

652 Microencapsulation of recombinant enzymes for application in accelerated cheese ripening. B. H. Lee*^{1,2}, ¹*Agriculture and Agri-Food Canada, Food R & D Centre, St-Hyacinthe, QC, Canada*, ²*McGill University, Montreal, QC, Canada*.

Direct addition of enzymes to milk during cheesemaking appears to be the simplest method to accelerate cheese ripening. However, due to loss of most enzymes in whey, poor enzyme distribution, and texture defects by extensive proteolysis, alternative encapsulation technology can eliminate these defects. Previous approaches on the addition of free enzymes during salting stage and encapsulation of natural flavour-enhancing enzymes using different food gums and liposomes will be presented. In this study, aminopeptidase (PepN) of the cheese isolate,

Lactobacillus rhamnosus S93 was genetically overproduced up to a 1,000 fold, purified and encapsulated in chitosan-coated alginate beads to investigate the effects of Cheddar cheese ripening for 6 months. The encapsulation efficiency was above 90%, and the experimental cheeses received higher scores for sensory properties than the control cheese. The amounts of PTA-N and total FAA in the cheese with the encapsulated enzyme after 2 months of ripening were close to those of the control cheese after 6 months, suggesting the acceleration of about 4 months in proteolysis. Although this study was aimed to develop an encapsulation method which can affect Cheddar cheese ripening, it would be useful for other type of cheeses.

Key Words: microencapsulation, enzymes, cheese ripening

Dairy Foods: Milk Protein and Enzymes Symposium

653 Indigenous enzymes in mammalian milk: Scientific, technological and physiological significance. A. Kelly*, *University College Cork, Cork, Ireland*.

The milk of all mammals contains a range of enzymes with a range of roles and functions; even in more well-studied species such as the bovine, the exact number of enzymes, factors affecting their activity, and their exact significance remains poorly understood. Some enzymes are recognised to have roles, either positive or negative, in the quality of milk and dairy products, such as milk lipoprotein lipase and the alkaline protease, plasmin. Other enzymes are of major industrial significance not for their function but due to their exploitation as surrogate indicators of the efficiency of processing, such as the use of alkaline phosphatase as an index of the efficiency of pasteurisation. In physiological terms, the activity of many enzymes is susceptible to secretory disturbances in the udder, reflecting their cellular or blood origins, and so levels can vary with factors such as stress, lactation and, in particular, mastitis. This presentation will give a brief overview of the profile and significance of the principal enzymes in mammalian milk. In addition, the results of some new research on the enzyme system of the human milk will be discussed, for comparison with the proteolytic system of the bovine, and highlights some potential insights into the physiological significance of modulation of hydrolysis of proteins in milk in terms of enhancing protein digestibility for the neonate.

Key Words: milk, enzymes, proteins

654 Enzymes associated with the bovine milk-fat-globule membrane with special reference to xanthine oxidoreductase. J.-K. Jeong, J. Xu, and I. H. Mather*, *University of Maryland, College Park*.

The enzymes of bovine milk-fat-globule membrane (MFGM) will be reviewed, with particular emphasis on their cellular origin and functional attributes. Over forty enzymes have been identified in the MFGM, including hydrolases, transferases and oxidoreductases. Hydrolases constitute the most abundant class, within which there are a large number of GTPases in the rab family. These enzymes originate from multiple cellular sources, including the mammary epithelium and immune cells, and have diverse physiological functions in milk synthesis and secretion, membrane trafficking, and immunity. The most abundant enzyme in the MFGM of dairy cows and many other species is xanthine oxidoreductase (EC 1.17.1.4) (XOR), a redox enzyme in the molybdenum hydroxylase family. XOR constitutes approximately 20% of globule-associated protein and over 10% of isolated MFGM. Besides its function

as a purine oxidase, XOR, under certain physiological contexts can generate reactive oxygen and nitrogen species, which may function in the innate immune system and in signaling pathways. In addition, XOR binds with high affinity to the cytoplasmic domain of butyrophilin, the most abundant transmembrane protein in the bovine MFGM. Interactions between XOR and butyrophilin are postulated to be essential for formation of the MFGM, and the secretion of lipid droplets. Thus XOR is a multi-functional protein, required for diverse physiological processes in lactation and the immune system.

Key Words: xanthine oxidoreductase, butyrophilin 1A1, milk-lipid-globule membrane

655 Proteolytic enzymes associated with somatic cell count and their relevance in raw milk and dairy products. L. B. Larsen*, *Institute of Food Science, Faculty of Agricultural Sciences, Aarhus University, Denmark*.

Somatic cell count and mastitis are associated with increased activities of bovine proteases, such as plasmin, but also enzymes from somatic cells play a role. These cause proteolytic degradation of the caseins, which can lead to poorer quality of stored milk, and may also contribute to a lower cheese yield. Different proteolytic enzyme systems have been demonstrated in bovine milk, and include, apart from the plasmin system, lysosomal enzymes such as cathepsin B and D. It is, however, not clear to what extent the enzymes are actively secreted from the somatic cells, leaked from dead cells or to some extent secreted by the mammary tissue. This complex situation is reflected in the enzyme profile of e.g. the cathepsin D system, where both active enzymes and proenzymes have been demonstrated in purified preparations from milk, with procathepsin D being the major form present. The enzymes have been characterized in milk and their significance in some dairy products has been studied. The potential contribution of these different bovine enzymes to the proteolysis occurring in milk at acute mastitis as well as at low or moderately elevated somatic cell count has been further characterized by use of proteomic and peptidomic methods including 2D gel electrophoresis, LC MALDI spotting and MALDI ToF MS/MS of peptides and protein fragments. By these methods a range of casein-derived peptides, including some with apparent bioactive properties, were identified in the different milk types. Based in these identifications possibly responsible proteases have been suggested, and these included plasmin, cathepsin B, D and leucocyte elastase, in addition to apparent amino- and carboxypeptidase activities.

Key Words: cell count, proteolysis, MALDI ToF

656 Lipases and lipolysis in milk and dairy products. H. C. Deeth*, *School of Land, Crop and Food Sciences, University of Queensland, Brisbane, Queensland, Australia.*

The enzyme, lipase, which catalyses the hydrolysis of triglycerides to free fatty acids, partial glycerides and, in some cases, glycerol, is a constant concern in the dairy industry. It presents in different forms but chiefly as the indigenous milk lipoprotein lipase and a raft of lipases produced by contaminating microorganisms in milk and dairy products. Lipases can cause flavour problems as well as texture problems such as inhibition of foaming of milk used for making cappuccino coffee. However, not all lipases are detrimental to the quality of dairy products. For example, they play a very important role in the typical flavour of many cheeses. While much is now known about the nature of these enzymes and their action in milk and dairy products, lipase-related problems continually arise. This paper discusses some case studies where lipase has been involved and outlines the causes and solutions to the problems in the light of this knowledge.

Key Words: lipase, lipolysis, dairy industry

657 Native proteases in milk: Current knowledge and relevance to dairy industry. B. Ismail*¹ and S. Nielsen², ¹*University of Minnesota, St. Paul,* ²*Purdue University, West Lafayette, IN.*

Plasmin is by far the predominant and most completely studied endogenous protease in bovine milk, so will be the primary focus of this talk on native proteases in milk. The hydrolysis of milk proteins by proteases, such as plasmin, affects the texture and flavor of dairy products, to have either beneficial or detrimental effects, depending on the extent of hydrolysis and type of dairy product. Plasmin is part of a complex protease-protease inhibitor system in milk that consists of active (plasmin) and inactive (plasminogen) forms of the enzyme, activators, and inhibitors. Considerable research has been done to isolate and characterize components of the plasmin system, determine how they interact, assess thermal stability, and develop and compare quantitation methods. Additionally, several studies have been carried out on the plasmin system activity and interactions as affected by cow characteristics, milk storage, processing conditions, bacterial proteases, and various milk proteins such as β -lactoglobulin and κ -casein. Depending on the end use, researchers have focused on either enhancing or minimizing the activity of plasmin system components. The intent has been to control protease activity in casein and whey fractions, depending on the food or ingredient application. Controlling the activity of endogenous milk proteases, such as plasmin, has great potential to improve dairy product quality and reduce their processing costs.

Key Words: milk proteases, plasmin, endogenous enzymes

Extension Education: Symposium: Models for Dairy Production Decision Making

658 To keep or cull a cow: An economic decision. A. De Vries*, *University of Florida, Gainesville.*

Dairy cow culling decisions are economic decisions. When a cow is culled, the producer expects to be better off without the cow. When no replacement is available, the open cow is often kept as long as her milk income exceeds her variable cost. When replacements are available, the typical recommendation is to keep the slot full, meaning that the culled cow should be immediately replaced by another cow, often a calving heifer. Alternatively, calving heifers may accelerate cow culling when space is limited. The decision to keep the current cow as long as her milk income exceeds her variable cost is then no longer optimal because earlier replacement might improve the profitability of the slot. When groups are overcrowded, culling might improve the performance of the remaining cows. In many of these situations, cash flow projections of the keep and cull decision may support the quality of decision making. Accurate cash flow projections are difficult to make without the help of computers and modeling. This difficulty arises because cash flow projections involve sequential cows in the same stall with differences in milk production, stage of lactation, and reproductive status, among others. Further, decisions in the future may or may not be optimized. Algorithms have been designed that provide cash flow projections of both the keep and cull decision for individual cows. Differences of the net present values of such projections allow for ranking of cows for future profitability, and hence identification of cows recommended for culling. Such algorithms have not been widely used on dairy farms because they were either not available, there was a lack of understanding of the calculations, or producers did not see the need for decision support. Newer algorithms allow for more accurate cash flow predictions based on daily updated cow performance data. They also allow for more insight in the calculations. Further, multiple decisions may be optimized simultane-

ously, such as insemination decisions, dry-off decisions, and replacement decisions. Collectively they provide added value to increasing amounts of data that is becoming available at a more rapid pace.

Key Words: culling, model, economics

659 Modeling the economic impact of reproductive change. M. W. Overton*, *University of Georgia, Athens.*

A spreadsheet-based model using partial budgeting was used to develop a stochastic simulation approach to estimate the economic benefit of improved reproductive performance in U.S. dairy herds. Through simulation, a herd is calved and followed through lactation. Time dependent culling risks and pregnancy rates for each cycle were used to project cumulative pregnancy rates following twelve potential breeding cycles. Distributions were fit to describe the potential cycle-specific conception and insemination risks and overall 305-day ME milk production for Holstein cows. Farm-level prices for milk, calves, market cows, and replacement animals from January 2007 through February 2009 were used to fit distributions for predicting future returns. Values associated with changes in pregnancy rate were obtained by comparison of a simulated reproductive management program with a simple estrus detection-based AI program. Final results were obtained by running 1000 iterations through the use of simulation software and are displayed as probability distributions, with a mean expected value and a 90% expected range. Sources of revenue include annualized milk per cow per day, annualized values of calves produced, and the annualized value of the market cows. Expenses include annualized replacement costs, the marginal feed consumed by cows producing marginal milk, feed consumed by additional non-lactating cows, additional costs for