lipolysis. The aim of our study was to develop an efficient hydro-gel enzyme encapsulation process for the controlled release of flavour-enhancing enzymes into the cheese matrix, and to study proteolysis during cheese ripening using a reverse-phase HPLC. Flavourzyme (commercial protease-peptidase enzyme mixture from Novozymes Pty. Ltd) when encapsulated using 1.8% (w/v) alginate and polymerised in 0.1M calcium chloride solution and 0.1% (w/v) chitosan solution, produced 72% encapsulation efficiency. When the hydro-gel capsules containing enzymes were incorporated during cheese manufacture, the enzymes were released and accelerated cheese ripening. Four batches of cheddar cheese were prepared without enzyme (control) and with encapsulated enzyme. Sampling was carried out during various stages of cheese ripening (0, 1, 7 and 10 weeks) to assess ripening. Free amino acid, water soluble and insoluble fractions of nitrogenous compounds in the ripening cheese were separately analysed by RP-HPLC and SDS-PAGE. Addition of encapsulated enzyme accelerated the cheese proteolysis by producing a large number of low to medium size molecular mass peptides in the water soluble nitrogenous fraction, some of which were unique for the enzyme treated cheese. In contrast, analysis of water insoluble nitrogenous fraction showed that hydrolysis of casein after 70 days of maturity was greater in enzyme treated cheese. Particularly, beta-casein degradation was rapid compared to alpha-casein and kappa-casein in the enzyme treated cheese. This study showed that alginate microcapsules can be effectively used to release flavour enhancing enzymes into cheese matrix to accelerate ripening and improve the flavour profile of cheddar cheese

Key Words: Cheddar Cheese, Microencapsulation, Accelerated Cheese Ripening

Dairy Foods: Milk Proteins and Enzymes: Proteomics and Milk

827 Recent developments in proteomics: Implications for dairy protein research. P. Qi*, *USDA-ARS-ERRC*, *Wyndmoor, PA*.

Proteomics, the systematic study of the identities, quantities, structures, and biochemical and cellular functions of all proteins in a cell, tissue or organism, promised a rapid transformation for biological and medical research in the post-genomic era. Tremendous progress has been made over the past decade in this highly mass spectrometry dependent discipline of systems biology. Proteomics is regarded as a comprehensive research tool not only capable of identifying and quantifying large sets of proteins but also can be used to determine their localization, interactions, modifications, activities, and functions. Recent developments in proteomics research that include protein separation methods, mass spectrometric instrumentation, computational analysis, and integrative databases will be reviewed with the focus on post-translational modifications. In light of the current status and the perspectives of proteomics applications, we will discuss the implications for future dairy protein research suc as structural and functional studies of milk fat globule membrane proteins and other low abundance yet biologically important proteins in milk and dairy products.

Key Words: Proteomics, Post-translational Modification, Milk Proteins

828 Quantitative proteomic analysis of bacterial enzymes released in cheese during ripening. V. Gagnaire, D. Molle, J. Jardin, and S. Lortal*, *INRA*, *Rennes, France*.

Bacterial ecosystems contribute to the cheese ripening not only during their growth but also after their lysis, through the release of numerous proteins in the cheese curd, including enzymes. A prefractionation method allowing the isolation of bacterial proteins from cheese extracts was previously developed as well as a proteomic qualitative survey during Swiss cheese ripening (Gagnaire et al., 2004. Int. J. Food Microbiol.,94,185). Numerous peptidases coming from the lactic starters were identified on 2D-gel electrophoresis as well as many glycolytic enzymes and stress proteins. This new insight even if very informative provided only qualitative data. The aim of this work, using new iTRAQ technique (isobaric tagging reagent for quantitative proteomic analysis), was to provide quantitative proteomic data about the bacterial proteins released at different stages of ripening. This technique is based upon chemically tagging the N-terminus of peptides generated from protein digests, which are then fractionated by nanoLC and directly analysed by tandem mass spectrometry.

Experimental Swiss cheeses were performed in our laboratory using microfiltered milk in order to remove the initial bacterial raw milk contamination. Thermophilic lactic acid bacteria (L. helveticus LH1 and S. thermophilus ST20) and propionibacteria (P. freudenreichii P23) were used as starters. At four times of the ripening (day one, entrance, middle and end of the warm room ripening), cheese aqueous extracts were prepared and fractionated to separate bacterial proteins. To standardize the protein content of each sample before proteomic analysis, a total amino acid analysis was performed. The standardized samples were i) analysed by 2D-electrophoresis for qualitative analysis and ii) submitted to trypsinolysis. Each tryptic hydrolysate was labelled with a specific iTRAQ tag (one tag per ripening time) and submitted to a LC-ESI-MS/MS analysis. This technique provided the identification of the bacterial proteins released and their respective abundance at different times of the ripening.

Key Words: Proteomic, Cheese, Enzyme