Gas Chromatography Olfactometry (GCO). Pasture and breed effects on the aroma milk profile were statistically evaluated considering more then 100 chemical odour active compounds (VOCs) detected at least one time. These compounds were grouped into chemical and odour classes. The following chemical classes were considered: acid, alcohol, ester, aldehyde, aromatic hydrocarbon, ketone, furane, lactone, peroxide, pyrazine, pyrrole, sulfur, terpene, and thiazole. Odours were classified as: toasty, buttery, butyric, floral, herbaceous, fried, fruity, potato, milky, mushroom, plastic, and stable. There were more total VOCs ($P<0.001$) in P1 milks (35 VOCs) compared to P0 milks (28 VOCs). There were more ester ($P<0.05$), pyrrole ($P<0.001$) and sulphur ($P<0.001$) compounds in P1 milk samples relative to P0. More often "fruity", "potato" and "stable" odour classes were smelled in P1 compared to P0 samples. The higher frequency of "stable" odours in P1 relative to P0 might be in part explained by climatic and hygienic conditions. Cows breed made no difference for any of the tested chemical and odour classes.

Notes

Poster A16

A field study on Sicilian dairy farms: pasture and cattle breed effect on milk volatiles composition detected by Smart Nose®.

G. Belvedere[1,*], T. Rapisarda[1], I. Schadt[1], C. Guardiano[1], G. Azzaro[1], G. Licitra[1,2] and S. Carpino[1].

[1]*CoRFiLaC, Regione Siciliana, s.p. 25 km 5 Ragusa Mare, Ragusa, Italy;*

[2]*DISPA, Catania University, via Valdisavoia 5, 97100 Catania, Italy.*

belvedere@corfilac.it

The aim of the present study was to evaluate whether there are differences in milk volatiles "fingerprint" due to pasture feeding and cow's breed. Four Sicilian dairy farms were selected, two with both Holsteins (H) and Brown Swiss (BS) cows and two with only local breed Modicana (M) cows. Bulk milk of each breed per farm was sampled four times per experimental period (P1 = spring, P0 = summer, and P2 = autumn). Samplings within period occurred with weekly intervals. Pasture was available in P1 and P2 but not in P0. Periods P1 and P2 differed by botanical composition and plant maturity. During P0 cows were grazing stubble. Additional hay and concentrate was supplemented during all periods. Pasture intakes were calculated using CPM-Dairy®. Milk samples (three replicates per sample) were analyzed by a MS-based Electronic Nose (SMart Nose®) in the mass-to-charge (m/z) range of 10 to 160 amu. The data were statistically evaluated by Principal Component Analysis. Volatiles profiles of samples from P1 and P2 compared to P0 were discriminated, but not between P1 and P2. There was apparently less variation in volatiles during P2 compared to P1. Milk volatile profiles collected at the last sampling day, during P1, except for volatiles composition from M milk, were similar to all milk samples collected during P0. At this stage, plants were very mature and almost dry and chemical composition might have been similar to stubble composition. In all three periods, also when pasture was not available during P0, volatiles composition of M milk was different from H and BS milk. Differences during P1 and P2 might be explained, at least in part, by the higher average pasture intake of M (62.1 dry matter) relative to the BS and H (19.2 dry matter).

Poster A19

Aminotransferase and aminopeptidase activity in Leuconostoc strains isolated from traditional cheese DL-starter.

T. B. Pedersen and Y. Ardö.

Department of Food Science, Faculty of Life Sciences, University of Copenhagen, Denmark.

tbpe@life.ku.dk

The Leuconostoc genera are heterofermetative bacteria that are present in mesophillic DL-starters, where they contribute among others to the formation of holes in Danbo and Gouda type cheeses, by the degradation of citrate. Although they are present in both the cheeses and starter cultures little is known about their role in cheese ripening. It has been suggested that their primary role is to ensure a balanced growth of *Lactococcus lactis* (cit+) strains. Leuconostoc grow poorly in milk, which could be due to lack of proteolytic activity and it depends on peptides provided by e.g. *Lactococcus*.

The objective of this work is to establish more basic knowledge of the role of the Leuconostoc genera in cheese ripening. The enzymatic activity of aminotransferases and aminopeptidases are studied in selected *Leuconostoc mesenteroides* subsp. *cremoris* and *Leuconostoc pseudomesenteroides* strains which were isolated from traditional undefined cheese DL-starters. The strains are grown in MRS and the enzyme activities are analyzed in crude cell free extracts by measuring the absorbance from enzymatic reactions using a microtitre plate scanner spectrophotometer.

From the results of the specificity of the enzyme activity, strains could be selected and used as adjunct cultures in cheese.

Notes

Poster A20

Comparative analysis of dairy and non-dairy lactic acid bacteria as potential flavour cultures for cheese production

D. Cavanagh[1, 2], J.A. Hannon[1], G. Fitzgerald[2], K.N. Kilcawley[1] and O. McAuliffe[1].

[1]*Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland;*

[2]*Department of Microbiology, University College, Cork, Ireland.*

Olivia.McAuliffe@teagasc.ie

Lactic acid bacteria isolated from non-dairy environmental niches posses more diverse metabolic capabilities than those of dairy origin. In terms of cheese production, this may be harnessed to develop cheeses of more unique and desirable flavour. The objective of this study was to investigate the potential of cultures of lactic acid bacteria isolated from the wider non-dairy environment to produce flavour volatile compounds compared to those of dairy origin with the view to assess their potential to be used as cheese adjunct cultures. Strains of lactic acid bacteria were isolated from silage and cheese samples of commercial and artisanal origin. For bacterial identification, API tests and genus-specific PCR were employed, resulting in the identification of a number of strains of *Lactobacillus*, *Lactococcus* and *Streptococcus thermophilus*. Cultures were initially compared by sensory analysis and milk acidifying activity, and strains of interest were selected for further analysis by volatile compound analysis using gas chromatography mass spectrometry (GC-MS). The proteinase, peptidase and amino acid degrading activities of selected cultures were also examined. It was found that while dairy cultures were more adept at lowering the pH of milk than those of non-dairy origin, as expected, non-dairy cultures exhibited more diverse flavour profiles when grown in milk compared to those of industrial or dairy origin. The aminopeptidase activity of cultures was also found to vary between those of non-dairy and dairy origin. The results indicate the potential array of metabolic activities within strains from non-dairy niches that could be harnessed to improve the performance of mixed culture systems in terms of flavour and other important technological properties.

Poster A21

The flavour potential of encapsulated bacterial cell free extract in attenuated yeast.

A.B. Yarlagadda[1,*], M.G. Wilkinson[2]., J.A. Hannon[1], D.K. Hickey[2], V. Chaurin[1] and K.N. Kilcawley[1].

[1]*Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland;*

[2]*Department of Life Sciences, University of Limerick, Castletroy, Limerick, Ireland.*

Anil.Yarlagadda@teagasc.ie

Enzyme encapsulation has a wide range of potential applications, one of which is to enhance biochemical activity to direct and control flavour development. In this study a cell free extract from *Lactococcus lactis* subsp. *cremoris* AM2 was encapsulated in attenuated yeast - *Yarrowia lipolytica*. Concentrated yeast cells were attenuated by microfluidization to create populations of predominantly non-viable permeabilized cells, which were stabilized by freeze drying. These cells were rehydrated with bacterial cell free extract under controlled conditions. Encapsulation efficiency, yeast morphology and stability were assessed using confocal microscopy and flow cell cytometry. Encapsulated cell free extract in attenuated yeast was added under controlled conditions and compared to cell free extract and rehydrated yeast cells separately. General aminopeptidase, post proline dipeptidyl aminopeptidase, and glutamate dehydrogenase activity were monitored over incubation at 20°C for 10 days. This study demonstrated that attenuated yeast cells can be used to encapsulate bacterial cell free extracts. As the mechanism is natural and food grade it has potential in a wide range of applications. In addition, the combination of yeast and bacterial cell free extract in a single package delivers more complex flavour characteristics than the use of bacterial cell free extracts or yeast separately.

Notes

Poster A22

A model system for rapidly screening whole and attenuated LAB for cheese flavour potential.

C. Healy[1], J.A. Hannon[1], P.L.H. McSweeney[2] and K.N. Kilcawley[1].

[1]*Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland;*

[2]*School of Food and Nutritional Sciences, University College, Cork, Ireland.*

colette.healy@teagasc.ie

Lactic acid bacteria (LAB) have a significant role in cheese flavour development primarily through metabolic activity and release of intracellular enzymes. The majority of starter cultures used in cheese making belong to the genera *Lactococcus*, *Lactobacillus*, *Streptococcus* and Leuconostocs. Since the biodiversity between strains and within genera in terms of flavour producing capacity is significant, it is beneficial to utilise models that can rapidly assess their flavour potential. LAB only metabolise free amino acids under carbohydrate starvation conditions. Therefore suitable models require significant levels of free amino acids, to be free from contaminating bacteria and have low levels of carbohydrates. Thus commercially available lactose free UHT milk was evaluated for this purpose. The milk was added to sterile laboratory fermentation vessels and hydrolysed to a specific level of proteolysis using a commercial proteinase under optimal conditions for 24hrs and subsequently heat-inactivated. LAB was grown under optimal conditions in 10% RSM and a portion attenuated by microfluidization. The flavour potential of whole and attenuated cells of LAB from the same batch was compared using the model system for 10 days under controlled conditions. Samples were analysed for cell counts, degree of proteolysis, peptidase, glutamate dehydrogenase, amino acid transferase activities and at 10 days for sensory analysis and volatile cheese flavour compounds (head space solid phase micro extraction gas chromatographic mass spectrometry). Results have shown that cell viability decreases over incubation and that results were strain dependent. Attenuated cells were found to have significantly more cheese flavour (sensory & HS-SPME GCMS) than whole cells of the same strain. These results have highlighted the benefit of the model and the importance of attenuated LAB for cheese flavour development.

Poster A23

Development of enzyme modified cheese utilising attenuated LAB strains.

C. Healy[1], J.A. Hannon[1], P.L.H. McSweeney[2] and K.N. Kilcawley[1].

[1]*Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland;*

[2]*School of Food and Nutritional Sciences, University College, Cork, Ireland.*

kieran.kilcawley@teagasc.ie

Cheese flavour concentrates, such as enzyme-modified cheese (EMC) are an important component in many convenience foods. EMC have traditionally been produced by enzymatic hydrolysis of cheese curd. However, in many natural cheeses, most cheese flavour compounds are produced by the metabolism of free amino and free fatty acids by lactic acid bacteria (LAB). Therefore traditionally produced EMC often lack the sophisticated complex flavour profiles of natural cheese. Utilization of LAB during EMC production can be problematic because (a)-LAB will not readily metabolise free amino and free fatty acids to cheese flavour compounds if sugars are readily available as an energy source; (b)-the metabolism of lactose to lactic acid by LAB reduces the pH below the optimal pH for many of the key flavour producing enzymatic reactions and (c)-longer incubation periods required to enable live cells to grow, lyse and metabolise substrate increasing the risk of microbial contamination and the cost of production. To alleviate these issues concentrated attenuated LAB were utilized rather than 100% viable whole LAB. The LAB were grown and attenuated by microfluidization to create populations of live, dead and permeabilized cells (permeabilized cells are damaged non-viable cells but remain metabolically active). The EMC substrate consisted of a blend of dairy ingredients to a specific composition, heat treated to eliminate contaminating bacteria and spores and pre-hydrolysed with a commercial proteinase to a specific degree of proteolysis. Attenuated LAB were added under controlled conditions, cell counts, key enzymatic activities, degree of proteolysis, acid degree value, and volatile cheese flavour compounds were analysed over incubation. The sensory characteristics of final products were compared to natural cheeses using ranked preference sensory analysis. This study has highlighted the potential of attenuated LAB for use in the development of natural "clean label" EMC.

Notes

Poster A25

Identification of the main esterase involved in lipolysis by *Propionibacterium freudenreichii*

M.C. Abeijón Mukdsi[1,2], Falentin H.[3,4], M.-B. Maillard[3,4], R.B. Medina[1,2], S. Parayre,[3,4], S. Lortal[3,4] and A. Thierry [3,4]

[1]*Centro de Referencia para Lactobacilos (CERELA-CONICET), Chacabuco 145, Tucumán (4000), Argentina;*

[2]*Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 471, Tucumán (4000), Argentina;*

[3]*INRA, UMR1253, Science et Technologie du Lait et de l'OEuf (STLO), F-35042 Rennes, France;*

[4]*Agrocampus Ouest, UMR1253, STLO, F-35042 Rennes, France;1INRA-Agrocampus Ouest, UMR1253, STLO, F35042 Rennes, France.*

Free fatty acids are important flavour compounds in cheese, where they contribute to pungent, rancid, cheese, and fruity notes. They mainly result from the lipolytic activity of cheese micro-organisms. *Propionibacterium freudenreichii*, a species used as a ripening culture in Swiss cheese, is the main agent of Swiss cheese lipolysis, with 96% of the free fatty acids released during the ripening resulting from *P. freudenreichii* activity. Our aim was to identify the most probable lipolytic esterase(s) involved in cheese lipolysis by *P. freudenreichii*. Since cheese lipolysis mainly occurs during *P. freudenreichii* growth, we focused our study on surface-exposed or secreted esterases. Out of the twelve putative esterases previously predicted from the genome sequence of *P. freudenreichii* CIRM-BIA1, the lipolytic esterase PF#279 was shown to be secreted, and the putative esterase PF#774 was predicted to be anchored in the plasma membrane. To evaluate the respective role of these two proteins in lipolysis, *P. freudenreichii* CIRM-BIA1 was knocked out and then complemented for the genes encoding these two proteins, separately. Each of these genes was also over expressed in *P. freudenreichii* CIRM-BIA1. All these genetically modified strains were assessed for their lipolytic activity during their growth in a medium containing an emulsion of milk fat. Results showed that the mutants over-expressing either PF#279 or PF#774 released three times more free fatty acids compared to the wild-type strain. However, only the mutants inactivated for PF#279 were affected in their lipolytic activity, suggesting that PF#279 is the main lipolytic esterase involved in milk fat hydrolysis in *P. freudenreichii* CIRM-BIA1 and is a key component in Swiss cheese lipolysis.

Poster A26

Helveticin type bacteriocins contribute to the the autolytic phenotype of *Lactobacillus helveticus* DPC4571

Lydia Slattery[1,3], John O'Callaghan[3], Gerald F. Fitzgerald[1,2], Tom. P. Beresford[3] and R. Paul Ross[2,3]

[1]*Department of Microbiology University College Cork,*

[2]*Alimentary Pharmabiotic Centre, University Cork,*

[3]*Moorepark Food Research Centre, Teagasc, Moorepark, Fermoy, Co. Cork.*

tom.beresford@teagasc.ie

The strain *Lactobacillus helveticus* DPC4571 has emerged as a promising flavour adjunct culture for Cheddar cheese given that it is consistently associated with improved flavour development. Autolysis is a key contributor to the release of intracellular enzymes which results in faster ripening and improved flavour characteristics. It has been well established that *Lb. helveticus* DPC4571 undergoes rapid autolysis in cheese, and that autolysis plays a substantial role in the success of this strain as a cheese starter. DPC4571 cultures release substantial levels of cytoplasmic enzymes such as lactate dehydrogenase during late exponential growth and flow cytometry indicates that the cultures contain a high proportion of non-viable cells. The genome sequence of DPC4571 was searched for genes that had potential lytic functions and a number of candidate genes were identified. This study describes a direct link between autolysis of *Lb. helveticus* DPC4571 and the activity of two genes (lhv_0086 and lhv_1632) encoding helveticin type bacteriocins. The construction of two antisense plasmids that prevented expression of the helveticins revealed that they play a prominent role in the instigation of cell lysis as strains containing the helveticin antisense plasmids did not release cytoplasmic enzymes during mid to late exponential phase growth and flow cytometric measurement of cell viability indicated that the number of non-viable cells present was reduced in comparison to the wild type DPC4571 cultures.

Poster A27

Expression of pyrrolidone carboxyl peptidase from *Lactobacillus helveticus* for enhanced production of pyroglutamic acid and gamma aminobutyric acid during cheese ripening

Lydia Slattery[1,3], John O'Callaghan[3], Gerald F. Fitzgerald[1,2], R. Paul Ross[2,3] and Tom. P. Beresford[3].

[1]*Department of Microbiology University College Cork,*

[2]*Alimentary Pharmabiotic Centre, University Cork,*

[3]*Moorepark Food Research Centre, Teagasc, Moorepark, Fermoy, Co. Cork.*

tom.beresford@teagasc.ie

Analysis of the genome sequence of *Lactobacillus helveticus* DPC4571 predicted that it produces a pyrrolidone carboxyl peptidase (Pcp) capable of cleaving pyroglutamic acid (PGA) from the N-terminus of polypeptide chains. The objective of this study was to isolate and characterise the Pcp enzyme in vitro and to analyse the effect of heterologous expression of this enzyme in *Lactococcus lactis* in laboratory scale cheesemaking.

The gene (pcp) encoding Pcp was identified based on homology to known pcp genes in other bacteria. The ability of this enzyme to generate free PGA from peptide substrates was confirmed heterologous expression in *Lc. lactis* and demonstrating activity against synthetic L-pyroglutamic acid 4-methyl-7-coumarinylamide hydrate (Pyr-AMC). The recombinant enzyme had a pH optimum of 7.5, similar to previously characterised Pcp proteins; however, unlike mammalian Pcp enzymes described by other workers the *Lb. helveticus* Pcp did not have an absolute requirement for a thiol reducing agent. Cheese produced with a lactococcal starter heterologously expressing Pcp contained substantial (70 µg/g) levels of PGA after 14 days of ripening which increased to 110 µg/g by day 28 of ripening. In contrast, no PGA was detected in cheese made using the vector control strain that did not contain the pcp gene.

Taken together, these data demonstrate that the cloned pcp gene produced an enzyme capable of releasing PGA both in vitro and in cheese and confirm the role of the Lb. helveticus Pcp enzyme in releasing PGA in ripening cheese

This is the first report of the heterologous expression of a Pcp enzyme from *Lb. helveticus* and also the first report of the effect of a heterologously expressed Pcp enzyme in the cheese matrix.

Poster A28

Heterologous expression of aminotransferase enzymes from *Lactobacillus helveticus* in Lactococcus lactis

E. Crowley[1], J. O'Callaghan[1,3], T.P. Beresford[1], R.P. Ross,[2,3] and G.F. Fitzgerald[1,3]

[1]*Department of Microbiology, University College Cork, Ireland.*

[2]*Moorepark Food Research Centre, Teagasc, Fermoy Co. Cork, Ireland.*

[3]*Alimentary Pharmabiotic Centre, University College Cork, Ireland.*

tom.beresford@teagasc.ie

The strain *Lactobacillus helveticus* DPC4571 has emerged as a promising flavour adjunct culture for Cheddar cheese and it is consistently associated with improved flavour development during cheese ripening. Amino acid catabolism by microbial enzymes released by the cheese starter culture(s) is an important contributor to the development of aroma compounds in ripened cheese. The primary step in the catabolism of most amino acids by the lactic acid bacteria is their transamination to alpha-ketoacids that are then further catabolised to aroma compounds. An *in silico* examination of the genome of *Lb. helveticus* DPC4571 identified 10 putative aminotransferase enzymes, two of which were selected for further study; a branched chain aminotransferase and an aspartate aminotransferase. Both enzymes were heterologously expressed in *Lactococcus lactis* and were characterised with respect to their pH and temperature optima and substrate range. The branched-chain amino acid aminotransferase lhv_1426 was most active against isoleucine, leucine and valine, while the aspartate or aromatic amino acid aminotransferase lhv_0910 degraded aspartate and was also capable of transaminating the aromatic amino acids phenylalanine, tyrosine and tryptophan. Both enzymes were active over a broad pH range. The optimum temperature for activity of lhv_1426 was 37°C while lhv_0910 was optimally active between 35-55°C. Both aminotransferases were also heterologously expressed in a strain of *L. lactis* suitable for use as a cheese starter and used in laboratory scale cheese production. Results from the cheese-making trials indicated that these aminotransferase enzymes can influence both the concentration and type of amino acids present during Cheddar cheese ripening.

THEME B:
Diversification

Abstracts for Poster Presentations

Notes

Poster B1

High pressure - treated starter in the manufacture of Feta cheese

D. Maniou[1], A. Tsala[1], E. Moschopoulou[1], M. Giannoglou[2], P. Taoukis[2] and G. Moatsou[1*].

[1]*Dept of Food Sci. & Technol., Agricultural Univ. Athens;*

[2]*School of Chemical Eng., National Technical Univ., Athens, Greece.*

mg@aua.gr

The use of high pressure-treated commercial DVI starter consisting of mesophilic and thermophilic strains was studied by assessing physicochemical and microbiological composition, proteolysis and enzymatic activities throughout ripening of Feta cheese. Cheeses were manufactured over three consecutive days using the untreated starter (control cheese A) and the same starter treated at 200 MPa, at 20 °C for 15 min (cheese B). Although mesophilic, thermophilic and NSLAB counts were similar in both cheeses throughout ripening, the acidification proceeded at first slowly in cheese B. This resulted in statistically significant lower mean total solids content of cheese B, i.e. 45.36% compared to 46.78% of cheese A at 15 d. At this stage of ripening, soluble N and TCA soluble N of cheese B were about 20% higher than that of control cheese A, differences being more intense in PTA soluble N. At 90 d, mean soluble N of cheeses A and B was 0.318% and 0.357% respectively. These findings were consistent with the enhancement of the area of small hydrophilic peptides in the chromatographic profiles of cheeses B. The activity of residual chymosin was not affected by the treatment of starter. The estimation of LDH activity gave no evidence that high pressure treatment of the starter increased autolysis. Although aminopeptidase activity followed the same trend in both cheeses during ripening, the cheese B values were always lower, e.g. 1.29 units per g of cheese in comparison to 1.67 of the control cheese at 90 d. These findings taken together, gave evidence that high pressure treatment of the starter could accelerate the ripening of this cheese variety, through a mechanism that is not related to autolysis enhancement. However, considering the observed retardation of acidification, its use as an adjunct rather than as regular starter for this cheese variety is proposed.

Poster B2

Flavour diversity and acceptance of Estonian cheeses.

K. Koppel[1] and E. Chambers[2].

[1]*Competence Center of Food and Fermentation Technologies, Tallin Estonia;*

[2]*Sensory Analysis Center, Kansas State University, USA.*

eciv@ksu.edu

The flavour and acceptance of locally manufactured cheeses in Estonia were studied. The samples included 36 cheeses, varying in texture, manufacturing technology, fat content, and additives. A highly trained descriptive sensory panel used 32 flavour attributes to describe the similarities and differences among the cheeses. Estonian cheese was described as milky and buttery, with sweet aromatics, with some samples having bite and butyric acid aromatics. The cheeses usually are not highly aged, and thus, often do not have dominant aged flavours found in cheeses from some other countries. Two major clusters of cheese were identified, the first containing samples that were characterized as mild, chalky and having sweet aromatics and the second containing cheeses that had pungent and butyric acid aromatics and some also were fermented, moldy, sweaty, or biting. Four cheeses, two milder and two stronger samples, were chosen for an acceptance study. One hundred and eleven consumers in Estonia tested the four cheeses. Cluster analysis of the consumers' liking scores indicated two clusters of consumers almost equivalent in size, one cluster liking cheese that had aged, pungent, astringent attributes, while the other cluster seemed to like cheese that were milder with more dairy and buttery flavours. The study provides information concerning cheese flavour and preferences in an area of Eastern Europe, which has been lacking.

Notes

Poster B3

Flavour characterization of diverse goat cheeses in the United States.

D.H. Chambers and M. Talavera-Bianch.

Department of Human Nutrition, Justin Hall Manhattan, Kansas State University, KS 66506, USA.

delores@ksu.edu

Goat cheeses have become more popular in recent years in the US. The objective of this study was to determine the diversity of artisan goat cheeses made in the United States. Highly trained sensory panelists reviewed previously established lexicons for cheese before establishing a lexicon of forty-three flavour attributes to represent the sensory characteristics for forty-seven artisan goat cheeses that were manufactured in different parts of the United States and included in this study. Twenty-seven attributes were present in a majority of samples and were able to describe most of the flavour characteristics. Other attributes were used occasionally to describe specific characteristics of certain cheeses. A large number of attributes were needed to describe flavour characteristics of cheese samples with more complex flavours (i.e. samples that were mold-ripened). Samples with milder characteristics (Chèvre-style) needed a reduced number of terms. Chèvre-style cheeses had lower intensities for most attributes. As expected, Feta-style cheeses generally were more salty and Cheddar-style cheeses were mostly nutty, waxy, and sweet. Mold-ripened cheeses had musty-like characteristics and were more pungent and sharp. These attributes had higher intensities specifically for blue-type cheeses. The goaty flavour attribute was higher for mold-ripened cheeses, but was characteristic of all the goat cheeses. This study can serve as a tool for goat cheese producers for development and quality control as it shows what types of flavours are typical of various types of goat cheese.

Poster B4

Fortification of milk for Cheddar cheese manufacture using skim milk powder.

A.C. Moynihan and P.L.H. McSweeney.

School of Food and Nutritional Sciences, University College, Cork, Ireland.

p.mcsweeney@ucc.ie

Using powders to fortify cheesemilk could have potential applications in ingredient cheese or to overcome problems caused by milk seasonality. The objective of this study was to make cheese fortified with skim milk powder (SMP) and to determine its effect on cheese properties. Skim milk (40 L) was fortified with 3.75 kg SMP making a milk stock with higher casein content and the casein to fat ratio was kept constant using cream. This mixture was divided and made to 50 L with pasteurised milk giving four cheese milks with casein levels of 2.61% (CSMP), 2.86% (LSMP), 3.22% (MSMP) and 3.83% (HSMP) and Cheddar cheese was made therefrom. Significant differences ($P<0.05$) were observed in cheese yields (9.76, 10.84, 12.06 and 14.99 kg/100kg milk for CSMP, LSMP, MSMP and HSMP, respectively). No significant difference ($P>0.05$) was observed between the cheeses in terms of moisture in non-fat substances. pH values were higher as SMP fortification level increased. Low levels of SMP had no significant ($P>0.05$) effect on the hardness values of the cheese throughout ripening compared to CSMP but higher level of addition of powder resulted in increased hardness. Meltability of all cheeses increased with ripening time but HSMP and MSMP melted less than CSMP and LSMP cheeses. As ripening progressed levels of proteolysis increased significantly ($P<0.05$) in all cheeses but higher levels of SMP resulted in slower proteolysis. Numbers of non-starter lactic acid bacteria (NSLAB) were higher in the fortified cheeses; as the powder level increased so did the numbers of NSLAB. Fortifying cheese with SMP had significant effects on the properties of the cheese without having major effects on its composition. Lower levels of fortification gives cheese with similar properties to the control but with increased yield.

Notes

Poster B7

The composition of Camembert cheese ripening cultures modulates both mycelial growth and appearance.

M.-H. Lessard, G. Bélanger, D. St-Gelais and S. Labrie.

Department of Food Sciences and Nutrition, (INAF/STELA) Université Laval;

Agriculture and Agri-Food Canada, (CRDA), Canada.

Steve.Labrie@fsaa.ulaval.ca

In bloomy-rind cheeses, ripening cultures composed of yeasts and molds are selected based on their contribution to the sensory properties of the cheese. Given the multicellular structure of fungal hyphae, colonies on agar medium rarely originate from single cells. Moreover, fungi tend to rapidly invade agar surfaces, covering small yeast colonies and resulting in an underestimation of their number. A real-time qPCR method was developed to quantify a mixed fungal community containing the most common dairy yeasts and molds used for bloomy-rind cheeses in Canada: *Penicillium camemberti*, *Geotrichum candidum*, *Debaryomyces hansenii*, and *Kluyveromyces lactis*. This community was monitored on soft cheese model curds prepared from an unsalted cheese lyophilisate that was rehydrated to a standard Camembert cheese composition (57% moisture and 3.5% salt-to-moisture ratio) and inoculated with mixed fungal ripening cultures. Our real-time TaqMan probe-based qPCR method was used to evaluate the growth dynamics of a ripening ecosystem combining *P. camemberti*, *G. candidum*, and *K. lactis*. *P. camemberti* and *G. candidum* dominated the ecosystem, while *K. lactis* was the least abundant. When *D. hansenii* was added to the fungal ecosystem, the yeast totally inhibited *K. lactis* and reduced the growth of *P. camemberti* and *G. candidum* 346- and 13-fold, respectively. The reduction of mycelium biomass density on the surface of the model curds from 386 to 175 mg/cm^2 corroborated this result. The thickness and colour of the rind was also affected by *D. hansenii*. This study demonstrated the pitfalls of culture-dependent methods and proposes a new real-time qPCR method to successfully quantify a complex fungal microflora in a model-curd simulating Camembert-type cheese. Real-time qPCR could be useful for monitoring fungal growth on bloomy-rind or smear cheese surfaces and detecting shifts in microflora composition.

Poster B8

Food safety and ancient cheesemaking technology in developing countries:

G. Licitra[*1,2], S. Mirabella[1], G. Belvedere[1], M. Marchese[1] and S. La Terra[1].

[1]*CoRFiLaC, Regione Siciliana, s.p. 25 km 5 Ragusa Mare, 97100 Ragusa, Italy;*

[2]*DISPA, Catania University, via Valdisavoia 5, 97100 Catania, Italy.*

licitrag@tin.it

Wagashi cheese is produced in Benin from women of the Peuhl ethnic group. It is characterized by the use of vegetable coagulant extracted from the latex of *Calotropis procera* which allows the coagulation of milk at temperatures higher than 70°C and raised over 90°C for 5 min until the curd fully separates from whey. This ancient technology makes the Wagashi cheese proteolytic activity very low, and allows the cheese to be boiled over and over again, every two days, for about 20 days since it has been produced, without any apparent modification of the cheese structure. Preliminary studies were carried out to verify the survival of pathogens just after the curd separation and after boiling, with negative response. To confirm the preliminary results, *Listeria monocytogenes* and *Salmonella typhimurium* were incolulated: A) in raw milk prior to cheesemaking; B) in Wagashi cheese after 24h that has been produced. Both experiments were carried out in triplicate. Experiment A was conducted by inoculating raw milk separately with strains of Listeria and Salmonella (~ 109 UFC/ml) before cheesemaking. In all cheeses produced from this milk Salmonella and Listeria were absent. Experiment B was carried out by inoculating strains of Listeria (~ 10^3 cfu/g) and Salmonella (~ 10^3 cfu/g) in 10 Wagashi cheeses produced according to the traditional process. After 24h from the inoculation, one cheese was sampled before boiling and analyses revealed the presence of both pathogens (~ 10^3 cfu/g). The remaining 9 cheeses were boiled and assigned to different boiling duration (15, 25, and 35 min) and temperature (65, 75, and 85 °C) treatments. Microbiological analyses confirmed that in all cheeses the inoculated strains of Salmonella and Listeria did not survive. According to the experimental results, conclusions are that this traditional technology guarantees the Wagashi cheese safety not only during cheesemaking but also from post contamination.

Notes

Poster B10

Development of a novel staining technique to determine localised variations in pH within cheese matrix microstructure using confocal scanning laser microscopy.

V. Chaurin[1], D.F.M. Daly[1,2], M.A. Fenelon[1], P.L.H. McSweeney[2] and J.J. Sheehan[1].

[1]*Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland;*

[2]*School of Food and Nutritional Sciences, University College, Cork, Ireland.*

diarmuid.sheehan@teagasc.ie

Analysis of commercial cheese samples by confocal scanning laser microscopy (CSLM) revealed a continuous open porous protein network structure containing; voids, varying levels of fat coalescence and differences in the distribution of the aqueous phase within the cheese blocks. Variations in moisture and fat distribution within the cheese matrix will have a direct effect on localised biochemistry within those areas e.g. lactate solubilises in the aqueous phase within the cheese matrix and thus has the potential to exert localised pH differences. The objective of this research was to develop a confocal scanning laser microscopy staining technique to explore localised variation in pH levels in cheese microstructure. The protein matrix of slices of commercial cheese samples was labelled with an aqueous solution of Fast Green FCF. This compound labels protein when excited at 633 nm. Areas of different pH on the cheese matrix were differentiated using a pH sensitive dye: Oregon Green 514 carboxylic acid which fluoresces when excited at 514 nm and becomes less fluorescent as pH decreases. The use of the two fluorescent probes allows the protein network and spatial variation of pH to be observed simultaneously. The method was successfully used to determine localised variations in pH of selected curd matrices. Such localised variations in pH levels observed within the samples may result in localised proteolysis patterns due to specific pH optima for both residual rennet activity and peptidase activities of lactic acid bacteria. In addition the technique may indirectly illustrate varying concentrations of solutes within the curd matrix aqueous phase which could influence patterns of the growth of starter and non starter bacteria. In conclusion, the development of this technique will contribute to the understanding of complex biochemical changes occurring at a localised level within the cheese matrix during manufacture and ripening processes.

Poster B11

Investigation into the influence of cheese microstructure and localised variations in cheese pH on the development of a pink discolouration defect in commercial cheese.

D.F.M. Daly[1,2], V. Chaurin[1], M.A. Fenelon[1], P.L.H. McSweeney[2] and J.J. Sheehan[1].

[1]*Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland;*

[2]*School of Food and Nutritional Sciences, University College, Cork, Ireland.*

diarmuid.sheehan@teagasc.ie

Pink discolouration defect results in a down-grading of ripened cheese varieties manufactured both with and without the colourant annatto. Discolouration may be evident on the surface, as a rim beneath the surface, or as an homogenous or dispersed discolouration within the cheese block. Many factors (e.g. redox potential, particular strains of lactobacilli, pH, oxidation of tyrosine and bixin, etc.,) have been associated with the defect but a knowledge gap relating to the underlying causes of the defect persists. The objective of this study was to investigate whether differences in cheese microstructure may be evident between cheeses with and without the defect. Commercial cheeses were sourced and comparable areas of cheese blocks with and without the defect were analysed by confocal laser scanning microscopy. Significant differences in microstructure between control and defect samples were observed; in particular a less homogenous distribution and far greater degree of fat coalescence in parallel with larger areas of moisture within the protein matrix of defect cheeses was observed in comparison to control equivalents. Greater levels of fat coalescence may result from interactions between mechanical and thermal treatments during cheese manufacture including curd temperature gradients arising from variable cumulative process heat loads, altered manufacture processes, seasonal differences in proportions of liquid and solid fat and variations in curd handling and pressing processes. This may result in altered light scattering properties of the cheese matrix. A novel localised microstructure pH differential staining technique was also developed and showed significantly lower pH levels at the fat-protein interface in comparison to the cheese matrix as a whole. Thus, differences in levels of fat coalescence may promote the pinking defect directly through altered light scattering properties or indirectly through localised variations in cheese pH, moisture and in other compositional and biochemical characteristics

Notes

Poster B12

Investigation of underlying microbial factors relating to the development of a pink discolouration defect commercial cheese.

D.F.M. Daly[1,2], D.K. Hickey[3], M.G. Wilkinson[3], V. Chaurin[1], M.A. Fenelon[1], P.L.H. McSweeney[2] and J.J. Sheehan[1].

[1]*Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland;*

[2]*School of Food and Nutritional Sciences, University College, Cork, Ireland;*

[3]*Department of Life Sciences, University of Limerick, Castletroy, Limerick, Ireland.*

diarmuid.sheehan@teagasc.ie

Pink discolouration in cheese resulting in commercial down-grading has been widely reported for different cheese-varieties. Many factors (e.g. differences in cheese redox potential, strains of lactobacilli, cheese pH, oxidation, etc.,) have been associated with the defect but none conclusively. The objective of this study was to investigate specific microbial factors potentially associated with this defect. Commercial cheese samples were obtained and analysis was undertaken in comparable areas of cheese blocks with and without the defect. Significantly lower levels of d-lactate ($P<0.05$) and higher levels of Arginine ($P < 0.05$) were observed in defect in comparison to control cheeses. Although such differences may be associated with differences in microbial populations or metabolic activities (e.g. racemisation activity or utilisation of Arginine as a carbon source by NSLAB), no significant differences were observed in mean viable counts of starter and NSLAB between control and defect cheeses. However, analysis by flow cell cytometry showed differences in profiles of live, dead or metabolically active cell populations between control and defect cheeses. Such differences may arise due to differences in the predominance of a particular population/species or also due to variations in levels of cell permeability. Provisional investigations using live/dead staining techniques with confocal scanning laser microscopy showed greater populations of permeabilised cells in defect in comparison to control cheeses. Such differences may potentially result in differing metabolic activities within the cheeses and thus potentially on development of the pink defect. In addition pyrosequencing techniques are currently being utilised to determine whether differences between populations of dominant and subdominant NSLAB micro-flora exist between control and defect cheeses. In conclusion, cheeses with development of a pink defect were observed to have greater populations of permeabilised cells with resultant variations in metabolic activities in comparison to control cheeses.

Poster B13

Preparation and use of carotenoid colours which mimic annatto for the use in Cheddar cheese without contamination of the whey waste stream.

N. Sexton and K. Dwane.

Cybercolors Ltd., Unit 8, Airport East Business Park, Farmers Cross, Ballygarvan, Co. Cork, Ireland

www.cybercolors.ie

Annatto, a colour extract prepared from the seeds of the annatto tree (Bixa orellana) has been used for centuries in the preparation of coloured Cheddar cheeses e.g. Cheddar, Red Leicester etc. The annatto pigment (norbixin) associates with the milk proteins and so colours both the casein (curd) and the whey proteins. This has the effect of producing a coloured whey by-product stream which is undesirable.

The aim of this work was to investigate the possibility of preparing a natural food colour which could colour be used to colour Cheddar cheese curd the same colour as annatto, but would specifically colour the cheese curd while leaving the whey stream uncoloured.

Natural Colour formulations were prepared using natural beta-carotene and paprika. These natural colours were formulated such that the natural colour would easily disperse in milk. Cheddar cheese trials were performed at 500 L and 2000 L pilot scale at the cheese processing hall at Moorepark Technology Ltd., Moorepark, Fermoy, Co Cork, Ireland. At each scale, three cheeses were designed as follows: (1) an uncoloured control in which no colour was added to the milk; (2) an annatto control in which a traditional annatto was added to the milk and (3) a new formulation of Natural Cheese colour WS1 which was added to the milk. The resultant cheese and whey streams were analysed for colour shade and colour content.

The results obtained for all trials were the same and are summarised as follows: (1) When no colour was added, the cheese was uncoloured and the whey streams were typical "green" colour as expected and no other colour was present in the whey stream; (2) When annatto was added as a red Cheddar colorant the cheese contained the typical orange colour associated with red Cheddar cheese. However, the whey stream was yellow in colour typically containing 15% of the annatto colour; (3) When Natural Cheese colour WS1 was added, the cheese contained the typical orange colour associated with red Cheddar cheese. However, the whey stream was typical of uncoloured whey streams ie the typical "green" colour and there was no other colour present.

In conclusion, the study highlights the preparation of a natural food colour of carotene and paprika which can be used to give Cheddar cheese its typical orange colour, but without colouring the whey stream with unwanted residual colorant. This natural cheese colour has been filed for patent world-wide.

Notes

THEME C:
Health & Nutrition/Fat and/or salt reduction

Abstracts for Poster Presentations

Notes

Poster C1

Effect of gum tragacanth on the rheological and functional properties of half-fat and full-fat Cheddar cheese.

D.R. Cooke[1], A. Khosrowshahi[2] and P.L.H. McSweeney[1].

[1]*School of Food and Nutritional Sciences, University College, Cork, Ireland;*

[2]*Department of Food Science and Engineering, Faculty of Agriculture, Urmia University, Urmia, Iran.*

p.mcsweeney@ucc.ie

Fat replacers can be used to improve the sensory and functional properties of reduced-fat cheeses. The aim of this study was to investigate the effect of gum tragacanth (GT) on the rheological, functional and sensory properties of half-fat and full-fat Cheddar cheese during ripening. Four Cheddar-style cheeses were made in triplicate: full-fat control (FFC); half-fat control (HFC); full-fat + GT (FFGum) and half-fat + GT (HFGum). Milk for the latter two cheeses was supplemented with GT at a level of 0.05%; all cheeses were ripened at 8°C for 10 months. Analyses carried out included texture profile analysis (TPA), Schreiber melting tests, dynamic small amplitude oscillatory rheology (DSAOR), pH 4.6-soluble N as a % of total N, urea-polyacrylamide gel electrophoresis, colour and sensory. GT addition caused an increase ($P<0.05$) in moisture-to-protein ratio and a decrease ($P<0.05$) in pH. GT appeared to increase proteolysis only in the FF cheeses. GT was successful in decreasing TPA hardness and springiness values during ripening. GT increased meltability in both the FFGum and HFGum cheese after 6 months of ripening. DSAOR showed that GT caused a decrease ($P<0.05$) in maximum loss tangent and an increase ($P<0.05$) in storage modulus at 75°C after 7 months of ripening. Results from a consumer ranking preference test showed that GT addition to the HFGum cheese was not successful in fully mimicking the sensory properties of the FFC. These results suggest that GT appears to be more suited to enhancing textural and functional properties in half-fat Cheddar cheese.

Poster C2

Effect pH and NaCl on the survival of probiotic cultures in a model cheese system.

B. Byrne, K. Jordan and T.P. Guinee.

Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland.

brian.byrne@teagasc.ie

Cheese is frequently used as a means of delivery of probiotic bacteria. The study investigated the effects of variations in pH and NaCl on the survival of the probiotic bacteria, *Bifidiobacteria* BB12 (BB12) and *Lactobacillus casei* LC-01 (LC-01) in cheese during storage. Model cheeses (~47% dry matter, protein-to-fat ratio ~1) were prepared in triplicate from concentrated dispersions of micellar casein in an aqueous-based solvent comprised of water, fat and lactose. The resultant dispersion, which was a liquid pre-cheese, was pasteurised at 80°C, and cooled to ~ 35°C. The pasteurised pre-cheese was subdivided into three sub-batches to which NaCl was added at 1.2, 2.2 or 3.5%. For each NaCl level, the pH was adjusted to 4.8, 5.3 or 5.8 using lactic acid. Each pre-cheese was inoculated with BB12 or LC-01 giving populations of ~ 10^7 – 10^8 cfu/g, and then set using rennet. The resultant cheeses were stored at 4°C, and enumerated for LC-01 and BB12 using selective agars. BB-12 cheeses maintained their target pH values and survival was independent of variations in NaCl and pH, with counts of ~10^7 – 10^8 cfu/g in all cheeses at 60 d. The pH of LC-01 cheeses with 1.2 or 2.2% NaCl reduced from the target values to ~ 5.3–4.8 at 90 d; there was no change in the pH of LC-01 cheeses with 3.5% NaCl. Counts of LC-01 in all cheeses remained at levels of greater than 10^6 cfu/g up to 90 d, but decreased as the initial pH decreased from ~ 5.8 to 4.8 and as NaCl increased from 1.2 to 3.5%. The results suggest that BB12 was tolerant of large variations of salt and pH, as found across the spectrum of different cheese varieties, and, hence, would be suited for use in the manufacture of probiotic cheese.

Notes

Poster C3

The composition of milk fat affects the functionalities of Swiss cheese.

R. Richoux[1], L. Aubert-Frogerais[1], J.R. Kerjean[1], P. Pradel[2], V. Briard-Bion[3,4] and C. Lopez[3,4].

[1]*Actilait, BP 50915, F-35009 Rennes cedex;*

[2]*INRA, UEMA, 15190 Marcenat;*

[3]*INRA, UMR1253 Science et Technologie du Lait et de l'Œuf (STLO), F-35042 Rennes, France;*

[4]*AGROCAMPUS-OUEST, UMR125, STLO, F-35042 Rennes, France;*

r.richoux@actilait.com

Milk fat is frequently decried because of its high content of saturated fatty acids. Hence, numerous studies have been conducted to improve the nutritional properties of dairy products by modifying the fatty acid profile. In France, the AGILAIT (1) project aimed to increase unsaturated fatty acids (UFA) content by modulating the diet of cows and to measure the technological properties of the milks.

In this context, five diets were compared for Swiss cheese making: i) maize silage (MS), ii) maize silage + extruded linseed at 2.5%, iii) maize silage + extruded linseed at 5% (MSLS5), iv) hay and v) spring pasture. To avoid cheese variability not linked to the fatty acids profile, we decided to standardise milk composition (fat, protein), milk microflora (thermisation, lysozyme) and the cheesemaking process. Only minor process adjustments were employed during cheesemaking. Three small scale Swiss cheeses (2 kg) per batch of milk and three replicates per diet were made and characterised.

Unsaturated fatty acids content increased from 22% (MS) to 42% (MSLS2) of the total fatty acids. The supramolecular organisation of lipids in cheese was influenced by the diet, the more unsaturated fat being the most destructurated during cheesemaking. Oiling-off and flowability of the Swiss cheese were positively correlated to the content of cis-UFA. Descriptive sensory analysis showed significant differences in texture but not in flavour. In particular, cheeses made from high unsaturated milk fat did not develop any defect related to lipolysis or oxidation of lipids.

UFA-enriched Swiss cheeses can be manufactured all along the year to contribute in the improvement of long-term health of the consumers.

(1) This work was carried out with the financial support of ANR (French National Research Agency; ANR-06-PNRA-012) and the French dairy industrials (ARILAIT Recherches / CNIEL).

Poster C4

The composition of milk fat affects the functionalities of Raclette cheese.

R. Richoux[1], L. Aubert-Frogerais[1], J.R. Kerjean[1], P. Pradel[2], V. Briard-Bion[3,4] and C. Lopez[3,4].

[1]*Actilait, BP 50915, F-35009 Rennes cedex;*

[2]*INRA, UEMA, 15190 Marcenat;*

[3]*INRA, UMR1253 Science et Technologie du Lait et de l'Œuf (STLO), F-35042 Rennes, France;*

[4]*AGROCAMPUS-OUEST, UMR125, STLO, F-35042 Rennes, France;*

r.richoux@actilait.com

Milk fat is frequently decried because of its high content of saturated fatty acids. Hence, numerous studies have been conducted to improve the nutritional properties of dairy products by modifying the fatty acid profile. In France, the AGILAIT (1) project aimed to increase unsaturated fatty acids (UFA) content by modulating the diet of cows and to measure the technological properties of the milks.

Semi-hard Raclette-type cheese were manufactured from milk obtained with nine diets: i) maize silage (MS), ii) MS + extruded linseed at 2,5%, iii) MS + extruded linseed at 5%, iv) hay and v) spring pasture, vi) MS + Linseed oil, vii) MS + Rapeseed oil, viii) grass silage (GS), ix) GS + Rapeseed oil (GSRO). To limit cheese variability, milk composition and microflora and the cheesemaking process were standardised. Three Raclette-cheeses per batch of milk and three replicates per diet were made and characterized.

UFA content increased from 22% (MS) to 51% (GSRO) of the total fatty acids. The oiling-off of the ripened cheeses were positively correlated (r^2=0,68) with UFA. Descriptive sensorial analysis showed that cheeses with high UFA content did not develop any defect related to lipolysis or oxidation, neither at 15°C nor in a hot melted form. In contrast, the texture was strongly affected at both temperatures, especially for firm, melting, unctuous and elastic items. Although the mouth-feel was significantly improved by the increase of UFA, an optimum was found, probably due to the strong oiling-off observed for the most UFA-enriched cheeses. UFA-enriched Raclette-cheeses can be manufactured all along the year.

(1) This work was carried out with the financial support of ANR (French National Research Agency; ANR-06-PNRA-012) and the French dairy industrials (ARILAIT Recherches / CNIEL).

Notes

Poster C5

A dairy vector exclusively fermented by dairy propionibacteria: a new model to study probiotic potentialities *in vivo*.

F. Cousin[1,2,3], S. Louesdon[1,2], L. Le Normand[4], G. Boudry[4], S. Jouan-Lanhouet[5], M.T. Dimanche-Boitrel[5] and G. Jan[1,2].

[1]*INRA, UMR1253 Science et Technologie du Lait et de l'Œuf (STLO), F-35042 Rennes, France;*

[2]*AGROCAMPUS-OUEST, UMR125, STLO, F-35042 Rennes, France;*

[3]*CNIEL/Syndifrais, 42 rue de Châteaudun, F-75314 Paris 09, France;*

[4]*INRA, UMR1079 SENAH, F-35590 Saint Gilles, France;*

[5]*EA SeRAIC, IRSET, Faculté de pharmacie, Université de Rennes 1, IFR 140 GFAS, F-35043, Rennes, France.*

fabien.cousin@rennes.inra.fr

Dairy propionibacteria, commonly used as cheese ripening starters, display probiotic potential. This includes modulation of the microbiota and epithelial proliferation/apoptosis equilibrium in the gut. They trigger apoptosis (programmed cell death) of colorectal cancer cells via the production of short chain fatty acids, propionate and acetate. Such effects require high populations of live and metabolically active propionibacteria in the colon. Fermented dairy products protect probiotic bacteria against digestive stresses and are suitable vectors for probiotic bacteria delivery. As cheese and fermented milks with dairy propionibacteria contain other microorganisms, the identification of specific beneficial effects of propionibacteria is limited. We aimed at 1) developing milk exclusively fermented by a dairy propionibacterium; 2) studying propionibacteria survival to digestive stress in this vector and 3) evaluating the pro-apoptotic potential of this fermented milk on HT-29 human colorectal cancer cells. Since dairy propionibacteria do not generally grow in milk, we determined their nutritional requirements with respect to carbon and nitrogen by supplementing milk ultrafiltrate with different concentrations of food grade lactate and casein hydrolysate. Milk supplemented with 50 mM lactate and 5 g/L casein hydrolysate allowed growth of all dairy propionibacteria studied, reaching populations of at least 10^9 cfu/mL. In this model fermented milk, dairy propionibacteria remained tolerant to digestive stress *in vitro* towards acid and bile salts challenge, and viable, during at least 15 days at 4°C. The most tolerant strain was used in an animal trial, reaching high concentrations in faeces and colon contents. Fermented milk supernatants were shown to induce the typical features of apoptosis of HT-29 cells. This work leads to a new food grade vector containing exclusively dairy propionibacteria, allowing preclinical and clinical trials. Such new fermented milk might be of interest as a functional food to prevent colorectal cancer or to potentialize therapeutic treatments.

Poster C6

Analysis of bifidogenic properties of a novel exopolysaccharide from *Weissella cibaria* and incorporation of the producer bacterium into milk.

K.M. Lynch[1*], S. Galle[2], E. K. Arendt[2] and A. Coffey[1].

[1]*Department of Biological Sciences, Cork Institute of Technology, Rossa Avenue, Bishopstown, Cork;*

[2]*Department of Food and Nutritional Sciences, University College Cork, Ireland.*

kieran.lynch@mycit.ie

Exopolysaccharides produced by lactic acid bacteria (LAB) are non-digestible by humans and are thus available for utilization by the resident microbes in the gut. The ability of known prebiotics such as inulin and galactooligosaccharides to modify the gut microbiota is well known. However, the potential of exopolysaccharides from food-fermenting LAB to affect such changes is less understood. A method for large-scale exopolysaccharide isolation and purification of a dextran from *Weissella cibaria* was developed and delivers up to 36 grams of purified exopolysaccharide per litre of bacterial culture from sucrose-supplemented MRS. The exopolysaccharide was used to evaluate its effect on a number of bifidobacterial species. This was done by comparing the growth of such strains in MRS to growth in modified media containing exopolysaccharide or various combinations of exopolysaccharide and glucose as carbon sources. Additionally, the exopolysaccharide-producer was added to milk, with a view to using the bacterium as an adjunct in fermented dairy products. Generally, it was observed that the EPS-producing *Weissella cibaria* grew in the milk environment, albeit slower than a *Lactococcus lactis* starter culture, but typically reaching levels of 10^8 cfu/mL after 24 hours. Additionally, EPS production in the milk environment was assessed. This EPS-producing strain may have applications as an adjunct in selected fermented dairy products.

Notes

Poster C8

UFA-enriched versus control mould-ripened soft cheeses: Research studies for health benefits.

J.-Y Gassi[1,2], M.-B. Maillard[1,2], V. Briard-Bion[1,2], E. Beaucher[1,2], B. Robert[1,2], R. Richoux[3], P Brunschwig[4], M. Fougère[5], J.-P. Guyonnet[6] and C. Lopez[1,2].

[1]*INRA, UMR1253 Science et Technologie du Lait et de l'Œuf (STLO), F-35042 Rennes, France;*

[2]*AGROCAMPUS-OUEST, UMR125, STLO, F-35042 Rennes, France;*

[3]*Actilait, BP 50915, F-35009 Rennes cedex;*

[4]*Institute de l'Elevage, UP Catl, F-49105 Angers, France;*

[5]*Chambre d'Agriculture, Ferme Expérimental, F-44590 Derval, France;*

[6]*CNIEL, F-75314 Paris, France.*

jean-yves.gassi@rennes.inra.fr

Nutritional recommendations concern the reduction in the consumption of certain saturated fatty acids (SFA), which are associated with the risk of cardio-vascular diseases, in favour of increased amounts of unsaturated fatty acids (UFA) in the human diet. In this context, adapting the FA profile of high-fat content dairy products such as cheeses is of considerable interest for industrialists and consumers. The objectives of this study were i) to produce an experimental UFA-enriched milk by adapting the cows diet (corn silage + grass silage + rapeseed) and to collect a control milk in a local dairy plant during winter season (corn silage based diet), ii) to evaluate the possibility of manufacturing mould-ripened soft cheeses, and iii) to characterize the physico-chemical and sensory properties of the UFA-enriched and control cheeses (project AGILAIT; ANR 06-PNRA-012). The biochemical composition of the milks and cheeses were determined and compared at each technological step. The sensory properties of the cheeses were evaluated. The dietary strategy investigated in this study allowed the reduction of SFA content from 71.2 ± 0.5% down to 60.3 ± 0.3%; the milks and corresponding cheeses had similar FA profiles. The fat / total nitrogen ratios of the cheese milks were standardized in order to avoid differences due to different fat and protein contents in the milks. Regardless of the FA composition, cheese yields were similar. Changes in the levels of proteolysis were similar during the 45 days of ripening. Increasing the UFA content affected the free FA profile released during lipolysis but the total free FA levels were similar. Sensory analysis did not reveal any significant differences particularly as regards to lipid oxidation. As a conclusion, the composition of milk fat can be improved to face new nutritional quality standards. The manufacture of UFA-enriched cheeses will contribute in the improvement of long-term health of consumers.

Poster C10

A field study on Sicilian dairy farms: pasture and cattle breed effect on PUFA composition in milk.

S. La Terra[1], V.M. Marino[1], I. Schadt[1], M. Caccamo[1], G. Azzaro[1], S. Carpino[1] and G. Licitra[1,2].

[1]*CoRFiLaC, Regione Siciliana, s.p. 25 km 5 Ragusa Mare, 97100 Ragusa, Italy;*

[2]*DISPA, Catania University, via Valdisavoia 5, 97100 Catania, Italy.*

laterra@corfilac.it

The objective of this study was to examine the effects of pasture feeding and cattle breed on polyunsaturated fatty acid (PUFA) concentrations in milk. Four Sicilian dairy farms were selected, two with both Holsteins (H) and Brown Swiss (BS) cows and two with only local breed Modicana (M)cows. Bulk milk of each breed per farm was sampled four times with weekly intervals, per two experimental period (P1 = spring, P0 = summer). Pasture was available in P1 but not in P0. During P0 cows were grazing stubble. Additional hay and concentrate was supplemented during all periods. Pasture intakes have been calculated using CPM-Dairy®. Milk Conjugated Linoleic Acid (CLA), Linolenic Acid (LNA) and Linoleic Acid (LA) and alpha-tocopherol were analysed by HPLC. Both, CLA and LNA contents in M milk were highest compared to BS and H milk ($P < 0.001$). Least square means ± standard errors (micrograms/g fat) in M, F, BS milk of CLA were 3675.8a ± 0.07, 2132.5b ± 0.06 and 2011.8b ± 0.06, respectively, and LNA 2.7a ± 0.09, 1.1b ± 0.08 and 1.2b ± 0.08, respectively. Least square means ± standard errors (micrograms/g fat) of milk LNA during P1 and P0 were 2.0a ± 0.07 and 1.1b ± 0.07, respectively. Milk LNA ($P < 0.001$), but not CLA ($P > 0.05$) was influenced by pasture feeding. Even though CLA contents between P0 and P1 milk was not statistically different in the current study, there was still some evidence for the importance of pasture intake. Breed effects might have been confounded by the higher average pasture intake of M (69.0 dry matter) relative to BS and H (21.8 dry matter) during P1. Milk alpha-tocopherol concentrations were positively related to CLA ($R2 = 0.43$) and LNA ($R2 = 0.34$) contents and alpha-tocopherol was affected by pasture intake.

Notes

Poster C11

A field study on Sicilian dairy farms: pasture and cattle breed effect on alpha-tocopherol and beta-carotene in milk.

V.M. Marino*[1], I. Schadt[1], S. La Terra[1], M. Caccamo[1], M. Manenti[1], G. Licitra[1,2] and S. Carpino[1].

[1]*CoRFiLaC, Regione Siciliana, s.p. 25 km 5 Ragusa Mare, 97100 Ragusa, Italy;*

[2]*DISPA, Catania University, via Valdisavoia 5, 97100 Catania, Italy.*

v.marino@corfilac.it

Factors such as breed and feed source can alter milk vitamin E and beta-carotene (AO). The objective of this study was to examine the effects of pasture feeding and cattle breed on AO concentrations in milk. Four Sicilian dairy farms were selected, two with both Holsteins (H) and Brown Swiss (BS) cows and two with only local breed Modicana (M) cows. Bulk milk of each breed per farm was sampled four times per experimental period (P1 = spring, P0 = summer, and P2 = autumn). Samplings within period occurred with weekly intervals. Pasture was available in P1 and P2 but not in P0. Periods P1 and P2 differed by botanical composition and plant maturity. During P0 cows were grazing stubble. Additional hay and concentrate was supplemented during all periods. Pasture intakes have been calculated using CPM-Dairy®. Milk AO contents were analysed by HPLC. Both, milk beta-carotene and alpha-tocopherol were highest during P1, lowest during P2 and intermediate during P0 ($P < 0.001$). Least square means (micrograms/g fat) during P1, P2 and P0 of beta-carotene were 9.68a, 0.84c, 2.45b, respectively, and relative to alpha-tocopherol 16.15a, 11.15c, 13.28b, respectively. Milk from BS contained more beta-carotene compared to H ($P < 0.05$), but alpha-tocopherol levels were not affected. Milks from M had the same levels of beta-carotene compared to BS cows and were the highest in alpha-tocopherol ($P<0.05$) compared to the milks from the other breeds. However, the latter results might have been confounded by the higher average pasture intake of M (62.1 dry matter) relative to the BS and H (19.2 dry matter). During P2 body resources first had to be restored before AO could be released into milk explaining low AO values. Pasture benefits relative to AO milk content might be obtained only after a minimum necessary exposure period of cows to pasture.

Poster C12

Safety of strains selected for dairy starter cultures.

V. Laffitte, B. Sauvion, M. Charron, F. Guillemoto, V. Martin, P. Zarzov, P. Horvath and C. Fremaux.

Danisco France SAS, BP10 ZA de Buxieres, 86220 Dange Saint Romain, France

christophe.fremaux@danisco.com

One result of the use of starter cultures in food is the consumption of micro-organisms by humans and the production in food matrix of metabolites. Despite centuries of safe use of starter cultures, recent scientific advances have shown some health threats related to starter cultures that have to be taken under consideration. Among metabolites of concern are toxins (mycotoxins) that have been known for a longtime and more recently biogenic amine production has been shown to be responsible for some food-borne diseases. Recently, as well the emergence and the spread of resistance to antimicrobials in bacteria, this poses a threat to human health. In an effort to reduce this spread, selection of micro-organisms to be used in food that are deprived of the genetic determinants for antimicrobial resistance is strongly recommended. As a consequence, the whole collection of Danisco industrial lactic acid bacteria was investigated for abnormal resistance to antibiotics. In addition, industrial Staphylococcal strains as well as moulds were tested for the production of toxins. Finally, based on the most recent genomics data, molecular methods were developed for the detection of a wide range of genes coding for either histidine decarboxylases or tyrosine decarboxylases; enzymes that are responsible for the production of histamine and tyramine, respectively. These methods were applied as well to the Danisco range of industrial lactic acid bacteria. Globally, this allows Danisco to continuously improve their Culture offer, with a focus on food and consumer safety.

Notes

Poster C14

Analysis of the factors that determine the chemical attributes of artisanal Ranchero cheese from central Mexico.

A.D. Solís-Méndez[1] J.G. Estrada-Flores[2] and O.A. Castelán-Ortega[1].

[1]*Faculty of Agricultural Sciences and*

[2]*Institute of Agricultural and Rural Sciences of the Universidad Autónoma del Estado de México.*

Instituto Literario no. 100, Colonia Centro, C.P. 50000, Toluca, México.

donaxi@gmail.com

The Artisan Ranchero Cheese (ARC) is one of the most popular cheeses from central Mexico; it has a great gastronomic and cultural value. The objective of the present study is to discuss the complex relationships between climate, land, grass, animal and know-how of cheesemakers, all of which determine the final chemical attributes of Artisan Ranchero Cheese. The fat, protein, moisture, pH, titratable acidity, ash and NaCl contents were determined in cheeses produced in 18 cheese workshops. Cheese samples were collected every two months over one year (May 2006 to April 2007), the composition of the raw milk used to manufacture ARC was also analyzed for its physicochemical attributes. Cheesemaking data during the manufacturing process and cheese yields were recorded. The cheesemakers from each community were classified into two groups according to the number of production blocks which are involved in the ARC process or Production Structure (PS). The first group was named Productive Unit with Complete Cycle (PUCC) and the second group Dairy Production Chain (DPCh). Cheesemakers in the PUCC group produced cheeses with a higher acidity and moisture content than the DPCh producers ($P<0.05$), because the latter group faces tougher demands for quality, good presentation and short delivery times. These factors mean that the manufacturing process is shorter, in particular small milk acidification periods. Average contents of protein 24.2 ±3.6, fat 21.6 ± 6.2, moisture 52.2 ± 6,7, ash 3.1 ± 1.9, NaCl 1.3± 0.58; pH 5.4 ± 0.4 and acidity D 18.1 ± 3.8 of ARC meet the standards established by Mexican regulations. Finally, it was observed that the quality of the ARC was better in the DPCh group because it has more interaction with the market and potential buyers, who request more quality than buyers in the PUCC group.

Poster C15

A diversity study of the Artisan Ranchero Cheese from Central Mexico.

A.D. Solís-Méndez[1], J.G. Estrada-Flores[2] and O.A. Castelán-Ortega[1].

[1]*Faculty of Agricultural Sciences and*

[2]*Institute of Agricultural and Rural Sciences of the Universidad Autónoma del Estado de México.*

Instituto Literario no. 100, Colonia Centro, C.P. 50000, Toluca, México.

donaxi@gmail.com

The objective of the present study is to determine the diversity in the textural profile of Artisan Ranchero Cheese (ARC), a popular soft cheese made with raw cow's milk, which is consumed fresh. These factors include climatic conditions, production zone, cheese supplies, cheesemaking procedure and chemical attributes. Texture attributes were determined by texture profile analysis method. The relationship between texture and chemical attributes of the ARC were determined by a Factor Analysis. An Agglomerative Hierarchical Clustering Analysis was used to differentiate the ARC manufactures into groups. Three groups were identified, production zone being the variable which explained the variation in the data. Finally, a Discriminant Analysis was performed in order to determine standardization of the ARC attributes through the sampling periods. 89% of cheese samples were correctly classified into their own cheese manufacture. This last analysis demonstrated that there is an important degree of standardisation of the cheesemaking procedure within each cheese manufacture.

Notes

Poster C17

Biogenic amines content in accelerated ripened Edam cheese – a preliminary study.

F. Buňka, L. Buňková, V. Pachlová and R. Flasarová.

Tomas Bata University in Zlin, nam. T. G. Masaryka 5555, 76001 Zlin; Czech Republic.

bunka@ft.utb.cz

Cheese characteristics develop during the ripening process; 6-8 weeks of maturation is necessary for Edam. The elevation of temperature is regarded as a simple method of acceleration of the maturation of cheese. Biogenic amines are low-molecular compounds formed predominantly by microbial decarboxylation of free amino-acids. Many lactic acid bacteria including starter cultures possess decarboxylases. The objective of this study was to investigate the influence of acceleration by elevated temperatures on production of selected biogenic amines. Six blocks of commercial Edam cheese made from pasteurised milk (52% w/w dry matter and 15% w/w fat; ~1.4 kg; ~9cm height, 9cm width and 15cm depth) were sampled before brining. On the second day (after brining and packing into Cryovac), two blocks were put into a ripening cellar (10±2°C; control) for 56 days and the other blocks were placed into ripening chamber (16±1°C) for 28 days. Three batches were tested. A central belt (2.5cm depth) was cut crosswise each cheese block and a core (approx. 2cm) was collected. The core was lyophilized. Concentrations of free amino acids (FAA) and biogenic amines (histamine, tyramine, putrescine, cadaverine) were determinated using ion-exchange chromatography (AAA400; Ingos, Czech Republic; n=36). After 56 d of ripening, FAA concentration in core of controls had the similar level of FAA in the accelerated samples ripened for 28 days. Histamine was not detected. After 56 d of ripening, the amount of tyramine, putrescine and cadaverine in core of controls was 48.5±3.1mg/kg; 54.7±2.9mg/kg and 12.8±1.7mg/kg, respectively. After 28 d, the concentrations of tyramine, putrescine and cadaverine in core of accelerated cheese reached higher values (71.1±5.6mg/kg, 79.2±5.1mg/kg and 16.3±0.9mg/kg, respectively; $P<0.05$) compared to the levels found in controls (ripened for 56 days). Elevated ripening temperature caused greater production of selected biogenic amines. Therefore, these processes should be studied more intensively.

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Notes

THEME D:
Cheese Quality to Concepts

Abstracts for Poster Presentations

Notes

Poster D1

Standardization of the lactose content of milk concentrate.

T. Aaltonen.

Valio LTD, R&D Center, PO Box 30, FI-00039 Valio, Finland;
Department of Food and Environmental Sciences, Division of Food Technology, PO Box 66, FI-00014 Finland, University of Helsinki, Finland.

terhi.aaltonen@valio.fi

Filtration techniques can be used in cheese processing to attain a standardized and more energy-efficient process. Microfiltration can be used to concentrate the basic components of cheese, fat and casein. The disadvantages of whey proteins can be avoided by removing them from the retentate with a diafiltration step. Reducing the lactose content is necessary if the retentate is used in cheese production without traditional curd formation and whey draining. The idea of this study was to determine how microfiltration and diafiltration of standardized milk affects the lactose content of retentate and how a second diafiltration with brine reduces the lactose content of the retentate. 600 kg of pasteurized and standardized (fat:protein ratio 0.7) milk was used as a feed in microfiltration. Milk was concentrated to a concentration factor of 4 and whey proteins were removed by a first diafiltration step where 1000 kg of ultrafiltered milk permeate was used as diawater. The lactose content was reduced with a second diafiltration step were 170 kg brine was used as diawater. The content of protein, total solids, whey protein and lactose were followed during filtration. Four separate filtrations were performed. It was found that the lactose content of the retentate reduced significantly ($P<0.001$) during milk concentration. Whey protein removal with milk ultrafiltered permeate had no effect on the lactose content of the retentate. After the second diafiltration, statistically significant ($P<0.001$) differences were observed in the lactose content. It was also found that the lactose content reduced linearly as a function of the diawater content in the second diafiltration with a strong correlation ($R2 = 0.95$). The content of lactose in the retentate decreased during milk concentration. However, this reduction may be too small if the retentate is used as the starting material for the production of full concentrated cheeses. Diafiltration may be used to reduce the lactose content of retentate to get optimal lactose content for cheese-making.

Poster D2

Relationship between technological steps and behaviour of French goat cheeses for culinary applications.

P. Barrucand and K. Raynal-Ljutovac.

Actilait, BP 49, F-17700 Surgères, France, France.

p.barrucand@actilait.com

The use of goats cheese as an ingredient in ready made meals is an area of growing value. However, a lack of data concerning the cooking properties of pure goats milk cheeses, mainly of lactic type in France, could impede the development of such applications. A first analysis of the culinary behaviour of French goats cheeses currently sold was performed through the development or adaptation of methods such as specific sensory analysis tests generated by a panel of experts, cooking tests coupled with photographs allowing a computer evaluation of spreading and rheological protocols. The colouring, spreading, texture and flavour after cooking, have been studied on raw milk cheeses either fresh or ripened with *Geotrichum candidum* as the main surface ripening strain and pasteurized milk cheeses either fresh or ripened with *Penicillium camemberti* as the main surface ripening strain. Some semi hard cheeses (Tome) were also evaluated. The biochemical composition of the cheeses was also assessed. Significant differences in colour and to a lesser extent in spreading were found between the studied cheeses but differences concerning the appearance were also detected with the occurrence of holes for most trade products. The last step was intended, through the completion of experimental cheese makings in controlled conditions, to highlight the main factors determining the cooking ability of cheeses and hence to obtain a better understanding of the mechanisms involved. These first data will help manufacturers: i) to solve the current problems observed with these products, ii) to establish the range of culinary uses of their products and, iii) to move towards new applications.

Notes

Poster D5

Repeatability and reproducibility of RP-HPLC profiles of proteolysis in cheese.

E. Parente[1], H. Patel[2], V. Caldeo[2], P. Piraino[3] and P.L.H. McSweeney[2].

[1]*Consiglio Nazionale Delle Ricerche, Italy.*

[2]*School of Food and Nutritional Sciences, University College, Cork, Ireland;*

[3]*Statistical Consulting, Rende, Italy.*

The assessment of proteolysis by RP-HPLC of soluble nitrogen cheese extracts is the most important technique to evaluate authenticity and quality of cheese. The aim of this study was to evaluate by a combination of univariate and multivariate statistical methods the repeatability and reproducibility of the measurements and the contribution of differences in sample/treatment, extraction, and the effect of the chromatographic run. For this purpose data were used from a pilot plant experiment (108 chromatograms) in which 4 treatments were used in the production of Cheddar cheese and for which replicate cheese trials, with replicate extraction for each sample and replicate injections for each extract were available. When all measurements were subjected to principal component analysis (PCA) a significant scatter was observed in replicates for extracts of the same cheese. However, using ANOVA on component scores it was still possible to identify significant differences among cheeses and the contribution due to replicate cheesemaking trials. Results from the partial least square discriminant analysis (PLS-DA) showed that the variance due to cheese treatment was relatively small but allowed identification of the variables which contributed to this variation, and quantification of the relative contribution of cheese type, replicate cheesemaking trial, extraction and injection. Moreover, a second set of data of replicate injections of the same extract of a single cheese was analyzed to evaluate the degradation of column performance over time. Results provide insights into the real discriminatory power of RP-HPLC of cheese extracts and information which may be used to improve experimental designs in both process control and process optimization.

Poster D7

Bacteriophages in cheese manufacturing: characterization and control procedures.

C. Barthod, A. Pulli, M.-A. Poisson, C. Ribiollet, C. Rambeaux and E. Jamet.

Actilait, The French dairy Institute, BP 49, F-17700 Surgères, France

e.jamet@actilait.com

Bacteriophages are a group of viruses that only infect specific bacteria and were discovered in the early 20th century primarily through the study of pathogenic bacteria. This infection often leads to the death of the infected bacteria (lytic cycle) or to the introduction of phage genetic material into the chromosome of the bacteria (lysogenic cycle). Like all viruses, phages have a rudimentary biological organization and depend on the functions of their host (infection) for their propagation and their long-term survival. Without hosted bacteria, they have no metabolic activity. In contrast, when in contact with their host bacterium, they can adsorb onto the surface and inject their genetic material. The host cells then stop their normal metabolic activity, synthesize new phages and finally lyse, releasing newly formed phages.

All industrial sectors which use bacteria in their processes are vulnerable to phages infections and in particular the dairy sector where an estimated 0.5 to 10% of fermentations using lactic acid bacteria are affected, generating a large number of flavours, texture and appearance defects in the dairy products.

Their introduction of phages into cheese plants is generally due to the raw materials (raw milk in our case) but also via the inoculation of lysogenic strains. Dispersal within the production tool is often very fast through the liquids used (water rinsing) and / or products (whey). It is now known that the strains present in modern dairies are highly heat-tolerant (resistance to pasteurization, for example) as well as high dynamic pressures. Resistance to cleaning and disinfection is also increasingly being described.

This poster makes a focus about the tools available for the fast detection, characterization and identification of bacteriophages in fermentations context (dairy or not) and to propose a complete procedure for analysis of phages infection in cheeses factories.

Notes

Poster D9

Effect of added biopolymers on the properties of a model cheese system.

I. Piska and T.P. Guinee.

Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland.

ivo.piska@teagasc.ie

Manufacture of high moisture ingredient cheeses with desired textural properties is a challenging task. When the moisture content of cheese is increased, the concentration of the polymer casein network is reduced and the cheese tends to become more fluid and less elastic. These changes generally lead to softer, stickier cheese which is less suited as an ingredient owing to its poor shreddability and sliceability. To compensate for the latter deficiencies, biopolymers are often used to introduce an additional network structure in the cheese system and, to thereby augment the degree of matrix structure in the resultant gel-based composite. The aim of the present study was to assess the effects of a range of added biopolymers (negatively charged hydrocolloids such as pectin, alginate, kappa-carrageenan, and neutral hydrocolloids such as LBG and guar gum) on the properties of a high moisture model cheese system. Model cheeses (~ 42 % dry matter) were prepared from liquid pre-cheese formulated by dispersing and blending milk protein, fat, lactose and water to the final cheese composition. The resultant pre-cheese was pasteurised at 80 °C, cooled to ~ 35 °C, and gelled by the addition of rennet and glucono-delta-lactone and incubating at 32°C. The biopolymers, added typically at a level of 0.5% (w/w), either along with milk protein prior to dispersion or to the milk protein dispersion just prior to pasteurisation. Following gelation and storage at 4 °C for 1-4 d, the model cheeses were analysed for composition, rheological properties, microstructure and flowability on heating.

Hydrocolloids were found to markedly alter rheology and melting properties of the model cheeses. However, these effects of hydrocolloid were highly dependent on hydrocolloid type (charged, neutral), sequence of addition, and addition of ions (calcium or potassium).

Poster D10

Effect of disodium phosphate (DSP) level on the chemical and physical properties of processed cheese (PC).

T.P. Guinee and B.T. O'Kennedy.

Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland.

tim.guinee@teagasc.ie

Emulsifying salts are added to PC formulations to promote hydration of the cheese protein (calcium phosphate para-casein) which emulsifies free fat released during processing. The current study investigated the effect of altering DSP, a commonly-used emulsifying salt, from 0.35 to 3.4% (w/w), on the chemical and functional properties of PC. PCs were formulated in triplicate from Cheddar cheese, DSP, preservative and water and standardised with respect to intact casein content (~ 91 %), moisture (~ 48.2%, w/w), protein-to-fat ratio (~ 0.75), calcium-casein ratio (33.8 mg/g), and pH (~ 5.75). Addition of DSP at 0.35% was insufficient to enable formation of a stable PC, as reflected by the presence of large quantities of free water and oil in the processed formulation. Stable PCs were formed at DSP levels greater than, or equal to, 0.75% (w/w), with 0.75% DSP giving PC that was notably more liquid on completion of processing than PCs with higher DSP levels. Increasing DSP from 0.75 to 3.4% (w/w) significantly increased storage modulus (G') at 25 °C, firmness and fracture stress of the unheated PC, and reduced the degree of flowability and fluidity (loss tangent at 80 °C) of the heated PC. The latter changes, on increasing DSP, coincided with significant increases in water soluble protein (WSP, from 35 to 80% of total protein) and ratio of water soluble phosphate-to-WSP, and a significant reduction in the ratio of water-soluble calcium-to-WSP (from ~ 18 to 5 mg/g). Simultaneously, the ratio of water-insoluble calcium plus phosphorous to water-insoluble protein increased significantly as DSP was increased. The results suggest that added DSP sequesters para-casein bound calcium and phosphate as insoluble calcium phosphate complexes/inclusions, to a degree that increases with DSP level. Hence, the calcium-to-para-casein ratio decreased while the proportion of para-casein solubilised increased significantly as the DSP level was increased.

Notes

Poster D11

Aspartase activity of dairy propionibacteria in Maasdam and industrial Swiss-cheese.

A.L. Jacquet[1], L. Aubert-Frogerais[1], R. Richoux[1], A. Leclerc[2], R. Lemée[2], J.R. Kerjean[1], N. Roland[2].

[1]*Actilait, BP 50915, F-35009 Rennes cedex;*

[2]*Laboratoires Standa, 68 rue Robert Kaskoreff, F-14050 Caen cedex 4.*

r.richoux@actilait.com

During Swiss-type cheeses ripening, *Propionibacterium freudenreichii* converts lactate into CO2, acetate and propionate, which contributes to the opening and flavour formation. The co-metabolism of lactate and aspartate is known to produce more CO2 than the sole lactate metabolism. This strain dependent aspartase activity is linked to the cheese aroma intensity but also to the sensitivity to secondary fermentation defects. However, cheesemakers often consider that this aspartase activity only concerns cheese varieties with low lactate content, e.g. traditional Emmental or Maasdam cheese.

To clarify this point, experimental minicheeses (1kg) were made from microfiltered (1.4µm) milk with high or low lactate content at their removal from the warm room: > 800 mg/100g vs ≈0 mg/100g. Two aspartase positive (Asp+) and two aspartase negative (Asp-) *P. freudenreichii* were tested in two cheese-models: i) hard cooked and ii) semi-hard non-cooked cheeeses. During ripening, the aspartase activity was monitored by measuring free aspartate and asparagine, volatile fatty acids, succinate and lactate.

Regardless of the cheese variety inoculated with Asp- strains, aspartate increased regularly from the start of the warm room and during the cold room, whereas with Asp + strains, it decreased or stabilized during the warm room and only slightly accumulated during cold storage.

Aspartate metabolism was related to an increase in succinate content in the semi-hard cheese. No aspartase activity was noticed in cheeses inoculated with Asp- strains, since similar evolution was observed in control cheese without PAB inoculation.

Both Asp+ and only one Asp- strains utilized asparagine during warm room ripening. This activity, particularly obvious in semi-hard cheese due to a longer ripening time, provides additional aspartate available for Asp+ strains or which accumulated in excess in cheese made with Asp- and Asn+ strains.

Assessment of the aspartase activity of Propionibacteria used for the ripening of industrial cheeses is thus a useful parameter to better control opening and quality of cheese.

Poster D12

Development of Generic Cheese Models.

R. Richoux, A.L. Jacquet, L. Aubert-Frogerais and J.R. Kerjean.

Actilait, BP 50915, F-35009 Rennes cedex, France

jrkerjean@actilait.com

Cheese experiments need reproducible and realistic cheese manufacturing methodologies to provide valuable results. Indeed, the statistical power (1-n) and the number of replicates required to highlight differences between treatments are greatly dependent on the reproducibility of the model. The aim of this work was to develop and assess the reproducibility of cheese generic models manufactured with controlled microflora (MF 1.4µm) and with actual technologies (strong heat treatment of the milk, protein enrichment).

Five generic cheese models were developed by Actilait, including hard-cheese (Swiss-cheese), semi-hard cheeses (Maasdam, Raclette) and soft cheeses (Brie-style, Camembert). Around 30 replicates were performed per model in order to check (assess) their reproducibility.

Depending on the model, 100 to 120 control variables and state variables were registered. This includes the milk composition, the manufacturing parameters, the composition of the curd at moulding, the composition of the green cheese at Day 1, the whey composition, yields, composition and functional properties of the ripened cheeses. The distribution and the dispersion parameters were examined for each variable. The coefficients of variation of reproducibility were around 1%-1.5%. These values of reproducibility are better than those generally found in cheesemaking experiments. Despite the great attention paid to the standardization of milk, most of the residual variability of the models was linked to the one of the cheesemilk. Indeed, the mapping drawn by the Principal Component Analysis clustered the cheeses according to the batch of milk.

Notes

Poster D15

Isolation and characterization of virus-like particles in *Penicillium camemberti*.

G. Petit and S. Labrie.

Department of Food Sciences and Nutrition, Nutraceuticals and Functional Foods Institute (INAF);

STELA Dairy Research Centre, Université Laval, Quebec City, QC, Canada.

Steve.Labrie@fsaa.ulaval.ca

Penicillium camemberti (syn. *P. candidum* and *P. caseicola*) is used as a ripening culture for the production of bloomy rind-type cheeses like Brie and Camembert. *P. camemberti* is a major contributor to the formation of the thin, white and uniform layer on the cheese surface and also contributes to the organoleptic quality of the cheese. The growth of *P. camemberti* and the development of flavour can vary during ripening. Moreover, we know that processes and ripening conditions influence the activity of *P. camemberti.* The presence of mycoviruses (fungal viruses) may also be one factor that contributes to these variations. Mycoviruses have been discovered in some species of the genus Penicillium, but never in species of dairy interest. The objective of this study was to demonstrate that six *P. camemberti* strains, including three industrial strains from different culture suppliers and three isolated from manufactured soft cheeses, contain mycoviruses. Ribosomal DNA ITS1-5.8S-ITS2 and partial β-tubulin sequencing was used to confirm the genus and species of the *P. camemberti* strains. Virus-like particles (VLPs) were then purified by ultracentrifugation on a sucrose gradient density. Purified VLPs were stained with uranyl acetate or phosphotungstic acid and examined by transmission electron microscopy. VLPs were detected in all six *P. camemberti* strains. Particles were not enveloped, had icosahedral symmetry, and were 45 ± 3 nm in diameter. The nucleic acid was extracted and digested with ribonuclease to determine whether the VLP genomes were composed of DNA or RNA. Results showed that all VLPs contained at least four RNA fragments. Efforts are currently underway to sequence the genomes in order to determine VLPs affiliation to a mycovirus family.

Poster D16

Does cow teat skin act as a reservoir of microbial diversity having an interest for cheesemaking?

I. Verdier-Metz[1], F. Monsallier[1], G. Gagne[2], S. Bornes[2], P. Veisseire[2], C. Delbès-Paus[1] and M.-C. Montel[1].

[1]INRA, UR545, Recherches Fromagères, F-15000 Aurillac, France;

[2]Clermont Université, Université d'Auvergne, Laboratoire de Biologie, IUT Aurillac, BP 10448, F-6300, Clermont-Ferrand, France.

isabelle.verdier-metz@clermont.inra.fr

The microbial diversity of milk contributes to the diversity and richness of the sensory properties of cheeses. A better knowledge of the reservoirs of biodiversity in a farm environment will help to maintain microbial milk diversity whilst eliminating pathogens. The aim of this work was to evaluate whether the teat can act as a source of microbial diversity for raw milk cheeses. Four farms located in the department of Cantal (France) were studied. They were selected according to the type of cattle housing and bedding used but also on the microbial characteristics of the teat skin. The diversity of the microbial community on cow teat skin was evaluated using a culture-dependent method based on the use of different dairy dedicated media followed by the identification of isolates by 16S rDNA sequencing. This was combined with a direct molecular approach by cloning and 16S rDNA sequencing. This study highlighted the large diversity of the bacterial community that may be found on teat skin. Altogether, 29 bacterial species were identified using a culture-dependent method and 36 using a culture-independent method. Eight bacterial genera (*Arthrobacter*, *Curtobacterium*, *Microbacterium*, *Aerococcus*, *Bacillus*, *Enterococcus*, *Staphylococcus* and *Streptococcus*) and ten species were found by both methods. Firmicutes was the most prevalent phylum found in common. The direct method highlighted the prevalence of Clostridiales whereas *Staphylococcus* sp. and *Aerococcus* sp. were dominant using the culture-based method. It may be noted that the composition of the microbial community of teat skin varied qualitatively and quantitatively from one farm to another. Teat skin could enrich milk with *Staphylococci*, *Corynebacteria* and *Leucobacter* which all contribute to the sensorial properties of cheese

Notes

Poster D17

Escherichia coli O26:H11 inhibition by *Hafnia alvei* in an uncooked pressed cheese.

C. Delbès-Paus[1], S. Miszczycha[2], S. Ganet[2], P. Veisseire[3], C. Callon[1], D. Thévenot[2] and M.-C. Montel[1].

[1]*INRA, UR545 Recherches Fromagères, 15000 Aurillac, France;*

[2]*VetAgroSup, UR CALYTISS, 1 avenue Bourgelat, 69280 Marcy l'Etoile, France;*

[3]*Laboratoire de Biologie, Clermont Université, Université d'Auvergne, IUT Aurillac, BP 10448, F-63000, Clermont-Ferrand, France.*

cdelbes@clermont.inra.fr

Shigatoxin-producing *Escherichia coli*, especially serotype O157:H7 but also O26:H11, are an important cause of foodborne disease. Its capacity to grow and survive in cheeses in the presence of a competing flora is not well known. Hafniae are among the Enterobacteriaceae most commonly isolated from raw milk and cheese that could impact *E. coli* O26:H11 behaviour. The aim of this work was to evaluate the potential of Hafniae, in interaction with a complex microbial consortium, to inhibit the growth of *E. coli* O26:H11 in cheese. *E. coli* O26:H11 (eae+, stx1+) and Hafniae strains (two H. alvei strains, one H. paralvei strain) were co-inoculated into pasteurized milk at concentrations of 10^2 cfu/mL and 10^3-10^6 cfu/mL respectively, together with a technological consortium representative of the major groups of microrganisms commonly found in raw milk cheese. Uncooked pressed model cheeses were prepared. Microbial counts and organic acids concentrations in the cheese core were determined after 1, 8, 18 and 28 days of ripening. *E. coli* O26:H11 counts on day 1 in cheese inoculated with the starter and the technological consortium was around 10^5 cfu/mL. It was ~1 log cfu/mL lower when an Hafnia strain was inoculated at 10^6 cfu/mL into milk. Then the technological consortium and Hafnia operated synergistically to reduce it further by 0.5-1 log cfu/mL by the end of ripening. Slightly lower pH values (-0.07 pH units) and low amounts of acetate (<1 g/kg) and succinate (<0.5 g/kg) were found on day 1 in cheeses inoculated with Hafnia at 10^3 and 10^6 cfu/mL. The inhibition of *E. coli* O26:H11 growth by Hafnia during the first day was not correlated with pH values nor the production of organic acids. The potential role of hydrogen peroxide or bacteriocin-like molecules in the inhibition will be further investigated.

Poster D18

Variation of microbial flora, intracellular enzymes and compositional indices in commercial Cheddar cheese blocks during ripening.

D.K. Hickey[1], T.P. Guinee[2] and M.G. Wilkinson[1]

1Department of Life Sciences, University of Limerick, Castletroy, Limerick, Ireland;

[2]*Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland.*

Martin.Wilkinson@ul.ie

Commercially, Cheddar cheese is typically produced as large, 20 kg rectangular blocks. Within commercial blocks localised variations in composition may influence microbial death rate, autolysis, enzymatic activity and consequently flavour development during ripening. In the Irish context, little is known regarding the extent of these variations throughout commercial cheese blocks manufactured using a commercial culture preparation containing a mixture of lactococci, thermophillic lactobacilli and *Streptococcus thermophilus*. Fresh Cheddar cheese blocks (20 kg) were sourced from a leading Irish manufacturer on two occasions and stored at 8 °C for 9 months. After different ripening times, separate blocks were monitored for microbial populations, intracellular enzymes and compositional parameters at five different locations along a diagonal from the top face (through the centre) to the bottom face. Starter and non-starter lactic acid bacteria (NSLAB) populations varied across individual blocks with maximum differences of 1.7 $\log_{10}$ cfu/g and 3.2 $\log_{10}$ cfu/g, respectively. Pep X and Pep N activities tended to be higher throughout ripening towards the centre of the block than at the surface and may reflect species-specific differences in permeabilisation/autolysis. Consequently, secondary proteolysis as measured by PTA-soluble N and free amino acids, was also generally higher towards the centre of the block. As ripening progressed, water activity tended to be lower in the centre of the block. Variations in composition also occurred across individual blocks, with maximum differences amounting to 1.3%, 0.16% and 0.1 units for moisture, salt and pH, respectively. Additionally, compositional differences were observed between different blocks from the same batch. Overall, this study has demonstrated that variation in a number of factors influencing cheese quality can occur within a 20 kg commercial Cheddar cheese block during ripening.

Notes

Poster D20

Validation of X-ray computed tomography for the quantitative measurement of the eye volume of cheese.

W. Bisig, D. Guggisberg, M.-T. Fröhlich-Wyder, M. Greco and D. Wechsler.

Agroscope Liebefeld-Posieux research station ALP,CH-3003 Bern, Switzerland.

walter.bisig@alp.admin.ch

X-ray computed tomography (CT) is widely used in medicine but also more and more in technical applications such as weld verification, structural mechanics or in archaeology. CT technology allows to distinguish materials of different density as they block the X-ray beam accordingly.

Eye formation is an important quality aspect of Swiss-type cheese and also of many semi-hard cheeses. The existing two-dimensional X-ray system only allows a semi-quantitative measurement of the eye-volume. To find differences in the activity of metabolic pathways with gas formation of different microorganisms in cheese for the development of eye forming cultures and to investigate other influences on eye formation, CT was applied to quantitatively determine the eye volume. A Philips CT system was used in collaboration with the veterinary hospital of Bern University. To be able to quantify the eye volume, specially designed software had to be developed. As the CT system recognises the density difference between gas and cheese body, the total volume of the cheese can be calculated and also the total eye volume inside the cheese. The CT system was validated with cheese made with the inclusion of hollow polypropylene balls of a diameter of 10 and 20 mm respectively. Six normally eyeless Gruyere type hard cheeses per ball diameter with an increasing number of balls and hollow volumes from 0 to 33.3 ml and 0 to 309.1 ml respectively were produced and ripened for 30 days within a plastic film. The eye volume measured and calculated with the CT system showed correlation factors of 0.9912 and 0.9976 respectively in comparison with the given hollow volume of the added balls. The study showed that X-ray computed tomography is well suited to measure quantitatively the eye volume of semi-hard and hard cheeses in a non-invasive manner.

Poster D21

The MicroCheese: a high-throughput screening tool for risk and benefit assessment.

L.A. Hazelwood[1,2], Z. Kruijswijk[1], J.D. Hoolwerf[1], W.J.M. Engels[1], and J.A. Wouters[1]

[1]NIZO food research, Kernhemseweg 2, 6718 ZB Ede, The Netherlands;

[2]Top Institute Food and Nutrition (TIFN), Nieuwe Kanaal 9a, 6709 PA Wageningen, The Netherlands

lucie.hazelwood@nizo.nl

The awareness of the influence of diet and food on health is continuously increasing in society. In cheese, salt is an important part of the taste, and also has a preservation function within the product. The challenge for cheese manufacturers is to develop novel cheese products that are lower in salt. However, for consumer acceptance the cheese should remain palatable and safe for the consumer. For acceleration of new product development the food industry is highly interested in food model systems that predict effects of formulation changes on risks and health benefits. In addition to the challenge of salt reduction, the application of live probiotic strains in cheese products is an attractive approach for companies wishing to claim health benefits in cheese. In this case, it is vital to select a probiotic strain which survives throughout the whole ripening and storage period. In the context of the DREAM project ("Design and development of realistic food models with well characterized micro- and macro-structure and composition") funded by the European Union, the MicroCheese model[1] has been applied as tool for risk and benefit assessment in cheese. For this purpose, a representative spoilage organism (*Clostridium tyrobutyricum* NIZO570) and the commercial probiotic strain (*Bifidobacterium lactis* Bb12) were assessed for their outgrowth and survival in MicroCheeses (200 mg, produced in 96-wells format) of varying salt contents. Survival of these organisms was monitored during cheese ripening as a function of salt content. The results obtained confirm that the MicroCheese is a suitable screening tool for risk and benefit assessment studies.

[1]*Bachmann H, Kruijswijk Z, Molenaar D, Kleerebezem M, van Hylckama Vlieg JE. 2009.* A high-throughput cheese manufacturing model for effective cheese starter culture screening. *J Dairy Sci. 92(12):5868-82.*

Notes

Poster D22

Multilevel approach indicates high clonal and functional diversity in Gouda cheese starter culture.

O.E. Kutahya[1,2], I. van Alen[1,3], P. Janssen[1,3], L.A. Hazelwood[1,3], M. Kleerebezem[1,2,3] and E.J. Smid[1,4].

[1]*Top Institute Food and Nutrition (TIFN), Nieuwe Kanaal 9a, 6709 PA Wageningen, The Netherlands;*

[2] *Laboratory of Microbiology, Wageningen University, 6703 HB, Wageningen, The Netherlands;*

[3]*NIZO Food Research BV, 6710 BA Ede, The Netherlands;*

[4]*Laboratory of Food Microbiology, Wageningen University, 6700 EV, Wageningen, The Netherlands.*

oylum.kutahya@nizo.nl

Gouda cheese starters are undefined cultures of closely related strains belonging to the species *Lactococcus lactis* and *Leuconostoc mesenteroides*. Several strains with different metabolic characteristics in proteolysis, citrate metabolism as well as phage and salt resistance coexist. Cheese fermentation exerts many stress conditions like temperature fluctuations, continuous pH change, carbohydrate starvation, salt stress due to brining and phage predation. These stress factors shape the population composition and determine the overall functionality of the starter during the entire course of fermentation. The functional diversity is not only linked with chromosome-associated genomic diversity. Also mobile genetic elements, like plasmids and phage predation play a crucial role. Therefore, we set out to explore the diversity in terms of genotypes, plasmid profiles, and phage resistance to understand the forces shaping the composition of the starter culture in an industrial setting.

A collection of isolates has been generated from different time points during cheese making covering the entire spectrum of strain diversity. Amplified fragment length polymorphism (AFLP) was used for fingerprinting of the isolates. 8 different genotypes have been identified on basis of the AFLP profiles and the plasmid diversity was found to be uncoupled to chromosomal diversity with 13 unique profiles. The major population shifts occurred after the brining step and the plasmid profiles associated with *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* strains were enriched during ripening period. Additionally, the phage sensitivity was investigated on different isolates of each genotype using bacteriophages originating from the starter culture. Strains within the same AFLP types showed different sensitivity levels to the phages isolated from their AFLP type strain. The diversity of phage sensitivity within the same genotype suggests robustness against clonal sweeps.

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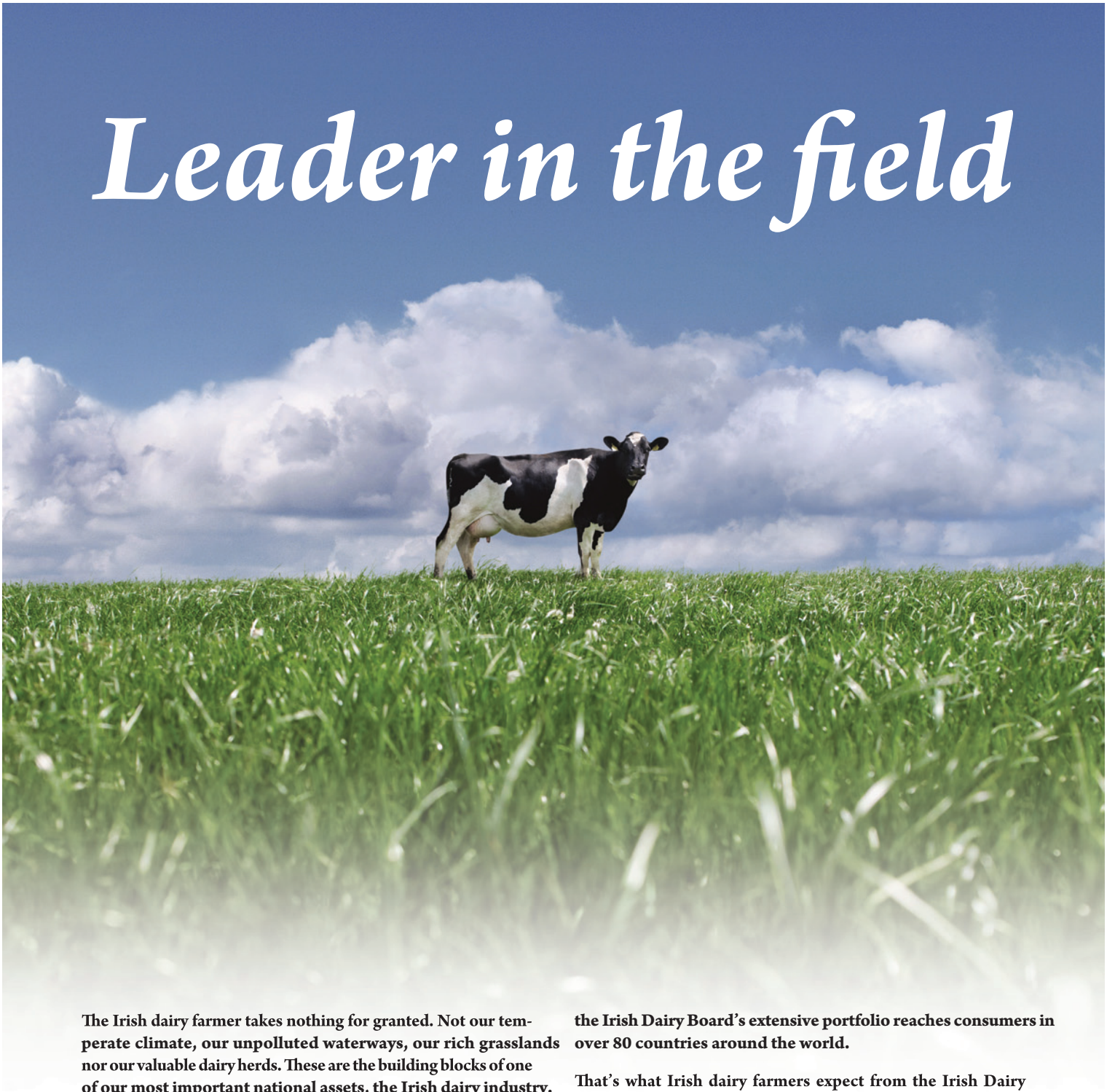

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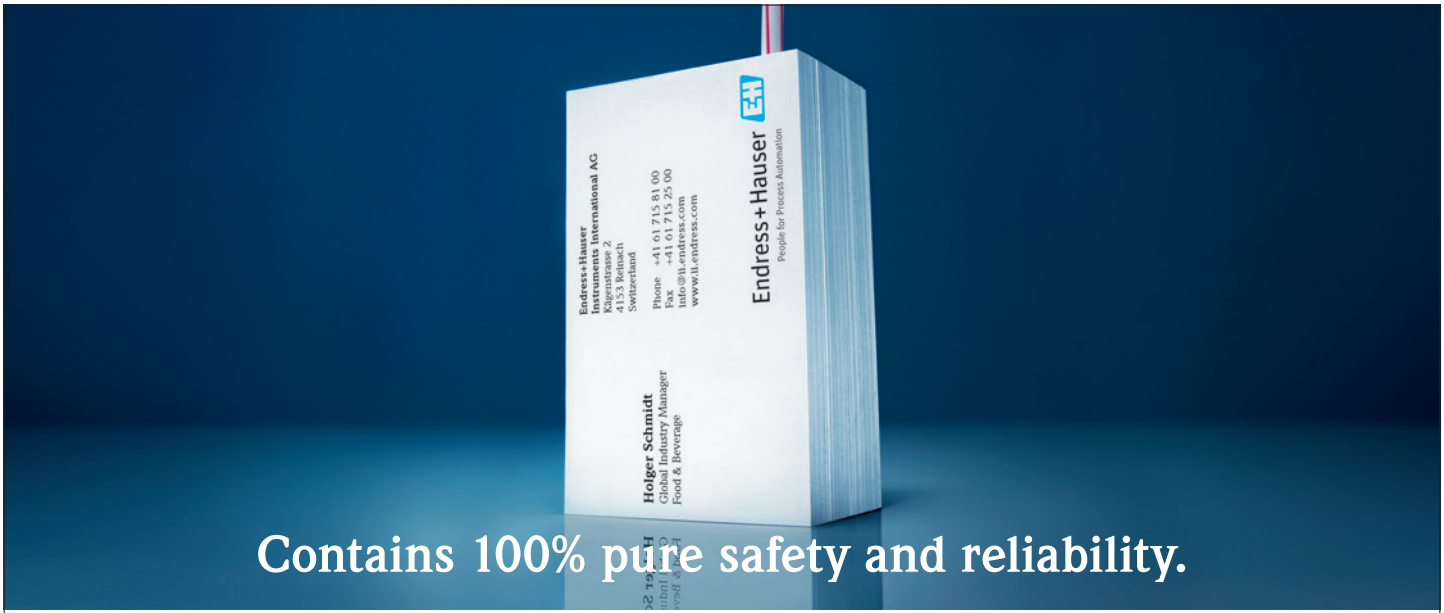

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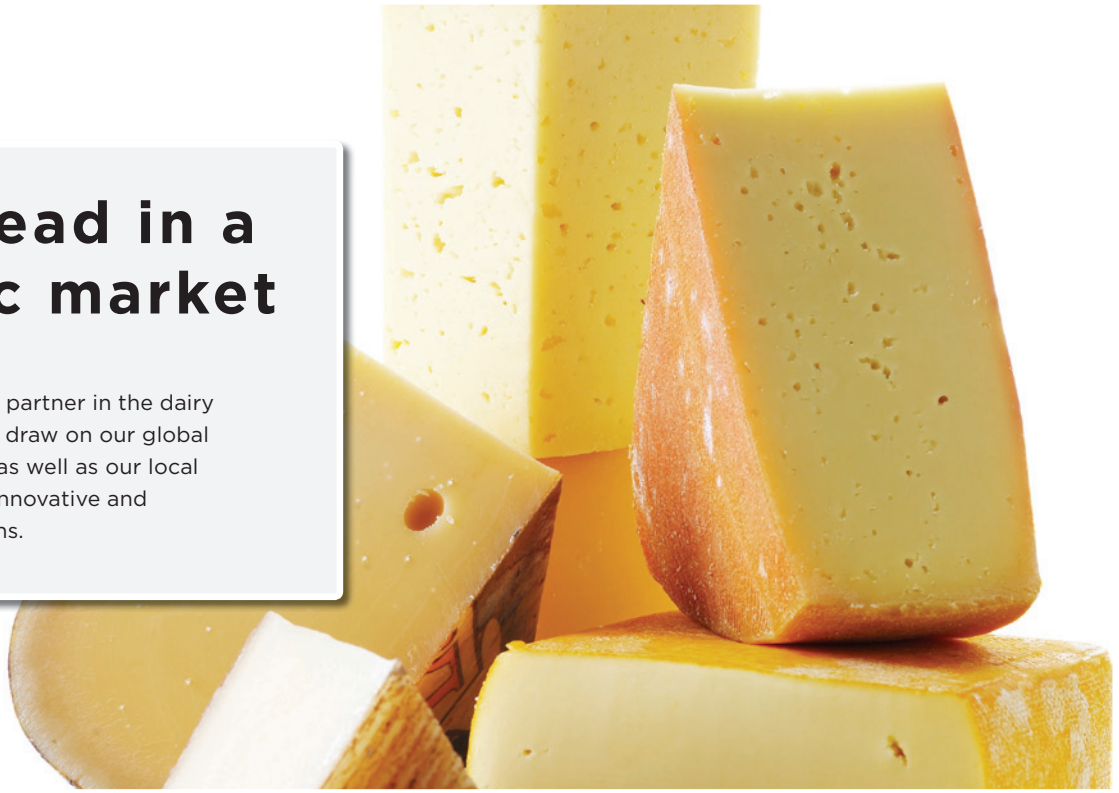

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