Fractionation of a-lactalbumin (a-la) and β-lactoglobulin (β-lg), the 2 major whey proteins (WP), is quite challenging to scale-up by using eco-efficient technologies due to their similar molecular weight (14.2 and 18.36 kDa, respectively). The proposed approach was to evaluate the difference of baro-sensitivity of a-la and β-lg under high hydrostatic pressure (HHP) treatment and their capacity to form specific aggregates when combined to casein (CN), used as a ligand in this study. The objectives were to (1) evaluate the impact of pressure and time intensity on WP, (2) compare the use of 2 different type of CN: micellar (MC) and isoelectric (IC) as ligand to generate specific interactions with WP, and (3) determine optimal parameters of pressure/time combination and type of CN to improve WP fractionation. Model protein solutions composed of a-la, β-lg and CN (IC or MC) (2.5 mg/mL for each protein) were pressurized (200, 400 and 600 MPa during 100, 200 and 300 s) using an hydrostatic pressurization unit (Hiperbaric 135, Burgos, Spain). Proteins were characterized by HPLC and PAGE. Protein aggregation was studied (size and composition) using a high-performance size exclusion chromatography (HPSEC). Response surface model demonstrated that an optimal pressure/time of 600 MPa – 300s allowed to generate the highest a-la enriched fraction (85.04% and 79.79% purity with a protein recovery of 77.39% and 82.76%, respectively for IC and MC). Analysis by HPSEC showed that 2 main categories of aggregates were generated: (1) aα1-CN, aα2-CN and a large portion of β-lg mainly linked by disulfide bonds, and (2) aα1-CN, aα2-CN and a low amount of β-lg supposedly linked by hydrophobic interactions. Our experiments performed on dairy model solutions demonstrated that using HHP, an eco-efficient process, was suitable to generate specific aggregation of β-lg with CN ligand due to the difference of baro-sensitivity. Optimal pressurization parameters allowed to generate an a-la-enriched fraction with purity of 85.04%. The approach suggested that HHP could be used as pre-treatment for a new method of a-la fractionation from whey.

Key Words: high hydrostatic pressure, purification, whey protein

Using only the intact CN bands divided by the total of all protein bands (i.e., CN%TP) and 2) using the sum of CN and CNPP divided by the total of all protein bands (i.e., CN+CNPP%TP). A t-test was performed to determine if there was difference between the mean CN%TP given by Kjeldahl versus the 2 estimates made using SDS-PAGE. The Kjeldahl CN%TP (mean = 81.05%, SD = 2.35) was higher (P < 0.05) than the SDS-PAGE CN%TP (mean = 75.80%, SD = 4.37). However, no difference (P > 0.05) was detected between Kjeldahl CN%TP (mean = 81.05%, SD = 2.35) and SDS-PAGE CN+CNPP%TP (mean = 80.42%, SD = 2.99). If the goal is to achieve agreement between results of Kjeldahl and SDS PAGE to estimate CN%TP for cheese yield, then the sum of the area of intact CN bands plus CNPP bands should be expressed as percentage of all protein bands. If the goal is to determine the extent of proteolytic damage that may relate to flavor defects and changes in functionality in dairy products, then the increase in SDS PAGE CNPP%TP is probably a more sensitive metric than Kjeldahl CN%TP.

Key Words: Kjeldahl, SDS-PAGE, casein

Iron is deficient in the milk of most dairy species including cows and goats; therefore, iron fortification is desirable in milk and dairy products to increase dietary iron levels. Ferrous sulfate (FeSO4) is reportedly the most preferable form of iron salts for iron fortification as it provides high bioavailability. No report has been available for iron recovery and microstructural distribution of iron in FeSO4 fortified cheeses, especially in caprine cheeses. The purposes of this study were to determine the recovery of iron, and locate microstructural loci of iron in iron-fortified caprine milk cheeses. Three batches of 3 types of goat Cheddar cheeses were manufactured, and stored at 2 temperatures (4°C and ~18°C) for 0, 2 and 4 mo. Three cheese types were control cheese (CC) without Fe addition, and 2 types of iron fortified cheeses with regular ferrous sulfate (RFS) and large microencapsulated ferrous sulfate (LMFS) by 8.23g and 9.03g per 9 kg cheese, respectively, considering 16% Fe in FeSO4 for both types of fortifications. All cheese samples were analyzed for microstructure and Fe loci of samples by scanning electron microscopy (SEM, S-3400N II, Hitachi, Japan). SEM samples were initially fixed with 2% glutaraldehyde and 1% of osmium tetroxide (OsO4) in 0.05 M phosphate buffer for 10 min, followed by a series of acetone dehydrations with increased concentrations and times, placed in liquid CO2 for critical point drying, and then were gold coated by sputter coating. Results showed that iron contents of CC, RFS and LMFS cheeses were 0.0162, 0.822, 0.932 mg Fe/g cheese, respectively, indicating that Fe levels substantially increased iron in both fortified cheeses. Respective Fe recovery rates for LMFS and RFS cheeses were 73.5 and 71.9%. Cheese microstructures revealed that LMFS contained smaller, elongated Fe particles. The aggregated iron particles became clearly visible from proteolyzed casein networks as storage time advanced. It was concluded that SEM analysis was able to identify iron loci and its concentration in the Fe fortified caprine cheeses.

Key Words: goat cheese, iron fortification, scanning electron microscopy
In vivo digestion of a model infant formula in piglets: an Leloir pathway, highlights the involvement of this pathway for EPS and WPI. The upregulation of galactokinase, a key enzyme involved in the biosynthesis of glycogen, hexokinase, and phosphofructokinase, and protein sources (lactalbumin hydrolysate, casein hydrolysate, whey protein isolate) on the growth, EPS production and EPS gene expression of ST1275 in M17 media and in reconstituted skim milk (RSM), respectively. The EPS production by ST1275 was studied at different time intervals from 0 to 48 h. When grown in M17 supplemented with different sugars, ST1275 produced significantly high amount of EPS (630 mg/L) in sucrose (1%) supplemented M17 in 12 h at 37°C when compared with glucose and lactose supplemented M17. Interestingly, the pH was found to remain stable at 5.5 in lactose supplemented media from 12 h, when the pH dropped to 4.5 in the presence of other sugars. The lactic acid production was further validated using HPLC. In case of protein sources, EPS production was significantly increased when RSM was fortified with 0.5% whey protein isolate (826 mg/L) and casein hydrolysate (740 mg/L) in 12 h at 37°C in pH uncontrolled fermentation. The gene expression studies were also performed using q-PCR to investigate the regulatory genes involved in EPS production. It was observed that the expression of genes that resulted in amino sugar synthesis like galactokinase (GK), glutamine-fructose-6-phosphate transaminase, and UDP-glucose pyrophosphorylase have significantly increased in the presence of sucrose and WPI. The upregulation of galactokinase, a key enzyme involved in the Leloir pathway, highlights the involvement of this pathway for EPS production in ST1275.

Key Words: exopolysaccharide, gene expression, q-PCR

In vivo digestion of a model infant formula in piglets: Protein digestion pattern and physiological responses. N. R. Tari*1, M. Z. Fan2, and M. Corredig1,2. 1Department of Food Science, University of Guelph, Guelph, ON, Canada, 2Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada. Gay Lea Foods Research and Development, Guelph, ON, Canada.

The objective of this work was to better understand the effect of milk protein composition and specifically, β-casein (β-cas) on the digestion behavior of a model infant formula. Three diets with similar metabolizable energy, total protein and other essential nutrients were formulated to meet specific nutritional requirements for 3 groups of 8 piglets, as an established model for human infants. The control diet contained whey proteins (WP), while 2 others contained 40:60 casein/WP ratio, but differing by 24% in β-cas amount. To modify the β-cas ratio, microfiltration was conducted on skim milk either at 7 or 22°C. The study was carried out in 2 blocks, euthanizing the piglets at 21 d of age, after either 60 or 120 min from the last meal. Electrophoretic analysis of digestate samples after the gastric stage showed a hard aggregate, with caseins extensively hydrolysed to peptides, while β-lg and α-la were still largely intact after 60 or 120 min of digestion. The microstructure and rheological properties of the gastric digesta were also distinct between the WP control and the 2 cas/WP model formulas. A wide pH range between 4.4 to 5.8 was measured for the piglets’ gastric contents, with no significant difference observed between diets nor digestion times. Piglets fed the formula containing caseins resulted in a higher (P < 0.05) average daily gain and daily food intake compared with the WP control formula. Food conversion efficiency was not significantly different among the 3 formulas. Piglets fed the higher β-casein formula showed a significantly lower secretion of IL-10, but higher section of IL-6 and TNF-a inflammatory cytokines in the proximal jejunum, compared with other diets. It was concluded that milk caseins, specifically β-cas, not only affect the physical properties of the gastric digesta but may also contribute to health performance and regulation of physiological responses of neonates. The research demonstrated the potential to design a new generation of infant formula based on microfiltered milk concentrates.

Key Words: milk proteins, in vivo digestion, infant formula