560 Physico-chemical characterization of casein micelles cross-linked by genipin. N. Nogueira Silva[1], A. F. de Carvalho[2], M. Piot[1], and F. Gaucheron*[1], [1]*INRA UMR STLO, Agrocampus-Ouest, Rennes, France,* [2]*University of Vicosa, Minas Gerais, Brazil.*

The creation of new structures-functionalities of casein micelles is a challenge for the dairy sector. In this context, the new physico-chemical properties of casein micelles (CM) were evaluated after their reactions with genipin (5, 10 and 25 m*M*) in buffer solution (25 m*M* HEPES and 2 m*M* $CaCl_2$, pH 7.15) at 50°C during 24 h. The reaction was spectroscopically monitored between 190 and 900 nm. The reacted products were evaluated by reversed-phase liquid chromatography (RP-LC) and electrophoresis. The reaction level was estimated by the measurement of the available lysine and arginine. The consequences of the reaction on the physico-chemical characteristics of CM were investigated by measuring their zeta potentials, size, hydrations, viscosity, and surface tension. The reaction between genipin and casein molecules was characterized by formation of blue pigmented products, presenting a maximum absorption at 600 nm. The RP-LC profiles showed that above 5 mM of genipin, individual casein molecules were not separated. Electrophoresis revealed that casein molecules formed polymers with molecular weights greater than 200 kDa. Lysine was mainly involved in cross-linking, with a minor participation of arginine. By comparing to control sample, the % reduction in the concentrations of lysine and arginine reached 95 and 12%, respectively, for 25 m*M* of genipin added. The zeta potential and hydration values of CM were gradually reduced, indicating changes in their surface properties. The size distribution did not revealed major changes regarding the diameter of the CM. Internal cross-linking was confirmed by submitting the CM to dissociation conditions. Regarding the viscosity, all the samples behaved as Newtonian fluids, nevertheless the values decreased progressively according to cross-linking intensity. Contrarily, the final values of surface tension as well as the adsorption rates at air/water interfaces increased gradually. Thanks to this new reagent, it was possible to create originally modified CM. Further researches are necessary to elucidate the modifications in their internal structure and the consequences in their functional properties.

Key Words: casein micelle, genipin, structure

561 Destabilization of UHT milk induced by different strains of *Pseudomonas fluorescens*: Role of AprX protease. F. Bagliniere[1], G. Tanguy[1], A. Mateos[2], J. Jardin[1], F. Rousseau[1], B. Robert[1], G. Humbert[2], A. Dary[2], J. L. Gaillard[3], C. Amiel[3], and F. Gaucheron*[1], [1]*INRA 1253 UMR STLO, Agrocampus-Ouest, Rennes, France,* [2]*URAFPA, University of Nancy, Nancy, France,* [3]*ERPCB, University of Caen, Caen, France.*

Destabilization of UHT milk (gelation or sedimentation) due to the proteolysis of casein micelles can be observed. In this context, the objectives of this work were to (1) Appreciate the variability of this destabilization with 9 strains of *Pseudomonas fluorescens*; and (2) Understand the physico-chemical modifications of casein micelles induced by these strains and also by AprX, an extracellular protease produced by one of these strains (*Pseudomonas fluorescens* F). Before UHT treatment, raw milk was inoculated either by different strains of *Pseudomonas fluoresecens* or by the purified AprX protease at different concentrations. Milk destabilization was then determined at macroscopic, colloidal and molecular levels. For experiments testing the strain variability in their proteolytic activities, 5 on the 9 strains were highly destabilizing. For experiments with the purified AprX enzyme, destabilizations were also observed. In all these cases, instabilities were visual (presence of sediment) and increasing as a function of time (after several days or weeks depending on the strains and the concentrations of added enzyme). The analyses of the destabilized UHT milks revealed the presence of aggregates. The zeta potentials and hydrations of casein micelles decreased. At molecular level, we noticed a significant proteolysis. The determination of one part of the released peptides, by liquid chromatography coupled to mass spectrometry, indicated that the α_{S1}-, α_{S2}-, β- and κ-caseins were hydrolyzed with a quantitative preference for β-casein. The nature of the different peptides released was similar for all destabilized UHT milks. The decrease in the stability of casein micelles will be discussed in relation with the modifications of structure and the observed proteolysis. Potential application of this research in term of detection of unstable UHT milks will be proposed knowing that this enzyme is heat-resistant and its implication in the destabilization of UHT milk during its storage is often evoked.

Key Words: casein micelles, UHT, *Pseudomonas*

562 Effect of adding chelators during skim milk powder manufacturing on the physico-chemical properties. V. Sikand*[1], P. Tong[1], S. Vink[1], A. Zeng[1], K. Sikand[1], and S. Roy[2], [1]*Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo,* [2]*Statistics Department, California Polytechnic State University, San Luis Obispo.*

Functional properties of skim milk powders (SMP) can be tailored by processing treatments before spray drying. The objective of this study was to determine the effect of chelator addition during SMP manufacture on reconstituted SMP's solubility, opacity and heat stability (HS). This study was conducted by adding 5, 15 and 25 m*M* sodium citrate dihydrate (SCD), sodium polyphosphate (SPP) and disodium EDTA (DSE) respectively to skim milk concentrate and adjusting its pH to 6.65 before spray drying. Samples were tested for solubility index (SI) and reconstituted to contain 9% total solids and tested for opacity using a colorimeter. Heat stability was determined by measuring the heat coagulation time (time for visible flocculation at 140°C). Samples were adjusted to pH 7.0 with either 0.1 *N* NaOH or 0.1 *N* HCl for HS measurements. Lower values for SI were observed for samples with 5 m*M* SPP and DSE (0.13 mL) as compared with control samples or samples with 5 m*M* SCD (0.3mL). Furthermore, lower SI values were observed with an increasing level of chelating agents regardless of chelator type. A decreased opacity (L* value) or an increase in the lightness of samples was found with increasing levels of mineral chelating salt treatment ($P < 0.001$) and may be associated with dissociation of caseins from micelles. The extent of increased lightness was dependent on concentration and type of chelator. Heat stability studies showed that SMP samples (pH 7.0) treated with 5 m*M* DSE or 5 m*M* SCD had higher HS (>30 min) than HS of 5 m*M* SPP samples (16 min) and HS of control samples (10 min) ($P < 0.001$). Samples with 15 m*M* SPP showed significantly higher HS (20 min) as compared with samples with 15 m*M* SCD or 15 m*M* DSE (<7 min). Samples showed poor HS (<3 min) regardless of chelator type at 25mM chelator usage level.

Key Words: SMP, chelator, solubility

563 Effect of succinylation of skim milk on its plasmin-induced hydrolysis. H. Bhatt*[2,1], A. Cucheval[1], C. Coker[1], H. Patel[3], A. Carr[2], and R. Bennett[2], [1]*Fonterra Research & Development Centre, Palmerston North, Manawatu, New Zealand,* [2]*Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, Manawatu, New Zealand,* [3]*Dairy Science Department, South Dakota State University, Brookings.*

One of the biggest causes of a reduction in the shelf life of dairy products, e.g., through proteolysis, gelation, and bitterness, is the presence of plasmin and its heat stability. It is therefore crucial to control the activity of plasmin in dairy products. Several approaches have been investigated in pure casein system; the present work explores the use of succinylation for the inhibition of plasmin-induced hydrolysis in a skim milk (miceller) system. Protein modification was achieved by attaching a succinate group at the ε-amino group of lysine residues, leading to the formation of succinyl-lysine. The target sites were identified using liquid chromatography-tandem mass spectrometry. The effects of different levels of succinylation on the particle size in skim milk and on the dissociation of casein from the casein micelles were determined using a Zetasizer and sodium dodecyl sulfate PAGE, respectively. The subsequent plasmin hydrolysis was monitored by quantifying the hydrolyzed product using reverse-phase high performance liquid chromatography. Although succinylation had an inhibitory effect on plasmin-induced hydrolysis, similar to that in a pure β-casein system, the trend was not linear. The non-linearity was explained by 2 competing effects. (1) Dissociation of β-casein from the casein micelle resulted in an increase in the micelle size, extensive unfolding, and expansion of the polypeptide chain on succinylation, which collectively reduced steric hindrance and made the protein more readily hydrolyzed by plasmin. (2) The formation of succinyl-lysine rendered β-casein unrecognizable to the substrate-binding pocket of plasmin, as observed for the pure β-casein system. The latter effect dominated the overall behavior and ultimately resulted in a decrease in the rate of hydrolysis with an increase in the level of succinylation. These results indicated the importance of miceller structure and it is possible to control plasmin-induced hydrolysis of milk proteins by succinylation. The present work can be useful in developing food grade approach i.e lactosylation, to control plasmin-induced hydrolysis.

Key Words: plasmin, succinylation, skim milk

564 Antioxidative activity and resilience of Cheddar and Edam whey as determined from total radical trapping potentials (TRAP). Z. Z. Haque*, D. Mukherjee, S. Mukherjee, and S. Chang, *Mississippi State University, Mississippi State.*

Both Cheddar and Edam whey (CW and EW, respectively) possess remarkable antioxidant properties, and thus can potentially be used as components of edible coating films for preserving a variety of food items from oxidative degradation. In the current study, we have investigated the antioxidative activity and persistence of EW and CW based on total radical trapping potentials (TRAP), with an objective of identifying the one with better suitability to be used for preservative purposes. We hypothesize that these 2 types of sweet whey differ considerably regarding their TRAP efficiencies based on differences in processing conditions and reported peptide content. Hydroxyl radicals generated in vitro by pyrolysis of ABAP (2,2′-Azobis[2-methylpropionamidine] dihydrochloride) and unquenched radicals detected by chemiluminescence of luminal was used to determine the TRAP of the whey samples (0.25, 0.5, 1, 2 and 3%, w/v). When generation of the hydroxyl radicals as indicated by the lack of chemiluminescence of control, a second luminescence curve was initiated by reinduction of pyrolysis of ABAP. The luminescence of the test samples at the time point of luminescence maximum of the control ($Lu_{Max}C$) was ascertained to derive of the luminescence at maximum free radical generation (Lu_{MaxFR}). This was done for both the initial (Ini) and final (fin) reactions. The difference between ${}_{Ini}Lu_{MaxFR}$ and ${}_{Fin}Lu_{MaxFR}$ gave the resilience whereas ${}_{Ini}Lu_{MaxFR}$ by itself gave the antioxidative activity of the test sample. The ${}_{Fin}Lu_{max}C$ of EW was observed at 16:30 min in the second reaction and found to be 1279.2 RLU (relative light unit), whereas those of the samples at various concentrations were 182.1, 173.8, 114.4, 86.9 and 84.8 RLUs, respectively. For CW, ${}_{Fin}Lu_{Max}C$ was recorded at 19:30 min and noted to be 1140.3 RLU, whereas ${}_{Fin}Lu_{Max}$ of the different samples were 654.4, 288.9, 235.5, 118.9 and 95.2 RLUs, respectively.The study exhibits the markedly enhanced antioxidative efficacy of EW both in terms of activity and resilience. It is also showed that both EW and CW show a dose-dependent increase in TRAP efficiency.

Key Words: chemiluminescence, free radical, antioxidant.

565 Effect of detergents on the antioxidative efficacy of sweet whey. Z. Z. Haque*, D. Mukherjee, and S. Chang, *Mississippi State University, Mississippi State.*

This study was conducted as part of an investigation to understand the molecular basis for the antioxidative efficacy of similar sweet wheys, Cheddar and Edam whey (CW and EW, respectively). The hypothesis was that the reportedly higher process induced peptide content of CW than EW would make it behave differently. Whey samples (1, w/v) in combination with various concentrations (nil, 0.01, 0.02, 0.05 and 0.1%, w/v). Non-ionic detergent Triton X-100 (TX100) was subjected to hydroxyl radicals generated in vitro by pyrolysis of 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (ABAP). Radicals, that were not quenched by the test samples, were detected by chemiluminescence of luminal. When generation of the hydroxyl radicals had ceased, as evidenced by the lack of bioluminescence of the control, a second luminescence curve was generated by reinduction of pyrolysis of another doze of ABAP. The luminescenceluminescence of the test samples at the time point of luminescence maximum of the control ($Lu_{Max}C$) was ascertained recorded to derive of the luminescence at maximum free radical generation (Lu_{MaxFR}) at . This was done for both the initial (Ini) and second final (Fin) ABAP induced reactions. The difference between ${}_{Ini}Lu_{MaxFR}$ and ${}_{Fin}Lu_{MaxFR}$ gave the resilience whereas ${}_{Ini}Lu_{MaxFR}$ by itself gave the antioxidative activity of the test samples. The IniLuMaxC in case of CW of 746.2 relative light unit (RLU) was observed at 25:30 min. The neutral detergent TX100 had a significantly detrimental effect at all concentrations of CW studied both in terms of antioxidative activity and resilience. However, in case of EW, this trend was reversed and all TX100 containing samples showed better antioxidative activity and resilience. This study shows that detergents induced change in association tendency affected antioxidative efficacy of whey differently depending on the type of whey.

Key Words: antioxidative acitivity, antioxidative resilience, Edam whey