AEDIL DAIRY

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Online Symposium Program

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SESSION 1

The Role of Milk and Dairy Products in Healthy and Sustainable Diets

24 June 2024, 15:00-17:25 CEST

Chair: Seamus O'Mahony (University College Cork, Ireland)

Sustainable healthy diets are dietary patterns that promote all dimensions of individuals' health and wellbeing, have low environmental pressure and impact, are accessible, affordable, safe and equitable and are culturally acceptable. A challenge for the dairy sector is that, very often in public debate, this is over simplified to focus solely on environmental impact at a product level, thereby ignoring the dietary level, as well as economic and social factors. A more holistic approach to sustainable and healthy diets, particularly when included in circular rather than linear food systems, clearly highlights the importance of milk and dairy products in a sustainable and healthy diet. Key aspects in this are the nutrient density, the high digestibility and bioavailability, and also the affordability and social acceptance, combined with an ever-decreasing footprint. The role of milk and dairy products in a sustainable and healthy diet should, as such, be the foundation of a demand driven dairy chain.

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15:00- 15:10	**Symposium opening, welcome and introduction** Lilia Ahrné, *University of Copenhagen, Denmark* & Seamus O'Mahony, *University College Cork, Ireland*
15:10- 15:35	**Linking nutrition and environmental impact: Challenges and opportunities** Adam Drewnowski *University of Washington, USA*
15:35- 15:45	**Examination of concentrate protein supplement level of cows on milk composition, functionality, and metabolism in early, mid and late periods of lactation** Nisha Suthar *University College Cork, Cork, Ireland*
15:45- 16:10	**Is there a role for dairy in a sustainable healthy diet?** Mairead Kiely *University College Cork, Ireland*
16:10- 16:20	**Unlocking Dairy's Potential: Single-Step Milk Fat Globule Separation for Sustainable Production** Ayushi Kapoor *Indian Institute of Technology Roorkee, India*
16:20- 16:35	**Break**
16:35- 16:45	**Synergizing sensory analysis and consumer science for sustainable innovation in dairy production** Bruno Domingues Galli *Fondazione Edmund Mach, Italy*
16:45- 17:10	**Delivering nutrition through dairy & plant-based products** Joeska Husny *Nestlé Product Technology Center, Switzerland*
17:10- 17:20	**Differential behaviour of *Lactobacillus helveticus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* in camel and bovine milk fermentation: Growth, acidification, and proteolytic activities** Kobika Chelladuri *United Arab Emirates University, United Arab Emirates*
17:20- 17:25	**Final remarks and closing** Seamus O'Mahony *University College Cork, Ireland*

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Examination of concentrate protein supplement level of cows on milk composition, functionality, and metabolism in early, mid and late periods of lactation

Nisha Suthar[1], Rochelle Van Emmenis[2], Raghunath Pariyani[1], James A. O'Mahony[1], Denis Lynch[3], Lorraine Bateman[3], Michael Dineen[2], Tom F. O'Callaghan[1]

[1] School of Food and Nutritional Sciences, University College Cork, Cork, Ireland
[2] Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland
[3] School of Chemistry, University College Cork, Cork, Ireland

Abstract

This study examined the effect of crude protein (CP) feeding strategies to improve nitrogen use efficiency (NUE) of cows in pasture-based dairy production systems and their implications on the quality of milk. Three experiments were established across early, mid and late lactation where 92, 100 and 88 cows, respectively, were blocked based on pre-experimental milk production and parity and then randomly assigned to 1 of 4 dietary treatments. The dietary treatments were pasture supplemented with; 1) a 17% CP concentrate (H); 2) a 13% CP concentrate (M); 3) a 9.5% CP concentrate (L); and 4) a 9.5% CP concentrate containing rumen-protected methionine (8.0 g/day absorbable met) and lysine (7.2 g/day absorbable lys; L-AA). Within treatment, cows were sub-grouped to provide three representative bulk milk samples. Milk collection was carried out on three occasions in each experiment. Milk analysis included compositional quality using a ProFoss, N fractions using the Kjeldahl method, functional characteristics including heat stability and rennet coagulation properties and the milk metabolome was assessed using ^{1}H-NMR. Dietary treatment had no significant effect on the majority of components measured throughout lactation with the exception of gel strength in the late lactation period; however, stage of lactation was demonstrated to have significant effect on the majority of components. Results suggest that reducing concentrate supplement crude protein concentration to improve NUE, does not appear to have a negative effect on the composition and processability of milk.

Practical Relevance

The study provides insight into how altering crude protein levels in cows diets affects milk composition, functional properties and milk metabolome. This will provide valuable information for dairy farmers and industrial stakeholders seeking to improve efficiency without compromising milk quality. Also, it addresses a key concern in dairy production, offering practical implications for optimizing feed management strategies to achieve both economical and environmental sustainability.

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Unlocking Dairy's Potential: Single-Step Milk Fat Globule Separation for Sustainable Production

Ayushi Kapoor, Kiran Ambatipudi

Department of Biosciences and Bioengineering, Indian Institute of Technology Roorkee, Roorkee, Uttarakhand, India, 247667

Abstract

Microfiltration of milk has garnered considerable attention within the dairy sector due to its capacity to differentially isolate and concentrate bioactive constituents from milk, facilitating their application in fortifying various food products. One such bioactive compound is the milk fat globule, which harbors potential health advantages. Unfortunately, the current multi-step separation process leads to the structural and functional impairments, loss of membrane phospholipids, prolonged processing times and elevated recovery costs. To address these issues, a single-step method was devised based on size for the separation of smaller milk fat globules from cow and buffalo milk. This separation process involved the utilization of a porous polysulfone membrane within a cross-flow microfiltration system. We synthesized an asymmetric polysulfone membrane with an average pore size of 0.8 ± 0.03 µm through the phase inversion technique. Subsequently, the membrane was characterized, assessing its surface and cross-section morphology, hydrophilicity, porosity, thermos-mechanical properties and water contact angle. The membrane exhibited good performance in terms of flux values, with the initial milk permeate flux ranging from 296 to 613 L/m^{2}h at different transmembrane pressures (e.g., 0.2, 0.4, 0.6 bar) for cow and buffalo milk. This flux exhibited a gradual decline owing to the concentration polarization, which eventually stabilized. Particularly noteworthy is the membrane's exceptional resistance to fouling at lower transmembrane pressures, with a flux recovery rate ranging from 81.56-83 % over the three cycles. Microscopic examination of separated milk fat globule confirmed the successful size-based separation, yielded intact globule membranes with higher phospholipid retention, and a well-balanced mass distribution. These findings represent, to the best of our knowledge, the first comprehensive analysis of membrane fouling and reusability in the selective isolation of milk fat globules.

Practical Relevance

The practical relevance of this work lies in its potential to revolutionize milk processing within

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the dairy industry. Microfiltration offers a promising avenue for selectively isolating and concentrating bioactive components from milk, such as milk fat globules, which are known to possess health-enhancing properties. The current multi-step separation processes used often result in structural and functional impairments, loss of valuable membrane phospholipids, extended processing times, and increased costs.

To address these challenges, the study proposes a single-step microfiltration method based on size separation, utilizing a specialized porous polysulfone membrane. This innovative approach allows for the efficient separation of smaller milk fat globules from cow and buffalo milk, circumventing the limitations of traditional methods. By synthesizing an asymmetric polysulfone membrane with precise pore size control, we have achieved remarkable performance in terms of flux values and resistance to fouling.

The significance of this work extends beyond laboratory experimentation. The successful development and characterization of the membrane, along with its demonstrated effectiveness in separating milk fat globules, pave the way for practical applications within the dairy industry. By improving the efficiency and efficacy of milk processing, this technology holds the potential to reduce production costs, enhance product quality, and broaden the range of functional food products available to consumers.

Furthermore, the comprehensive analysis of membrane fouling and reusability presented in this study represents a critical advancement in the field. By addressing key operational challenges associated with membrane-based processes, such as fouling and flux decline, this work has contributed valuable insights that can inform future developments in milk processing technologies.

In summary, the findings of this study offer tangible benefits to both the dairy industry and consumers. By optimizing the microfiltration process for selectively isolating milk fat globules, this research opens new avenues for the development of functional foods and nutritional products with enhanced health benefits, ultimately improving the overall sustainability and competitiveness of the dairy sector.

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Synergizing sensory analysis and consumer science for sustainable innovation in dairy production

Bruno Domingues Galli, Franco Biasioli, Isabella Endrizzi

OnFoods project – Spoke 4, Research and Innovation Center, Fondazione Edmund Mach, San Michele all'Adige, Italy

Abstract

Sensory analysis and consumer science play crucial roles in advancing the sustainable production of novel dairy products in response to industry challenges. Sensory analysis evaluates attributes like taste, texture, and aroma, guiding product development to meet consumer preferences. Concurrently, consumer science examines attitudes toward sustainability and nutritional demands, informing marketing strategies and product positioning. Integrating these disciplines empowers producers to tailor dairy products for sustainability, optimizing resource use and minimizing waste. This approach fosters innovations such as eco-friendly packaging and improved animal welfare standards. By leveraging sensory analysis and consumer science, dairy producers can meet consumer demands for quality while contributing to broader sustainability objectives.

Practical Relevance

Integrating sensory analysis & consumer science in dairy production enhances product quality, meets consumer demands, & drives sustainability. It optimizes resources, reduces waste, & improves animal welfare, ensuring industry competitiveness & environmental stewardship.

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Differential behaviour of *Lactobacillus helveticus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* in camel and bovine milk fermentation: Growth, acidification, and proteolytic activities

Kobika Chelladuri, Santhoshani Warakaulle, Sifatun Nesa Ali, Mutamed Ayyash, Afaf Kamal-Eldin

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Abstract

In this research, low-fat camel milk (CM) and bovine milk (BM) were fermented using *Lactobacillus helveticus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Streptococcus thermophilus* at varying temperatures (28°C, 35°C, and 42°C) for 96 hours. The bacterial counts, titratable acidity (TA), pH levels, changes in lactose and organic acids, and proteolytic activity were analysed. Our findings showed that these factors increased slightly as the temperature rose for each type of bacteria. While the growth of bacteria was consistent in both CM and BM, there were differences in acidification and proteolysis. Both types of milk were negatively correlated with TA (%) and pH, indicating that similar buffering capacities. *L. helveticus* behaved more similarly to *S. thermophilus* than *L. bulgaricus*, especially in terms of proteolysis and acidification. This study reveals valuable information about the complex relationships between bacteria and various milk substrates, providing valuable knowledge that could improve fermentation techniques in the dairy industry.

Practical Relevance

The results suggest fermentation of camel and bovine milk with of *L. helveticus* might lead to the production of health promoting dairy products.

SESSION 2

Delivering Nutrition with Dairy

25 June 2024, 15:00-17:20 CEST

Chair: Thom Huppertz (FrieslandCampina, The Netherlands & University College Cork, Ireland)

Milk and dairy products provide nutrition to billions of people worldwide. In this perspective, both the macronutrients, e.g., protein, fat and carbohydrate, but also the micronutrients, e.g., salts and vitamins, are crucial. Nutrition can be delivered the form of dairy products such as milk, cheese, or yoghurt, but also via the isolation of components from milk and dairy products and subsequently applying them as ingredients in a wide variety of products. In all cases, it is not only the nutrients themselves, but also the product matrix in which nutrients are present. This product matrix is determined by all the components and their interactions, which are strongly affected by processing. In this session, delivering nutrition with dairy is explored.

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15:00- 15:05	**Welcome and introduction** Thom Huppertz *FrieslandCampina, The Netherlands & University College Cork, Ireland*
15:05- 15:30	**Post-prandial Protein handling: You are what you eat** Luc van Loon *Maastricht University, The Netherlands*
15:30- 15:40	**Do we really know how much protein is in milk?** Tim Hoekstra *University College Cork, Ireland*
15:40- 16:05	**Structure, function and digestion of lipids in human milk and infant formula** Wei Wei *Jiangnan University, China*
16:05- 16:15	**Temperature and pore size effects on efficient fat separation from raw milk using hydrophilic silicon carbide ceramic membranes** Tobias Roland Dons *University of Copenhagen, Denmark*
16:15- 16:30	**Break**
16:30- 16:40	**Development of glycation and cross-links during storage of pre- and post-hydrolyzed lactose-free UHT milk** Lotte Juul Knudsen *Aarhus University, Denmark*
16:40- 17:05	**Benefits of galacto-oligosaccharides: From discovery to application** Andre Groeneveld *FrieslandCampina, The Netherlands*
17:05- 17:15	**In situ emulsion characterization utilizing microfluidics-based technologies: A case study of hybrid, plant-dairy based protein systems** Dionysios D. Neofytos *Aarhus University, Denmark*
17:15- 17:20	**Final remarks and closing** Thom Huppertz *FrieslandCampina, The Netherlands & University College Cork, Ireland*

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Do we really know how much protein is in milk?

Tim Hoekstra[1], Elaine K. McCarthy[2], Tom F. O'Callaghan[2], Noel A. McCarthy[1]

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[2] School of Food & Nutritional Sciences, University College Cork

Abstract
The Kjeldahl & Dumas methods are widely utilised throughout the food industry to measure protein, spanning the fields of proximate analysis, research, nutrition, regulations and economics. However, these methods only measure nitrogen – protein content is estimated by utilising nitrogen to protein conversion factors (NPCFs). Conversion factors vary, depending on protein source (i.e animal vs plant proteins), and their appropriateness has been the topic of much debate and scientific discussion. Unfortunately, in many cases, these factors still do not give an accurate representation of protein content, often due to erroneous assumptions on the content of non-protein nitrogen in foods. This brief talk will provide a synopsis on the origin, underlying scientific basis and misunderstanding of the NPCFs used in the Soy and Dairy industries. Furthermore this presentation will offer some insight and considerations to improve the accuracy of these factors, based on physical purification, amino acid hydrolysis and protein primary sequencing from an extensive review of the existing literature. Finally, this talk will briefly discuss the implications a change to these factors may have to the wider food industry.

Practical Relevance
Nitrogen to protein conversion factors directly influence how much protein is quantified in foods and nutritional ingredients. This therefore has direct and significant economic and regulatory implications. There is significant evidence to suggest nitrogen to protein conversion factors could be improved to fully take into account differences in primary and tertiary structures, as well as non-protein sources of nitrogen, to better reflect the true value of proteins from different sources with significant economic consequences.

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Temperature and pore size effects on efficient fat separation from raw milk using hydrophilic silicon carbide ceramic membranes

Tobias Roland Dons[1], Victor Candelario Leal[2], Ulf Andersen[3], Lilia Ahrné[1]

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[2] University of Seville, Seville, Spain
[3] Arla Foods amba, Skejby, Denmark

Abstract
Traditionally fat has been separated from milk through centrifugation by a cream separator. This process induces mechanical stress consequently disrupting the milk fat globule membrane resulting in loss of nutritional components decreasing the quality of milk. Ceramic Silicon Carbide (SiC) membranes are suitable material for the separation of fat from raw milk due to their hydrophilicity.
This work will be presented the potential of SiC membranes at varying temperature and pore sizes for separating fat from raw milk. The separation performance of milk fat globules (MFGs) was investigated at different temperatures (15, 25, 35 and 50 °C) and varying pore sizes (SiC 1.4 µm, SiC 0.5 µm and Zirconia 0.06 µm). Furthermore, combinations of SiC 1.4 and 0.5 µm were used to fractionate MFG based on size.
The process showed excellent yield in terms of fat retention ranging from 88 – 98 % with filtration times varying from 12 – 106 minutes producing a fat rich retentate (cream), while shifting the protein profile and produce a skim milk-like permeate. At 50 °C the separation performances showed an increased distribution of larger MFGs in the permeate stream. Micrographs using confocal laser scanning microscopy depicted intact MFGs in the retentate obtained. Though the further fractionated 0.5 µm permeate stream showed coalescence and aggregation of MFGs probably induced by the local pore pressure in the membrane. Further, the 0.5 µm permeate showed a shift from 80:20 to 50:50 casein-to-whey ratio. Lastly scanning electron microscopy and elemental composition analysis by energy dispersive X-ray revealed no irreversible fouling.
The findings provide valuable insights into the structural and compositional aspects of the separated components.

Practical Relevance
Microfiltration may be an alternative process to fat separation from raw milk. In this study, for the first time was demonstrated that effective milk fat separation, and retentate and permeate streams with variable characteristics, in terms of composition and integrity of the milk fat globules can be obtained by using silicon carbide support membranes with different sizes and materials. The results obtained, provide new insights for industrial use of membrane technology to separate milk.

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Development of glycation and cross-links during storage of pre- and post-hydrolyzed lactose-free UHT milk

Lotte Juul Knudsen[1,2], Søren Drud-Heydary Nielsen[1,2], Peter Dekker[3], Daniel Otzen[4], Valentin Rauh[5], Lotte Bach Larsen[1,2]

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[2] *CiFOOD Aarhus University Centre for Innovative Food Research, Agro Food Park 48, 8200 Aarhus N, Denmark*
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[5] *Arla Foods Innovation Centre, Agro Food Park 19, 8200 Aarhus N, Denmark*

Abstract
During processing and storage of lactose-hydrolyzed UHT milk, non-reducible covalent protein aggregation can occur, which can be derived from Maillard or dehydroalanine (DHA) pathways. To study the development of these pathways in relation to processing and storage, lactase was added to semi-skimmed milk, either prior to UHT (pre-hydrolysis) or after UHT treatment (post-hydrolysis). Then milk samples were stored at 25 °C or 35 °C for up to one year. Process- and storage-induced glycation was investigated by liquid chromatography-mass spectrometry. Results showed that pre-hydrolysis lactose-free milk had higher levels of glycation at initiation of storage compared to post-hydrolyzed milk, which on the other hand increased to these same levels during storage. Protein molecular changes from Maillard or cross-link formation from DHA pathway was further investigated via multiple reaction monitoring, and showed pre-hydrolyzed milk to contain higher levels of markers related to the Maillard reaction, whereas post-hydrolyzed and non-hydrolyzed milk had higher concentrations of DHA pathway related cross-links.

Practical Relevance
The purpose of studying glycation and development of Maillard and DHA pathways is to gain insight into the molecular mechanisms occurring in lactose-hydrolyzed UHT milk relative to processing strategy. This knowledge can be used when considering the hydrolysis strategy for processing of lactose-hydrolyzed milk in relation to storage stability of the milk.

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In situ emulsion characterization utilizing microfluidics-based technologies: A case study of hybrid, plant-dairy based protein systems

Dionysios D. Neofytos, Katherine F.Grasberger, Anders Holste, Sandra B. Gregersen, Milena Corredig

Department of Food Science, CiFOOD Center for Innovative Foods, Aarhus University, Aarhus, Denmark

Abstract

Combining animal and plant-based proteins offers a promising solution to address challenges in sustainability and nutrition. The aim of this work was to develop a novel approach to understand the emulsification properties of mixed plant and dairy protein systems across multiple time and length scales. The ability of whey protein and faba bean isolates to stabilize water-oil interfaces was studied at both single and hybrid protein systems. Classic pendant drop tensiometry was coupled with time-resolved, label-free droplet morphometry characterization,utilizing microfluidics-based technologies with a view to explore systems adsorption dynamics, droplet formation, and droplets resistance to short-term coalescence. Our approach demonstrates that label-free, in situ microfluidic-based analysis can provide dynamic insights into protein adsorption and droplet stability at time and length scales more comparable to that of industrial emulsification processes. It was shown that heteroprotein association affects both droplet size during formation and the shape eccentricity, as well as droplets resistance to short-term coalescence with respect to protein ratio. The findings suggest that blending whey protein isolate with faba protein isolate leads to systems characterized by modified interfacial properties, potentially enhancing emulsion stability and resistance to coalescence compared to using faba protein isolate alone.

Practical Relevance

The methodology employed unveils how protein blending can influence interfacial properties of emulsions and demonstrates how the presented complementary techniques can be utilized to obtain practical insights into emulsified systems properties. This can be applied for obtaining new knowledge on plant and dairy proteins hybrid systems,providing the tools for the design of hybrid plant and dairy emulsion-based products with enhanced properties.

SESSION 3

Milk: Designed to Deliver

26 June 2024, 15:00-17:20 CEST

Chair: Ulf Andersen (Arla Foods amba, Denmark)

Key in the ability of milk and dairy products to function as excellent food matrices is the fact that milk, in essence, is designed to deliver. It is the sole source of nutrition for the neonate and contains essential structural elements, e.g., in the form of casein micelles and milk fat globules, which deliver a multitude of nutrients, including salts, vitamins and proteins. In addition, colloidal stability of these structure elements in the gastro-intestinal tract also leads to important control of the kinetics of digestion and release of nutrients, enabling maximum utilization of nutrients from milk and dairy products. Hence, understanding of these key structure elements and their biological function, and their interaction with (micro-)nutrients is key to creating products that fit in a healthy and sustainable diet.

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15:00- 15:05	**Welcome and introduction** Ulf Andersen *Arla Foods amba, Denmark*
15:05- 15:30	**Reassembling of human casein micelles** Peng Zhou *Jiangnan University, China*
15:30- 15:40	**Artificial casein micelles and the road towards animal-free cheese** Laurens J. Antuma *Wageningen University & Research, The Netherlands*
15:40- 15:50	**Optimizing the stability of chilled drinking yogurt through various prebiotic additions** Lindayani *Soegijapranata Catholic University, Indonesia*
15:50- 16:15	**Calcium sequestering salts in dairy systems: Impact and understanding** Thom Huppertz *FrieslandCampina, The Netherlands & University College Cork, Ireland*
16:15- 16:30	**Break**
16:30- 16:40	**Impact of processing on protease activities in bovine milk** Tiziana Racca *University College Cork, Ireland*
16:40- 16:50	**Characterising the Irish milk metabolome for improved dairy processability and traceability** Paula Rojas Gómez *University College Cork, Ireland*
16:50- 17:15	**Oligosaccharides and glycoconjugates in milk and dairy co-products** Daniela Barille *UC Davis, USA*
17:15- 17:20	**Final remarks and closing** Ulf Andersen *Arla Foods amba, Denmark*

Artificial casein micelles and the road towards animal-free cheese

Laurens J. Antuma, Remko M. Boom, Julia K. Keppler

Laboratory of Food Process Engineering, Wageningen University & Research

Abstract

The recombinant production of caseins through precision fermentation offers the opportunities to formulate high-quality animal-free cheese. However, recombinant caseins are produced individually may need to be assembled into the micellar structures in which they occur in milk to allow the efficient production of animal-free cheese.

In our research, we found that casein micelle formation occurs upon inducing calcium phosphate phase separation in the presence of at least two phosphorylated caseins. On this basis, we developed novel processes that allow the efficient, fast, and continuous production of artificial casein micelles on industrial scale. Furthermore, we found that the properties and functionality of artificial casein micelles can be tailored by varying the processing conditions (i.e. pH, temperature, time), whereby micelles can be obtained with improved cheesemaking properties compared to natural bovine casein micelles. Our research thus paves the way for the efficient future production of animal-free cheese from recombinant casein.

Practical Relevance

Precision fermentation of caseins offers food manufacturers the opportunity to produce high-quality vegan cheese, but there are currently no guidelines regarding which caseins should be produced, how these caseins can be assembled into micelles efficiently and how functional these micelles are. Our research generates such guidelines that enable efficient production of future animal-free cheese.

Optimizing the stability of chilled drinking yogurt through various prebiotic additions

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[3] PT Global Dairi Alami (Milk Life®)

Abstract
This study aimed to identify effective prebiotics for maintaining the stability of chilled drinking yogurt during storage. Three types of prebiotics - inulin, fructooligosaccharides (FOS), and polydextrose (PDX) - were added to chiller yogurt fermented by *L. bulgaricus* and *S. thermophilus* starter cultures (3% w/v) for 8 hours at 43 oC. The sample with PDX was selected for stability testing due to its highest lactic acid bacteria (LAB) count, low viscosity, and highest fiber content among the prebiotics.
Over a five-week chilled storage period, the PDX-added sample showed a significant decrease in LAB count and pH ($p<0.05$), while maintaining stable viscosity. However, its LAB count (7.30×107 CFU/ml) was higher than samples without prebiotics (6.63×107 CFU/ml), suggesting improved viability during storage with prebiotic addition. Based on mold and yeast counts and compliance with Indonesian national standards (SNI) for chilled drinking yogurt, the shelf-life of PDX-added chilled yogurt was estimated to be three weeks. Sensory evaluation by a selected panel confirmed product acceptance. Future research may focus on optimizing production hygiene to extend product shelf life.

Practical Relevance
This collaborative research between the dairy industry and academia addresses the challenge of maintaining cultural viability in chilled-drinking yogurt. Prebiotics, acting as fuel for gut microbiota, enhance culture stability and promote health benefits. The study screens prebiotics for fermented milk suitability, assessing their physicochemical and microbial stability.

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Impact of processing on protease activities in bovine milk

Tiziana Racca[1], David Goulding[2], Jonathan O'Regan[2], Michael Affolter[3], Anthony J. O'Donoghue[4], Alan L. Kelly[1]

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Abstract

***Objectives**:* The primary objective of the study was to develop enzymatic methods to measure protease activities in bovine milk, with a major focus on cathepsin D and cathepsin B, in addition to plasmin.

***Methods**:* Twenty-six bovine milk samples differing in somatic cell count (SCC) were provided by a local farm (Ireland), portions of which were (1) pasteurized or (2) treated with aprotinin (inhibitor of plasmin). All samples were incubated at 37°C for 7 days and analyzed throughout the incubation period.

***Results**:* Assays methodologies based on tailored synthetic substrates for cathepsin D and cathepsin B were developed. Significant decreases in cathepsin D and cathepsin B activities ($p<0.05$) were observed in all the pasteurized samples compared to raw milk before the beginning of incubation. In contrast, plasmin activity increased significantly over the first 24h of incubation in pasteurized milk ($p<0.05$). No correlation was observed between SCC and cathepsin D activity. Cathepsin B activity was found to be significantly lower in samples with medium SCC (1-4 x105 cells mL-1) compared to samples with low and high SCC ($p<0.05$). The correlation between SCC and plasmin activity will be presented, as well as the significance of changes in activities of all three proteases over incubation.

***Conclusions**:* Enzymatic assays represent a key tool to investigate enzymatic activity in milk. Activities of cathepsin B and D may be less relevant following pasteurization and they are, perhaps surprisingly, not correlated with SCC.

Practical Relevance

Understanding the role of these proteases in milk and the factors that influence their activity will give more insight about milk quality and nutritional properties.

Characterising the Irish milk metabolome for improved dairy processability and traceability

P. Rojas Gómez[1], R. Pariyani[1], M. Dineen5, E. Roche5, D. Lynch[2,3], A.R. Maguire[2], F. Buckley[4,5], N.A. McCarthy[5], J.A. O'Mahony[1], L.M. Bateman[2,3], T.F. O'Callaghan[1]

[1] University College Cork, School of Food & Nutritional Sciences
[2] University College Cork, School of Chemistry
[3] University College Cork, School of Pharmacy
[4] University College Cork, School of Biological, Earth and Environmental Sciences (BEES)
[5] Teagasc – The Irish Agriculture & Food Development Authority

Abstract
MetaBó-Bainne project aims to understand the factors affecting and affected by the milk metabolome. This research investigated how seasonality in pasture-based spring calving dairy systems affects the milk metabolome, and its consequent effects on techno-functional properties of milk, such as heat stability and gelation. Weekly raw milk from 10 farms bulk tanks and shop bought pasteurised milk from 3 brands were collected over 41 weeks (n=533). Macronutrient composition, gelation properties, and heat stability of these samples were determined, as well as metabolomic profile using proton nuclear magnetic resonance (H-NMR) with subsequent profiling utilizing Chenomx. >20,000 data points have been gathered from Irish milks, comprising metabolites, macronutrient components, and techno-functional characteristics across lactation. ~40 metabolites from diverse chemical classes such as amino acids, carbohydrates, fatty acids, and organic acids have been quantified. Multivariate analysis of data to date has demonstrated an evolution of the milk metabolome as lactation progresses. On-going statistical analysis aims to understand the intrinsic and extrinsic factors affecting the milk metabolome and its prediction potential for techno-functional properties of milk.

Practical Relevance
The use of metabolomics for understanding the milk metabolome represents a significant advancement in the field of dairy science. Understanding the interaction between intrinsic and extrinsic factors and the milk metabolome can provide insights into strategies for optimizing techno-functional properties, enhancing authenticity and traceability, and improving processing efficiency.

SESSION 4

Processing of Milk and Dairy Products: For Safe, Stable and Nutritious Products

27 June 2024, 15:00-17:25 CEST

Chair: Effie Tsakalidou (Agricultural University of Athens, Greece)

Within the dairy chain, processing of milk plays an extremely important role. First and foremost, it is required to improve the safety and extent the shelf-life of products, thereby ensuring that products can be safely distributed all over the world. This shelf-life extension can be achieved through heat treatment, but also through fermentation or drying. In addition, processing is also important to ensure that a dairy matrix is created which is preferable by consumers, which can be digested and from which nutrients bioavailable. Such processing can include similar steps as for shelf-life extension, but also other processing techniques, including e.g., non-thermal processing.

15:00- 15:05	**Welcome and introduction** Effie Tsakalidou *Agricultural University of Athens, Greece*
15:05- 15:30	**Producing dairy proteins by precision fermentation: From science to success** Marcel Wubbolts *Vivici, The Netherlands*
15:30- 15:40	**Effect of incubation conditions on the enzymatic cross-linking of casein nanoparticles** Kristin Eichelberger *Technische Universität Dresden, Germany*
15:40- 16:05	**Mass balance and yield considerations during microfiltration of skim milk** John Tobin *Teagasc Food Research Centre, Ireland*
16:05- 16:15	**Rethink the effect of lactose crystallization on storage stability for milk powder** Xiàowěi Qí *University of Copenhagen, Denmark*
16:15- 16:30	**Break**
16:30- 16:55	**Structure formation in yoghurt gels** John Lucey *University of Wisconsin-Madison, USA*
16:55- 17:05	**Microstructure and textural properties of heat and acid-induced milk gels produced at different acidification temperature and acidulants** Zhe Cheng *University of Copenhagen, Denmark*
17:05- 17:15	**Dynamic microstructural and rheological analysis of cheese under controlled temperature sweep** Gaurav Kr Deshwal *Teagasc Food Research Centre, Ireland; Wageningen University, the Netherlands*
17:15- 17:25	**Final remarks, closing and announcement of the Dairy Academy 2025** Effie Tsakalidou, *Agricultural University of Athens, Greece* & Lilia Ahrné, *University of Copenhagen, Denmark*

Effect of incubation conditions on the enzymatic cross-linking of casein nanoparticles

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Abstract

The self-association and cross-linking of non-micellar casein to hydrated nanoparticles with microbial Transglutaminase (mTGase) is affected by casein concentration, ion concentration and incubation temperature. The aim of this work is to produce defined particles by varying the incubation conditions during cross-linking. The resulting particles will then be tested for their suitability as pickering stabilisers in emulsions. Pickering stabilisers, which are solid particles, can be used instead of interfacial active substances and are characterised by their long-term stabilisation. In this study, solutions of β-casein-rich acid casein (β-NaCn), produced from reconstituted skim milk by diafiltration and acid precipitation, or commercial acid casein (cNaCn) in demineralised water were adjusted to pH 6.6 by NaOH. NaCn samples with concentrations between 5 and 50 g/kg were cross-linked with 3 U mTGase per g protein by 40 °C for 0 h (control) until 96 h. In a trial with lower casein concentrations, the incubation temperature was additionally varied. NaCl and $CaCl_2$ were added to the solutions before incubation to analyse their effect on particle size. SDS-PAGE was used to roughly follow changes in the particle size. The emulsions were prepared from the NaCn solutions and rapeseed oil using Ultra-Turrax and analysed with regard to their stability.

Practical Relevance

The aim of the work is to determine the potential of cross-linking with mTGase for controlling particle size and particle properties by varying casein concentration, temperature and ion concentration. The suitability of these cross-linked casein nanoparticles as pickering stabilisers in multiphase systems is investigated on the basis of emulsion stability. This will determine their potential use as a replacement for conventional stabilisers in various food systems.

Rethink the effect of lactose crystallization on storage stability for milk powder

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Abstract

Milk powder is a good alternative for fresh milk for improving good nutrition intake, especially in the developing world. Milk powders, as for all food products in the global market, might be exposed to challenging transportation and storage conditions. Storage stability deteriorates under undesirable conditions. Quality reconstitution is required by consumers and evaluated as one of the top stabilities that industry cares about.

Lactose crystallization during storage has been shown to be a major impacting factor for quality. Amorphous lactose forms continuous phase together with protein, that embeds fat globules when formulation contains fat. When storage temperature exceeds glass transition temperature, the amorphous lactose turns to rubbery and can further form crystalline lactose. Glass transition is broadly recognized as the event should to inhibited in lactose crystallization for maintaining good quality of milk powder. Lactose crystallization is time dependency, the kinetic of lactose crystallization varies at different storage conditions and affected by formulation, and the formed crystalline lactose can be at different phases. For milk powders resemble formulation of commercial powders, the kinetic of lactose crystallization towards describing the formed crystalline lactose, has been studied only for skim milk powder (SMP). Moreover, due to incompletion of crystallization at low relative humidity (below 66% RH), the kinetic at low RH is unknown. Consequently, to which extend of lactose crystallization would really affect reconstitution is yet unknown and the underlined mechanism is inexplicit.

This work is to characterize the evolvement of lactose crystallization at industry relevant conditions and relate lactose crystallization to reconstitution in milk powder with commercial interests.

Three types of commercial milk powders, skim milk powder (SMP), whole milk powder (WMP) and fat filled milk powder (FFMP), were exposed to relative humidity from 23% to 75% at temperature in the range from 25 °C to 45 °C. Dynamic vapour sorption (DVS) was used for obtaining sorption isotherm to preliminary

compare lactose crystallization. Long-term storage lasted for 50 days to 4 months were carried out under constant RH and temperature. During the long-term storage, complimentary techniques, including Differential Scanning Calorimetry (DSC), Powder X-ray diffraction (XRD), Small/Wide Angle X-ray Scattering (S/WAXS), Fourier-Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and Laser Diffraction Particle Analyser, were used to characterize lactose crystallization, microstructure, reconstitution and other physico-chemical properties.

Glass transition temperature decreased with the increase of absorbed water. WMP and FFMP were estimated reaching glass transition faster than SMP based on faster water absorption at the same RH. Lactose crystallized as anhydrous α/β-lactose (1:4) firstly irrespective of RH and powder formulation. At high RH (64% for SMP and FFMP, and 75% for WMP), lactose converted to α-lactose monohydrate. The higher RH, the faster phase conversion. The appearance of crystalline lactose in SMP was later than WMP and FFMP, which was most obvious at 43% RH. On the opposite, the growth rate of crystalline lactose was faster in SMP than in WMP and FFMP. Phase conversion were quite comparable in SMP and FFMP, but much slower in WMP at the same RH. Temperature did not affect the final phase of crystalline lactose in FFMP after 4 months of storage.

Solubility of powder stored at 25 °C, was not affected by glass transition irrespective of RH and powder formulation. It was the formation of crystalline lactose affecting solubility as evaluated by Mastersizer. Interestingly, even though the lactose had completed crystallization in FFMP at 25 °C and 43% RH, the solubility of this powder was comparable to that of fresh powder when evaluation was carried out by Turbiscan and visual impression. By SEM and FTIR spectra, we found this FFMP had remained similar microstructure, surface hydrophilicity and surface fat content to that of fresh powder. By XRD and SEM, crystal size of this FFMP was estimated below 100 nm. Moreover, we found microstructure turned rougher, surface hydrophilicity decreased and surface fat content increased when crystal size increased in FFMPs stored at 25 °C from 43% to 75% RH. These results indicate the size of crystalline lactose is an important parameter of lactose relating the physiochemical change of milk powder.

In conclusion, WMP and FFMP have earlier glass transition and appearance of crystalline lactose than SMP, whereas, they have slower growth of crystalline lactose than SMP. The higher RH and temperature, the faster kinetic. It is the formation of crystalline lactose, the later event after glass transition in lactose crystallization, affecting solubility of milk powder. The size of crystalline lactose is the critical feature of lactose crystallization that affects the microstructure and surface properties, thus relating to reconstitution.

Practical Relevance

This work generates state diagram of lactose for SMP, WMP and FFMP, which can be a reference for evaluating storage stability of different type of milk powder. The new knowledge of the relationship between lactose crystallization and reconstitution can potentially expand control space beyond glass transition by controlling the kinetic of lactose crystallization.

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Microstructure and textural properties of heat and acid-induced milk gels produced at different acidification temperature and acidulants

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Abstract
Heat-acid coagulated cheeses, such as Paneer, are cookable and can substitute meat in traditional cuisine. The acidification temperature and type of acid play a crucial role in determining the yield as well as the gel structure. In this study, the composition, texture, and microstructure of cheese produced by acidifying milk using citric acid, lactic acid, and HCl at 60 to 90 °C were investigated. Increasing the temperature reduced yield regardless of the acids, due to lower moisture retention. As the temperature increased, Young's modulus and hardness also increased, primarily due to extensive protein aggregation and calcium recovery. CSLM images revealed the presence of smaller fat globules and pores in 90 °C citric acid cheeses, contributing to a more compact protein network. Results of low-field NMR corroborated these results, by a reduction of proton mobility. The findings provide valuable insights for the modulation of textural and physicochemical properties of cookable cheeses.

Practical Relevance
The knowledge of this study can be exploited for the production of milk protein-based acidied gels having consistent and tailored physicochemical and textural properties.

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Dynamic microstructural and rheological analysis of cheese under controlled temperature sweep

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Abstract

Confocal laser scanning microscopy (CLSM), Raman micro-spectroscopy and rheological analysis were used to assess the changes in natural and processed cheese during heating from 5 to 95°C, at 2°C per min. Processed cheese with less than 10% fat showed no substantial structural changes in CLSM micrographs which were consistent with less than 1 value of loss tangent during temperature sweep. Starch containing processed cheese showed uniform fat distribution and cohesive protein network, causing reduced cheese flow. Cheese samples with fat content equal to and higher than 25% showed fat coalescence, which finally merged into large fat pools of diameter 105-225 µm. Mobility ratio based on ratio of Raman spectral peak intensities at 2885 to 2850 cm^{-1} provided information about the melting transitions of lipids. With increasing temperature, cheese samples with fat content >25% showed a decrease in peak intensities of 2885 cm^{-1} and a noticeable increase in 2850 cm^{-1}. CLSM micrographs and rheological analysis correlated well and described the changes in fat globule size and structural breakdown. Raman micro-spectroscopy was useful in characterizing the changes in fat at molecular level.

Practical Relevance

The combination of dynamic in situ confocal laser scanning microscopy and rheological analysis will help in understanding the melting behaviour of different cheese matrix during heating at micro- and macroscopic scale. Raman spectral region corresponding to 2800-3000 cm^{-1} can be used to track fat melting in cheese samples with minimum 10% fat.

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