E/P treated heifers ($p < 0.01$); E and E/P treated heifers did not differ. This RPA will be used to further evaluate the possible role of ER α receptor in regulating important processes such as mammary gland development.

Key Words: Bovine, Steroid, Gene Expression

1355 Effects of chronic in vitro growth hormone treatment on insulin receptor substrates and PI3 kinase in adipose tissue. F. Castro*[1], E. Delgado[2], and D. Lanna[2], [1]*University of California, Davis/ CA/ USA,* [2]*Esalq-USP/ SP/ Brazil.*

Growth hormone (GH) has profound effects on carbohydrate and lipid metabolism, including a reduction in adipose tissue sensitivity to insulin. The objective of the present study was to characterize some components that may be involved in the cellular mechanism of this insulin resistance. Concentrations of insulin receptor substrates (IRS) including IRS-1, IRS-3 and phosphatidylinositol 3-kinase (PI3K); phosphorylation of IRS-3; and association of IRS-1/PI3K and IRS-3/PI3K were evaluated in adipose tissue cultured in the absence or presence of GH. Male Wistar rats had their epididymal fat pads removed and placed in medium 199. In a first protocol adipose tissue explants were incubated in medium 199 for 48h with: a) 100 ng/ml of insulin plus 10 nM dexamethasone or b) 100 ng/ml of insulin plus 10 nM dexamethasone and 100 ng/ml of hGH (human GH). The second protocol evaluated the effects of chronic exposure to hGH on adipose tissue response to short-term insulin stimulus. Explants were incubated for 24h in medium 199 with: a) no additions or b) 100 ng/ml hGH. After 24h, half of the explants were stimulated for 20min with 1μg/ml of insulin and homogenized in extraction buffer. The concentration, phosphorylation state and association of the proteins were studied using western blots. In adipocytes exposed to hGH for 48 hours, the amounts of the IRS-1, IRS-3 and PI3K decreased by 336% ($P<.01$, n=6), 278% ($P<.05$, n=7) and 268% ($P<.05$, n=7), respectively. Consistent with effects observed after 48 hours, adipose tissue treated with hGH for 24h had concentrations of IRS-1, IRS-3 and PI3K decreased by 294% ($P<.01$, n=5), 153% ($P<.01$, n=8) and 295% ($P<.01$, n=6), respectively. Short-term insulin stimulation increased degree of phosphorylation and associations of IRSs ($P<.01$). Short-term insulin effects were altered by chronic in vitro incubation with hGH, including: amount of phosphorylated IRS-3 was reduced by 286% ($P<.05$, n=7), and the amounts of IRS-1 and IRS-3 associated with PI3K were reduced by 4420% ($P<.10$, n=6) and 284% ($P<.01$, n=6), respectively. Results of this study suggest that chronic GH treatment in vitro alters the early steps of insulin signal transduction in rat adipose tissue, including decreased IRS-3 concentration.

Key Words: Insulin, Growth Hormone, Insulin Receptor Substrate (IRS)

Milk Protein and Enzymes

1356 Characterization of carbohydrate structure of MUC1 and MUCX in porcine and bovine milk by exoglycosidase treatment and lectin blot test. C. Liu*, A.K. Erickson, and D.H. Francis, *South Dakota State University, Brookings, SD.*

Mucins are glycoproteins characterized by a high level of O-linked glycosylation of their core proteins. Two types of mucins, MUC1 and MUCX, are found to be present in porcine and bovine milk. Little information is available about their carbohydrate portion. This study employed exoglycosidase treatment together with lectin binding studies to determine the carbohydrate structure of MUC1 and MUCX in porcine and bovine milk. Treatment with neuraminidase reduced the mobility of both mucins on SDS gels in both species, indicating the existence of terminal sialic acid (NeuAc) residues; treatment with β-galactosidase had no effect on the mobility of native mucins but decreased the mobility of neuraminidase-treated mucins, suggesting terminal β-D-galactose occurs more commonly in the penultimate position. Twenty five lectins were used to further identify certain carbohydrate stucture associated with mucin core proteins. Regardless of species and mucin type, the presence of both N-linked and O-linked oligosaccharide chains was implicated by the binding of Concanavalin A and Jacalin. The presence of terminal N-acetyglucosamine (GlcNAc) was indicated by the binding of soybean agglutinin lectin (SBA) and *Vicia villosa* lectin (VVA), which both bind to MUC1 less strongly than to MUCX,implying a difference in terminal GalNAc abundance. Some carbohydrate structures were found to be species-specific and mucin type-specific. Bovine MUC1 contained exposed β-D-Gal-β(1,3)-D-GalNAc (T antigen) [peanut agglutinin (PNA) binding] while porcine MUC1 did not; terminal α(2,6)-linked NeuAc [elderberry bark lectin (EBL)] was found present in porcine MUC1 while it was lacking in bovine MUC1. Both porcine MUCX and Bovine MUCX contained exposed T antigen structure (PNA binding) and N-acetyglucosamine (GlcNAc) [*Solanum tuberosum* lectin (STL) binding]; however, bovine MUCX had α(2,6)-linked NeuAc [*Maackia amurensis* lectin II (MAAL II)] and terminal α(2,6)-linked NeuAc which were absent in porcine MUCX. The complexity and diversity of mucin glycosylation imply the functional importance of mucin carbohydrate. Further studies on the carbohydrate structure of milk mucins may contribute to an understanding of their possible functions between mother and young.

Key Words: Bovine milk mucins, Porcine milk mucins, Carbohydrate structure

1357 Structural studies of bovine β-casein by CD, FTIR and molecular modeling. P. X. Qi* and H. M. Farrell, Jr., *USDA-ARS-ERRC, Wyndmoor, PA, USA.*

The caseins of milk form micelles to carry the otherwise insoluble calcium and phosphate which are indispensable nutrients for humans. To better understand the molecular basis for the calcium-phosphate transport complex, we studied the major component of this complex: β-casein. The assumption that β-casein is #rheomorphic# remains controversial. In this work, we report our studies on the association reaction of β-casein to address the question of whether or not a conformational change in the monomer precedes aggregation, or occurs as a result of aggregation. Circular dichroism (CD) and Fourier transformation infrared (FTIR) spectroscopies were used to investigate the temperature-induced changes in the secondary structure of the β-casein under physiological relevant conditions (water, pH and low ionic strength). The degree of self-association under these conditions was assessed by analytical ultra centrifugation. CD and FTIR spectroscopies, as well as secondary structure predictions suggest the possible existence of polyproline II left-handed helices in β-casein. These short helices may play an important role in the self-association process. Furthermore, CD and FTIR results show that the β-casein may fold considerably prior to self-association, but may further respond to close packing in the polymer. The binding of β-casein to hydrophobic probe 1-anilino-8-naphthalenesulfonate (ANS) indicates it may be a molten globule-like protein. Molecular modeling techniques were used to not only generate a three-dimensional structure but also provide dynamic information for the self-association process as well as its function in calcium transporting.

Key Words: β-Casein, Structure, Polyproline II

1358 Conformational change in alpha-lacatalbumin produces an alternative biological function. K Stokes and B Alston-Mills*, *North Carolina State University, Raleigh, North Carolina, USA.*

Research within the past 10 years has suggested that the structure of alpha-lactalbumin is essential to its function, both as the modifier protein in the lactose synthase complex and as a modulator of mammary epithelial cell (MEC) proliferation. To directly test this hypothesis, we treated 3 mammary cell lines with 5 lots of bovine alpha-lactalbumin that differed in purity and tertiary conformation. Protein purity was determined by one- and two-dimensional PAGE and UV-spectroscopy and protein fold was determined by both flourescence and circular dichroism. Two lots were purchased from Sigma (98H7003 and 99H7029) and used without further purification. PAGE revealed several minor impurities in lot 98H7003, but the major species was alpha-lactalbumin. Two wild-type (wt) recombinant lots were expressed, folded and purified: lot wt01 was pure and correctly folded and lot wt02 was pure, but misfolded. A third recombinant protein, D87A, was pure, but unable to bind calcium and lacked a well-defined tertiary structure. Correctly folded native alpha-lactalbumin (99H7029) and wild-type (wt01) and mutant (D87A) recombinant proteins did induce inhibition of MEC

proliferation. Only the misfolded native (98H7003) and wild-type recombinant (wt02) proteins significantly inhibited the proliferation of the MECs (95% confidence). After 24 hour treatment with the misfolded, native protein (1mg/ml), cell proliferation was reduced by 87%, 90% and 14% in the MCF-10a, MCF-7 and MDA-MB-231 cell lines, respectively. Moreover, recombinant lot wt02 (1mg/ml) also significantly inhibited the proliferation of MCF-10a and MDA-MB-231 by 12% and 21%, respectively. Circular dichroism revealed that wt02 had increasd helical content which greatly contributed to its altered secondary structure. Alpha-lactalbumin protein in native and various non-native conforamtions have been isolated from various milks. The data presented here is in agreement with published reports and suggest that changes in the secondary of alpha-lactalbumin can produce a conformation that modulates cell proliferation *in vitro*.

Key Words: alpha-lactalbumin, protein conformation, cell prolieration

1359 Estrogen response of the human lactoferrin promoter in mammary gland cells. K Stokes[1], C Teng[2], and B Alston-Mills*[1], [1]*North Carolina State University, Raleigh, North Carolina USA,* [2]*National Institutes of Environmental Health Sciences, Research Triangle Park, North Carolina USA*.

It is well documented that the expression of *lactoferrin*in the uterus is induced by estrogen. However, little data is available concerning the regulation of *lactoferrin*expression in the mammary gland. We employed transient transfection assays using mammary epithelial cell lines (MEC) to determine whether diethylstilbestrol (DES), a potent environmental estrogen, could stimulate human *lactoferrin*promoter activity *in vitro*. The estrogen response is mediated by the estrogen receptor (ER) through the estrogen response element (ERE) an possibly the estrogen receptor related receptor (ERR) through the steroid factor response element (SFRE). We demonstrated expression of these receptors in the human MECs MCF-7 and MCF-10a using RT-PCR methods. Both cell lines endogenously express ERR-alpha but only the MCF-7 cells express ER-alpha. Co-transfection of the reporter constructs and the ER-alpha expression vector, followed by 24 hour DES (10nM) treatment stimulated *lactoferrin*promoter activity by 7-fold in the MCF-7 cells and 3-fold in the MCF-10a cells. Mutational analysis of the potential cis-regulatory elements revealed that both elements are essential for maximal estrogen response. Mutation of the ERE decreased DES-induced promoter activity to 3-fold in MCF-7 cells and mutation of the SFRE completely abolished DES-induced promoter activity. The same trend was observed in the MCF-10a cells. These data suggest that *lactoferrin*is an estrogen-inducible gene in the mammary gland as well as the uterus and support recent reports of cross-talk between ER and ERR during estrogen signaling.

Key Words: human lactoferrin, gene regulation, diethylstilbestrol

1360 Disulfide bonding patterns between β-lactoglobulin and κ-casein in a heated and spray-dried milk-model. A. Bienvenue*[1], C.S. Norris[2], M.J. Boland[2], L.K. Creamer[2], and R. Jimenez-Flores[1], [1]*DPTC, California Polytechnic State University, San Luis Obispo, CA,* [2]*New Zealand Dairy Research Institute, Palmerston North, New Zealand*.

The heat treatment used during the manufacturing of milk powder causes protein interactions that define functionality (Singh, H. & Creamer, L.K. 1991). Moreover, the genetic variant of β-lactoglobulin (β-LG) in homozygous milk influences dramatically its properties during heat processing (Hill, J.P. et al. 1998, US Patent 5,850,804). To better understand the effects of processing on the functionality of milk powder we characterized at the molecular level the protein-protein interactions after heating and drying. The objective of the project was to determine the position of the heat-induced disulfide interchange between β-LG and κ-casein (κ-CN). The powder sample was produced in the pilot plant of the Cal Poly DPTC in San Luis Obispo, CA. A simplified milk system was created by mixing affinity-purified β-LG (β-LG genetic variant AB) to casein micelles obtained by filtration of raw milk (κ-CN variant AA; β-LG variant BB) through a 0.1mm pore size ceramic membrane. The mixture was heated at 90°C for 15 minutes and spray dried. The high molecular weight molecules were segregated by size exclusion chromatography (Rasmussen, L.K. & Petersen, T.E., 1991), identified by SDS-PAGE, and hydrolyzed by trypsin. The native and disulfide-bond-reduced hydrolysates were analyzed by HPLC-MS at the NZDRI Palmerston North, NZ. Our SDS-PAGE analysis shows that we isolated a disulfide-linked protein polymer that contained predominantly κ-CN and β-LG. By comparing our mass spectroscopy results to tryptic digest data banks; we identified 42 peptide fragments including 11 disulfide-linked peptides. We identified three different types of disulfide links: 1) The expected (Rasmussen et al. 1992) intermolecular bridges between two κ-CN molecules connected κ-CN Cys11 to κ-CN Cys11 and κ-CN Cys88 to κ-CN Cys11. 2) The heat induced association of two β-LG linked β-LG AA Cys66 to β-LG BB Cys106/119/121 and β-LG Cys160 to β-LG BB Cys106/119/121. 3) The heat-induced covalent bonding between β-LG and κ-CN involving κ-CN Cys88 to β-LG Cys66, κ-CN Cys11 to β-LG Cys160, and κ-CN Cys11 to β-LG BB Cys106/119/121. These peptides aid in the elucidation of protein interactions in dried milk.

Key Words: Heat-interactions, Disulfide bonds, Milk Powder

1361 Interactions between β-lactoglobulin and xanthan gum studied by capillary electrophoresis. M. Girard*, S.L. Turgeon, and S.F. Gauthier, *Universite Laval, Quebec, Canada*.

Protein-polysaccharide interactions are of great interest to the food industry. The polysaccharide xanthan gum is often incorporated in dairy products and can interact, under certain conditions, with β-lactoglobulin, the major whey protein. Because these interactions can influence the behaviour of the biopolymers, they must be investigated. The aim of this work was to determine the strength and nature of the interactions under different conditions. A capillary electrophoresis technique has recently been developed to measure the interactions between homogeneous molecular weight polymers. This technique, known as Frontal Analysis Continuous Capillary Electrophoresis (FACCE), has been applied successfully to the study of interactions between xanthan gum, a heterogeneous biopolymer, and β-lactoglobulin. With FACCE technique, it was possible to determine precisely the amount of proteins that was not bound to the xanthan gum. The pH values studied were 5 and 6. The impact of the molecular weight on the interactions was assessed using native ($5.36*10^7$ Da) and microfluidized ($3.25*10^7$ Da) xanthan gum. Binding constants and binding site sizes were obtained with modified Scatchard plots. The binding constants were $8.96*10^4$ M-1 and $5.11*10^4$ M-1 for β-lactoglobulin/xanthan gum and $7.49*10^3$ M-1 and $3.08*10^3$ M-1 for β-lactoglobulin/microfluidized xanthan gum, at pH 5 and 6, respectively. The effect of the pH on the binding constants revealed the existence of electrostatic interactions between the biopolymers. Lower molecular weight xanthan gum had weaker interactions with β-lactoglobulin. The shape of the binding isotherms showed that the xanthan gum became saturated with proteins at a 10/1 protein/polysaccharide ratio.

Key Words: β-lactoglobulin, Xanthan Gum, Interactions

1362 Coisolation of Plasmin/Plasminogen with Xanthine Oxidoreductase. D.A. Clare*, G.L. Catignani, and H.E. Swaisgood, *Southeast Dairy Foods Research Center, Dept. of Food Science, NCSU, Raleigh, N.C.*

Bovine milk xanthine oxidoreductase (XOX/XDH) was purified from fresh cream using a new combination of experimental methods. The initial goal was to maximize retention of xanthine dehydrogenase (XDH) activity during this process; thus, we added 50 mM dithiothreitol (DTT) at the beginning step. Ion exchange chromatography was performed using DEAE and hydroxyapatite resins, respectively. Peak protein fractions, as determined by measuring the A_{280nm} of the eluate, were evaluated for their purity by SDS-PAGE. XDH activity was monitored using hypoxanthine and nitro blue tetrazolium (NBT). Additional assays, with xanthine and nicotinamide adenine dinucleotide (NAD+), were also made with the most highly purified samples. Xanthine oxidase (XOX) activity was detected using hypoxanthine and molecular oxygen. Over time, we noted the slow appearance of smaller molecular weight protein bands, and their numbers increased during storage at refrigeration temperatures even in the presence of a high salt concentration (0.2M). We tested these samples for protease activity using polyacrylamide gels that were incorporated with casein. These zymograms (InVitrogen, Inc.) also contained an azure dye in the background of the gel to contrast the cleared zone produced by hydrolysis of casein. We have identified plasmin (PL) and plasminogen (PG) in most of the highly active XOX/XDH fractions using Western blotting techniques and an antibody specific for plasminogen. Plasmin enzymatic activity was measured using the substrate, tosyl-glycyl-prolyl-lysine-4-nitroaniline acetate (Chromozym PL

kit; Roche, Inc.). Three commercial preparations of XOX were also assayed for the presence of PL/PG, and each showed varying degrees of copurification as defined by the ratio of XOX to PL activity. The implications of these findings will be presented.

Key Words: Enzymes, Milk Proteins, Protease Activity

1363 Identification of catalytic amino acid residues at the active site of mouse glucosidase II. Jie Feng[1] and Inder K. Vijay*[1], [1] *University of Maryland.*

Following the action of glucosidase I to clip the terminal α1,2-linked glucose, glucosidase II sequentially cleaves the two inner α1,3-linked glucosyl residues from the oligosaccharide, Glcα1,2Glcα1,3Glcα1,3Man9GlcNAc2, of the incipient glycoprotein during its biosynthesis. A model has been proposed in which the action of glucosidase II, in conjunction with calnexin and calreticulin, has been shown to facilitate the proper folding of N-linked glycoproteins in the secretory pathway. The enzyme is a heterodimeric protein in which α subunit has the catalytic activity, while β subunit provides the ER localization signal. Sequence alignments and analysis showed that the enzyme belongs to the glycoside hydrolase Family 31 with a putative active site that contains the DMNE motif. To obtain experimental evidence in support of this hypothesis, mouse glucosidase II gene was used to express the enzyme as a histidine-tagged fusion protein in Sf9 insect cells. The enzyme was expressed by co-infecting the cells with recombinant baculoviruses containing the genes of α and β subunits, and partially purified by Ni+-agarose affinity chromatography. The enzyme expressed as fusion protein is catalytically active. It is a high mannose glycoprotein as it bound to concanavalin A-Sepharose beads, and its electrophoretic mobility was altered by treatment with endoglycosidase H. A total of nine mutations were carried out in the DMNE motif. The D564N, E567Q and scramble mutants completely lost the enzyme activity, while the other mutants, D564E, E567D, M565N566/AA, D-E-exchange, MN/AA-D-E-exchange, and F571A retained over 70% of the enzyme activity. The results indicate that the D564MNE567 motif is within the active site of the enzyme and Asp564 and Glu567 represent the acid-base catalyst. This work was supported by N.I.H. grant GM59943.

Key Words: glucosidase II, active site

1364 Stimulation of the functional expression of glucosidase I by calnexin and identification of catalytic Amino acids at its active site. Xaio-Lian Zhang[1] and Inder K. Vijay*[1], [1] *University of Maryland.*

The cDNA of rat glucosidase I was incorporated in-frame into pcDNA3.1A (-) MycHis and pBlueBacHis2A and expressed in COS7 and Sf9 cells, respectively. The fusion protein of the enzyme was expressed as a doublet of 93 and 95 kDa, which merged into a single 93 kDa polypeptide after deglycosylation with N-glycanase. The expressed fusion proteins had low catalytic activity. It was speculated that incomplete folding may be the cause. Co-expression of several molecular chaperones was investigated to enhance folding of the over-expressed enzyme. Among the chaperones examined, calnexin gave the best results. A physical association between calnexin and glucosidase I was shown by co-immunoprecipitation of both the enzyme and calnexin from cell lysates. Mutational analysis of amino acid residues within the previously-proposed substrate-binding, #glucosyl motif#, E592RHLD596LRCW, showed that aspartate and glutamate within this motif serve as the acid-base pair for the catalytic activity of the enzyme. Importantly, a double switch mutation, in which the positions of aspartate and glutamate within the motif are exchanged, retains full enzyme activity. Thus, the active site is flexible and engages in a dynamic interaction with the substrate. Glucosidase I cloned in higher eukaryotes also shows a highly conserved N-glycosylation sequon, NHT in close vicinity to the glucosyl motif. Mutation of asparagine to glutamine in this sequon of rat cDNA of the enzyme gave expression to non-glycosylated protein in COS7 cells. The non-glycosylated mutant enzyme still retained >90% of the activity of the WT enzyme, consistent with our earlier observation on the enzyme purified from the rat mammary gland. Calnexin has been reported to be a lectin-chaperone and shown to interact primarily with the glucose-containing oligosaccharide of nascent glycoproteins. Significantly, it was shown to associate with the non-glycosylated form of the enzyme, albeit weakly. (Supported by N.I.H. grant GM59943 and CGP of MAES).

Key Words: glucosidase I, active site

1365 Photoaffinity labeling of the active site of glucosidase I. A.V. Romaniouk[1], A. Silva[1], and I.K. Vijay*[1], [1] *University of Maryland.*

N-glycosylation represents one of the most common protein modifications of eukaryotic cells. Regulation of glycosylation is critical for normal physiology and for many pathological conditions. Glucosidase I (Glc I) clips the terminal α1,2-linked glucose of Glc3Man9GlcNAc2-polypeptide and triggers the post-translational folding and maturation of the incipient N-linked glycoprotein. It is, therefore, critically positioned to control the assembly of this important class of glycoproteins. Our laboratory has been investigating the biosynthesis and regulation of mammary glycoproteins for a number of years. Recently, we identified and proposed ERHLDLRCW as the substrate binding motif in the active site of mammalian glucosidase I. To probe the active site of the enzyme, we synthesized several novel photoactive reagents of 1-deoxynojirimycin (DNM); N-alanyl DNM, N-glycyl-DNM, N-carboxyethyl-DNM and the photoactive compound (4-[p-azidosalicylamido]butylamido-pentyl-1-DNM (ASBA-P-DNM). All DNM compounds were analyzed as potential inhibitors of Glc I, and ASBA-P-DNM was shown to be the most effective inhibitor of the enzyme. [I125]ASBA-P-DNM was used to photo label the purified Glc I enzyme from the bovine mammary gland. Mass spectroscopic analysis of the specifically tagged peptide of Glc I along with the information from the cDNA sequence and the substrate-binding motif will be used to map the architecture of the enzyme. (Supported by NIH grant GM59943)

Key Words: glucosidase I, photoaffinity labeling, active site

1366 Molecular models for bovine α_{s2}-casein. P.D. Hoagland* and H.M. Farrell, Jr., *USDA ERRC, Wyndmoor PA.*

To enable future molecular modeling of the casein sub-micelle, models for α_{s2}-casein were constructed to complement existing models for α_{s1}-, β-, and κ-caseins. Predicted helical structure, based on theoretical and experimental evidence, was used to organize the secondary structure of the seed model into five domains. Each domain was modeled using standard minimization and dynamics methods to first identify possible local interactions. The entire protein was then modeled using a negatively charged N-terminal domain [f1-f75], a spacer helix [f76-f90], a neutral hydrophobic domain [f91-f128], a polar/ionic [f129-f143] domain, a long spacer helix [f144-f176], and a positively charged C-terminal domain [f177-f207]. The models favored electrostatic interactions between the positive C-terminal domain with the negative N-terminal domain. These interactions arose from phosphoserine-arginine/lysine ionic pairs. They contributed greatly to the large negative minimization energy. The central hydrophobic domain [f91-f128] was directed to the exterior by its neighboring short spacer helix. An internal disulfide bond between Cys36 and Cys40 did not significantly alter the secondary structure of the N-terminal domain. This bond was located in an exterior loop that formed as a result of neighboring phosphoserine cluster, [f56-f58, f61], interactions with the local Arg45 and distant Arg160, Arg170 and Arg207. In general, ionic interactions observed in models built *in vacuo* persisted after solvation with water. The organization of these domains can be expected to contribute to the observed functional properties of α_{s2}-casein. Through ionic interactions, it can self-associate or form tight complexes with the other casein proteins. In the presence of Ca++ α_{s2}-casein can help assemble and stabilize casein sub-micelles through its 2 clusters of phosphoserine in the N-terminal domain.

Key Words: α_{s2}-casein, molecular model, phosphoserine

1367 Effect of sugars on milk protein gels and stabilized-milk protein emulsion gels. L Matia* and E Dickinson, *University of Leeds, Leeds, U.K.*

The effect of sugars in dairy products and their aggregation processes needs further investigation to improve the understanding on interrelations among water, proteins, lipids and sugars used in so many formulations. The influence on rheological and microstructural properties of sucrose (0-66% w/v)or a mixture of sucrose + glucose (76% w/v) in the aqueous phase of oil-in-water emulsions and emulsion gels based on sodium caseinate (1.4% w/v) at *n*-tetradecane (30% v/v) has been investigated. The influence of sucrose (0-70% w/v) on pure caseinate gels (3%

w/v) has also been studied. The development of a three-dimensional network, following slow acidification on addition of glucono-δ-lactone granules (0.3 g GDL / g protein), was followed through the resulting increase in the small-deformation shear moduli ($G'$and $G"$)at 25.0°C. Sugar concentrations greater than 60% w/v led to larger emulsion droplets. Added sugar was found to increase substantially the elastic modulus and to reduce gelation times in all cases for emulsion and protein gels, especially at a high sugar /protein ratio. Sugar was also found to affect the large deformation rheology, promoting strain-weakening and a shorter linear regime. Confocal scanning laser microscopy has been used to detect the emulsion droplet network (dyed with Nile Red) or the caseinate network (dyed with Rhodamine B). High sucrose concentration seemed to affect the microstructure of emulsion and protein gels, leading to a more uniform network compared to sugar-free systems. In general, the pore size distribution of caseinate gels decreased with increasing sucrose concentration and different correlations from image analysis have been obtained.

Key Words: Sugar, Acid-induced gelation, Caseinate gels

1368 Impact of genetic variants of β-lactoglobulin on their binding capacity to peptide β-LG 102-105. I. Noiseux*, S.L. Turgeon, and S.F. Gauthier, *Centre STELA, Universite Laval.*

In previous work, it was demonstrated that some peptides can bind to β-lactoglobulin (β-LG) under specific physicochemical conditions, and that material absorbing at 214 nm was released from the protein following its interaction with hydrophobic peptides β-LG 102-105 and α-$_{S1}$-CN 23-34. The aim of the present study was to evaluate the binding capacity of β-LG AB, A and B for the peptide β-LG 102-105, an hydrophobic peptide of 554.7 Da. This peptide (1000 μM) was combined with three concentrations (0, 50 and 100 μM) of β-LG A, β-LG B and two different mix of β-LG AB, using different physicochemical conditions: pH 6.8 and 8.0, temperature of 25 and 40°C, and buffer molarity of 0.05 and 0.10 M. After overnight contact between β-LG and peptide, solutions were filtered (Microcon, MWCO 10 KDa) and the amount of unbound peptide was quantified by RP-HPLC (column C-18). Results indicated that β-LG variant A bound 16 to 92% of the peptide β-LG 102-105 depending of physicochemical conditions under study, whereas the binding capacity of β-LG B and β-LG AB for this peptide varied from 0 to 48%. All the β-LG variants leaded to the release of material absorbing at 214 nm following their interaction to the peptide, but material released from β-LG A was different than those observed for variant B or for the mix AB. The higher binding capacity of β-LG A compared to β-LG B could be explained by the different conformation of the EF loop adopted by these variants at neutral pH.

Key Words: β-Lactoglobulin, Variant, Peptide

1369 Study on the molecular mass changes of bovine k-casein glycomacropeptide and on its separation in ultrafiltration with and without application of electric fields. M.-J. Michel*[1], Y. Pouliot[1], M. Britten[2], I. Noel[1], and R. Lebrun[3], [1]*Dairy Research Center STELA, Universite Laval,* [2]*Food Research and Development Center, FRDC,* [3]*Laboratory of engineering of membrane process, UQTR.*

Glycomacropeptide (GMP) constitutes a potential ingredient for the development of functional foods or nutraceuticals. The use of membrane separations for the purification of GMP at large scale is however limited by the heterogeneity of its molecular mass. GMP has a molecular mass of 7 kDa but polymeric forms of GMP can have mass greater than 40 kDa. Variations of the molecular weight of GMP must be limited in order to facilitate its passage on the side permeate during the ultrafiltration of whey. The objective of our work was to identify the physicochemical conditions limiting polymerization. GMP was prepared from the hydrolysis of sodium caseinate by chymosin (60 min, 35°C, pH 6.6). The enzymatic reaction was stopped by an adjustment at pH 4,6 and a heating (1 min, 80°C). The peptide was filtered, dialyzed (MWCO 3,5 kDa) and freeze-dried. An isoelectric point of 4.18 was determined experimentally. The molecular association behavior of GMP was studied as a function of its pH (3.5 to 7.5), ionic strength (0.02 to 0.14), temperature (5, 25 and 50°C) and peptide concentration (0.15 to 2.00% w/v). Size exclusion chromatography (HPSEC) showed that the size of the GMP was minimal at pH lower than 4.0, at 50°C and at ionic strength of 0.14. Various fractions separated by HPSEC were analyzed in reversed phase chromatography (RP-HPLC). No evidence was found that the associative behavior of GMP would be related to its level of glycosylation. Model solutions of 0.1% (w/v) GMP at pH 3.0 and 4.6 were ultrafiltered using 10 or 30 kDa UF-membranes. Superimposing an electrical field (20 or 40 V/m, anode on the permeate side) at pH 3.0 and 4.6 had limited impact on the transmission of GMP but improved UF-flux.

Key Words: glycomacropeptide, ultrafiltration, electric fields

1370 Characterization of heat-induced whey protein-anionic surfactant complexes. H.J. Giroux* and M. Britten, *FRDC, Agriculture and Agri-Food Canada, St-Hyacinthe, Qc, Canada.*

Low molecular weight surfactants interact with proteins and modify their structure, thermal stability and functional properties. Three anionic surfactants (sodium dodecyl sulfate (SDS), sodium stearoyl-2 lactylate (SSL) and diacetyl tartaric acid ester of monoglyceride (DATEM)) were mixed with whey protein dispersions at concentrations ranging from 0 to 640 μmol/g protein. Thermal behavior was studied by differential scanning calorimetry at pH 7.5. Protein surfactant mixtures were heated at 75°C for 30 min (pH 7.5) and the resulting complexes were analyzed. Zeta potential was determined by microelectrophoresis. Surface tension was measured using du Nouy ring method. Protein solubility profile was established between pH 2 and 6. Denaturation temperature of whey protein increased with anionic surfactant concentration and reached a plateau value at concentrations larger than 80 μmol/g protein. Maximum denaturation temperatures for protein dispersions mixed with SDS, SSL and DATEM were respectively 85.0±0.3, 81.7±0.7 and 77.4±0.6°C. Zeta potential of heat-induced complexes decreased with increasing surfactant concentration and leveled off at concentrations larger than 320 μmol/g protein. The surface tension of mixed dispersions, was measured as a function of surfactant concentration. The profiles before heating showed a transition associated with the unfolding of the protein structure. After heat treatment, this transition disappeared except for low protein concentration. Heat induced complexes showed low solubility between pH 4.8 and 5.4. The pH where minimal solubility occurred shifted to lower values when the concentration of surfactant increased. This shift resulted from the effect of negatively charged surfactants on the isoelectric point of complexes. The high pH portion of the solubility profile suggests that DATEM, unlike SDS or SSL, does not adsorb extensively to whey proteins. Our results confirm that hydrophobic interactions is the main factor responsible for the formation of heat-induced complex between whey protein and anionic surfactants.

Key Words: Whey protein, Surfactants, Heat

1371 Characterization of bovine lactoferrin isolates by cation-exchange chromatography. G. Brisson*[1], M. Britten[2], and Y. Pouliot[1], [1]*Dairy Research Centre (STELA), Laval University, Quebec (Quebec), Canada,* [2]*Food Research and Development Centre (FRDC), St-Hyacinthe (Quebec), Canada.*

Lactoferrin isolates (>85% of total proteins) are sold as bioactive components for use as nutraceuticals. Lactoferrin is an iron-binding glycoprotein that can be purified from bovine milk or from cheese whey. Two isomeric forms of lactoferrin have been reported in bovine milk, revealing differences in one glycosylation site. The objective of the present work was to investigate the performance of a cation-exchange chromatography technique using a S-HyperD column for the characterization of the lactoferrin profile in bovine commercial lactoferrin isolates. The lactoferrin elution profile was obtained using a 50 mM phosphate buffer (pH 7.0) with a sodium chloride (1.0 M) sequential step gradient. Three commercial bovine lactoferrin isolates were studied and each was fully separated into three main peaks. Peak fractions were analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and reverse phase chromatography (RP-HPLC) and all three peaks were identified as lactoferrin. The first two peaks are associated to the two different glycosylated isomeric forms of lactoferrin in milk, as assessed by SDS-PAGE. The third peak detected is corresponding to another lactoferrin molecular form. The nature of this form needs to be further investigated but we suggest that is corresponding to differences in lactoferrin glycosylation level, possibly related to the raw material used for the purification. The experimental conditions used in our study have allowed the detection of minor fractions of lactoferrin isomeric forms.

Key Words: Lactoferrin, Cation-exchange chromatography