MILK PROTEIN AND ENZYMES

1278 (T167) Separation and quantification of major milk proteins in different species by reversed phase high performance liquid chromatography. L. Ma, D. P. Bu*, J. Q. Wang, and J. T. Chen, *State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

To detect milk proteins, reversed phase high performance liquid chromatography (RP-HPLC) (Waters 2695 Series chromatograph, USA) was successfully performed to separate and quantify the major proteins from cow (n = 20), goat (n = 20), buffalo (n = 20) and yak (n = 20) milk which were collected around the fourth month of lactation for each species from February to April 2013 within 30 min. Descriptive statistics and Duncan's multiple comparison of protein contents were obtained by SAS 9.1 via GLM model (SAS Institute Inc., Cary, NC). Main protein data sets were analyzed by PCA using the Unscrambler 9.8 (CAMO software AS, Oslo, Norway). Results showed that the contents (g/L) of milk total protein were significantly different in milk from yak (62.87), buffalo (56.09), goat (42.83), and cow (37.35), respectively. Average content of major protein fractions obtained were all converted by purity of individual protein standard (Sigma-Aldrich, St. Louis, MO) to assess the real individual protein content. Composition (g/L) of yak milk contained the highest κ-CN content (9.80), and this was significantly higher than in buffalo, cow, and goat milk (7.35, 6.24 and 5.56). Content (g/L) of α-CN in yak milk (16.14) was significantly higher than those for buffalo, cow, and goat milk (11.53, 11.81, and 9.48). The β-CN content (g/L) was similar in yak and buffalo milk (23.34, 22.68), but higher than in goat and cow milk (20.53, 13.31). The β-LgB quantification (g/L) in goat and buffalo milk was similar (3.69, 3.88), significantly lower than in yak milk (9.46), but higher than in cow milk (1.19). The α-La content (g/L) was highest in buffalo milk (7.96), while significantly lower in cow, goat, and yak milk (1.45, 1.28 and 1.47). Cow milk contained highest content (g/L) of β-LgA (2.15), and this was significantly higher than that in buffalo, yak, and goat milk (0.13, 0.12 and 0.14). And different chromatographic profiles were obtained for them. The data of milk protein can be differentiated according to animal species by principal component analysis (PCA). It was concluded that major milk proteins from different animals had special profiles.

Key Words: milk protein, reversed phase high performance liquid chromatography, species

1279 (T168) Size distribution of casein micelles in milk from dairy cows with different crossbreding levels of Holstein-Zebu cattle. D. R. Freitas[1], M. M. Santoro[1], F. N. Souza[1], C. V. Ladeira[1], M. O. Leite[2], C. F. A. M. Penna[2], S. A. Diniz[1], M. X. Silva[1], J. P. Haddad[1], L. M. Fonseca[1], and M. P. Cerqueira[*2], *[1]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [2]Universidade Federal de Minas Gerais (School of Veterinary Medicine), Belo Horizonte, Brazil.*

Over the years, there has been an increasing interest in milk casein, because of their industrial importance. Casein micelle structure is crucial for gel formation in cheese and yogurt, and it is correlated with milk stability after of heating, freezing or drying. The present study evaluated the casein micelles mean size distribution in raw milk from crossbred Holstein-Zebu dairy cows, as follows: 1/2 Holstein-Zebu (n = 41); 9/16 Holstein-Zebu (n = 21); 5/8 Holstein-Zebu (n = 31); 3/4 Holstein-Zebu (n = 29); 7/8 Holstein-Zebu (n = 51); and 15/16 Holstein-Zebu (n = 27). The milk composition (protein, lactose, fat, nonfat and total solid contents), somatic cell count score, age, d in milk, and milk production were also recorded. Size of casein micelles was determined by Photon Correlation Spectroscopy (PCS) after milk fat removal by centrifugation. The mean size of the casein micelles was 170.22 + 21.18 nm (121.8– 235.6 nm). The logistic regression analysis was adjusted ($P < 0.20$) for days in milk, crossbreeding levels of Holstein-Zebu cattle, age, milk production, somatic cell count score, urea levels, fat, protein, lactose, and nonfat milk solids. The final model of the logistic regression analysis ($P < 0.05$) showed that the mean size of the casein micelles was associated with the crossbreeding levels of Holstein-Zebu cattle (odds ratio: 0.27; $P = 0.001$), somatic cell count score (odds ratio: -1.40; $P = 0.001$) and nonfat contents (odds ratio: −6.55; $P = 0.033$). To the best of our knowledge, this is the first study that access the effect of the crossbreeding levels of Holstein-Zebu on the mean diameter of casein micelles. This fact has important implications to production of milk-based products, and thus economic output of the dairy industry.

Key Words: casein, milk quality, raw milk

1280 (T169) Comparative analysis of immunoglobulin and lactoferrin in bovine milk from different species. J. T. Chen[1,2], L. Ma[1], J. Q. Wang[1], Y. X. Yang[1], and D. P. Bu[*1], *[1]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]College of Animal Science and Technology, Gansu Agricultural University, Lanzhou, China.*

Immune active components in milk play an important role in the host defence. Bovine milk from different speices has its own character. The objective of the current study was to char-

acterize the concentrations of immunoglobulins (IgA, IgG, IgM) and lactoferrin in bovine milk samples of different species. Sandwich enzyme-linked immunosorbent assay (ELISA) was performed according to the manufacturers instructions to quantify the immunoglobulins and lactoferrin (Lf) contents in yak milk (n = 20), buffalo milk (n = 20) and Holstein cow milk (n = 20) samples, and correlation analysis was completed. The results showed that immunoglobulins and lactoferrin in yak milk, buffalo milk, and Holstein cow milk samples had linear relationsship between concentration and absorbance values, and the standard curve equation R was greater than 0.99. There were significant differences of IgA, IgG, IgM and Lf in different milk samples ($P < 0.05$). The contents of IgA, IgG, IgM and Lf in different species bovine milk samples showed remarkable individual differences. IgA and IgG concentrations in yak milk were significant higher than in the other two kinds of milk. While IgM and Lf in Holstein cow milk were higher than others. In addtion to negative correlation between IgA and IgM existed only in Holstein cow milk samples, the trends of IgA, IgG, IgM and Lf in the same kinds of bovine milk all showed a positive correlation. The results indicated that the content of immunoglobulins and lactoferrin in different species milk samples were influenced by genetic factors and had distinctive characteristics. According to the correlation of immunoglobulins and lactoferrin, forecasting the content of immunoglobulins and lactoferrin within species may be possible.

Key Words: bovine milk, immunoglobulin, lactoferrin

1281 (T170) Effect of thermal conditions on the concentration of biological active whey protein in cow milk.

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Biological active whey protein in cow milk play an important role in the health of human, which is sensitive to thermal conditions during dairy processing. The objective of the present study was to investigate the variation of mainly immune active proteins concentration in cow milk, which was heated by reference commercial pasteurized milk processing technical specification of China dairy industry standards. The contents of the immunoglobulins (IgG, IgM, IgA), lactoferrin (Lf) and bovine serum albumin (BSA) were determined after different thermal processing by Sandwich enzyme-linked immunosorbent assay (ELISA), respectively. The results showed that pre-heat treatment and homogenizing had relatively mild effects on immune active protein concentration. From 75 to 115°C with holding times for 15 sec, immune active proteins contents were decreased significantly. The concentration of immune active proteins remained fairly constant and lightly decreased over time when heating temperature maintained at 85°C. Immune active proteins contents were extremely low and the diversity among different treatments was small at heating temperatures ranging from 125 to 145°C for 4s. When heated at 138°C for 4s, 6s and 8s, concentrations of immune active proteins had shown no significant difference. When milk heated at UHT and superheated conditions, treatment of raw milk at 140°C for 4s had a relatively higher content. These finding indicated that the appropriate condition of pasteurization heating at 75°C for 15s, and treatment at 140°C for 4s are fit for UHT processing. Moreover, there is no need for superheated which has no benefit for dairy nutritional properties.

Key Words: cow milk, biological active whey protein, heat-stability

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1282 (T171) Effect of extraction methods on the 2-DE map of whey proteome in cow milk.

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For all proteomic approaches, protein extraction and sample preparation are the most critical steps for optimal results. However, protein isolation is often complicated. The objective of this study was to investigate the effect of extraction methods on two-dimensional electrophoresis (2-DE) maps of whey proteome in cow milk and explore an optimal eatraction method. Samples were extracted from cow milk by different isoelectric precipitations and ultracentrifugation methods. SDS-PAGE and 2-DE maps were analysised by QuantityOne v4.62 and PDQuest 8.0 software, respectively. The results of 2-DE maps showed that milk whey protein could be effectively extracted by the methods above with less background, no significant strips and good repeatability. However, there were still some residual caseins appearing in each map. 2-DE maps of whey protein refined by isoelectric precipitations were relatively informative compared with the gel maps obtained by ultracentrifugation. Moreover, the richness of different whey proteins in various maps extracted by the method adjusting pH to 4.6 as isoelectric point is slightly higher than adjusting pH to 4.8. The results indicated that adjusting pH to 4.6 as isoelectric point to extract whey protein has some advantages than the other two methods. However, all the methods used in this research could effectively remove high abundant casein to improve the detection sensitivity of low abundance proteins, which could provide some useful information for the futher research on whey protein proteomic of cow milk.

Key Words: whey protein, extraction methods, two-dimensional electrophoresis maps

1283 (T172) Effect of metabolic acidosis in lactating dairy cows on concentration of milk proteins.

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Milk casein subunits (α, β and κ-casein) play a role to avoid the formation of toxic amyloid fibrils and maintain the milk thermal stability. When milk is produced by healthy cows fibrils are not formed because an alternative aggregation pathway is followed that results in formation of the casein micelle. However, nutritional disorders of lactating cows may negatively influence on composition and quality of milk. Thus, the present study aimed to evaluate the effect of the dietary cation-anion difference on concentrations of milk subunits of casein and whey proteins. Sixteen Holstein cows were distributed in four contemporary 4 × 4 Latin square, consisting of four periods of 21 d. Cows were distributed to four treatments according to the dietary cation-anion difference (DCAD): 1) +290 mEq/kg of DM; 2) +192 mEq/kg of DM; 3) +98 mEq/kg of DM; 4) −71 mEq/kg of DM. The cows were fed total mixed ration and the DCAD was calculated according to the contents of Na, K, Cl, and S of the diets. Individual milk samples were collected for determination of the concentrations of α (α-CN), β (β-CN) and κ-casein (κ-CN), α-lactalbumin (α-La) and β-lactoglobulin (β-Lg), by high performance liquid chromatography. The results were analyzed using the PROC MIXED of SAS (2001) (α = 0.05). The DCAD reduced linearly the concentration of κ-CN [κ-CN = 5.02(0.30)–0.00098(0.0004)*DCAD] as well as increased the concentration of β-LG [β-LG = 1.41(0.16) + 0.00047(0.0002)*DCAD] (Table 1283). The κ-CN and β-LG have a potential tendency to assemble into toxic amyloid fibrils. Thus, as a consequence of metabolic acidosis due to reduction of DCAD, β-LG may be associated with the κ-CN in the micelle and the milk stability during the heat treatment may be reduced.

Key Words: casein, DCAD, milk stability

Table 1283. Effect of dietary cation-anion difference (DCAD) on the milk proteins concentration (mg/ml of milk)

Milk proteins	DCAD (mEq/Kg of DM)					*P*		
	-71	98	192	290	*SEM*	*Linear*	*Quadratic*	*Cubic*
α-CN	6.38	6.36	6.52	6.17	0.12	0.404	0.165	0.118
β-CN	7.34	7.29	7.38	7.10	0.19	0.423	0.482	0.449
κ-CN	5.07	4.96	4.95	4.68	0.15	**0.031**	0.361	0.478
β-LG	1.41	1.45	1.46	1.59	0.08	**0.040**	0.330	0.539
α-LA	0.84	0.76	0.74	0.77	0.05	0.115	0.172	0.815

1284 (T173) Process optimization for production of whey protein hydrolysate from cheese whey having antioxidant property.

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Oxidative stress, the increased production of reactive oxygen species and reactive nitrogen species combined with overtaking endogenous antioxidant defense mechanisms, is a significant causative factor for the initiation or progression of several life style mediated diseases. Dietary consumption of antioxidants appears to benefit endogenous antioxidant defense strategies in the fight against oxidative stress. Cheese whey is a rich by-product in nutritional terms: possessing high biological value components, excellent functional properties, and an inert flavour profile. In particular, biological activities of whey proteins and their hydrolysates have received more attention in recent years. Peptides generated from whey protein hydrolysis have antioxidant properties and it depends on the protease specificity as well as hydrolysis conditions. Currently, data on the hydrolysis conditions for the direct production of hydrolysate from whey is lacking. So in the present study, mozzarella cheese whey was ultrafiltrated to concentrate the protein content and the retentate after preheat treatment was hydrolyzed using commercial food grade enzyme alcalase. Response Surface Methodology (RSM) was applied to optimize the hydrolysis conditions, including incubation time, hydrolysis temperature and pH with the purpose of obtaining the most powerful antioxidant hydrolyzate from whey proteins. A central composite circumscribed (CCC) design was employed to study the effect of the experimental variables on the antioxidant activity determined by 2, 2'-azinobis (3-ethylenebenzothiazoline-6-sulphonic acid) ($ABTS^{\cdot+}$) radical scavenging activity and the parameters of the model were estimated by multiple linear regression. The highest antioxidant activity (1.18 ± 0.015μM of trolox/mg of protein) was found in retentate hydrolyzed for 8 h at pH 9 and temperature 55°C. Seven β-lactoglobulin derived peptides were identified by RP-HPLC-MS/MS in this hydrolyzate. Amino acid composition of the peptides DTDYK f(96–100) and VLDTDYK f(94–100) indicating their important contribution on antioxidant property of whey protein hydrolysate (WPH) was evaluated. Hydrolysis of ultrafiltrated retentate of whey can be an energy and cost effective method for the direct production of WPH from whey compared to the industrial production of WPH from Whey protein concentrate. This study suggests that WPH with good nutritional and biological properties can be effectively used in health promoting foods as a biofunctional ingredient.

Key Words: whey protein hydrolysate, antioxidant property, bioactive peptides

1285 (T174) The effect of heat and extraction technique on β-lactoglobulin hydrolysis. C. Kembel[*1], and R. Jimenez-Flores[2], *[1]California Polytechnic State University, San Luis Obispo, [2]Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo.*

Whey proteins are an abundant source of biologically active peptides that have a diverse set of properties. One limitation in the production of novel peptides is the native folding of the proteins secondary and tertiary structure. β-Lactoglobulin is a barreled protein with a hydrophobic core capable of binding other proteins and fatty acids. Due to its prevalence in whey, it represents an important source of bioactive peptides. Presenting a method for the production of novel peptides by native whey protein change in conformation will bring value added products to market. Previous studies have shown the resistance of isolated, native, β-lactoglobulin to trypsin hydrolysis in protein-lipid emulsions. However incorporation of washed cream (40% fat) into this system (natural emulsion from cream) significantly changes the hydrolysis patterns of β-lactoglobulin. The hydrolysis patterns of β-lactoglobulin was evaluated using electrospray ionization mass spectrometry (ESI-MS) after three heat treatments (none, 50°C– 20 min, and 70°C–20 minutes) and four extraction methods (none, supercritical carbon dioxide, hexane, and Folch). Although hydrolysis was improved compared to native β-lactoglobulin, the degree of hydrolysis did not exceed 42% in any of the treatments. Substitution of purified native β-lactoglobulin with whey protein isolate (WPI) in the 40% cream solution significantly increased the number and type of β-lactoglobulin peptides released when subjected to various heat treatments and fat extraction methods. The degree of hydrolysis reached a maximum 64% and there appeared to be sequential, predictable peptide release depending on the heat treatment and extraction technique used. The heat treatment and extraction method change the absorption and spreading process of β-lactoglobulin within and along the MFGM and thus, effecting which peptides are available and released by the enzymatic action.

Key Words: β-lactoglobulin, peptides, mass spectrometry

1286 (T175) Evaluation of the viscosity profile during simulated conditions of thermal processing. A. Souza[1], L. C. Junior[2], R. Stephani[1], M. Pinto[3], A. Carvalho[*3], Perrone[3], and R. Costa[2], *[1]Gemacom Tech, Juiz de Fora, Brazil, [2]EPAMIG, Juiz de Fora, Brazil, [3]Federal University of Viçosa, Brazil.*

The understanding about milk protein interactions provides better cost/benefit ratio to food industry by using each type of protein within the desired characteristics in final product. Denaturation and interaction of different proteins occur in different way and intensity when pH value varies accordingly to the medium in which they are located. This study aimed to verify the influence of whey protein/casein interaction in the evolution of viscosity at different pH (6.0, 6.5 and 7.0) values using the Rapid Viscosity Analyzer (RVA) (model 45000, Perten Instruments, Sweden) as thermal processing simulator. Samples of commercial whey protein concentrate (WPC) and milk protein concentrate (MPC) were analyzed. Thermal denaturation and level of protein interaction were measured by RVA. Further, fat, protein, ash, lactose, pH, protein concentration and moisture were performed according to procedures described by the Association of Analytical Communities (AOAC, 2005). The solid-liquid concentration of the dispersion measured in the RVA was 0.3 gg^{-1} dry weight in water. Viscosity was as low as possible in low pH and high MPC/WPC level. At pH 7.0 and 100% whey protein concentrate results in higher final viscosity. At pH 6.0 and 100% milk protein concentrate results in lower final viscosity. High viscosity can be related with favoured κ-CN-whey protein complex formation at high pGH (Anema, 2008). Further, increased viscosity in treatments with high proportion of WPC can be explained by the difference in size of aggregates formed between whey proteins and/or κ-CN-whey proteins and by the higher degree of denaturation at higher pH values. References: (1) Anema, S.G. On heating milk κ-casein from the casein micelles can precede interactions with the denatured whey proteins. Reference: *Journal of Dairy Research,* 75, p. 415–421, 2008; (2) AOAC International. Official Methods of Analysis of AOAC International. United States, 2005.

Key Words: protein denaturation, pH, RVA

1287 (T176) Viscosity measurement of solutions composed by whey protein using a rapid viscosity analyser (RVA). M. Alves[1], M. Martins, P. H. Junior, R. Moreira, G. Mendes, M. Pinto, Perrone, and A. Carvalho[*], *Federal University of Viçosa, Brazil.*

The evaluation of the viscosity performance of protein solutions used as ingredients in food is essential for this application in the food production. The Rapid ViscoAnalyzer (RVA) is a rotational viscometer capable of continuously measuring the viscosity of a sample under controlled temperature conditions (Booth & Bason, 2007). Using a RVA, the current study aimed to evaluate the viscosity of solutions prepared from whey protein concentrates (WPCs) produced from milk whey samples initially subjected to thermal treatment 72°C for 15 s (*P*T72) or microfiltration (0.8 μm- MF0.8 or 1.4 μm- MF1.4). Each treatment was ultrafiltered and subjected to vacuum evaporation using a rotary evaporator and dried in a spray dryer. The resulting WPCs were evaluated for their content of fat, total solids, moisture, ashes, and total protein. Furthermore, water activity (Aw) of each WPC was measured. The solutions prepared from the WPCs were also evaluated for their viscosity. Data analysis was performed using Statistical Analysis System v9.2 software (SAS Institute Inc., 2006). The WPCs presented

composition compatible with the international standards, with a significant difference ($P < 0.05$) for fat concentration. Viscographic profiles indicated that WPCs produced from microfiltered whey had higher viscosities than those subjected to heat treatment. In addition, 10 min was determined to be the optimal length of time for heat treatment to maximise WPCs viscosity. The WPC solutions obtained from pasteurized whey showed lower viscosities than solutions obtained from microfiltration whey. This result demonstrates the importance of technological choices on the behavior of the WPC and further, WPC can be design for different food applications. Finally, a rapid viscosity analyzer was demonstrated to be an appropriate tool to study the application of whey proteins in food systems.

Key Words: milk protein, microfiltration, RVA