



Global Cheese TECHNOLOGY FORUM

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Speaker Abstracts

SESSION 2 - DAIRY INGREDIENTS FOR CHEESE

Use of MPC or Micellar Casein as an Ingredient in Process Cheese or in Cheese Milk Extension

Lloyd Metzger, South Dakota State University

Milk protein concentrate (MPC) manufacture utilizes ultrafiltration to produce partially or completely delactosed high protein dairy ingredients. A variety of products are available in the liquid or dried form and range in protein content on a dry basis from 56 to 85%. The protein in MPC is a mixture of native casein and whey protein at a concentration of 80 and 20% of the total protein, respectively. Essentially, MPC is skim milk powder with a portion of the lactose and soluble minerals removed. Consequently, MPC has an advantage over condensed skim milk or skim milk powder in applications that require a maximum amount of highly soluble protein and a limited amount of lactose and is routinely used as an ingredient in process cheese products or to fortify milk prior to natural cheese manufacture. Micellar casein concentrate (MCC) is similar to MPC except it is produced by microfiltration of skim milk instead of ultrafiltration. Microfiltration of skim milk results in the removal of whey protein, lactose and soluble minerals. Consequently micellar casein has an increased ratio of casein to whey protein as compared to MPC. MCC typically has >92% casein as a percentage of protein and greater than 80% protein on a dry basis. As a result of the increased ratio of casein: total protein in MCC, when it is used in process cheese, a modification in functional properties (firmness, melt and viscosity) is observed relative to MPC. These modified functional properties provide increased flexibility in a process cheese formation and provides an opportunity to optimize the cost of formation. Additionally MCC can be used to fortify milk prior to natural cheese production. In this application MCC again has advantages as compared to MPC that result in increased yield and improved ripening.

Standardization of Lactose to Protein Ratio and Effect on Quality of Cheese

John Lucey, University of Wisconsin-Madison, Center for Dairy Research

Controlling the acidity in the cheesemaking process is critical to achieving the desired texture, functionality and flavor. Excessive acidity is a common undesirable attribute in finished cheeses, and can lead to other issues like impaired melt, crumbly texture and increased risks of defects like calcium lactate crystal formation. To try to control acid development during cheesemaking steps like curd washing, whey dilution and rinsing are often used. A new approach to this issue involves control the exact level of lactose in the original cheesemilk, and standardizing milk to a specific lactose to protein ratio. Protein contributes to buffering and is thus involved in dictating final pH of the cheese. Membrane filtration can be used to standardize the lactose level, while fortifying the protein content. Various examples of this approach will be discussed, as well as the potential benefits.

Optimizing Microfiltration for Maximum Removal of Whey Proteins

Steve Beckman, South Dakota State University

Microfiltration (MF) of skim milk is used to create Micellar Casein (MC) concentrates from MF retentate. Uses for MC include fortification of cheesemilk, and addition into process cheese formulations. Efficient fractionation of whey proteins (WP) from skim milk during MF is highly desired by MF operators. Factors affecting WP passage during skim MF include crossflow velocity (CFV), and pressures. Commonly, spiral-wound (SW) MF of milk is conducted at low differential pressures, 100 kPa (14 psi), with low CFV. Low velocity can lead to increased fouling and increased rejection of WP.

Increasing CFV while maintaining transmembrane pressure could positively impact WP fractionation during MF due to less fouling. One method to achieve this is to control the membrane flux, and to increase the CFV. Therefore, the objective of this work was to evaluate the impact of high CFV and controlling permeate flux during SW MF on the fractionation of WP from skim. Skim milk was MF (0.5 μm pore size, 60°F) in batch mode using two schemes, constant pressure (CP) and controlled flux (CF). In both schemes, concentration factor was 4.14x, and diafiltration water, 132.5% of feed mass, was added during MF. Constant pressure MF held constant the baseline and differential pressures, and allowed permeate to freely exit. Controlled flux MF held pressures the same as CP, however permeate was throttled to maintain a set flow at each stage during MF. Samples of retentate and permeate were collected during MF to assess the passage of protein into permeate. Initial results indicate a higher recovery of WP in MF permeate for the CF process compared to CP. We believe that the higher CFV at low transmembrane pressures during CF MF allowed more WP to pass into permeate because of reduced surface fouling compared to CP MF.

SESSION 3 - UNDERSTANDING CHEESE MICROBIOLOGY

Understanding How Pathogens Invade Cheese Microbial Communities

Manon Morin, University of California San Diego

Microbial invasion of cheese-associated microbiomes by pathogenic or spoilage strains is a common threat in the cheese industry. In addition to major health issues, they constitute an important economic loss. During invasion, pathogenic or spoilage species have to overcome natural barriers presented by the local cheese-associated microbiome and the cheese physiochemical characteristics. Understanding how invading strains and local microbiomes interact and how these interactions determine the invasion outcome is key for our control of microbial invasion. Here, we used a synthetic invasion model based on the invasion of bloomy-rind cheeses by *E. coli* to investigate mechanisms at work during invasion. The local community was reduced to its three main members: *Hafnia alvei*, *Geotrichum candidum* and *Penicillium camemberti* grown in a “lab-made” cheese medium. *E. coli* K-12 was introduced as the invader. *E. coli* was grown either alone, with the complete community or in pairwise conditions with each community member. The presence of different species significantly decreased *E. coli* growth on the cheese environment. We used a method adapted from Transposon sequencing (TnSeq) to identify the genetic basis of *E. coli* growth in the different contexts. These genetic requirements include genes involved in interactions with the local community and with the environment. We

identified essential requirements associated with physiochemical parameters such as the high osmolarity of cheese, low iron availability and low amino acid availability.

Comparing the genetic basis for interactions during pairwise growth and during growth within the complete community, we found two categories of interactions: interactions facilitating the invader growth (positive interactions) and interactions inhibiting the invader growth (negative interactions). Positive interactions were mostly associated to cross-feeding of amino-acids while negative interactions were associated to social behavior traits and response to toxic stress. In-depth analysis allowed us to determine what community member(s) were involved in each interaction. In conclusion, investigating molecular mechanisms underlying microbial interactions during invasion provided a better understanding of the processes determining invasion outcome.

Role of *Streptococcus thermophilus* in Modern Day Cheese Making

Prof. Sylvain Moineau, Université Laval

Cheese manufacture requires the inoculation of milk with carefully selected bacterial cells (starter culture) to properly control the fermentation and to obtain high-quality products. Because 10^{11} bacteria are needed to produce 1 kg of cheese, lactic acid bacteria are of obvious considerable interest to the dairy industry. *Streptococcus thermophilus* is one of the industrially relevant Gram-positive bacteria added to milk to manufacture several cheeses. It is primarily used as an acidifier in cheese technologies that use a higher temperature profile. The adaptation of traditionally lower temperature to higher temperature to make cheeses can also require *S. thermophilus* cultures. In these cases, *S. thermophilus* will be used with other cultures to increase speed of acidification, shorten cheesemaking time, which can lead to increased productivity. It is well documented that increased productivity within existing manufacturing facilities may lead to milk fermentation failures or delays due to bacterial viruses (phages) that destroy the added starter cultures. Viruses are now recognized as the most abundant biological entities on the planet and display a remarkable genetic diversity. Thus, it is natural to find them in cheese manufacturing facilities. Hence, the importance of using systematic anti-phage controls to prevent losses and low-quality products. Indeed, constant phage monitoring and stringent application of the appropriate control measures are indispensable to avoid “phage attacks”.

In this seminar, sources of phage contamination and phage detection methods will be described, with an emphasis on the recent emergence of new phages infecting *S. thermophilus*. Latest discoveries related to the exploitation of CRISPR-Cas systems to develop robust natural bacteriophage-resistant *S. thermophilus* strains will also be highlighted. The presence of CRISPR arrays in these cultures also offers a unique opportunity to type *S. thermophilus* strains to improve starter culture rotation. Finally, future directions will also be discussed.

Update on *Lactobacillus wasatchensis* as a NSLAB Causing Slits and Gassy Cheese

Craig Oberg Ph.D., Weber State University

A recently identified obligately heterofermentative nonstarter lactic acid bacteria (NSLAB), *Lactobacillus wasatchensis*, is responsible for late gas production in commercially aged cheese. *Lactobacillus wasatchensis* can grow at the salt concentration and ripening temperature of aging cheese, and, preferentially, metabolizes ribose released by starter culture lysis. When *Lb. wasatchensis* was added to milk prior to experimental cheesemaking trials it produced gas during aging, especially if a hexose sugar (i.e., galactose) was available and/or if an elevated ripening temperature (12°C vs. 6°C) was used. Additional cheesemaking trials, using *Streptococcus thermophilus* as the starter resulted in extensive gas production when *Lb. wasatchensis* was added during cheesemaking.

A survey of commercial cheeses obtained across the U.S. revealed *Lb. wasatchensis* was present in almost all aged cheeses exhibiting late gas defect, but not from any non-gassy aged cheeses. Rep-PCR analysis of these *Lb. wasatchensis* isolates revealed strain variations, which clustered based on geographic region. Specific PCR primers developed allow identification and possible enumeration of *Lb. wasatchensis* strains while excluding other NSLAB cultures common in aging cheese. Use of bio-protective adjunct lactic acid bacteria cultures shows promise for reducing late gas defect. Since recent studies show *Lb. wasatchensis* cannot survive pasteurization, it enters the cheesemaking process through other avenues, suggesting enhanced sanitation could be an effective deterrent. Additional practices that may decrease its occurrence include limiting hexose sugars in the cheese, reducing ripening temperature, and monitoring feed for *Lb. wasatchensis* contamination. These studies indicate *Lb. wasatchensis* is a previously undetected contributor to late gas defect in ripening cheese and that the defect is more pronounced during elevated ripening temperatures and/or when *S. thermophilus* is used in the starter culture.

SESSION 4 - NEW CHEESE MANUFACTURING TECHNOLOGIES

The Use of Preacidification When Making Cheese With Membrane Concentrated Milk

Dean Sommer, University of Wisconsin Center for Dairy Research

The use of membrane concentrated milk is becoming increasingly common in the cheese industry. With the recent communication by the U.S. Food and Drug Administration of their intent to exercise enforcement discretion regarding the use and labeling of UF milk and UF nonfat milk in the manufacture of standardized cheeses and related cheese products, it is likely that this trend will continue.

However, when using membrane concentrated milks in cheese making, make procedure changes need to be made to account for the additional protein and bound calcium in the system. Not taking this into account can lead to undesirable textural, flavor, and functional attributes in the finished cheeses. This presentation will look at the value of preacidification to avoid some of these undesirable cheese attributes when using membrane concentrated milk.

Salt Considerations for Cheese Making

Janice Johnson, PhD, Cargill, Inc.

Milk, enzymes, culture and salt are the only four ingredients allowed in the production of natural cheeses. Considering salt, it plays a critical role in developing the desired characteristics of cheeses. The functional roles of salt in cheese include microbial management (impacts water activity), altering enzyme activity, removing whey from the curds (syneresis), altering protein structure (impacts texture) and taste. There are many factors to consider when handling and applying salt to cheese in order to optimize production and quality.

The addition of salt to cheese depends on the type of cheese. Methods to apply salt to cheese include direct addition to the curds (e.g. Cheddar), absorption through brine application (e.g. Feta) or a combination of the two methods (e.g. Mozzarella). In the case of direct salt addition, the physical properties of salt have been shown to influence the amount of salt uptake into the cheese. Maximizing salt absorption can be important considering the amount of salt that can end up in the whey.

It is important to consider the characteristics and handling of salt in order to minimize production issues. Salt is hygroscopic, which can create clumping issues that impact salt flow and salt distribution on the curds. Various additives can be added to help minimize caking of the salt. Pneumatic conveying of salt can lead to particle size separation and breakdown of salt particles. Engineering design of the conveying system and the dense phase conveyance of the salt can help minimize salt degradation. Type of salt crystal, as determined by type of production, can also help minimize salt breakdown.

Types of salts can also be used to help with product differentiation. Marketing trends can be leveraged to help differentiate cheese products in the markets. Current trends include clean label, sodium reduction and sea salt.

Mathematical Modeling of Salt Transport in Cheese Curd

Meghan Keck, Massey University

Sodium chloride is a key ingredient in cheese, effecting preservation, texture and functionality, and flavour. Understanding the mechanisms of moisture and salt transport within fresh cheese curds is essential to optimize production and consistency. Current models for salt transport in cheeses have primarily used an unsteady-state Fickian diffusion approach. While simple, these models fail to account for the underlying mechanisms and interactions that control the rate of salt uptake into cheese, such as the effects of local moisture content and syneresis. A fundamentally-derived, one-dimensional mathematical model has been developed to model the uptake and transport of salt and moisture through fresh, dry-salted cheese curds by defining the curd as a proteinaceous matrix with whey-filled, interconnected pores and accounting for the effects of osmotic pressure induced moisture expulsion. The model provides an essential first step to describing the mechanisms that control the uptake and transport of salt and moisture during the salting process of cheese making. This model has the potential to be used in industry to reduce salt and moisture content variation in final cheese products, and optimize the conditions to control salt whey losses.

SESSION 5 - WHEY PROCESSING / PRODUCTS / APPLICATIONS

Functionalization of Whey Protein by Reactive Supercritical Fluid Extrusion

Sy Rizvi, Cornell University

Whey proteins are widely used in a variety of food formulations and constitute a significant share of the dairy ingredients market. Their modification under precisely superimposed processing conditions have the potential to impart in them a wide range of improved functional properties and make them more attractive beyond their well-recognized nutritional values. These two completely distinct roles are indeed likely to enhance the protein's use in clean label food formulations.

WPC-80 based formulations were extruded in a high-pressure extruder at 90 °C in the pH range of 2.89 to 8.16 with 1% (dry feed basis) SC-CO₂ injected as a pH modifier and a blowing agent. The average specific mechanical energy (SME) input for the process was 57 Wh/ kg. The resulting texturized WPC (tWPC) extrudates were found to be strongly dependent on the pH and SC-CO₂ levels used during extrusion. The highest apparent viscosity ($\eta=933$ mPa·s) and elastic modulus ($G'=10$ kPa) values were observed in the tWPC produced at extremely acidic condition (pH 2.89) and were significantly higher than those exhibited by the un-extruded control samples. The 20% (w/w) tWPC dispersion exhibited a highly viscous and creamy texture with particle size in the micron-range (mean diameter ~ 5 μ m) which could serve as a thickening/gelling agent in food formulations over a wide range of temperatures. A homogeneous gel-like emulsion of creamy consistency was also successfully produced by incorporation of corn oil. Only 4% (w/w) tWPC was needed to emulsify 80% corn oil and it showed a higher thermal stability upon heating to 85 °C. The tWPC showed excellent emulsifying properties (emulsion activity index, EAI, = 431 m² g⁻¹, emulsion stability index, ESI, = 13,500 h) compared to the commercial WPC-80. These unique properties have not been previously reported for whey protein concentrates and will be discussed in more details.

Integrating Membrane Processing to Produce Milk Fractions for Making Ideal Whey & Infant Formula

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Bovine milk is the most commonly-used starting material for the development of protein ingredients for application in infant formula (IF) products. However, total protein, casein:whey protein ratio and ash levels are all significantly higher in bovine milk than in human milk. Formulation strategies designed to 'humanise' the protein profile of IFs are very much informed by the latest research on breast milk biochemistry and typically involve recombination of skim milk and demineralised whey to help achieve the highly-regulated limits for protein content, amino acid profile and mineral profile. These demanding targets have fuelled intensive research in recent years aimed at developing enriched and purified whey protein (e.g., α -lactalbumin-, lactoferrin- and osteopontin-enriched whey protein concentrates/isolates) and casein (e.g., β -casein-enriched native whey) fractions and modified (e.g., selectively hydrolysed) milk protein in-

gredients for use in IF applications. The traditional model for the formulation of infant nutrition products, whereby dried milk protein ingredients are recombined in water with oils, carbohydrates and micronutrients, is currently the focus of a significant rethink, and this presentation will outline how integrated membrane filtration approaches are allowing for new possibilities in the development of novel milk protein ingredients for use in the next generation of IF products. The casein-dominant retentate fraction which is a co-product of ideal whey and IF ingredient manufacture therefrom using such an approach, has several interesting, and yet to be fully realised, functionalities and applications and some recent work in this area will also be presented.

Efficiency of Whey Protein Passage During Ceramic Microfiltration for the Production of Whey Protein Isolate

Brandon Carter, North Carolina State University

Microfiltration (MF) is a commonly used technology in the dairy industry for removing bacteria, fractionating casein and serum proteins, and defatting of whey. In the production of whey protein isolate (WPI), microfiltration or anion exchange is required to achieve protein concentrations above 90%. When using microfiltration, a co product is produced called whey protein phospholipid concentrate (WPPC). As an ingredient manufacturer, WPI is sold for a higher premium than WPPC, thus improving whey protein passage during microfiltration will increase protein yields and profit. Spiral MF membranes protein rejection has been reported as 50-60%. In contrast, ceramic MF has been shown to remove 95% of serum proteins in a 3x three stage process.

The objective of this study was to determine if 95% removal of whey proteins is achievable with fluid whey as the starting material under the same conditions as has been reported with skim milk. Microfiltration of whey was done with Cheddar cheese by mass balance approach. Fluid whey was produced from standard Cheddar cheese make and 375 kg of pasteurized, fat-separated whey was subject to ceramic MF. The experiment was repeated 3 times with a 0.1 micron ceramic UTP membrane. Samples of retentate and permeate were collected at each stage and analyzed for total protein, as well as protein profile. Protein profile was evaluated using a reversed phase C4 UPLC column with a photodiode array detector. Peak areas were calculated from standards of individual proteins to track and quantify individual whey proteins in the retentate and permeate. Results were compared to previous published research from skim milk. Approximately 80% of the whey protein was removed in 3 stages of filtration. Protein removal was 15% lower than values reported from skim milk. This research demonstrates that higher WPI yields can be achieved through 0.1 micron ceramic MF membrane.

SESSION 6 - UNDERSTANDING CHEESE CHEMISTRY

Impact of Cooker/Stretcher Thermo-Mechanical Conditions on Fat and Moisture of Mozzarella Cheese

Michel Britten⁽¹⁾, Vincent Banville⁽²⁾, Pierre Morin⁽²⁾, Yves Pouliot⁽³⁾

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Thermo-mechanical treatment is a critical step in the production of pasta filata cheeses. This plasticising and kneading treatment of fresh curd in hot water or brine, gives the cheese its fibrous structure. During the process, partial loss of curd constituents in hot brine is observed, which affects both the cheese composition and yields.

A lab-scale approach was used to measure the effect of curd stretching conditions on mass balance and cheese characteristics. A Farinograph™ equipment was used to process 500g batches of curd with continuous monitoring of torque and curd temperature. In the present study, the influence of mixer wall temperature (60, 65, or 70 °C), mixing speeds (6, 9, or 12 rpm) and mixing time (3 or 6 min) were tested according to a complete factorial design. Specific mechanical energy, heat load index and final curd temperature were calculated from data collected during curd stretching and used as predictive parameters for cheese properties.

On average, 10% of curd mass was lost during curd stretching and considerable changes in cheese composition were observed depending on the mixing conditions. Fat loss was strongly correlated to the mechanical energy imparted during process and the proportion of expressible serum in young cheese increased with heat load and final curd temperature. Curd stretching conditions induced changes in cheese microstructure but the rheological properties were not significantly affected.

The use of the Farinograph™ unit as a lab scale model for the curd stretching process allowed strict control of temperature, time, and speed of mixing and facilitated mass balance calculations. This approach can provide additional knowledge for a better control cheese yield and composition.

Whey Expulsion from Cheese Curd as a Function of Protein Concentration, Temperature and Cut Size

Ram Panthi, J.J.(Diarmuid) Sheehan and Donald McMahon

Utah State University and Teagasc Food Research Centre Moorepark, Ireland

Increasing the protein in milk via ultrafiltration (UF) can allow higher yields per vat of cheese and allow greater consistency in cheese composition to be achieved by standardizing each vat of milk to a constant protein and fat level. However, the cheese making process is influenced by protein level with respect to curd formation and whey expulsion. Milk was standardized to protein levels of 4, 5 and 6% by combining ~3X UF retentate with skim milk and UF permeate, with a protein-to-fat ratio of 1.0. Milk was renneted at 28, 32 and 36°C and development of curd firmness was monitored by

rheometry and the curd cut at a firmness of ~35 kPa. Samples of curd were collected 5 min after cutting the curd and then every 10 minutes during forework, cooking to 37°C, and a whey dilution step, and moisture content measured gravimetrically. Increasing protein from 4 to 6% only slightly shortened the time before coagulation was observed (by ~15%) but had a large effect on the rate at which curd firming occurred. Compared to having a cut window (the time when the curd firmness was between 35 and 50 kPa) of 3.45 minutes with 4% protein milk at 32°C, increasing protein shortened the cut window to 1.1 minutes at 6% protein. Increasing set temperature to 36°C further shortened the cut window to 2.0 and 0.8 minutes, respectively. Lowering the temperature to 28°C lengthened the cut window to 6.9 and 2.0 minutes, respectively. As expected, cutting the curd at a small size (6 mm compared to 12 and 18 mm) increased the rate of whey expulsion. Whey expulsion from the curd with higher protein levels was slower than at 4% protein, but this has to take into consideration that the initial curd moisture is also lower so less moisture needs to be expelled. At higher set temperatures faster whey expulsion occurs but since all curds were cooked to a constant temperature of 37°C, the curd formed at the lower temperature underwent a greater change in temperature during cooking and so overall they had similar total extent of whey expulsion.