822 Whey protein nanoparticles prepared by desolvation: Encapsulation capacity and interfacial activity. I. Gülseren* and M. Corredig, *University of Guelph, Dept. of Food Science, Guelph, Ontario, Canada.*

Whey protein nanoparticles were prepared using a desolvation method, using ethanol as solvent. Physical characterization including particle size characteristics, encapsulation capacity for a model hydrophilic molecule (Brilliant Blue FCF), and interfacial activity at the oil-water interface were carried out, after diluting the samples in acidic conditions (pH 3). The particle size distribution was determined using dynamic light scattering technique, and particle diameter was between a few nm up to about 100 nm. Solvent composition was highly influential on the average particle size and its distribution. The release of the Brilliant Blue from the nanoparticles with storage was quantified using UV-Visible spectrophotometry, after separation of the whey protein particles by centrifugation. Both unloaded and loaded nanoparticles were stable at room temperature after one month of storage, and little release of the encapsulated dye molecules was observed. The interfacial properties of the unloaded nanoparticles were also tested using drop tensiometry, and it was shown that nanoparticle formation only slightly affected the interfacial activity.

Key words: whey protein nanoparticles, desolvation, encapsulation

823 Comparative proteomic analysis of whey proteins between healthy and subclinical mastitic cows. J. Bian, Q.-Z. Li*, and X.-J. Gao, *Key Laboratory of Dairy Science of Ministry of Education, Northeast Agricultural University, P.R. China.*

This study was to investigate different whey protein contents between healthy dairy cows and subclinical mastitical dairy cows, and to explore the mechanism of pathogenesis. This study presented changes of the whey proteins from healthy dairy cows and subclinical mastitical dairy cows by using 2-DE. After the protein stained with coomassie blue G-250, 21 differential expression protein spots were detected by ImageMaster 2D Platinum 6.0 software, then 10 protein spots were analyzed by using the MALDI-TOF/MS. Meanwhile. Proteins were identified by using the Mascot software to search the NCBInr database and the swiss-port database.8 proteins were identified successfully. In cows with subclinical mastitis, 3 proteins were downregulated: Alpha-S1 casein precursor, Chain D of bovine β-Lactoglobulin A and Chain B of the bovine β 1,4 galactosyltransferase catalytic domain ; 5 proteins were upregulated:Serotransferrin precursor, α-1-acid glycoprotein, β 2- microglobulin, Complement C3 precursor and Cytokeratin 9, these proteins were involved in signal transduction, binding and transportion and immune defense activity. The α-1-acid glycoprotein expression in whey of cows with subclinical mastitis was 2.55-fold higher than the healthy dairy cows confirmed by ELISA protocol. The results provided valuable information for the investigation on mechanism of the subclinical mammitis of dairy cows and potential protein targets for treatment.

Key words: subclinical mastitis, whey protein, comparative proteome

824 Controlling whey proteins spontaneous self assembly. T. Croguennec*[1], D. Salvatore[2], T. Nicolai[3], V. Forge[2], and S. Bouhallab[1], *[1]UMR 1253, INRA- Agrocampus Ouest, Science et Technologie du Lait et de l'Oeuf, Rennes, France, [2]Laboratoire de Chimie et Biologie des Métaux, CEA-Grenoble, Grenoble, France, [3]UMR CNRS-Université du Maine, Polymères, Colloides, Interfaces, Le Mans, France.*

Sustainability in food manufacture involves a profound reasoning of the way food are produced. Reducing energy input during food processing and optimizing ingredient formulation to meet both sensorial acceptance and nutritional benefit through a controlled release of macro- and micro-nutriments constitute great challenges for food industries. Controlled self-assembly of molecules into biomaterials throughout bottom-up approach is a promising way to achieve these goals. Because of their omnipresence in food systems, whey proteins are the focus of many attempts for their use as building blocks for such biomaterials. For instance, the apo form of α-lactalbumin (α-LA) and β-lactoglobulin are able to self-assemble into well-defined microspheres in the presence of an oppositely charged protein such as lysozyme (LYS). Formation and destabilization of microspheres are inducible by changing the physicochemical conditions. Because of this reversibility, such microspheres could be used to trap, protect during processing and storage, carry and deliver bioactives. However, to complete this challenge, a perfect understanding of protein assembly and disassembly mechanisms are necessary. In this communication we address the mechanism of formation of microspheres of α-LA and LYS from the molecular scale to the microspheres. One of the first events in the mechanism of formation of microspheres is a specific interaction between α-LA and LYS leading to a heterodimer. Probably throughout charge screening, α-LA/LYS heterodimers rapidly self-assembled into nanometer-sized aggregates. These small entities further aggregate into clusters following a diffusion limited mechanism (DLCA) and fuse upon physical contact into spherical microspheres. The driving force for the reorganization of the clusters into microspheres is suggested to be the decrease of the total surface free energy. However, the reorganization of the clusters was only inducible when the temperature was increased above 30°C, temperature above which α-LA adopt a molten globule structure. We put forward that the higher flexibility of α-LA above 30°C may facilitate clusters–microspheres transition.

Key words: self assembly, α-lactalbumin, microsphere

825 Study of the combined acidification and rennet gelation behavior of casein micelles using single *Streptococcus thermophilus* strains, with high or very low exopolysaccharide production. Z. Miao*, E. Kristo, and M. Corredig, *University of Guelph, Guelph, Ontario, Canada.*

The effect of the presence of exopolysaccharide (EPS) produced by lactic acid bacteria on the gelation properties of caseins is still largely unknown. Objective of this work was to study the gelation behavior of caseins during renneting, after controlled fermentation with high-EPS producing or very low-EPS producing *S. thermophilus* cultures. Fresh skim milk was fermented with either a high ropy exopolysaccharide-producing strain (CHCC-5086, Chr. Hansen); or a very low EPS producing strain (CHCC-742, Chr. Hansen), as control. The inoculated skim milk was incubated at 40°C and rennet was added when a pH of 6.2 was reached. The gelation behavior was followed using rheology, diffusing wave spectroscopy (DWS) and confocal microscopy. Milk containing the high-EPS producing strain showed a significantly higher viscosity than the control during fermentation up to pH 6.2. After addition of rennet, the gelation point occurred earlier (at significantly higher pH values) in milk fermented with high-EPS producing strain compared with milk fermented with control strain. Confocal

microscopy also showed differences in the microstructure between the gels, with larger pores in the presence of EPS. These results further our fundamental understanding of the effect of EPS on the beginning stages of the rennet-induced gelation.

Key words: exopolysaccharides, casein, gelation

826 In situ structural investigations of the milk fat globule membrane revealing heterogeneities and sphingomyelin-rich domains. C. Lopez*, *INRA-STLO, Rennes, France.*

In recent years, the milk fat globule membrane (MFGM) has attracted the attention of scientists and industrials because of its interesting functional, nutritional and health properties. These properties of the MFGM result from its chemical composition, but they may also be influenced by the capacity of the MFGM components to be specifically structured. Various models of the MFGM have been proposed, all of them presenting polar lipids as a homogenous 2D solvent for membrane proteins (Singer & Nicholson fluid mosaic model). However, the structure of the MFGM still remains the least understood aspect of milk. The objective of this work was to investigate the organization of the MFGM in situ in milk, using confocal laser scanning microscopy and adapted fluorescent dyes (exogenous polar lipid, lectins). For the first time, our work revealed i) the lateral segregation of sphingomyelin (SM; 20 to 25% of milk polar lipids) in rigid liquid-ordered domains surrounded by the fluid matrix of the glycerophospholipids (PC, PE, PI, PS), ii) that the SM-rich domains are of micronic size with a circular shape for bovine milk, iii) that the SM-rich domains diffuse in the plane of the outer bilayer of the MFGM as a function of time, iv) that the SM-rich domains are devoid of proteins. These SM-rich domains have been characterized whatever the size of milk fat globules and in milks from various species. As a conclusion, we showed that the MFGM is a heterogeneous and highly dynamic biophysical system. Moreover, on the basis of our experimental results, we proposed a new model for the organization of the MFGM. These original results raised the question of the role played by these SM-rich domains on the functional and nutritional properties of milk fat globules. Directions for future research studies will be discussed.

Key words: milk fat globule membrane, sphingomyelin, confocal microscopy

827 Fractionation of glycomacropeptide and beta lactoglobulin using positively charged ultrafiltration membranes in staged configurations. S. Gemili* and M. R. Etzel, *University of Wisconsin-Madison, Madison.*

Membrane ultrafiltration is a commonly used method for the concentration and diafiltration of protein solutions. For fractionation of proteins, ultrafiltration is not commonly used, because it lacks the separation capability of chromatographic methods. The selectivity of membrane ultrafiltration is increased dramatically when a charge is added to the membrane surface. Configuring the membranes in stages also increases the selectivity of the separation. Using membrane ultrafiltration instead of chromatography for protein fractionation has advantages for the dairy industry of a lower cost of manufacture resulting from decreased buffer consumption and the use of existing installed membrane equipment. In this study, positively charged cross-flow ultrafiltration membranes having a 300 or 30 kDa molecular weight cut off were used to fractionate glycomacropeptide (GMP) and β lactoglobulin (BLG). Sieving coefficients of GMP and BLG were determined experimentally by ultrafiltration of binary protein mixtures. These sieving coefficients were used to calculate purity and yield of GMP products from one-stage and 2-stage membrane configurations using mass balance models. Flow configurations that allowed for recycle of by-product streams were compared with cases without recycle. Different volume concentration factors (ratio of permeate to feed solution) ranging from 0.01% to 80% were also included in the evaluation. A ratio of 80% gave the highest GMP purity (97%) using a 300 kDa membrane in the first stage and a 30 kDa membrane in the second. However, this configuration gave the lowest GMP yield (21%) in the permeate stream. In contrast, a one-stage configuration using a 300 kDa membrane gave a 60% yield of GMP in the permeate stream at a purity of 74%. In conclusion, GMP fractionation can be accomplished using positively charged ultrafiltration membranes. Different flow configurations and membranes having different molecular weight cut offs can be used to balance yield and purity.

Key words: ultrafiltration, fractionation, glycomacropeptide

828 Antimicrobial role of serum amyloid A3 in goat milk. A. Domènech*[1], J. G. Raynes[2], A. Arís[1], A. Bach[1,3], and A. Serrano[1], *[1]Department of Ruminant Production, IRTA, Caldes de Montbui, Spain, [2]Immunology Unit, Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom, [3]ICREA, Barcelona, Spain.*

Milk is an important source of antimicrobial compounds. Serum Amyloid A3 (SAA3) is an acute phase protein which level is particularly high in colostrum and milk during mastitis, suggesting a potential protective role against infections. The aim of this study was the recombinant production of the milk goat SAA3 (gM-SAA3) and to evaluate 2 possible mechanisms of antimicrobial activity, directly preventing bacterial gastrointestinal adhesion and indirectly participating in macrophage-phagocytosis activation. Previous studies have explored some of these roles using either just fragments of the bovine milk-derived protein or the human plasma circulating form (SAA1). The recombinant gM-SAA3 was cloned from goat milk through RNA isolation, retrotranscription and specific amplification. Recombinant expression was achieved using the pET101 TOPO-cloning. For gastrointestinal assays, differentiated intestinal Caco-2 cells were incubated in 6-duplicates during 1h with 30 µg/mL of gM-SAA3 prior 2 h infection with 10^6 cfu/well of enteropathogenic *Escherichia coli* (EPEC). Phosphate buffer saline (PBS) and 10^8 cfu/well of *Lactobacillus rhamnosus* were used as negative and positive controls. Bacterial adhesion was quantified by viable cell counts. Human blood and goat milk macrophages were isolated using histopaque gradient density or conventional centrifugation. Macrophages were incubated for 1 h with 3 and 30 µg/mL gM-SAA3, PBS or human serum along with fluorescent labeled *E. coli* BL21-GFP+. Phagocytosis was evaluated using confocal microscopy and quantified as the association index (AI): number of fluorescent bacteria per 100 macrophages. Soluble gM-SAA3 was successfully expressed at 0.5 mg/ml. In gastrointestinal assays, a 70% decrease ($P < 0.001$) in EPEC binding was observed after incubation with gM-SAA3. Also, gM-SAA3 increased ($P < 0.05$) the AI in both human (62 ± 11%) and goat (72 ± 17%) macrophages, compared with PBS (9.8 ± 8% and 18 ± 15% respectively). These results confirm that the whole gM-SAA3 protein may play an active role in the newborn's defense via milk intake and it could participate in the clearance of bacteria through macrophage activation.

Key words: SAA, antibacterial, phagocytosis