728 Effect of milk processing on the MFGM proteins and phospholipids. X. ElÍas-Argote* and R. Jiménez-Flores, *California Polytechnic State University, San Luis Obispo.*

MFGM phospholipids (PLs) and proteins have been ascribed antimicrobial and antiviral properties, as well as anticancer and antihypercholesterolemic activities. However, few studies have highlighted the effects of processing on the MFGM constituents and the repercussion it may have on their functionality. In this study, we have applied analytical and proteomic techniques to analyze the protein and lipid profile in the MFGM throughout milk processing. Milk was collected/treated at 6 different points: before reaching the storage unit (35°C), storage temperature (4°C), batch, high temperature short time (HTST), and ultra high temperature (UHT) pasteurizations, and by pulsed light treatment. MFGM proteins were analyzed using 1D and 2D-PAGE and LC-MS. Over 117 proteins were identified using HPLC-MS, and their relative quantities at different motilities in the SDS gel were analyzed using Scaffold 3 and Delta2D_4. As the heat treatment increased, more protein aggregates were observed, especially in the UHT milk, where caseins, lactoferrin, and guanine nucleotide-binding proteins were detected in the high molecular weight region (>150 kDa). Also, low heat at a prolonged time (63°C, 30min) resulted in more MFGM proteins being released into the skim milk. In addition, the protein profile for the light pulse treatment was similar to the 4°C and HTST samples. In contrast, variation in the phospholipid composition of the membrane throughout milk processing was not statistically significant ($P > 0.05$). From this study, the best method to preserve milk bioactivity might be HTST, which reduces pathogenic microorganisms and still preserves most of the MFGM proteins. In regard to functionality, formation of aggregates might reduce the full potential of a nutraceutical MFGM protein, thus more studies are needed to create processing methods that retain the proteins' native state, including the consideration of pulsed light treatment as a new pasteurization method.

Key words: MFGM, proteomics, phospholipids

729 Focus on milk fat globule membrane proteins from goat milk. C. Cebo*[1], C. Henry[2], S. Truchet[3], F. Bouvier[4], H. Caillat[5], and P. Martin[1], *[1]INRA, UMR1313 Unité Génétique Animale et Biologie Intégrative, Jouy-en-Josas, France, [2]INRA, Plateforme PAPSSO (Plateforme d'Analyse Protéomique Paris Sud Ouest), F-Jouy-en-Josas, France, [3]INRA, Unité Génomique et Physiologie de la Lactation, Jouy-en-Josas, France, [4]UE332 Domaine de Bourges, Osmoy, France, [5]INRA, UR631 Station d'Amélioration Génétique des Animaux, Castanet-Tolosan, France.*

Fat is present in milk as droplets of triglycerides surrounded by a complex membrane deriving from the mammary epithelial cell and called the Milk Fat Globule Membrane (MFGM). In-depth proteomic studies have been published for bovine MFGM proteins. However, to date, only sparse studies exist on MFGM proteins from non-cow milks. The objective of this study was thus to investigate the protein composition of the goat Milk Fat Globule Membrane. MFGM proteins from goat milk were separated by 6% and 10% SDS-PAGE and Coomassie or Periodic Acid / Schiff (PAS) stained. Most of MFGM proteins (mucin-1, fatty acid synthase, xanthine oxidase, butyrophilin, lactadherin and adipophilin) already described in cow milk were identified in goat milk using peptide mass fingerprinting. A prominent difference between the cow and the goat species was demonstrated for lactadherin. Indeed, we have shown that lactadherin from goat milk appears as a single polypeptide chain in 6% SDS PAGE whereas 2 polypeptide chains are easily identified in cattle. In addition, goat MFGM proteins were subjected to 1D-LC-MS/MS (one dimensional gel coupled to tandem mass spectrometry) analysis. Twenty-five µg MFGM proteins were separated in 10% SDS-PAGE and Coomassie stained. The lane was divided in 20 slices and each slice was digested by trypsin and subjected to LC/MS/MS analysis. This approach led us to identify - with at least 2 unique peptides (Bos Taurus Database)- more than 160 proteins associated with the goat Milk Fat Globule Membrane. Interestingly, integrative analysis of MFGM-associated proteins using DAVID Bioinformatics Resources demonstrated that identified biological processes are not only connected with lipid metabolic processes or exocytosis-related biological processes, but also with G-protein receptor signaling pathway, translation, or regulation of apoptosis, as previously demonstrated for MFGM proteins from bovine milk. These findings may help in the understanding of lipid droplet formation in the goat species, where an apocrine mechanism for milk secretion is hypothesized.

Key words: milk fat globule membrane, goat, lactadherin

730 Identification of major milk fat globule membrane proteins from pony mare′s milk highlights the molecular diversity of lactadherin across species. C. Cebo*[1], E. Rebours[1], C. Henry[2], S. Makhzami[1], P. Cosette[3], and P. Martin[1], *[1]UMR1313 Unité Génétique Animale et Biologie Intégrative, Jouy-en-Josas, France, [2]INRA, Plateforme PAPSSO (Plateforme d'Analyse Protéomique Paris Sud Ouest), Jouy-en-Josas, France, [3]UMR6270 CNRS, Université de Rouen, Plateforme Protéomique de l'IFRMP23, Mont-Saint-Aignan Cedex, France.*

Although numerous studies have been devoted to the soluble fraction, namely caseins and whey proteins, to date, little is known about the milk fat globule membrane (MFGM) fraction from mare′s milk. Using mass spectrometry, most of MFGM already described in cow or goat milks were identified in mare′s milk. Prominent differences through species were highlighted for lactadherin. Indeed, whereas one or 2 polypeptide chains are respectively identified by peptide mass fingerprinting matrix-assisted laser desorption/ionization-time of flight (PMF MALDI-TOF) analysis for caprine and bovine lactadherin, lactadherin from mare′s milk appears as 3 polypeptide chains in 6% SDS PAGE. Digestion of MFGM proteins from mare′s milk with Peptide N-glycosidase F (PNGase F) revealed that the existence of 3 distinct polypeptide chains for equine lactadherin could not be solely explained by differential N-glycosylation of a single polypeptide chain. On the other hand, polymerase chain reaction (PCR) experiments on lactadherin transcripts isolated from milk fat globules revealed that splicing events occur on lactadherin from mammary gland with the existence of 2 distinct lactadherin transcripts in the horse species. Cloning and sequencing of both lactadherin transcripts revealed the existence of a cryptic splicing site usage located at the end of exon 5 of equine lactadherin and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) analyses confirmed the existence of 2 lactadherin variants in the MFGM from mare′s milk. Interestingly, this cryptic splicing event led to the suppression of a putative N-glycosylation site in the protein. Whatever, we demonstrate here that expression of lactadherin is species-dependent, therefore questioning about of the precise function of these different isoforms in mammary gland biology across species.

Key words: milk fat globule membrane, lactadherin, cryptic splicing site

731 Effect of methane emission reducing diet on coagulation properties of bovine milk. A. Aprianita*[1], O. N. Donkor[1], P. J. Moate[2], M. J. Auldist[2], J. S. Greenwood[2], W. J. Wales[2], and T. Vasiljevic[1], *[1]School of Biomedical and Health Sciences, Faculty of Health, Engineering and Science, Victoria University, Melbourne, Victoria, Australia, [2]Department of Primary Industries, Ellinbank, Victoria, Australia.*

The effects of methane emission reducing diets on coagulation properties of bovine milk were investigated. The treatment diets included supplementation with fat, tannin or combination of fat and tannin to a normal diet which also served as a control. The obtained milk samples were skimmed, standardized (C/*F* = 0.7), homogenized (25 MPa), and heat treated (60°C; 30 min). Subsequently, glucono-delta-lactone (GDL) (2.2%) or commercially available rennet (0.2 mL/L) was added to induce gel formation. For rennet-gel, calcium chloride (0.02%) was added before rennet addition. Both types of gel were analyzed for rheological parameters (small amplitude oscillatory and large deformation), syneresis, permeability, and microstructural characteristics. This study indicated that fat or tannin supplementation could improve gelatinization characteristics of acid milk gel by increasing storage modulus (G'), gel hardness and reducing time of gelatinization. Addition of tannin enhanced the elastic property of gel greater in comparison to that of fat; while combination of fat-tannin did not alter G' value. Supplementation of fat, tannin, or combination of fat and tannin slightly increased syneresis of acid milk gel. This was confirmed by shift angle and permeability values. The presence of fat during rennet induced coagulation had a substantial impact on the properties of the gel. Addition of fat alone or in combination with tannin increased G' and reduced gelation time. In contrast, tannin supplementation impaired gelatinization by reducing G' and increasing gelation time. All types of diet also slightly increased syneresis of milk gel, with tannin giving the highest impact. This study showed that milk obtained from cows fed a methane emission reducing diet had altered coagulation properties that were apparently dependent on the supplement.

Key words: methane emission reducing diet, milk properties, coagulation

732 Development of a method to determine the susceptibility of raw milk to oxidation. J. K. Amamcharla* and L. E. Metzger, *Midwest Dairy Foods Research Center, Dairy Science Department, South Dakota State University, Brookings.*

The initial quality of raw milk plays a critical role in the consumer acceptability of pasteurized milk. In recent years especially in the Midwest region, numerous cases have been reported where pasteurized milk is susceptible to spontaneous oxidation. The objective of the present work was to investigate the applicability of Ferric Reducing Antioxidant Power (FRAP) assay for identification of raw milk that are susceptible to oxidation. In this study, the FRAP assay was modified for analysis of raw milk. A FRAP working reagent consisting of 300 mmol/L of acetate buffer (pH 3.6), 20 mmol/L of ferric chloride, and 10 mmol/L of 2, 4, 6-tripyridyl-s-triazine made up in 40 mmol/L of hydrochloric acid was used. All 3 solutions were mixed in the ratio 10:1:1. To measure the FRAP value, 0.3 mL of milk was mixed with 4.5 mL of FRAP reagent and incubated at 37°C for 4 min. After the incubation, the sample was filtered using a 0.45µm syringe filter to remove precipitated protein. Absorbance at 593 nm was measured on the filtrate relative to the FRAP reagent as a blank. The FRAP value was calculated using ferrous sulfate calibration standards (50–600µ mol/L). Raw milk samples were collected from 6 individual cows. Each of the 6 samples was divided into 4 sub-samples. Three of the sub-samples were spiked with either 0.1 ppm of copper, 7.5 IU/quart of vitamin E, or both. The remaining fourth sub-sample served as a control. Each of the sub-samples was again divided into 3 equal parts and kept refrigerated. On each experimental day, one sub-sample was withdrawn and analyzed using the FRAP assay. The data was analyzed as repeated measures design using the GLM procedure in SAS. The treatment (presence or absence of copper or vitamin E), time and their interaction significantly ($P \ll 0.05$) influenced the FRAP value of raw milk. Moreover, the average percent reduction in FRAP value by the end of 48 h was found to be 27, 54, 28, and 47% for control, copper, vitamin E, and copper + vitamin E spiked samples, respectively. Overall the FRAP assay shows potential in the identification of milks susceptible to oxidation.

Key words: milk oxidation, ferric reducing antioxidant power (FRAP)

733 Measurement of a milk gelation time constant using laser-scanning fluorescence confocal microscopy and image processing techniques. R. Hennessy*[1] and R. Jimenez-Flores[2], *[1]Cal Poly Biomedical Engineering, San Luis Obispo, [2]Cal Poly, DPTC, San Luis Obispo.*

The gelation kinetics of milk can dictate how nutrients are absorbed after ingestion and are therefore important when determining the nutritional benefit of a dairy product. Current methods to measure gelation kinetics, such as near-infrared spectroscopy and rheology, are destructive and only provide one-dimensional data, while other methods, such as the Berridge clotting time method, are subjective because they depend on an operator's skill. A 2-dimensional, non-destructive, objective measurement technique is needed to accurately quantify the gelation kinetics of milk. The purpose of the present study was to investigate the ability of laser-scanning fluorescence confocal microscopy (LSFCM) to measure gelation kinetics. In this study, a mixture of raw milk and chymosin was imaged using LSFCM. The milk was stained with the fluorescent markers Nile red, which stains lipids, and fast green FCF, which stains proteins. Once chymosin was added to the raw milk, images were captured every 5 seconds for 30 minutes. Because gelation causes the milk to change from a liquid to a solid, the instantaneous gelation rate could be estimated by calculating the mean difference between successive images (R). As the milk begins to gel, the movement of the lipids and proteins eventually ceases, and the mean difference between successive frames eventually reaches zero. R was plotted versus time and fit to the curve B=e exp-kT[Ch]t, where B is the initial value of R, T is the temperature of the milk at the time the images were acquired, [Ch] is the concentration of chymosin, t is the time, and k is the gelation time constant of the milk. The gelation time constant, k, was then used to characterize the gelation kinetics. Because this method is able to account for the initial rate of the gelation process, the chymosin concentration, and the temperature when calculating the gelation time constant, it shows promise as a technique to measure and compare the intrinsic gelation characteristics for different milk varieties.

Key words: milk coagulation, gelation kinetics, confocal microscopy

734 Mid-infrared predictions of lactoferrin content in bovine milk. H. Soyeurt*[1,2], C. Bastin[1], F. Colinet[1], V. Arnould[1,3], D. Berry[4],

E. Wall[5], N. Gengler[1,2], P. Dardenne[6], and S. McParland[4], *[1]University of Liège, Gembloux Agro-Bio Tech, Animal Science Unit, Gembloux, Namur, Belgium, [2]National Fund for Scientific Research, Brussels, Brussels, Belgium, [3]CONVIS Herdbuch, Ettelbruck, Luxembourg, [4]Animal and Grassland Research and Innovation Centre, Teagasc, Fermoy, Cork, Ireland, [5]Animal and Grassland Research and Innovation Centre, Teagasc, Penicuik, Midlothian, UK, [6]Agricultural Walloon Research Centre, Quality Department, Gembloux, Namur, Gembloux.*

Lactoferrin (LF) is a glycoprotein present in milk and active in the immune system of cows and humans. Therefore, an inexpensive and rapid analysis to quantify this protein is desirable. A previous study reported the potential to quantify LF from the mid-infrared (MIR) spectrometry from 69 milk samples. Through the European Robust-Milk project (www.robustmilk.eu), 3,606 milk samples were collected in Belgium, Ireland, and Scotland from individual cows and analyzed using a MIR MilkoScanFT6000 spectrometer. Milk LF content was quantified using ELISA in duplicate. Average ELISA data with a CV lower than 5% were used. After the detection of spectral and ELISA outliers, the calibration set contained 2,499 samples. An equation to predict LF content from MIR was developed using partial least squared regression. A first derivative pre-treatment of spectra was used to correct the baseline drift. To improve the repeatability of the spectral data, a file which contained the spectra of samples analyzed on 5 spectrometers was used during the calibration. The lactoferrin mean was 159.28 mg/l of milk with a SD of 97.21 mg/l of milk. The calibration (C) coefficient of determination (R^2) was equal to 0.73 with a standard error (SE) of calibration of 50.54 mg/l of milk. A cross-validation (CV) was used to assess the robustness of the equation. R^2 CV was 0.72 with a SE-CV of 51.16 mg/l of milk. An external validation (V) was conducted on 150 milk samples collected in Belgium. The SE of prediction (SEP) was 59.17 mg/L of milk. The similarity between R^2 C and R^2CV as well as between SE-C and SE-CV and between SE-CV and SEP confirms the equations developed are robust. The correlation between predicted and measured LF values was 0.71. This lower value compared with the one obtained from the calibration set (0.85) could be explained by the low ELISA reproducibility (16.24% ± 25.51%). If the developed equation is used to clean the validation data set, a total of 16 samples can be deleted. The validation coefficient for these 134 samples increased to 0.82. From these results, the developed equation could be used for screening the dairy cow population for breeding purposes.

Key words: milk, lactoferrin, infrared

735 First assessment of diffusion coefficients in model cheese by fluorescence recovery after photobleaching (FRAP) analysis. J. Floury*[1,2], M. N. Madec[2], M. H. F. Famelart[2], S. Jeanson[2], and S. Lortal[2], *[1]Agrocampus Ouest, UMR1253, Rennes, France, [2]INRA, UMR1253, Rennes, France.*

In cheese technology, mass transfer of solutes like salt, moisture and metabolites, is very important for the final quality of cheese, through the control of the brining and ripening processes. Numerous studies have reported salt and water transfer in cheese, but very few have dealt with the mass transfer properties of other solutes in cheese. Most of the reported diffusion coefficients have been obtained by macroscopic and destructive methods. The objective of the study was to develop, for the first time, the FRAP technique that allows in situ measurements of diffusion properties at the microscopic scale inside cheese. The effect of the matrix microstructure on mass transfer properties of small solutes was also studied. A model matrix based on ultrafiltrated milk, mimicking soft-type cheese, was used. Its structure was modified by adding gelatine and analyzed by confocal microscopy and rheological measurements. Two different sizes of FITC-dextran molecules (4 and 20 kDa) were chosen as models of small migrant solutes. Diffusion coefficients were estimated with a new modeling approach which allows to take into account diffusion of the molecules during the bleach phase. The two FITC-dextrans were able to migrate in the model cheese network, but their mobility is reduced compared to water: diffusion coefficient values were equal to 68 ±9 $\mu m^2/s$ for the 4kDa and 23±3 $\mu m^2/s$ for the 20kDa dextran. The composition of the matrix has a great influence on the mass transfer properties. The diffusion coefficients of the dextrans were reduced by a factor 3 in the model cheese with gelatin. This result was explained by structural measurements: gelatin led to a more heterogeneous microstructure than the UF model cheese that increased the global length path of the migrating solutes. This study shows the power and the potentiality of the FRAP technique to study mass transfer properties of fluorescent solutes in complex food matrices such as cheeses.

Key words: mass transfer, FRAP, modeling