

## Development of a milk processing sector model

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A processing model was developed to simulate milk manufacturing in the Irish Dairy Industry. An annualised approach with a monthly time step was developed that simulates (i) milk receiving, (ii) milk assembly and (iii) product manufacture. The model estimates the product yield, net milk value and component values of milk each month of the year based on milk quantity, composition and product portfolio. Final product specifications of cheese, butter, skim and whole milk powders, liquid milk and casein are met through milk separation into skim milk and cream. The volumes of whole milk, cream and skim milk are reconstituted in differing proportions to meet final product specifications. Excess cream or skim milk from the processes are used in other product manufacture. Volume-related costs, including milk collection, assembly and processing costs per litre, are calculated. Product-related costs, including processing costs per tonne, packaging, storage, distribution and marketing, are quantified. Operating costs, incurred irrespective of milk received and processing activities, are included in the model on a fixed rate basis. The net milk value is estimated as the difference between the sale value and total costs. Monthly component values of fat and protein are estimated from the marginal rate of technical substitution. The model was applied to quantify the effect of three cow breed's (Jersey (J), Holstein Friesian (HF) and New Zealand (NZ)) on net milk value. The net milk value of 1,000 L of milk, producing 30% cheese (€3,831/t), 20% whole (€2,343/t), 25% skim milk powder (€2,374/t) and 25% butter (€2,591/t) was €352, €281 and €311 for the J, HF and NZ respectively.

## Assessment of techno-economic status of creameries in Hisar- A case study

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The demand of milk of city dwellers in India is largely met by vendors who collect whole milk from the surrounding villages. Some of the vendors get a part of their milk skimmed and sell cream to make more profits, unscrupulously. Some part time or full time operators have installed cream separators for this purpose and have thus found gainful employment in this process. They separate cream for a charge and purchase it at cheaper rates from the vendors and convert it into *ghee*. This study was undertaken to assess the techno economic status of such units. Hisar city, a district headquarter in Northern India, was selected for this purpose. A total of 26 creameries could be identified, most of which were located on the entry points on the out skirts of the city. It was found that the operators had invested INR 11,150 to 1,53,200 on fixed assets like cream separator, utensils and construction etc. The variable costs per annum ranged from INR 80,453 to 7,69,529 and the total returns from INR 92,000 to 8,63,040 with net profits ranging from INR 8,313 to 1,36,204. The benefit/cost ratio ranged from 0.10 to 0.21. The man hours devoted per day were 3 to 10.5 and the operators worked on farm or grocery shops during vacant hours. The cream so obtained contained 63 to 71 percent fat and up to 97 percent of this milk fat could be recovered in *ghee*. *Ghee* was prepared by creamery butter method. Most of the *ghee* samples met the legal standards. Microbiological and chemical quality of incoming milk, skim milk, cream and *ghee* were also studied.

## **Physiological effects of season and parity on production and nutritional quality of milk in camel (*Camelus dromedaries*) under pastoral environment of Pakistan**

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Relatively little is known about the actual situation concerning milk production and quality in the Thal area of Pakistan, particularly when regarding seasonal variation in milk composition and with different parities. Therefore, this study was planned to evaluate the effect of season and parity on milk production and compositional quality of camels kept under pastoral environment of Pakistan. Based on purposive sampling method, 200 she-camels were selected in thal area (District Jhang) and their composite milk samples were collected. The research was carried out in two periods. The first period was the summer period covering May-June-July months and the second one was the winter period covering December-January-February months. The collected milk samples were analyzed through standard procedures in order to determine the percentages of milk fat, protein, lactose, acidity and solids not fat (SNF). Mean daily milk production and mean percentages of fat, protein, lactose, acidity and SNF were found to be  $5.50 \pm 0.18$  L and  $3.40 \pm 0.19\%$ ,  $3.30 \pm 0.11\%$ ,  $4.67 \pm 0.13\%$ ,  $0.20 \pm 0.01\%$  and  $9.56 \pm 0.18\%$ , respectively. Season of the year and parity imparted significant effect ( $p < 0.05$ ) on daily milk production. The values for milk production ( $6.20 \pm 0.20$  L), fat ( $3.98 \pm 0.23\%$ ), protein ( $3.43 \pm 0.16\%$ ), lactose ( $4.92 \pm 0.21\%$ ) and SNF ( $9.14 \pm 0.39\%$ ) were significantly higher ( $p < 0.05$ ) during winter season as compared to summer. In 3rd parity, significantly highest ( $p < 0.05$ ) daily milk production ( $6.59 \pm 0.30$  L) and percentages of fat ( $4.00 \pm 0.27\%$ ), protein ( $4.61 \pm 0.41\%$ ), lactose ( $5.50 \pm 0.36\%$ ) and SNF ( $11.33 \pm 0.39\%$ ) was observed whereas she-camels in 6th parity produced significantly lower ( $p < 0.01$ ) milk volume ( $2.90 \pm 0.56$  L) and percentages of fat ( $2.70 \pm 0.30\%$ ), protein ( $1.69 \pm 0.68\%$ ), lactose ( $3.79 \pm 0.39\%$ ) and SNF ( $8.02 \pm 0.48\%$ ). However, acidity of camel's milk was not influenced by season and parity. Efficient feeding strategies during scarcity periods and culling after 5th parity are imperative measures for getting maximum milk of high nutritive value from this novel animal.

## Static headspace analysis of volatile compounds released from $\beta$ -lactoglobulin-stabilized emulsions determined by the phase ratio variation method

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The volatile compounds release from oil in water emulsions stabilized by  $\beta$ -lactoglobulin ( $\beta$ -lg) was studied under equilibrium conditions. The influence of fat type and particle size was investigated using soy oil and milk fat. The release was compared using matrices such as water, oil and other emulsions. Gas-matrix partition coefficients ( $K_{gm}$ ) for five volatile compounds: 1-propanol, ethyl butyrate, heptanal, octanol and 2-decanone, were determined by static headspace gas chromatography. The compounds were chosen based on their polarity, vapour pressure and functional groups. Two methods to measure  $K_{gm}$  were compared. A direct method called vapour phase calibration (VPC) from a previous study was compared to the current experimental  $K_{gm}$  using the phase ratio variation (PRV) method and calculated  $K_{gm}$ . A good agreement between the two results was achieved. The PRV method was chosen as a simple and accurate indirect method to measure  $K_{gm}$  without using external calibration. An increase in retention for all volatile compounds was observed above oil and emulsions compared to water in the respective headspaces. Hydrophobic interactions between the volatile compounds and the oil phase were the main reason for the lower partition coefficients. Ethyl butyrate showed the highest  $K_{gm}$  above the emulsion whereas 2-decanone had the lowest. Emulsions made with milk fat compared to soy oil led to a lower  $K_{gm}$  for all compounds except 1-propanol. This can be explained by the propanol hydrophilic behaviour. The role of  $\beta$ -lg as an emulsifier, which is capable of bind volatile compounds in its hydrophobic cavity, was investigated by comparison with Tween 20 emulsifier which does not have such a cavity. Hydrophobic compounds were not released as much from  $\beta$ -lg emulsions compared to Tween 20 emulsions due to strong hydrophobic interactions with the protein. Similar results were observed for water with  $\beta$ -lg versus a water-only system. It can be concluded that the volatile release is influenced both by volatile compound properties and their affinity to the emulsion/matrix composition, structure and type of emulsifier. This study provides important understanding of the way volatile compounds release can be controlled using  $\beta$ -lg-stabilized emulsions as a food matrix platform.

## **A risk-based approach to assessing raw milk products in Australia**

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Australia is fortunate to have a very safe supply of milk and dairy products. Current regulations and practices in the Australian dairy industry ensure a high level of dairy product safety. These include through-chain control of food safety hazards by dairy businesses implementing a documented food safety program at the primary production, transport and processing stages. Milk that is to be sold as liquid milk or used in the manufacture of dairy products is required to be pasteurised (or equivalently processed). Alternative processing requirements to pasteurisation are permitted for cheese production including thermisation (in combination with ripening) and curd cooking (in combination with ripening and minimum moisture content) which allows for the sale of very hard grating cheeses produced from raw milk. In addition, the importation and sale of French Roquefort cheese and Swiss Gruyere, Sbrinz and Emmental is also permitted. Food Standards Australia New Zealand (FSANZ) is aware that there is some demand for a wider range of raw milk products to be made available in Australia and, in response, is assessing the current requirements for the sale of raw milk products. This work is not intended to challenge or alter the use of existing food safety mechanisms for dairy products, in particular pasteurisation. Rather, it is targeted at assessing the potential use of alternative production and processing techniques that provide for the same level of safety of dairy products for the Australian population. This presentation walks through the structured and scientific risk-based approach FSANZ has developed to assess the safety of raw milk products.

## Effect of consumption of kiwifruit on the gastric digestion of milk proteins

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Anecdotal evidence suggests that consumption of kiwifruit (*Actinidia deliciosa*) together with other food aids digestion. This is generally believed to be due to the presence of a high level of the proteolytic enzyme actinidin (EC 3.4.22.14), although this has not been substantiated. We have investigated the effect of addition of kiwifruit to caseins and whey proteins using both an *in vitro* model of gastric digestion and an *in vivo* model using laboratory rats. Samples were analysed by both polyacrylamide gel electrophoresis and reverse-phase HPLC. Results indicated very little effect on the proteins of whey protein isolate in both models, but a substantial effect on the digestion of the caseins. Casein digestion in the presence of kiwifruit gave differences in the rate of disappearance of the intact proteins, and in the peptides formed. Further analysis of the peptides is underway using Mass Spectrometry to determine bonds being cleaved by actinidin under gastric conditions, and preliminary results will be reported. Hydrolysis of caseins during digestion is known to give rise to a number of bioactive peptides. It will be of interest to see whether inclusion of actinidin in the diet changes the range and potential activities of these bioactives.

## **The influence of heat treatment on immunoglobulins concentration in milk**

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The biological function of immunoglobulins in milk and colostrum is to protect mammary gland against pathogens and to provide the calf with an immunological protection against surrounding pathogens. The effects of processing conditions on the immunoglobulins' concentration in milk have been the subject of many recent studies. During processing, the stability of the immunoglobulin in milk is influenced by thermal treatment. Some researchers' mentioned immunoglobulins activity was resistant after heat treatment. However, concern has been raised by other researchers, that the heat stability of immunoglobulins is sufficiently influenced. Milk rich in immunoglobulins may find beneficial applications in human health care, at the same time providing milk quality for a longer period of time. Therefore the aim of the present study was to evaluate the heat stability of immunoglobulins in milk.

A total 25 bulk milk samples were analyzed. The milk was obtained from healthy cows. Milk samples were heated in different regimes: 72°C 15-20 s, 78°C 15-20 s, 85°C 15-20 s, 95°C 15-20 s. The concentration of immunoglobulins (IgA, IgG, IgM) were determined by turbidimetric method. The conductivity of milk was detected with "Milk checker N 4L" to exclude the possibility to analyse milk obtained from mastitis cows. Descriptive statistics were carried out to determine the differences of IgA, IgG, IgM concentration by Microsoft Windows for SPSS software packages.

The concentration of IgA, IgG and IgM in fresh milk samples were accordingly - 0,125 gl<sup>-1</sup>, 0,633 gl<sup>-1</sup>, 0,081gl<sup>-1</sup>. The significant decrease of IgA and IgG concentration was determined after pasteurization at 85°C 15-20 s, accordingly it was decreased for 41,6% and for 50,55%. Lower heat stability was detected in IgM, significant IgM concentration decrease was determined already after pasteurization at 72°C 15-20 s, the concentration of IgM was decreased for 52,1%. The equal heat treatment regimes have different influence on IgA, IgG and IgM concentrations in milk.

The research results demonstrate – considerate thermal treatment of product provides keeping sufficient concentration of immunoglobulins in milk.

**Microencapsulation of *L. casei* using sodium caseinate-gellan gum-GDL complex followed by freeze drying to minimize cell destruction in *in-vitro* gastric model**

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Probiotic bacteria need to be consumed in high concentration to provide its physiological benefits but a large portion is destroyed during processing, storage and gastric transition. Microencapsulation has been extensively researched to improve survival of probiotic bacteria. In current study microencapsulation of *Lactobacillus casei* 431 has been carried out using sodium caseinate-gellan gum-GDL complex followed by freeze drying and the impact on survival of bacterial cells was studied using *in-vitro* gastric model. Encapsulation improved the viability of the probiotic cells in simulated gastric fluid (SGF). After 2 hours of incubation at 37°C, the reductions were  $4.56 \pm 0.47$  and  $3.03 \pm 0.33$  log CFU/gm for free cells and encapsulated wet cells respectively. Freeze drying of encapsulated cells caused loss of viability on an average  $1.7 \pm 0.18$  log CFU/gm. The viability after freeze drying improved significantly ( $p \leq 0.05$ ) when trehalose or lactose was used as compatible solute in the growth media and also as cryoprotectant during freeze drying. The log reduction in trehalose and lactose fortified samples were  $0.84 \pm 0.16$  and  $0.37 \pm 0.09$  log CFU/gm respectively. An additional gellan gum coating on the microcapsules improved the survival further; the log reduction in gellan coated trehalose and lactose fortified samples were  $0.53 \pm 0.16$  and  $0.13 \pm 0.06$  log CFU/gm respectively. Use of cryoprotectants and gellan coating improved the survival of bacterial cells in SGF. After 2 hours of incubation the log reductions were  $1.57 \pm 0.31$  and  $1.38 \pm 0.52$  for gellan coated trehalose and lactose fortified samples respectively, whereas without disaccharide fortification and gellan coating, the log reduction was  $2.28 \pm 0.27$ .

## **$\beta$ -galactosidase ( $\beta$ -gal) from the yeast *Rhodotorula ingeniosa* and its utilization in ice milk production**

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Among of 100 of yeast isolated from Egyptian soils and tested for their  $\beta$ -galactosidase ( $\beta$ -gal) activity, the highest producer was characterized and identified as *Rhodotorula ingeniosa*. This enzyme was found to be intracellularly produced, therefore permeabilization treatments to release the enzyme in a good yield was noticed when homogenization with sterile sea sand under cooling was employed as compared with chemical treatment. The enzyme was maximally produced when cellobiose at 1% final concentration and ammonium sulphate at 1.5 % final concentration were employed. The enzyme was partially purified with ammonium sulphate followed by Sephadex G-100 column chromatography. Some properties of the purified enzyme including the Effect of pHs, Effect of temperature, Effect of reaction periods, and Effect of metal ions on the activity were estimated. On the other hand the use of this enzyme in ice milk production was studied. Different concentration of the enzyme was added to fresh pasteurized milk. Results clearly indicate that the addition of the enzyme to ice milk mixes increased the sweetness although the organoleptic properties of the resultant ice milk was slightly decreased.

## **From HPLC to Microarray – Ripening control in cheese production**

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Each variety of cheese has its own characteristics like flavour, texture and appearance, which develops during its specific ripening process. To assure a maximum of quality and flavour development – which meets consumer interests - extended analytical controls are needed. Beside usual controls based on analytic chemistry, the experience of the cheese producer and sensory evaluation, a combination of instrumental-analytical methods, a computer-assisted evaluation of digital photography and chemometric techniques were used. For the optimization of different kinds of cheese production, chemical ripening indices like proteins, peptides, amino acids and carbohydrate metabolites were detected by RP-HPLC.

Based on this analytical design scopes like the influence of whey cream and DVS-cultures on cheese ripening, the formation of off-flavours, the determination of the stage of ripening and the development of the crack formation in different kinds of cheeses were investigated. The final correlations of analytical results as well as technological parameters with sensory characteristics were carried out by PCA and PLSR. The results led to an evaluation of maturation and corrective actions to avoid ripening defects.

As a new method a microarray in the form of a BioChip was developed to determine the amount of Leucin during ripening in water-soluble extracts of Swiss-type cheeses. The principle of this measurement is based on an adapted immunoassay, which was evaluated by RP-HPLC using OPA-pre-column derivatization. The spot intensity measured by the microarray increased in accordance with the ripening time and a correlation coefficient of  $R=0,990$  was obtained. So the detection of essential ripening parameters like amino acids by microarrays represents an innovative possibility to control the ripening process in a short time.

## **Production health benefit yoghurt supplemented with Rosemary and green tea extract as natural antioxidant**

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The development of the fermented products with health benefits helps the dairy industry increase its sales. The objective of this study was to use rosemary and green tea extracts with different concentrations as a part of water (v/v) used in reconstituting skim milk powder in processing yogurt 14% total solids. The organoleptic properties and antioxidant values were evaluated when fresh and along storage at 5±2°C for 12 days. The antioxidant values were monitored during storage estimated by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging, ferric reducing antioxidant power (FRAP) reducing activities and phenolic compound content. Results showed that sensory evaluation revealed that supplemented fresh yoghurt was highly accepted for both ingredients. Yoghurt supplemented with rosemary up to 8% and with green tea up to 6% was acceptable. Yogurt supplemented with green tea extract had the most powerful antioxidant activity; but yoghurt with rosemary extract showed the highest general acceptability.

## How to avoid sampling - *In-line* process monitoring of dairy products by Photon Density Wave spectroscopy

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Photon Density Wave (PDW) spectroscopy permits the dilution-free investigation of multiple light scattering, highly concentrated dispersions such as dairy products. Here, light scattering is directly related to casein micelles and fat globules and is expressed by absorption and reduced scattering coefficients. Their time-dependency, independently measured by PDW spectroscopy, is the key to the understanding of structural and compositional changes in dairy groceries. Due to its contamination-free fibre probe and its time resolution in the sub-minute regime, PDW spectroscopy is therefore of particular interest for *in-line* analytics and process control, exhibiting substantial potential for cost optimization in processing.

Systems investigated include self-acidification, rennet-coagulation and heat treatment of various types of milk. Here, remarkable changes of the reduced scattering coefficient do occur continuously in dependence on the according influencing quantity. For example, the isoelectric point of casein micelles and the pH of the dissolution of colloidal calcium phosphate as well as the melting of fat crystallites lead to distinct changes in the time-dependent spectra of the processes mentioned. Interestingly, in rennet-coagulation, even both optical coefficients are affected strongly as ripening proceeds.

As the reduced scattering coefficient also correlates with the fat content of dairy products, it can be used as a parameter for quality control. For example, this coefficient differs clearly with filling plant and filling time of a variety of UHT-milk samples. Finally, especially for particle sizing in highly turbid dairy products, the huge advantage of the dilution-free determination by PDW spectroscopy in contrast to other, dilution-based techniques is illustrated by the investigation of milk powder suspensions.

## Site direct mutagenesis of $\beta$ -lactoglobulin to verify the interaction of Lysine $\epsilon$ - amino groups with *Kluyveromyces lactis* $\beta$ -galactosidase

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Studies of the effect of  $\beta$ -lactoglobulin ( $\beta$ -lg) and the heat treatment of milk on the activity of *Kluyveromyces lactis*  $\beta$ -galactosidase have shown that  $\beta$ -lactoglobulin raises lactase activity through two different mechanisms, one of which depends on the release of sulfhydryl groups by heat treatment from the denatured protein, and the other one is a result of the ability of the native protein to bind the enzyme (Jiménez-Guzmán et al., 2006). Del Moral-Ramírez et al., (2008) found that lysine 47, 69, and 138 are the most exposed residues, thus they could participate in the interaction between both proteins and that this interaction is very likely to occur through the Lys  $\epsilon$ -amino groups of  $\beta$ -lactoglobulin. With the objective to verify the interaction and activating role of  $\beta$ -lg over *Kluyveromyces lactis*  $\beta$ -galactosidase, a synthetic gene and site-directed mutagenesis of  $\beta$ -lg with substituted lysine 47, 69 and 138 was constructed. The nucleotide sequence for the  $\beta$ -lac was generated by reverse-translating its amino acid sequence using an *E. coli* codon usage table for highly expressed *E. coli* genes and were constructed 12 oligonucleotides, those were ensemble by PCR. This construction was bound to the expression vector pET3 and cloned in *E. coli* BL21. All clones were sequenced at IBT-UNAM, Mexico. The design of degenerate oligonucleotides for the change in the positions 47, 69 y 138 was successful and the constructions were correctly cloned into *E. coli* BL21. One millilitre of LB media was inoculated with a single recombinant *E. coli* colony and induced with IPTG (DO600 = 0.4), then incubated at 30°C with shaking (200-225 rpm) over 4hr. The protein expression was evaluated by SDS-PAGE (Laemmli, 1970). Cells were sonicated over ice and a prominent band appeared in the gel at approximately 32 kDa. This molecular weight corresponds to that calculated for the bovine b-LGA-thioredoxin fusion protein, showing that the protein was correctly cloned and expressed in *E. coli*. The same was done for the mutant clones which also showed the band of the mutated  $\beta$ -lg at 32 kDa. Studies are being performed to demonstrate the effect of these mutated proteins on the activity of  $\beta$ -galactosidase.

## **Effect of pH on the peptide profile and angiotensin- I converting enzyme inhibitory activity in milk fermented by *Lactococcus lactis* NCFB712**

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Various studies have demonstrated that many peptides derived from milk proteins exert physiological activities when tested in vivo and in vitro. Antihypertensive are the most studied peptides, their activity is based mainly in the inhibition of angiotensin converting enzyme (ACE). These bioactive fragments can be released during milk fermentation by proteolytic microorganisms. The aim of this work was to evaluate the effect of pH on the production of antihypertensive peptides by *Lactococcus lactis* subsp. cremoris NCFB 712. Two fermentations were made, one controlling pH at 6.2 (pH control) and another without control. The proteolytic activity in the fermentation with pH control was higher than fermentation without control. Both fermentations showed differences in peptide profiles from the 8th hour of fermentation on, when the pH of both fermentations showed significant difference. Furthermore the fermentations without pH control presented a higher percentage of inhibition of ACE and lower IC50 than the fermentation with pH control. The molecular weight of peptides produced in both fermentations was compared with the molecular weight of known bioactive peptides, and when the pH of the fermentation was controlled more peptides with different bioactivities were found, while allowing pH to descend conducted to a greater amount of antihypertensive peptides. This effect could be attributed to the decrease of pH in the fermentation without control due to the inhibition of some proteases of *Lactococcus lactis* subsp. cremoris NCFB 712, leading to a more limited amount of peptides. Furthermore, the stability of the hydrolysates to digestive enzymes was evaluated, showing that when pH was controlled the hydrolysates were more resistant to digestion, probably because some of the antihypertensive activity in the hydrolysates without pH control was due to larger peptides, which are easily digested.

## Metal chelating activity in milk fermented by *Lactococcus lactis* NCFB712

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In the last decade a growing number of scientific evidence has revealed that many food proteins and peptides could exert specific biological activities in addition to their well established nutritional value. Bioactive peptides are specific protein fragments that positively impact body function and ultimately may influence human health. One of the most important bioactivity that these peptides exert is the metal chelating activity. These peptides have the ability to enhance the absorption of calcium and other bivalent minerals because of their capacity to form soluble complexes with  $\text{Ca}^{+2}$ ,  $\text{Fe}^{+2}$ ,  $\text{Mn}^{+2}$ . These peptides can be produced during milk fermentation by proteolytic microorganism. In this work the production of metal chelating peptides during the milk fermentation by *Lactococcus lactis* subsp. cremoris NCFB 712 was studied. Two fermentations were made, one controlling pH at 6.2 (pH control) and another without control. The proteolytic activity in the fermentation with pH control was higher than fermentation without control. The proteolytic activity in the fermentation with pH control was higher than fermentation without control. Both fermentations showed differences in peptide profiles from the 8th hour of fermentation on, when the pH of both fermentations showed significant difference. Metal chelating activity of the hydrolysates obtained was tested for Calcium ( $\text{Ca}^{+2}$ ) and Iron ( $\text{Fe}^{+2}$ ). The samples of the fermentation with pH control showed a greater capacity to chelate iron and calcium while the samples of the fermentation without control only showed the capacity to bind calcium. The higher concentration of chelated iron was 390  $\mu\text{g}/\text{mL}$  (fermentation with pH control) while the higher concentration of calcium bound found in the fermentation with pH control was nearly 325  $\mu\text{g}/\text{mL}$ . This effect could be attributed to the decrease of pH in the fermentation without control due to the inhibition of some proteases of *Lactococcus lactis* subsp. cremoris NCFB 712, leading to a more limited amount of peptides in these hydrolysates than in the pH control.

**Verocytotoxigenic *Escherichia coli* O157, O111, O26, O103, O145 in Irish dairy cattle and raw milk: prevalence and epidemiology of emergent strains**

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Verocytotoxigenic *Escherichia coli* (VTEC) have emerged as highly significant zoonotic threats to public health. Although it is well-known that clinically healthy animals can harbour VTEC in their faeces, the prevalence of emerging VTECs such as serogroups O26, O103, O111 and O145 is relatively unknown. The aim of this study was to establish the prevalence of verocytotoxin-producing *E. coli* in faeces of dairy cows and raw milk. One hundred and twenty farm visits were completed (60 farms in Cycle A and 60 farms in Cycle B) with 600 faecal swabs analysed, 120 liquid milk samples and 117 milk filters. Real-Time PCR (RTi-PCR) was used to detect verocytotoxin genes (*vt1* and *vt2*) and subsequently all *vt* positive samples were assayed by RTi-PCR for the serogroups *E. coli* O157, O111, O26, O103 and O145. All sero-group positive samples detected using RTi-PCR were then cultured using serogroup-specific immuno-magnetic separation. All positive isolates were then screened for the presence of confirmed *vt1* and *vt2*, the adhesion encoding *eaeA* and enterohaemolysin *hlyA* genes. The results showed that 64%, 36% and 69% of the fecal, milk and milk filter samples were *vt* positive by RTi-PCR, respectively. A total of 11.8%, 0.8% and 1.6%, respectively, were culture positive. Of the culture positive isolates, only 1.2% of the fecal isolates and none of the milk isolates were positive for virulence factors. Although no pathogenic VTEC was cultured in either milk samples or milk filters, the high occurrence of *vt* genes (by RTi-PCR) in the farm environment may present a potential risk for horizontal gene transfer into non-virulent strains.

## Moisture adsorption & thermodynamic characteristics of Basundi (Indian milk dessert)

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Basundi is a heat-desiccated, thickened milk dessert popular in India having white to caramel colour, creamy consistency with soft textured flakes uniformly suspended throughout the product matrix. The adsorption isotherms of Basundi were measured at the temperatures of 20°C, 30°C and 40°C and the relative humidity of 0.11 - 0.97. The isotherm thus obtained exhibit sigmoid shape which is typical for most food products and correspond to type – II. The equilibrium moisture content rose gradually at lower water activities (0.11 - 0.52) followed by a steep rise at higher water activities. At all temperatures, as the water activity increased EMC also increased. Out of various mathematical models BET, GAB & Caurie, models were tested to predict the experimental moisture adsorption data and to characterize the adsorption behaviour in Basundi. GAB equation showed good fit in the water activity range of 0.11 – 0.97 with the coefficient of regression obtained as 0.9988, 0.9542 and 9316 at 20°C, 30°C and 40°C respectively. GAB monolayer values decreases from 2.11 g of water/100 g of solids at 20°C to 1.54 g of water/100 g of solids at 40°C. The heat of adsorption decreased with increased in moisture content, initially rapidly up to 5.23 g of water/100 g of solids and later approached a constant value as this trend observed in many other dairy products. The net isosteric heat of sorption in Basundi powder ranged from 1.32 kJ/mol at 13.61 % moisture content to 46.71 kJ/mol at 2.39 % moisture content. The results would be useful in package design, shelf life prediction and optimizing drying conditions for Basundi.

## Sweet whey desalination by membrane processes

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Sweet whey is a by-product of cheese manufacturing. Its wider usage in food industry is limited by relatively high concentration of salts which has a negative effect on organoleptic properties. Electrodialysis presents an effective method of whey desalination when higher levels of desalination are demanded (50 % and more). Electrodialysis operates better at higher salt concentration. For this reason it is convenient to pre-concentrate whey to approx. 18-20 % of solids. It can be done either by evaporation or by pressure driven membrane processes (nanofiltration, reverse osmosis). The combination of nanofiltration and electrodialysis is interesting because monovalent ions are partially removed during concentration by nanofiltration.

The study is concentrated on deep desalination of whey (ash content lower than 1 % on total solids). Different kinds of defatted sweet whey were demineralized by electrodialysis: natural whey (5.5 % total solids), whey concentrated by evaporation (10 % and 18 % total solids), whey concentrated by nanofiltration (17% total solids) and whey concentrated by reverse osmosis (17 % total solids). Desalination was carried out in pilot electrodialysis units with membranes RALEX<sup>®</sup> CMH-PES and AMH-PES. Total membrane area was 4.08 m<sup>2</sup>. Desalination process was operated in batch mode. The combination of nanofiltration and electrodialysis was studied more thoroughly. Defatted natural sweet whey was concentrated by nanofiltration using pilot unit containing two pieces of 3.8"x38" elements with DOW<sup>™</sup> NF245 membranes (total membrane area of 14.8m<sup>2</sup>) and further desalinated by electrodialysis in pilot electrodialysis unit.

Composition of final product and energy requirements for desalination by electrodialysis were evaluated for each type of whey. The kinetics of the process (decrease of ash content and transport of individual ions) during desalination was studied for chosen types of sweet whey.

## **The principal acid phosphatase active molecules in raw skim milk is the bovine lactoferrin**

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The aim of this study was to determine the principal active molecules in the acid phosphatase (AcP) fraction of raw skim milk origin using chromatography technique and the matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-ToF-MS). The one peak of AcP active fraction was separated from high-performance size-exclusion chromatography (HPSEC) on TSKgel G 2000 SWXL. This active fraction consists of 3 tubes (tube no. 19-21) that were collected in one tube per 0.5 ml (total 1.5 ml). The molecular mass of the active molecules in individual column tubes was estimated to be around 80 and 15 kDa by silver staining of sodium dodecyl sulphate polyacrylamide gel electrophoresis. The first tube (no. 19) showed a single band and molecular mass estimated to be around 80 kDa. The last tubes (no. 21) showed a single band and molecular mass estimated to be around 15 kDa. The no. 20 tube contained the above-described two bands. The total activities of the individual three tubes were 9.65, 9.5, and 8.6 units, respectively. The unknown band of 80 kDa analyzed by protein sequencer, using Edman degradation methods and MALDI-ToF-MS; the first thirteen N-terminal amino acid sequence obtained were shown to be APRKNVRWXTIXQ and 9 peptide fragment signals (m/z) were detected in mass spectra, which corresponding to the bovine lactoferrin (LF). The peptide fragment signals of 15 kDa band were not in the protein database, though the result of the first N-terminal amino acid sequence (FETAAAFYYRQHMM) corresponded to bovine ribonuclease. These data suggested the presence of an AcP activity molecule other than LF in raw skim milk.

## **Development of production technology for compressed milk powder**

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Milk powder, especially infant formula is typically dissolved at a specified concentration. This requires the consumer to put a predetermined quantity of powder into a baby bottle using a scoop provided with the product. Complaints about the inconvenience of preparing infant formula are frequently heard, spilled powder and/or incorrect measurement, both resulting from use of the scoop.

The purpose of this study was to develop a compressed milk powder and related production technology in order to reduce the inconvenience of typical milk powder. The resulting compressed milk powder product, which dissolves easily, is the world's first such product. The basic production technology of this product consists of 1) molding milk powder by low compression pressure, 2) humidification by saturated vapour and 3) drying with hot air. Since no chemical additives are used, the chemical composition of the compressed milk powder is identical to that of the original milk powder. This means the compressed milk powder retains all the nutritional qualities. The important properties of the compressed milk powder are both ready solubility (complete dissolution by shaking in hot water for 10 seconds) and the strength (no breakage during commercial distribution). Although the compression process is similar to the tableting processes for pharmaceuticals or confectioneries, the compression pressure in our process is only 10% of that typically used. The compressed milk powder obtained by such low pressure is too brittle for practical use, but the strength of the product is increased by humidification followed by drying. During the humidification process, the powder particles located close to the surface of the compressed milk powder are partially dissolved resulting in bridging structures between the particles, leading to an increase in strength.

## Recovery of oligosaccharides from caprine milk whey

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Oligosaccharides are attracting increasing interest as prebiotic functional food ingredients as gut-health and well-being promoters. Caprine milk contains a significant concentration of these carbohydrates, most of which are lost in the whey during cheesemaking so exploitation of this potential source may add value and reduce environmental pollution.

Whey was pretreated by ultrafiltration (UF) at nominal molecular-weight-cut-off (MWCO) of 25 kDa (PCI type ES625) to remove proteins, fat globules and particulates. To separate the target oligosaccharides from the lower molecular weight molecules, the UF permeate was further processed using a tighter UF (TUF) membrane, 1kDa MWCO (TAMI Industries, type CERAM). Both permeates and retentates were analysed by high performance anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD) using a CarboPacth PA1 column (4x250 mm, Dionex Corporation) and by Capillary Electrophoresis (CE) using a Hewlett Packard HP3D CE system (Agilent Technologies) for oligosaccharide (OS) and carbohydrate (CHO) evaluation.

UF pretreatment left 80% of the initial CHO in permeate at a flux rate of 10 Lm<sup>-2</sup> hour<sup>-1</sup>. Less than 10% of CHO was retained with TUF, at a mean flux rate of 20 Lm<sup>-2</sup> hour<sup>-1</sup>. This retentate was submitted to a 4h hydrolysis and on second TUF at a mean flux rate of 28 Lm<sup>-2</sup> hour<sup>-1</sup>, less than 1% of CHO was retained.

CE electropherograms revealed the existence of an OS mixture in the final retentate, the components of which are to be identified and the fraction tested for its functionality in a model gut system.

## Bioactive peptides from red deer (*Cervus elaphus*) milk

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Milk proteins are considered the most important commercial source of bioactive peptides and such information on milk from other animal species is very limited. Deer milk and its products have traditionally been used for medicinal purposes. Deer milk has more solid content ( $25.7\pm 0.76\%$ ) compared to cow's milk ( $12.1\pm 0.01\%$ ). Deer milk contains on average  $8.8\pm 0.13\%$  total protein which is twice the levels found in cow's milk ( $4.1\pm 0.02\%$ ). Casein content in deer and cow's milk are  $8.7\pm 0.13\%$  and  $4.0\pm 0.02\%$  respectively. Hydrolysis of milk protein by digestive enzymes and fermentation to produce biologically active peptides was investigated in the present study. To simulate the digestion process, deer and cow's milk were hydrolysed using an *in vitro* digestion system. The digestion was performed in two steps; imitating both the human stomach (Pepsin, pH 2.5) and the duodenum (Corolase PP, pH 7.5). Fermentation studies were also carried out on deer and cow's milk using *Lactobacillus belburueckii* subsp *bulguricus*, *Streptococcus salivarius* subs *thermophilus* and *Lactobacillus casi* strain Shirota. Release of peptides during milk digestion and fermentation was quantified using OPA (*o-phthaldialdehyde*). Changes in protein & peptide profiles were compared by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The immunomodulatory activity will be compared for cow's and deer milk peptides after FPLC fractionation.

## **Doped diamond-like carbon (DLC) coated surfaces to reduce fouling from milk**

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In the dairy industry, fouling of processing surfaces is a common and unresolved problem. Surface modification, for example through the application of a surface coating, can alter the surface properties of a material, and may be a potential way to reduce fouling. Typical dairy plant stainless steel surfaces were modified by the deposition of doped Diamond-Like Carbon (DLC) films with varying concentration of doped elements. These modified surfaces were studied for their fouling behavior with milk at both laboratory and pilot scale. None of the doped DLC modified surfaces investigated in the study presented benefits in fouling reduction as compared to unmodified surface.

## **Enhanced product recovery from drying air in milk & dairy spray drying processes by advanced CIP bag filter technologies**

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The off gas in an industrial milk drying plant is containing significant portions of valuable product. The need for a solid/gas separation process with the best available particle retention efficiency is therefore driven by the emission legislation as well as the product recovery rate and the elimination of cross-contamination. Compared to other separation equipment, like cyclones, the technology of jet-pulse cleaned bag filters shows significant higher particle retention efficiency and reduced operation costs (e.g. energy demand). The milk & dairy industries require a flexible operation which allows a batch production of different products. Additionally, explosive atmospheres have to be considered. CIP filters will meet these demands and combine a hygienic CIP'able design with the advantages of the jet-pulse filter separation technology. Clean gas concentrations of less than  $10 \text{ mg/m}^3$ , corresponding with product recovery rates from the dryer outlet air of up to 99,98% are achieved. Application examples include the product recovery and dedusting of the world largest milk spray drying plant. Based on this state of the art technology, the design limits of spray tower CIP filters were moved by the help of extensive CFD studies. The design and validation of the so called "long bag filter technology" which means bag lengths of 8 m, will be discussed. The improved design offers further advantages by reducing product losses during CIP, lesser consumption of CIP fluid and increased gas volume flow per footprint area of the filter.

## Compact protein particles to improve protein-enriched foods

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Protein-enriched foods are prone to sensory and stability defects as a result of extensive protein-protein interactions. In liquid systems, these defects are typically related to the formation of aggregates, which sediment and are perceived as sandy. In semi-solid and solid protein-enriched products, strong protein networks are developed which are perceived as tough or rubbery.

Compact protein particles were employed to reduce protein-protein interactions and compared for efficacy in three model systems. Particles varied in reactivity, surface properties, density, and particle size and shape. Aggregated particles were prepared by heating proteins in suspension and by ageing whey protein isolate powders in controlled moisture environments. Microparticulated particles were prepared by shearing, drying, and grinding protein gels. Three model systems were developed: a protein drink with 8-12% protein, a semi-solid spread-type product with 20% total protein, and a chewable protein bar with 40-60% protein. The model systems were prepared at neutral pH, using whey protein isolate as the primary protein source, and processed according to industry standards for the product category.

Results show that compact protein particles can be an effective means to reduce modulus and fracture force in gelled systems. The type of particle, and in particular its reactivity with the product matrix determines the extent of the effect. Whey protein aggregates prepared by heating in solution actively participate in the protein gel matrix and yield products similar to untreated whey protein. Microparticulated whey proteins, in contrast, reduce gel strength, possibly by introducing fracture points. In drinks, pre-treated particles can be used to reduce the increase in viscosity observed during heat treatment. QDA sensory testing is currently underway to check the effect of particles on sensory quality defects and highlights clear differences between particle types.

## **Emulsification of fish oil and phytosterol esters in water using milk proteins and dietary fiber: emulsion properties and oxidation stability**

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Delivering bioactive lipid components have become a popular topic in pharmaceutical and food industries in current decades. However, most efforts were focus on finding suitable systems to deliver a single component, e.g. omega-3 fatty acids, pigment and flavours. This work aimed to deliver fish oil with phytosterol esters (PE) in oil-in-water emulsion system using whey protein isolate (WPI) and sodium caseinate (NaCA) or WPI and soluble corn dietary fiber (DF) as emulsifiers. Physiochemical properties of emulsions including creaming stability index (CSI), droplet size, zeta-potential, microencapsulation efficiency (ME), rheological behaviour and oxidation stability of emulsion were studied. The results of creaming stability index (CSI), droplet size and zeta-potential showed that all the formulations were quite stable. However, there was a slight increase in droplet size and CSI with the increase of PE concentration. The microencapsulation efficiencies were largely dependent on the core materials as it decreased with increasing PE concentration. Results indicated that WPI and NaCA or WPI and DF had different emulsifying ability toward different lipid types. Fish oil was more efficiently encapsulated as it contained more unsaturated fatty acids (55% EPA and DHA), consistent with data reported previously. The emulsifier systems could protect the oxidation of fish oil and PE as reflected by the peroxide and p-anisidine values.

## **Investigation of the effect of milk proteins on the thickness and lamellarity of liposome using small angle x-ray scattering**

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The insertion and orientation of protein in lipid bilayer is critically depend on the bilayer thickness. Therefore, investigating the lipid bilayer thickness is important to understand the influence of milk proteins on liposomes. Bilayer thickness is usually obtained from small angle X-ray scattering (SAXS) experiments and the method is currently in development. SAXS also provides an indication of the lamellarity of a liposome population.

In this study, liposomes were formed with different proportions of casein or whey protein isolate (WPI) added before or after liposome formation, in the range of protein/phospholipid ratio of 0.02 to 0.1(w/w). The bilayer thickness and lamellarity of the samples were then measured using SAXS.

The average bilayer thickness of  $\sim 4.5$  nm (given by  $2\pi/\Delta Q$ ) was observed for all liposome samples regardless the ratio of protein/phospholipid. Neither casein nor WPI was found to influence the thickness of liposome bilayers when they were added before or after liposome formation. However, addition of milk proteins before liposome formation caused significant changes in the scattering at low-Q region ( $Q < 2 \text{ nm}^{-1}$ ) particularly as the protein/phospholipid ratio increased. This implies that milk proteins were bound onto liposome membrane or there were particle-particle interactions. There was no sign of Bragg peaks in any of the liposome dispersions, suggesting that only unilamellar liposomes were present in the all sample dispersions. However, a large number of multilamellar liposomes were observed in transmission electron micrographs. This implies that the overall proportion of multilamellar liposomes in the whole sample is low ( $< 20\%$ ), or that the inter-lamellar spacing is not uniform.

## Specific physiological indicators of *Lactobacillus bulgaricus* CFL1 cryotolerance

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Lactic acid bacteria are of great importance in the dairy industry because of their central role in the manufacturing of cheeses and fermented milks. Freezing is widely used for their long-term preservation, but often affects their viability and technological properties, such as acidification activity, organoleptic, textural and preservative properties (1). The initial physiological state of the cells during fermentation is described to play a role in their further cryotolerance (2, 3). Nevertheless, even if this information gives a valuable explanation, it is not able to predict the cellular behaviour after freezing and during frozen storage. In this context, this study aimed at identifying some key cellular parameters during fermentation to play a role as indicators of the cryotolerance of a cold-sensitive lactic acid bacterium *Lb. bulgaricus* CFL1. We therefore analyzed the proteome, membrane fatty acid composition, and acidification activity of cells recovered in 10 various fermentation conditions (pH and fermentation time), by respectively using two-dimensional electrophoresis, gas chromatography and the Cinac system. Corresponding physiological parameters were related to the cryotolerance of the cells. Statistical data analyses made it possible to identify specific physiological indicators of *Lb. bulgaricus* CFL1 cryotolerance at the population level (low acidification activity), the membrane level (high levels of specific membrane fatty acids such as C16:0 and cycC19:0) and at the cytosolic level (over-synthesis of elongation factor P, adenylate kinase and of numerous proteins of energetic metabolism, or under-synthesis of the ribosomal protein 30SS1 and the ABC transporter ATPaseC). Finally, this work demonstrated that it is possible to predict *Lb. bulgaricus* CFL1 cryotolerance from its initial acidification activity and from specific initial cytosolic or membrane compositions.

## **Natural states changes of cows' and buffaloes' milk proteins induced by microbial transglutamise**

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The percent incorporation of some amino acids in milk protein as a result of cross-linking by Microbial Transglutaminase (MTGase) was investigated. Effect of MTGase on electrophoretic patterns, microstructure, micellar hydration and sedimentable solids of milk proteins as well as the viscosity of whole and skim Cows' and Buffaloes' milk was also studied. Incubation of milk with MTGase at 40°C for 1h prior to thermal inactivation (at 80°C/2min) resulted in a complete incorporation of Glutamine and Arginine in skim Cows' milk protein and Glycine and Valine in skim Buffaloes' milk protein. That treatment also induced reductions in levels of monomeric caseins ( $\alpha_1$ -,  $\beta$ -, and  $\kappa$ -caseins),  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin and an increase in the fractions of relatively low electrophoretic mobility. The effect of MTGase on the microstructure of treated samples was quite clear; the enzyme was capable of forming covalent linkages between protein molecules. The micellar hydration and viscosity of treated skim milk samples were markedly improved and were the highest between the samples makes it possible to produce different types of dairy products with low fat contents or a reduced content of non-fat solids.

## **Relationships between morphology and rehydration properties of milk powders**

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The influence of the physical properties or the chemical composition of a dairy powder on powder functionality has been quite extensively investigated. The surface of the powder is expected to play also an essential role. For example, the surface was systematically found different from the bulk composition. In the present study, we investigated the influence of milk powder morphology on functional properties. Very little work has been carried out in this field for food powders whereas the literature was extremely furnished for minerals powders. Indeed, the morphology of the powder is expected to play also an essential role and should certainly not be neglected.

Recent and novel advances in image analysis methodology have been useful for quantitative evaluation of morphology and structure of food materials. In this study, a laser diffraction system coupled with an image analysis processor (QICPIC, Sympatec) was used for measuring the shape of a representative number of particles (more than 90000 per experiment). The following morphological parameters were investigated: mean diameter, convexity, elongation, sphericity, aspect ratio, straightness, etc. Concurrently, some functional properties related to powder hydration were determined following the FIL standards (wetting, dispersibility, solubility). Five different milk powders (whole milk, skim milk, low-fat milk, native micellar casein and native whey isolate powders) presenting different particle sizes (355, 250, 150, 75 and 45µm) were studied.

As expected, the instant properties of the powders were found dependent on powder composition but also on powder morphology. Then, from multivariable data analysis (Unscrambler v7.6) tentative models were developed; the objective being to predict from morphology parameters the rehydration properties of the powders. In conclusion, the Qicpic equipment could be considered as an attractive instrument to evaluate the quality of the powders.

## **Electrical Resistance Tomography for monitoring and control in milk powder processing**

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Simplicity, strong construction, high speed potential, and low cost are the main characteristics of Electrical Resistance Tomography (ERT), one of the most common modalities in process tomography, providing the user with the ability to perform non-invasive, remote internal inspection through volume scanning. This study describes the validation of ERT for qualitative visualization, quantitative analysis and attaining informative data, such as total solids content distribution in the processing of milk to produce milk powder. Although various methods of concentration measurement have been proposed, most have disadvantages including inaccuracy and sampling problems. No other existing methods have the possibility of providing a continuous spatially distributed image of the data variation. Such a multidimensional picture of the dynamic state of the process provides for fundamental process monitoring and control.

The methodology used for the validation of ERT for monitoring milk was to develop an appropriate strategy for the novel application on milk and validating the methodology by testing it on various concentration samples. The validated strategy was then used for monitoring a milk mixing tank and two operating variations related to it. Colour-coded cross-sectional views of the total solids content distribution of the tank were developed in MATLAB. The milk solutions used in this work were recombined skim and whole milk solutions commensurate with milk solutions processed in the front end of milk powder production plants.

This method provides an accuracy of more than 96% in most cases, which is similar to the accuracy of current methods of off-line point measurement, with the valuable advantage of providing continuous multidimensional data output which is critical for process control applications.

## **DNA methylation is associated with a suppression of $\alpha$ S1-casein gene expression during involution and infection of the bovine mammary gland**

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Factors such as physiological state and udder health influence bovine milk production. This study examined the role of DNA methylation in the silencing of milk protein gene expression during involution and mastitis. Recent studies suggest that DNA methylation, a stable epigenetic event, may also play an acute regulatory role in gene transcription. Alveolar tissue was obtained from non-pregnant cows in mid-lactation slaughtered at 0, 6, 12, 18, 24, 36, 72 and 192h (n=6/group) after the last milking. Five lactating heifers were intramammary infused in an uninfected quarter with approximately 1,000-1,500 colony-forming units of a wild-type strain of *S. uberis*. Quantitative RT-PCR analysis showed  $\alpha$ S1-casein mRNA was down-regulated (P<0.01) in involuting compared to lactating tissue and in *S. uberis*-infected compared to non-infected tissue. To elucidate the silencing mechanisms of  $\alpha$ S1-casein a quantitative MassARRAY methylation analysis was carried out. There was an increase (P<0.05) in methylation levels of 3 CpG sites at a functional STAT5-binding site of the  $\alpha$ S1-casein-encoding gene by 192 h (32-53% methylation) compared to 18 h post-milking (15-28% methylation). In infected tissue, there was an increase (P<0.05) in methylation levels of these 3 CpG sites (28-68% methylation) compared to controls (10-25% methylation). Results suggest that alterations in methylation status at CpG sites at the functional STAT5 binding site in the  $\alpha$ S1-casein promoter are associated with the silencing of this gene during involution and mastitis. Understanding the role of DNA methylation in regulating milk production may result in novel approaches and/or technologies for enhancing the lifetime lactation performance of dairy cows through manipulating epigenetic mechanisms.

## **The study of lactic acid and mix fermentation of demineralised whey**

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The majority of fermented milk products are produced from whole or standardized milk. Sometimes sweet whey may be used. Fermented whey beverages usually have liquid consistence and a peculiar taste and aroma.

The purpose of our research was to study demineralised salt whey as a potential raw material for fermented beverages.

Different types of demineralised whey have been compared. Skim milk samples were used as reference. Pure cultures of lactic acid bacteria: *Lactococcus lactis*; *Lactobacillus acidophilus*; *Streptococcus thermophilus* and *Lactobacillus bulgaricum* have been used as starters.

Starter culture with microflora of traditional Russian fermented milk product «Ayran» has been chosen to research mix fermentation process. Ayran's microflora consists of mesophilic and thermophilic lactic acid bacteria and several types of yeasts.

According to the results obtained the fermentation processes proceeding in demineralised whey and skim milk were similar. Although in demineralised whey the increase of acidity was less intensive and the clot wasn't formed. Some differences in organoleptic parameters of whey and milk samples were observed as whey had a specific taste. In samples fermented by *Lb. Acidophilus*, *Str. thermophilus* and Ayran's microflora whey taste was less evident, these products had a pleasant sour-milk taste and aroma.

The analysis of whey samples with different demineralisation level showed that whey with demineralization level 70 % had best properties.

Thus, demineralisation of salt whey enhances the production of high quality fermented whey beverages.

## **Micro filtration - The efficient spiral polymeric option**

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Microfiltration-MF has been used for dairy processing needs since the early 90's, specifically to reduce fat and lipids sufficiently to allow for concentration of protein to an isolate level, WPI 90 and above. This application is best suited for spiral polymeric membrane technology as total cost of ownership has been proven superior to other technologies, even with a protein yield reduction of up to 40%.

As more systems were built, work started to greatly improve this process, reducing this loss as much as possible. This resulted in system design and element changes that reduced this loss to single digits, and additionally opened more application possibilities. This new application concept has proved itself for more than 6 years in some locations.

Today new opportunities for dairy products exist, the use of separated milk components as unique ingredients for more efficiently manufacturing such as with traditional cheese and the soft cheese food varieties, and exciting new products like MSP, the native milk or Serum Protein offering functional advantages not possible with WPC and WPI.

MF membrane technology is the backbone of this process separating milk components. Efficient MF membrane is the critical component in this opportunity, and the improvements made in the application of spiral MF membrane play a major role in the commercialization of this application.

This poster will review MF design and element changes that improve the separation efficiency of cross flow systems, and speak to the yield expectations that can be reached with appropriate designs, specifically for the whey fat removal and milk component separation processes mentioned above.

## Interactions of milk protein-stabilized oil-in-water emulsions in a sequential simulated oral-to-gastrointestinal model

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The aim of this study was to understand the behaviour of milk protein-stabilized emulsions (1.0 wt% protein) as they pass through a model oral-to-gastrointestinal system. Oil-in-water emulsions (20.0 wt% soy oil) stabilized by lactoferrin or  $\beta$ -lactoglobulin ( $\beta$ -lg) were prepared at pH 6.8 using a two-stage valve homogenizer to produce cationic or anionic interfaces respectively. Behaviour of the emulsions as influenced by sequential treatment with artificial saliva, simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) (modelling in terms of pH, electrolytes, surfactants and enzymes) was investigated. The emulsion droplets were characterized at each step of simulated digestion using light scattering, confocal laser scanning microscopy, free fatty acid release, and electrophoretic mobility measurements. Broadly, both the protein-stabilized emulsions showed extensive droplet flocculation, and some degree of coalescence followed by disruption of droplets as they passed through the *in vitro* digestion model. The  $\beta$ -lg-stabilized emulsion underwent charge reversal in presence of SGF due to the extremely acidic conditions (pH 1.5). Upon exposure to SIF (pH 7.5), both  $\beta$ -lg and lactoferrin-stabilized emulsion droplets became negatively charged. Except in the simulated oral environment, the initial charge of the interface had relatively limited influence on flocculation behaviour during the simulated digestion. In both the emulsions, competitive interfacial displacement of initial protein layer by bile salts and pancreatin-induced lipid digestion products (fatty acid, mono-and/or diglycerides) was confirmed. These results contribute to the knowledge of how structure and charge of the emulsified lipid droplets are influenced by digestion at various stages and thus might have important consequences for developing suitable microstructures for controlled delivery applications.

## Microfiltration (MF) of skim milk (SM) for separation of serum proteins (SP) from micellar casein

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The series of studies were carried out with the main goal to evaluate the performance of different MF membranes (ceramic 0.1  $\mu\text{m}$  uniform transmembrane pressure (UTP), ceramic 0.1  $\mu\text{m}$  graded permeability (GP) and polymeric 0.3  $\mu\text{m}$  spiral-wound (SW)) when used for separation of SP from micellar casein. Significant ( $P < 0.05$ ) differences in flux were detected between the systems with the highest flux observed for GP for all 3 stages (72.5, 84.5, 92.7  $\text{kg/m}^2$  per hour in 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> stage, respectively), followed by UTP (54.0, 54.0, 54.6  $\text{kg/m}^2$  per hour in 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> stage, respectively) and SW (14.4, 22.1, 32.6  $\text{kg/m}^2$  per hour in 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> stage, respectively). The SP removal was higher ( $P < 0.05$ ) in the 1<sup>st</sup> stage (continuous bleed and feed 3X microfiltration at 50°C) of the UTP system than GP (63.7 vs. 56.0 for UTP and GP, respectively) with 2<sup>nd</sup> and 3<sup>rd</sup> stage (diafiltration of the retentate diluted with pasteurized reverse osmosis water in 1:2 ratio) being higher ( $P < 0.05$ ) for GP system (26.7 vs. 21.9%, 13.8 vs. 9.7%, respectively). No difference ( $P > 0.05$ ) in cumulative percentage of SP removal was detected for GP and UTP membranes, 96.5 and 95.2%, respectively. In similar 3-stage MF, polymeric SW membrane removed 38.6, 20.8, and 10.9% of SP in the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> stages, respectively for an overall SP removal of 70.3%. GP membranes had a higher ( $P < 0.05$ ) SP removal rate ( $\text{kg/m}^2$  per hour) for 3 stages than UTP and SW membranes: GP 0.69 and UTP 0.45 and SW 0.12  $\text{kg/m}^2$  per hour.

## Isolation of a highly heat-resistant spore former from UHT-treated milk in Iran

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Highly heat resistant spores (HRS) which survive industrial sterilization or ultra-high temperature (UHT) have emerged an important problem in the dairy industry. After passing into the final product, these spores can germinate and grow during storage and cause the problem of nonsterility. These mesophilic bacteria which differentiate into HRS were detected first in UHT-treated milk from southern Europe, but the problem subsequently spread to countries in and outside Europe. During an investigation of sterility of UHT milk, a HRS former was isolated and characterized for the first time in Iran. The mean colony count of contaminated packages after 15 days incubation at 30°C was  $3.5 \times 10^4$  CFU/ml. This density did not affect the pH of the milk and neither did the sensory quality. Cells of isolated strain are gram positive, aerobic rods which produce subterminal oval endospores in swollen sporangia. The sequence of almost 1500 nucleotides of 16S rRNA gene was determined. The alignment of this sequence with described species showed less than 90% similarity to *Bacillus sporothermodurans*. Based on the obtained results the isolated strain could be a novel *Bacillus* species.

## ***In vivo* digestion of infant formula: protein digestion kinetics and release of bioactive peptides**

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Infant formula (IF) are major sources of proteins for the neonate when breast-feeding is not possible. During IF manufacture, protein structures are modified by processing conditions. Further changes of these modified structures in the digestive tract of a newborn remain unknown.

The aim of this work was to study IF milk proteins digestion over time in the stomach, jejunum and ileum of 21 piglets (taken as a model of human neonates), and to identify the bioactive peptides present in the small intestine.

An experimental IF reaching the nutritional requirements of the piglet was manufactured in a pilot plant. Piglets were fed the IF during 28 days and slaughtered 0h30, 1h30 and 3h30 after the last meal respectively. Contents of the stomach, jejunum and ileum were collected, weighed and pH was measured. Digestive contents were analysed for milk proteins and peptides using SDS-PAGE, ELISA, immunoblotting and mass spectrometry.

Half an hour after the last meal, caseins were extensively hydrolysed in the stomach. In contrast,  $\beta$ -lactoglobulin partially resisted to digestion whereas  $\alpha$ -lactalbumin showed an intermediate behaviour. Milk protein concentrations decreased, with time and along the GI tract. Surprisingly, large protein fragments were shown to resist digestion and were detected in the ileum.

Peptides resulting mainly from  $\beta$ -CN hydrolysis were identified by mass spectrometry in jejunum 0h30 and 1h30 after the last meal; some of them exhibit a biological activity like  $\beta$ -CN (f60-66), known to exert anti-hypertensive and opioid activities.

## **Sustainability, nutrition and health**

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Over the past several years, the topic of sustainability has garnered much attention among professional colleagues as well as consumers. Increasingly, considerations such as locally grown, eco-friendly, organic, or carbon footprint are factored into the debate regarding sustainable and responsible food choices. Individuals representing health care, food service management companies, chefs, and retail corporations as well as celebrities and politicians decry food production practices that exact a heavy environmental toll. Animal agriculture has been a favored target. Frequently, however, such discussions are cloaked in sensationalistic terms; misperceptions and inaccuracies around current agricultural practices are often propagated. Additionally, it is the rare discussion where the nutritional profiles of individual foods are considered in the context of the environmental costs.

Dairy Council of California (DCC) sought to broaden and balance the dialogue around sustainability, nutrition and health. In fall of 2009, DCC contracted with Marianne Smith-Edge, MS, RD, LD, FADA, a former president of the American Dietetic Association, to develop an online continuing education course on this topic in conjunction with dairy council staff.

The course includes definitions of sustainability and what this movement encompasses, the background of how the movement started, recent trends, how the consumer perceives sustainability, and the role of the practicing health professional. Sustainable practices are described from farm to retail to food service to what consumers can do at home, with an emphasis on *all* aspects of the sustainability chain rather than on one specific component. The slides include practical suggestions for how health professionals can encourage clients to consider the bigger picture of making sustainable food choices that include not just food production and packaging outcomes, but health outcomes as well.

This multi-media module includes a 45 minute PowerPoint slide presentation with audio and print PDF documents to review. Those documents include five reference articles and a consumer market research report for the Hartman Group. Additional references are included in a bibliography, as well as the examination which must be successfully completed. The course is approved for 4 hours of continuing education credit for registered dietitians, dietetic technicians registered, certified dietary managers and certified health education specialists.

Over 100 individuals have completed the course for continuing education credits in the few months since it was launched at the end of March, 2010. DCC will be collecting course evaluations from the administrators, Nutrition Dimensions, and will have that evaluation data available to share in a poster presentation in November, 2010.

## **The influence of whey protein and peptides on satiety in humans**

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The prevalence of obesity has increased rapidly in recent years. Obesity is associated with increased risk of disease such as type 2 diabetes, cardiovascular disease and hypertension. The mechanisms that regulate appetite and weight control, particularly satiety, may play a key role in the safe and effective treatment of obesity. It is widely believed that protein is more satiating than either carbohydrate or fat, which may help facilitate weight loss over the long term. However, within proteins, the effect on satiety appears to be dependent on the source of protein. There is some evidence that dairy whey protein produces a stronger effect on satiety compared to other protein sources. The ingestion of protein preloads reduces food intake at a later meal but is dependent on the time interval between the preload and the test meal. In the present study, a water control and two preloads were administered at 30, 60 and 120 minutes before an *ad libitum* single test meal. On 9 separate occasions, twenty-one healthy women received a standard breakfast, a preload drink and lunch. The preload drinks were presented as a milkshake with either maltodextrin carbohydrate or whey protein isolate. Satiety was determined subjectively using visual analogue scales (VAS) and objectively by measuring energy intake at the test meal. Significantly less energy was consumed at lunch after the whey protein preloads compared to the control and carbohydrate preloads at each time interval. However, this was not sufficient to show complete energy compensation. Satiety ratings varied significantly by preload type. These findings are in line with previous reports that protein is more satiating than carbohydrate.

## **Satiety effects of whey protein-enriched water beverages in overweight women: a dose response study**

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Protein is recognised as the most satiating macronutrient. There is currently little information on whether satiety is maintained when proteins are consumed as beverages. This study investigated the dose-response of whey protein-enriched water beverages on post-ingestive satiety and energy intake [EI] in overweight women.

Using a cross-over design 46 participants completed four 500mL pre-load treatments [0%,8kJ; 1%,93kJ; 2%,178kJ; 4% w/w,348kJ ClearProtein™] at least 3 days apart. Following a standard evening meal and breakfast, beverages were consumed 120 mins before an *ad libitum* lunch, where EI was measured. Feelings of hunger, fullness and satisfaction were measured using visual analogue scales (VAS).

There was a significant effect of beverage pre-load on hunger (treatment\*time;  $P=0.0074$ ), whereby the 1%, 2% and 4% protein treatments decreased hunger compared to the water control during the 2h following the beverage ( $P<0.05$ ). Similarly, fullness (treatment\*time;  $P=0.0020$ ) and satisfaction (treatment\*time;  $P=0.0356$ ) were significantly increased by 1% and 4% protein treatments. The 4% beverage decreased EI at the *ad lib* lunch by 8 percent compared to water control but did not reach statistical significance (0%,3028kJ; 1%,3080kJ; 2%, 2924kJ; 4%,2781kJ,  $P>0.05$ ).

Whey protein-enriched water beverages impacted positively on key measures of VAS-assessed satiety, but had no impact on EI.

*Parts of this research have been previously presented at the International Congress of Nutrition; Bangkok, October 2009*

## Development of a “shot” delivering 500 mg EPA: DHA per serving

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Omega 3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), provide a range of health benefits including reduction of the risk of coronary heart disease, and prevention of neurological and psychological disorder. The best source of omega-3 fatty acids is oily fish. The recommended daily intake of EPA: DHA varies from 450 mg (UK) to 520 mg (USA). However, most of the fortified food products available in the market contain only 50 – 80 mg omega-3 per serving. The major challenge for omega-3 fortification in food and beverage at higher level is the development of off-flavour due to oxidation of unsaturated fatty acids during processing and storage. To overcome this problem, a novel emulsion was developed, using milk proteins as natural antioxidants. A patented blend of sodium caseinate/whey protein mixed in specific combination and heat processed, was mixed with fish oil and homogenised to produce an emulsion (30% oil and 4% protein) [Patent: WO 2006/115420 (Singh, Ye and Zhu)]. This emulsion was formulated to a “shot” with other ingredients, such as, sugar, a combination of gums, foods grade acid, flavours, antioxidants, preservative and colour to deliver 500 mg EPA: DHA per 6 ml. The product did not have any perceivable fishy flavour. The product pH was adjusted to 3.2 – 3.4 and contained 2% milk protein. The process was designed to ensure minimum level of oxidation, and scaled-up to commercial plant level. The shot is packed in oxygen and moisture resistant sachets, and stable for 6 months under refrigeration. It can be consumed either directly or by mixing with yoghurt.

## **Immunomodulatory effects of probiotics bifidobacteria**

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Probiotics are currently defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host”. *Bifidobacterium longum* BB536 is a probiotic strain originated from a healthy infant and has been broadly applied in food industry. The present presentation shows the results of the efficacy of BB536 in the prevention of influenza virus infections, and in the treatment of allergic disorders.

In a pilot trial with elderly volunteers, we found that continuous administration of BB536 reduced the occurrence of influenza virus infection and fever as well as enhanced and maintained cellular immunity such as NK activity and the bactericidal activity of neutrophils. In mice, oral administration of BB536 suppressed the symptom development, body weight loss after influenza virus infection and virus proliferation in lung. These results demonstrate the efficacy of BB536 in protecting against influenza virus infection.

Japanese cedar pollinosis (JCPsis) is an immunoglobulin E -mediated type I allergy caused by exposure to Japanese cedar pollen. In human trials, BB536 alleviated subjective symptoms of JCPsis and suppressed a Th2-skewed immune response associated with pollen dispersion. Furthermore, it was found that some intestinal bacteria such as the *Bacteroides fragilis* group fluctuated significantly during the pollen seasons and BB536 intake suppressed the fluctuation. These results suggest the efficacy of BB536 in relieving JCPsis symptoms, probably through the modulation of Th2-skewed immune response and through the improvement of intestinal microbiota.

These studies suggest the immuno-modulating effects of probiotics bifidobacteria and its efficacy in the prevention of influenza virus infections and in the treatment of allergic disorders.

## **ACE inhibitory peptides of milk origin and their role in proliferation and differentiation of bone cells**

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Angiotensin Converting Enzyme (ACE) inhibitory peptides are the bioactive peptides, produced mostly from casein by trypsin digestion. These peptides mostly contain 2-12 amino acids and characterized by presence of hydrophobic amino acids at the C- terminal positions. Recently, it has been reported that ACE inhibitory peptides has a role in bone cell formation. In the present investigation, ACE inhibitory peptides from buffalo milk were isolated, partially characterized and their role in osteoblast proliferation and differentiation was studied. The ACE inhibitory peptides were isolated from different fractions of casein. The electrophoretic pattern revealed that ACE inhibitory peptides had molecular weight of  $\leq 3$ KD. The RP-HPLC profile of  $\alpha$ ,  $\beta$  and  $\kappa$  casein derived ACE inhibitory peptides showed 4, 6, and 5 major peaks, respectively. The RP-HPLC profile also revealed that the hydrophobic peptides obtained were more in  $\beta$  casein derived peptides as compared to  $\alpha$  and  $\kappa$ . *In vitro* culture of bone marrow cells in presence of ACE inhibitory peptides at 0.05 and 0.25mg/ml concentration stimulated the proliferation and differentiation of osteoblasts which was confirmed by Alizarin staining and MTT proliferation assay. Likewise, significant ( $p < 0.05$ ) increase in the m-RNA expression of osteoprotegerin, alkaline phosphatase and osteocalcin has also been observed in treatment groups as compared to the control. These results showed that the casein hydrolysates depicting ACE inhibitory peptides are anabolic to bone, an effect that is consequent upon their potent proliferative and differentiated actions in osteoblasts.

## **Consumer Education Project on the health benefits of dairy in South Africa – a multi disciplinary approach**

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The Consumer Education Project is an initiative by Milk South Africa that was formed to promote awareness of health and nutritional benefits of milk and other dairy products to South Africans. The initiative was inspired and shaped by the outcomes of a comprehensive market survey which was conducted to obtain a better understanding of current dairy consumption patterns by all population groups in South Africa.

South Africa is a developing country with a population of almost 50m people, consisting of four different population groups i.e. black African, coloured, Indian or Asian and white. Milk production in South Africa currently stands at approximately 2500m litres per annum.

The South African Advertising Research Foundation, has developed a marketing research tool, Living Standards Measure (LSM), which segments the population into 10 LSM groups people according to their standard of living. LSMs are used by marketers to gain a better idea of the socio-economic status of an individual or group.

The communication campaign consists of two elements i.e. general and specialised communication. General communication includes messages aimed at the public at large. Consumers that fall in the LSM 6-8 groups have been identified as the primary target audience. Consumers in the LSM 9-10 groups, with higher dairy consumption than in the lower LSM groups, are also targeted. Six key messages regarding the health and nutritional advantages of dairy products have been developed, which are translated into television and print advertisements, promotional articles and leaflets, including a website.

Specialised communication includes proactive and reactive messages aimed at opinion formers in the South African society i.e. health professionals such as doctors, dietitians and nurses. Latest research in dairy nutrition is translated in the editorial articles which are published in health journals. A school education campaign reaches school learners through educational material based on balanced nutrition, which is developed for the teacher to use in the classroom.

## **Yogurt in the diet may combat obesity**

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Based on the book “The Yogurt Diet,” by author Ana Luque, this poster will emphasize the great importance of dairy in the diet – specifically fermented dairy products – as more evidence emerges linking obesity to the quality of bacteria in the intestines. Luque’s book is based on the theory that the gut flora is the heart and soul of one’s health and plays a major role within the immune system. The connection between the variations in gut flora of obese and non-obese individuals may hold the answer to not only combating obesity, but a number of other illnesses as well.

Probiotic bacteria in the diet are absolutely essential to maintain overall health and weight. The Yogurt Diet recommends eating fermented dairy products three times a day along with a balanced, healthy diet.

GermS in the gut may play an important role in driving appetite, according to new research reported in the journal *Science* (March 4, 2010) at Emory University. Previous studies have shown that overweight people and those of normal weight harbor different types and amounts of microbes in the flora living naturally in the intestine. The studies also show that mice with an altered immune system – likely due to variations in gut flora – were fatter than healthy mice and had a collection of disorders, such as high blood pressure, cholesterol and insulin problems.

Altered immune systems in these mice were the result of different bacteria growing in their intestines than in those of normal rodents – driving larger appetites, metabolic syndrome and a low-grade inflammation believed to be a root cause of obesity. The addition of fermented dairy products to the diet has been shown to yield promising results.

## **Casein maintains higher post-absorptive muscle protein synthesis rates than milk soluble proteins in energy restricted rats**

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During obesity, severe energy restriction leads to fat mass loss but also to lean mass loss. Our aim was to compare the capacity of casein and milk soluble proteins (MSP) to modulate protein metabolism in order to limit energy restriction induced lean body mass loss. Male Wistar rats were fed ad libitum during 5 weeks with a high fat high sucrose diet and then were fed a restricted amount of a high protein (32 %) diet containing either casein, MSP, or a 50/50 mixture of both (n=10 per group) during 3 weeks. We followed food intake, body weight, nitrogen balance, creatinine and 3-methyl-histidine (3MH) excretion during energy restriction. After 3 weeks, in the post-absorptive state, we measured tissue weights, plasma metabolic parameters (amino acids, glucose, insulin, cholesterol, triglycerides), and in vivo liver and EDL muscle protein synthesis rates. Although there was only 4 to 11% differences, mean dry matter intakes over the restriction period were significantly different between groups ( g/d - mix:  $10.98 \pm 0.13$ ; casein:  $10.55 \pm 0.06$ ; MSP:  $9.78 \pm 0.10$ ). Comparing results obtained in casein and MSP rats, the only significant differences were a higher intestine weight, a higher plasma post-absorptive leucine concentration, a higher fecal nitrogen excretion, and higher EDL muscle post-absorptive protein synthesis rates in casein rats. Feeding a mixture of casein and MSP had similar effects than MSP feeding. In conclusion, although casein maintained higher rates of post-absorptive muscle protein synthesis, it was not sufficient to induce a better nitrogen balance or higher final muscle weights.

## Effects of dairy products on Crohn's Disease symptoms in an Auckland population

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Crohn's Disease (CD) is a form of inflammatory bowel disease which can affect any location of the gastrointestinal tract, resulting in considerable morbidity. Dairy products are often avoided by individuals with CD for fear of symptom aggravation. Unnecessary avoidance of dairy products may exacerbate the already elevated risk of decreased bone mineral density in this population. This study aimed to evaluate self-reported effects of dairy product consumption on symptoms in an adult CD population. This study was based on analysis of dietary questionnaires completed by adults with CD who participated in the 'Genes and Diet in Inflammatory Bowel Disease' study in Auckland, New Zealand. In the questionnaire, participants indicated whether particular foods had a beneficial, adverse or lack of effect on CD symptoms. Survey data for consumption of ruminant milk, yoghurt, butter, custard, ice cream, cream and cheese was evaluated. Dietary data for 165 participants (116 females, 49 males) was analysed. Of this sample, 72.5% reported experiencing no adverse reaction to products containing milk. When categorized by type of dairy product consumed, no difference in CD symptoms was associated with butter, standard cow's milk and reduced fat cow's milk in 71.5%, 64.2% and 58.2% of participants respectively. Dairy products most frequently associated with worsening CD symptoms were cream (43.6%), ice cream (37.6%) and cheese (34.5%). Conversely, yoghurt was perceived as beneficial in 14.5% of subjects. In conclusion, consumption of dairy products most frequently made no difference to CD symptoms within this Auckland sample. The range of beneficial and adverse CD affects reported for different dairy foods reflects the individual nature of dairy product tolerance in this clinical population.

## **Supplementation with complex milk lipid gangliosides in healthy infants**

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Gangliosides are complex glycosphingolipids which make up approximately 10% of brain lipids. They are found in serum and in many tissues in the body, however they are most concentrated in the brain. Gangliosides play a role in the formation of neuronal synapses and their functions in the process of neural transmission. Human milk contains higher levels of gangliosides than infant formula and may be conditionally essential. In fact breastfed infants have higher brain ganglioside levels than infants who were fed standard infant formula. This study aimed to determine the impact on ganglioside status in normal healthy infants who received infant formula supplemented with gangliosides from complex milk lipids, using a double blind randomized controlled clinical trial design. The control group (n=30) received standard infant formula and the treatment group (n=29) received the same formula supplemented with complex milk lipid to increase the ganglioside content to approximately 10ug/ml. The reference group (n=32) consisted of normal healthy exclusively breastfed infants. The impact of supplementation on cognitive development has been reported elsewhere. Ganglioside supplementation using complex milk lipids significantly increased ganglioside serum levels. This suggests that fortifying infant formula with a Complex Milk Lipid ingredient is a useful way to enhance ganglioside content in infant formula and to improve ganglioside status of young infants. This may in turn contribute to their improved cognitive development.

## Evaluation of Aflatoxin M1 in raw milk in west of Iran

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Aflatoxins may be produced by three species of *Aspergillus*– *A. flavus*, *A. parasiticus*, and rare *A. nomius*-that contaminate plants and its products. Aflatoxins are both acutely and chronically toxic for animals and humans, and can produce dangerous illnesses including acute liver damage, liver cirrhosis, tumor induction and are also teratogen. AFM1 is a minor metabolic product of *Aspergillus flavus* and *Aspergillus parasiticus*. However, it occurs in dairy products as a metabolite formed in cows from aflatoxin B1 contained feed. The aim of this study was to determine the AFM1 concentration in raw milk in west of Iran.

AFM1 was determined in the 80 samples of raw milk and described by National Standard No 4925, “Determination of AFM1 in milk and milk products” ISIRI, using ELISA and HPLC method to prove the results.

Aflatoxin M1 was found in 100% of the examined milk samples. 73 samples (91/25%) had contamination more than 50 ng/l of AFM1, 40 samples (50%) contained 50–100 ng/l, 33 samples (41/25%) contained more than 100 ng/l and the remaining, 8/75% of samples contained less than 50 ng/l of AFM1. In general, regardless of the 8/75% of the samples that are in borderline limit (45–50 ng/l), the amount of AFM1 in 91/25% of the samples was higher than the maximum tolerance limit (50 ng/l) accepted by European Union. Contamination with AFM1 is a serious problem for public health. Contamination with AFM1 in our region is more than standard levels. Infants and children are at greater risks. To achieve a low level of AFM1 in milk, cow feed samples from various cowsheds must be evaluated routinely for aflatoxin and those with excess contamination should be discarded to keep the dairy cow feeds away from fungal contamination as much as possible.

## **New method for preparation of lactose ureide from lactose or whey permeate**

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The feeding of non-protein-nitrogen (NPN) to ruminants is well documented. Urea is used for NPN source and has generally been aimed at simply reducing the release rate of ammonia. The lactose ureide (LU) is a compound which has received some attention as possible ruminant feed supplements, mainly because ammonia is released from them more slowly than from urea. On the other hand, LU is metabolized more slowly than free lactose would be. The present work was done to develop suitable preparative technique of LU from lactose or whey permeate as a starting materials.

The detection, estimation and identification of LU were carried out by TLC and MS spectrometry. Yield of LU were studied on the proportions of lactose and urea in the mixture and type of acid catalysis ( $H_2SO_4$ ,  $H_3PO_4$  and citric acid). Yield of LU crystal for given amounts of whey permeate using  $H_2SO_4$  was similar to that of  $H_3PO_4$ . In view of the safety, corrosion and pollution control, a study was made of the citric acid as catalysis. Almost similar yield was obtained when citric acid was employed, but reaction time was prolonged under the given conditions.

Most method reported for preparation of LU is intended for small-scale laboratory preparation. Lactose in whey permeate (approximately 90 % lactose in dry matter) may be of economic importance for ureide formation. It seems from the present study that LU could be formed by reacting whey permeate and urea which may be a potentially useful feed supplement for not only ruminant but also other mammals or chicken.

**Functional and microstructural properties of low fat mozzarella cheese as affected by exopolysaccharides-producing *S. thermophilus* and storage conditions**

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The objective of this work is to improve the functional properties of low fat mozzarella cheese and to extend its shelf-life via the use of exopolysaccharides (EPS) producing starter cultures and different freezing conditions. Conventional starter (non EPS producing *S. thermophilus* St CH-1 in association with *L. helveticus* Lh CH-5) with or without fat replacer (Simplese.S-100), and two EPS-producing strains of *S. thermophilus* SFi-12 and SFi-39, each in combination with non-EPS *L. helveticus* Lh CH-5 were used in manufacture of low fat Mozzarella cheese. The resultant cheeses were ripened at 4°C for 28 days. The freezing schemes were as follows: a) Cheese ripening for 14d at 4°C before freezing at -20°C for 60 days, then again ripening at 4°C for 14d., and b) Cheese freezing immediately after manufacturing at -20°C for 60 days then ripening at 4°C for 28d. The results showed that cheeses made with EPS-producing *S. thermophilus* had significantly ( $p < 0.05$ ) higher level of moisture and moisture in non fat substances (MNFS) as compared with non-EPS cheeses with or without simplese when fresh or during the refrigerated storage period. In addition, the meltability and fat leakage values of EPS-cheeses were the highest among all experimented cheeses and their interactions. However the results showed that frozen EPS cheeses retained the highest MNFS and TA at the end of frozen schemes. Meltability and fat leakage of experimented frozen cheeses have increased upon ripening at two stages or one stage. Scanning electron micrographs of the refrigerated cheeses showed that the EPS-cheese was porous and had an open texture with numerous voids, whereas the non EPS cheeses had a closed and compact protein matrix. There were no marked differences between the microstructure of refrigerated and frozen cheeses, which had a large voids in the cheese matrix.

## **Extraction, milk clotting activity measurements and purification of *Solanum dubium* Fresen (Gubbain) for cheesemaking**

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This investigation was carried out in order to extract a milk clotting enzyme from *Solanum dubium* Fresen, determine its milk clotting activity and purify the enzyme. The plant material used in this study was collected from Shambat area, Khartoum north, Sudan. The fruits, coats and seeds of *Solanum dubium* (Gubbain) were separated from each other, carefully cleaned and then coarsely powdered using an electric grinder. The enzyme was extracted using four methods, and the enzyme activity was determined. The proteolytic activity of the enzyme was measured and purification was carried out with ammonium sulphate (0-90% saturation). Results indicated that *Solanum* seed extracted with distilled water had the highest milk clotting activity and lowest coagulation time, while both *Solanum* seed and fruit extracted with 5% NaCl had the lowest activity and no clot was observed after 5 minutes. Ammonium sulphate saturation range of 40-50% for *Solanum* seed extracted with distilled water gave the highest milk clotting activity (827.58 su/ml), yield (2.7%) and purification fold (1.88). The partially purified enzyme was chromatographed in a column of Sephadex G-100 and the purification exhibited two peaks of proteolytic activity.

## Development of a novel multilocus sequence typing scheme for diversity analysis of *Geotrichum candidum* strains

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*Geotrichum candidum* is a dimorphic yeast commonly inoculated on surface-ripened cheeses. Technologically-relevant physiological attributes of *G. candidum* are strain-specific, so analytical tools are essential for intraspecific differentiation. The aim of the present study was to characterize the genetic relationship among 18 *G. candidum* strains isolated from various environmental niches and to evaluate the correlation with their environmental origins. We developed a novel multilocus sequence typing (MLST) scheme based on intragenic variations between six selected housekeeping genes (loci): *Ala1*, *Cdc19*, *Pgi1*, *Gln4*, *Pgm2* and *Erg10*. For the first time, we have determined the complete sequence of these genes for the 18 strains of *G. candidum*. Beforehand, strains were characterized by polymorphism analysis of the complete rDNA operon sequence and by random amplification of microsatellites by PCR (RAM-PCR). We determined that all *G. candidum* strains were closely related based on the rDNA sequence analysis and could be classified as members of de Hoog and Smith's Group 1. Four RAM-PCR primers were tested, but only (GATA)<sub>4</sub> generated distinguishable PCR patterns that allowed to differentiate 4 strains. In contrast, MLST showed an efficient and reliable discriminatory ability. Twenty-nine polymorphic sites were identified among 6 loci chosen for the MLST scheme. Three to 7 genotypes were observed at different loci. The sequence analysis of the contigs showed 11 sequence types, 8 of them represented by only one strain. A UPGMA dendrogram showing genetic relatedness among *G. candidum* strains revealed that they grouped according to their origin. MLST allows precise identification and characterization of the strains and easy comparison and exchange of results obtained in different laboratories.

## **Technological developments in Paneer, a soft variety of cheese**

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Paneer, a soft variety of cheese, is highly popular in Indian subcontinent. It is an acid coagulated dairy product consumed as fresh in various forms. The phenomenon of coagulation involves the formation of large structural aggregates of proteins in which milk fat and other colloidal and soluble milk solids are entrained with whey. Paneer on an average contains about 54% moisture, 25% fat, 17.5% proteins, 2% lactose and 1.5% minerals. It has many uses starting from its consumption in raw form to preparation of several varieties of culinary dishes and snacks. Good quality paneer is characterized by a white color, sweetish, mildly acidic, nutty flavor, spongy body and close knit fibrous texture. Buffalo milk paneer has all these attributes, hence preferred for paneer making. Good quality paneer is obtained by heating milk to about 90°C, acidifying the hot milk by adding citric acid solution followed by removal of whey and pressing of the curd before cooling the pressed mass in chilled water. Traditionally paneer is prepared by batch method. A lot of R & D work has been done on paneer that include development of a mechanized method, use of UF process, identification of cheaper coagulants and ingredients, manufacture of many variant of paneer including low fat paneer and shelf life extension. The use of UF retentate helped increasing the yield and could be amenable to develop long life paneer. The suitability of hydrochloric and phosphoric acid was also attempted with a view to replace costly citric acid as coagulant. The use of citric acid in naturally soured whey reduced the requirement of citric acid and increased the solids recovery without any loss of paneer quality. All these aspects will be discussed in the present presentation.

## **The synergistic effect of Xanthan and Locust Bean Gum in model processed cheese**

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The synergistic effect obtained by mixing Xanthan (X) and the galactomannan locust bean gum (LBG) was examined for a model processed cheese formulation. X and the blend of 50% X and 50% LBG (X: LBG blend) were used to modify the texture of model processed cheese. Model processed cheese made from rennet casein, soya oil, trisodium citrate and lactose was formulated on a small-scale (30g) using a cylindrical mixing vessel with a single stirrer that controlled the temperature, stirrer-speed and timing. The protein content of model processed cheese varied from 8.0 to 12.0% and the polysaccharide content varied from 0.0 to 2.0%. Trends in modulus of deformability, adhesion area, temperature sweep and microstructure were determined to evaluate the effect of polysaccharide on the texture of processed cheese. Modulus of deformability was higher at the same polysaccharide concentration by a factor of 1.2 to 1.6 for X: LBG blend than for X. This indicated a strong synergistic effect of the X: LBG blend over X. In addition, at the same polysaccharide concentration, the X: LBG blend had lower adhesion area than X. The flow behaviour of this cheese with X and X: LBG blend was studied over a temperature range of 5° C to 85 ° C. The crossover temperature ( $G' = G''$ ) was not significantly influenced by the type of polysaccharide. However, X: LBG blend had improved flow behaviour (higher values of  $\tan \delta / G'$ ) at higher temperature compared to X. The microstructure of the model processed cheese with polysaccharide was examined using confocal laser scanning microscopy with labelling for polysaccharide, protein and fat.

## **Monitoring starter culture activity and phage sensitivity – a comparison of conventional continuous pH measurement with fluorimetric and colourimetric microplate methodologies**

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The milk activity (lactic acid production rate) of *Lactococcus lactis* starter cultures is a key feature that determines their use for the manufacture of cheese and other fermented dairy products. The simplest laboratory tests to assess milk activity involve inoculation of a reconstituted skim milk medium with a strain, incubation over a cheese temperature profile and the determination of pH after a set time (e.g. 5 hours). Similar tests have been used to predict temperature and salt concentration effects and to monitor the effects of seasonal milk variation and starter culture manufacturing procedures (freezing, lyophilisation). A drawback of such tests is that the information gained from a single pH reading is limited. Continuous monitoring of lactic acid production is a preferable alternative, since it provides more information with the potential to calculate a range of kinetic parameters. In this investigation three methods for continuous monitoring of starter culture activity in milk were evaluated. The ELIT 8-channel ion/pH analyser is able to continuously monitor pH (NICO 2000 Ltd, Harrow, U.K.). The L\*a\*b\*SMART reflectance colourimeter system monitors the L\*a\*b\* value of milk containing a pH sensitive dye (LabSMART, L.L.C., Logan, Utah, USA). This system uses a flexible multi-sample layout capable of handling multiple 96-well microplates. The HydroPlate system uses 96-well microplates with integrated pH sensors (PreSens, Precision Sensing GmbH, Regensburg, Germany). An advantage of the 96-well plate systems is the high-throughput ability to screen large numbers of strains, strain variants or strain combinations for their milk activity and phage sensitivity.

## Effect of different processing methods on physicochemical and microbiological characteristics of Minas Frescal Cheese

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Fresh Minas Cheese (FMC) is widely produced and consumed in Brazil. Three procedures can be used to process FMC including ultrafiltration, direct acidification with lactic acid and fermentation promoted by lactic acid bacteria (LAB). The production process can cause a significant change in the texture and consistency, but also affect the type of microbial contaminants. In order to evaluate the influence of the production process in the physicochemical and microbiological properties of FMC, we evaluated 30 samples of six commercially available brands collecting two samples from each different production process of FMC in five repetitions. We evaluated the humidity, protein, fat, ashes and chloride content, and performed quantitative analysis for *Escherichia coli*, *Staphylococcus aureus* and detection analysis for *Listeria monocytogenes* and *Salmonella* sp. The results were submitted to an analysis of variance (ANOVA) and Turkey's test ( $P > 0.05$ ). The cheese produced using the ultrafiltration process differ from other cheese by having higher humidity and lower fat and protein content. In addition, no contaminants were detected in the cheese produced using the ultrafiltration process. The samples produced by both direct acidification and LAB were contaminated by *E. coli* with levels above  $5 \times 10^2$  CFU.g<sup>-1</sup> in 30% and 50 % of samples, respectively. *S. aureus* was detected with levels higher than  $10^3$  CFU.mL<sup>-1</sup> in 20% of samples produced according to the standard method of cheese production. *L. monocytogenes* was detected in one sample and no *Salmonella* sp. were detected. The results demonstrate that there is an absence of a standardized method of production of FMC and there is a need to increase hygiene and food quality control in the production.

## Characterization of the non starter lactic acid bacteria of Latvian cheeses

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Non starter lactic acid bacteria (NSLAB) are present at high numbers during ripening of Latvian Krievijas cheese (Dutch type cheese variety). Some authors have summarized that the NSLAB have been identified in >50 varieties of cheese (Beresford, 2004). The diversity of NSLAB of Latvian Krievijas cheese was studied in two factories over a two month period during summer and winter time. A total of 10 isolates of NSLAB from milk and Krievijas cheeses were characterized at the species level. Over 90% of the isolates were either *Lactobacillus curvatus* and *Lactobacillus plantarum* or *Lactobacillus helveticus*. The isolates from the two factories differed from one another and varied slowly over the two month period. Although the different species have different growth characteristics (specific growth rate, acidification ability and final cell number), they well adapted to changing environmental parameters of ripening cheese (carbohydrate limitation, low temperatures and water activities).

All isolates were biochemically characterized as homofermentative and facultative heterofermentative lactobacilli. The isolates found in the cheeses all came from raw milk and from environment of cheese plant.

NSLAB do not contribute to acid production during cheese manufacture, but impact on flavor development in ripening cheese. The analyses of volatile compounds as well as sensory analysis showed that the taste and smell of analyzed cheese samples were significantly different.

## **Interaction between pathogens and lactic acid starters during milk fermentation**

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Food Standards Australia New Zealand (FSANZ) is currently developing a Standard for the production of raw milk products, including cheeses. A major focus for the development of the Standard has been the consideration of the risk associated with the presence of microbiological pathogens in raw milk and their behaviour during cheese production and maturation. A category approach has been proposed where cheeses may be grouped according to risk. This may include consideration of the physico-chemical characteristics of the cheese and the potential for growth of pathogens. A starting point for determining the risk category of a raw milk cheese is consideration of the interaction between lactic acid starters and pathogens during curd formation. Typically, lactic acid starters are added at about 1% (about  $10^6$ - $10^7$  cfu/ml) of the milk volume for cheese production. As the fermentation progresses the concentration of the lactic starters increases until the stationary phase is reached and no more growth occurs. A common hypothesis applied in food microbiology, known as the Jameson Effect, suggests that the growth of all micro-organisms stops when the micro-organism with the greatest concentration enters the stationary phase. This hypothesis is tested for a variety of lactic starter strains and pathogens grown in skim milk. A combination of the Baranyi primary growth models for the micro-organisms and the Torrestiana pH model for acidification are used to determine the values of the key variables, including time to reach the stationary phase and maximum rate of pH drop. Analysis of the experimental data suggests that the Jameson Effect doesn't always apply for pathogens grown with lactic acid starters. This approach illustrates the application of modelling for predicting the behaviour of pathogens during cheese production.

## **Analytical methods for quality assurance in cheese production and ripening**

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The quality of cheese during ripening is mainly influenced by manufacturing methods, types of culture, enzyme activity and ripening conditions. To ensure consumer acceptance an efficient control of these factors which affect the typical flavour, the texture and the appearance of the final product is required.

For the evaluation of ripening defects and the optimization of different cheese productions instrumental-analytical methods in combination with chemometric techniques - based on Principal Component Analysis and Partial Least Square Regression - were used. In addition a computer-assisted evaluation of digital photography of eye and split formation was applied. The detection of chemical ripening parameters, e.g. proteins, peptides, amino acids and carbohydrate metabolites was carried out with RP-HPLC. The analytical results as well as the technological parameters were correlated with sensory characteristics like eye and split formation, stage of ripening or bitterness to provide a possibility to evaluate the maturation and to take corrective measures.

In addition a new method based on the detection of ripening parameters by microarray is being developed. By this process of controlling cheese ripening microarrays offer a possibility to detect essential ripening parameters like amino acids and other proteolytic products in time to control and change the production if necessary. Due to successful pre-experiments with glutamine, which showed that a microarray detection in milky matrix is possible and that the spot intensity increased during the ripening process, an adapted immunoassay for the quantitative detection of leucine was developed. Thus, the examination of Swiss-type-cheese showed a significant increase in the concentration of leucine during ripening.

## **Development of a microarray for the assessment of proteolytical cheese ripening**

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Microarray techniques are currently applied in the fields of molecular biology and genetics. Because of the various advantages, e. g. the massive parallel throughput, a minimum of time for investigation as well as a reduced consumption of reagents during the analytical process, this application for food technology, especially for quality assurance of cheese ripening, is of interest. The detection of essential ripening parameters like amino acids by microarrays represent an innovative possibility to control the ripening process and allow producers a quick change in production to avoid ripening defects.

In cooperation with the Fraunhofer-Institute for Biomedical Engineering experiments for the detection of amino acids by microarray have been carried out. After the successful determination of glutamine in spiked milk samples by the use of enzymatic reaction with a peroxidase-label, water-soluble extracts of Swiss-type cheese were investigated. The extracts were mixed with a glutamine antibody spotted on a chip and subsequently detected by fluorescent secondary antibody. As a result the spot intensity increased with the amount of glutamine during ripening, which showed that the ripening process can be described qualitatively.

In addition and for the further quantification of amino acids by antibodies an adapted immunoassay for the detection of leucine was developed. The measurements of water-soluble cheese extracts were also evaluated by a validated RP-HPLC-method, using OPA-pre-column derivatization. Comparing these measurements a correlation coefficient of  $R=0,986$  was obtained. However in contrast to the chromatographic-method the immunoassay method resulted in higher absolute values, which can be explained by possible reactions of the antibodies with other amino acids or leucine containing oligopeptides.

## Elucidating flavour development in cheese using new and classical approaches

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Flavour development in cheese is primarily determined by numerous bacterial metabolic processes involving extensive and complex catabolism of milk constituents. Although some of these metabolic processes have been well described, little is known about the relative importance of these processes or if other, as yet unidentified, pathways are involved. Proteomic technologies provide an opportunity to investigate a broad range of metabolic pathways without pre-supposing which of them are influential.

To elucidate the molecular basis of flavour and aroma development resulting from certain *Lb. helveticus* strains, cheese was manufactured with a range of strains. The sensory characteristics of the cheeses were assessed and multivariate statistical techniques were used to classify strains into two groups according to the sensory profiles they imparted to the cheese. Elucidation of the molecular basis of the different flavour profiles given by the strains in the two groups utilized a dual approach that combined determination of the biochemical characteristics of the cheese with a proteomic comparison of the strains. With respect to the former, cheeses were characterised for free amino acid content, peptide profiles, volatiles and strain autolysis throughout cheese maturation. For the proteomic comparison, the acidic and basic proteome of each of the strains, which had been assigned to one of two flavour groups, were compared using 2-D Fluorescence Difference Gel Electrophoresis (DIGE). Differentially abundant proteins were identified by nano-LC-MSMS. The findings of the biochemical and proteomic comparisons are discussed.

## **Effect of Basil seed gum (BSG) on microstructure, rheology and melting properties of processed cheese**

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Basil seed, traditionally used to treat some diseases is currently incorporated into drinks and icy products in many Asian countries as a source of dietary fibre. The polysaccharides extracted from Basil seed have been shown to comprise a mixture of glucomannan, xylan and glucan. Previously, we have reported the optimum gum extraction conditions from basil seeds. Furthermore, we have reported rheological properties of BSG solutions as function of concentration, temperature. We have also shown that BSG is a surface active gum which shows high potential as a novel emulsifying hydrocolloid. Using hydrocolloids in processed cheese formulations can effectively reduce total cost due to less used dairy proteins. The objective of this study was to evaluate the effect of BSG concentration (0-1%) on protein reduction (12-6%) in model processed cheese. Model processed cheese was formulated using a Rapid Visco Analyser. The effects of BSG on viscoelastic properties of the processed cheese were studied based on storage modulus ( $G'$ ) and phase angle ( $\delta$ ). The fracture stress and fracture strain of the processed cheeses were determined to study their mechanical properties. The microstructure of processed cheeses with different concentrations of BSG was studied using CLSM method. The crossover temperature (where  $G' = G''$ ) was increased as the BSG concentration increased from 0 to 1%. Addition of BSG into process cheese completely changed its microstructure, textural and melting properties.

## **Effects of acidification on the rheology of rennet induced curd made from buffalo milk**

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Oscillatory dynamic rheology was used to measure the curd firmness ( $G^*$ ), overall viscoelasticity ( $\tan \delta$ ) and total yield stress ( $\sigma$ ) of the rennet induced curds made from pasteurized whole buffalo milk at differing pH values (6.6- 5.6). The dynamic moduli ( $G'$ ,  $G''$ ) increased with increasing time after chymosin addition at initial pH values of 6.6 to 6.0 and attained maximum value after 90 minutes. This is may be due to reduction in electrostatic repulsion of the micelles and a slight decrease in the total amount of the casein bound calcium. The maximum gel strength obtained after 90 minutes and decreased with decreasing pH from 6.0 to 5.6. This is assumed to be due to the excessive loss of casein bound calcium from casein micelles at the lower pH values. Loss tangent increased with the reduction in milk pH during the acidification process. After 95 minutes of chymosin addition, rennet curds were subjected to constant low shearing force to break up the system. The measured yield stress is an indicator of the overall interaction of the curd components and below pH 6.0 decreases with decreasing pH. This resulted in a significant loss of casein bound calcium associated with the loss of “cross linking” between the casein micelles.

The structure and casein bound calcium in the actual casein particles are major determinants for the production of good quality buffalo curd. It is postulated that any alteration of casein micelle structure will result in changes to the physicochemical and rheological properties of buffalo rennet curds with subsequent changes in the overall quality of mozzarella produced.

## **Persistent strains of *Listeria monocytogenes* in the Farmhouse cheese environment**

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*Listeria monocytogenes* is of great concern in the speciality dairy industry because of its high mortality rate of 30% in susceptible groups of the young, old and immunocompromised. Although generally considered ubiquitous in the environment, the ability of some strains to persist in food manufacturing environments facilitates its transfer from the environment to food. The aim of this study was to determine the degree of persistence of *L. monocytogenes* strains in the Irish farmhouse cheese manufacturing environment. The cheesemaking environments of fifteen cheesemaking facilities were sampled on a monthly basis for twelve months. *L. monocytogenes* were isolated by the ISO 11290 method. Isolates were confirmed as *L. monocytogenes* by PCR, serotyped and the strains compared by PFGE. Forty seven different pulsotypes were identified. None of the pulsotypes were identified at more than one cheesemaking facility. Only 3 persistent pulsotypes were found at 2 facilities. Persistence was determined as repeated isolation of an indistinguishable strain for a time period of more than two months. The significance of this result is that 87% of the cheesemaking facilities tested do not contain persistent strains of *L. monocytogenes*, indicating that the normal hygiene practices used are adequate. Thus the risk of *L. monocytogenes* on the food product is reduced.

## Various aminotransferase and hydroxy acid dehydrogenase activities of cheese related *Lactobacillus helveticus* strains

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*Lactobacillus helveticus* contribute to flavour formation in cheese by amino acid catabolism that begins with a transamination to  $\alpha$ -keto acids, which are degraded further to volatile compounds or hydroxy acids. High activity of the hydroxy acid dehydrogenase (HADH) may decrease the amount of volatile and flavour compounds formed from  $\alpha$ -keto acids. *Lb. helveticus* strains have typically aminotransferase (AT) activity on aromatic amino acids (e.g. Phe) and branched-chain amino acids (e.g. Leu), and the activity is highest against the aromatic. The objective of this work is to investigate AT and HADH activities in the catabolism of the amino acids Leu and Phe by analysing genetically different *Lb. helveticus* strains. The activities are analyzed in crude cell free extracts by measuring the absorbances from enzymatic reactions using a microtitre plate scanner spectrophotometer. Preliminary results show a variation between strains in the AT activities against Leu and Phe, while generally, the HADH activity against  $\beta$ -phenylpyruvic acid, the  $\alpha$ -keto acid from Phe, are 100 times higher than the activity against  $\alpha$ -ketoisocaproic acid, from transamination of Leu. These results will be confirmed by further analysis of several cheese related *Lb. helveticus* strains. It could be concluded that a much higher amount of volatile compounds from Leu than from Phe should be expected to be formed in cheese.

## The effect of added calf lipase on the lipolysis during the ripening of ultrafiltered Feta cheese

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The effects of a commercial pregastric lipase enzyme (calf lipase) on the free fatty acid profile of Iranian UF (ultrafiltered)-Feta cheese during ripening period were investigated. Sensory evaluation and physico-chemical analysis of cheese samples were also performed. Commercial pregastric lipase (at the levels of 0, 2, 4 and 6 g 100 kg<sup>-1</sup> of retentate) together with rennet was added to retentate. The main components (fat, total solids, salt, total nitrogen and water soluble nitrogen), pH value and free fatty acids were analyzed in cheese samples after 3, 20, 40 and 60 days of ripening. With an increase in lipase level and ripening period, the main components of cheese did not change significantly but water soluble nitrogen increased during ripening period ( $P < 0.05$ ). With an increase in the pregastric lipase level, the percentages of C<sub>4:0</sub>-C<sub>8:0</sub> fatty acids decreased significantly while that of C<sub>12:0</sub>-C<sub>18:0</sub> and C<sub>18:1</sub> increased. The percentages of C<sub>10:0</sub>, C<sub>18:2</sub> and C<sub>18:3</sub> did not change. With an increase in ripening period, the percentages of C<sub>4:0</sub>-C<sub>14:0</sub> decreased while the percentages of C<sub>16:0</sub>, C<sub>18:0</sub>, C<sub>18:1</sub> and C<sub>18:2</sub> increased ( $p < 0.05$ ) and C<sub>18:3</sub> percentage did not show any changes. Overall, sensory scores (appearance, flavour, body and texture and piquant taste) indicated that combination of 20 days with 6 g 100kg<sup>-1</sup>, 40 days with 4 g 100kg<sup>-1</sup> and 60 days with 2 g 100kg<sup>-1</sup> retentate were the best ripening conditions. The addition of lipase to cheese milk could be recommended for the acceleration of flavour development in UF-Feta cheese over a short ripening period.

## **Modeling the safety of mould cheeses with regard to *Listeria monocytogenes***

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Predictive microbiology is a new tool enabling predicting the behavior of microorganisms, especially the foodborne pathogens, in foods during production and storage. Predictive models describing the growth of *Listeria monocytogenes* have been generated in a response to such factors as temperature, water activity, organic acids and others. Most of these models have been generated in microbiological media, which do not include the interaction among foodborne pathogen with other microorganisms present in food, as well as with chemical composition of foods. The purpose of this work was to study the growth of *Listeria monocytogenes* in mould cheeses at isothermal conditions (3, 6, 9, 12, 15°C). Data were then fitted into primary models (Baranyi, logistic and modified Gompertz function). Measures of goodness-of-fit such as mean square error (MSE) and Akaike's information criteria were used to compare primary models performances. On the basis of these criteria, model which described growth data the best was chosen. Specific growth rates were analyzed as a function of storage temperature using Ratkowsky, polynomial and Arrhenius models. Mathematical validation (Bias and Accuracy factors) was performed in order to evaluate the goodness-of-fit of generated models. It was concluded that usefulness of predictive models describing the growth of foodborne pathogens need to be checked with the validation studies carried out in the food products of interest. Predictive models generated on microbiological media do not describe the behaviour of microorganisms accurately, and it is worth investing into dairy products-specific predictive models.

## Comparison of the kinetics and specificity of camel and calf chymosin on bovine $\kappa$ -casein

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The principal idea of using renneting enzymes in cheese making is to coagulate the milk by the specific cleavage of the Phe<sub>105</sub>-Met<sub>106</sub> bond of  $\kappa$ -casein. In addition, rennets contribute to casein breakdown during ripening and thereby directly to the development of cheese texture and indirectly to cheese flavour. However, the extent of this casein breakdown depends on the rennet type.

A high ratio between clotting and proteolytic efficiency is generally beneficial, and calf chymosin has for a long time been the preferred coagulant. Camel chymosin, however, has been demonstrated recently to provide a sevenfold higher ratio of clotting to proteolytic activity in bovine milk as compared to calf chymosin (Kappeler *et al.*, 2006). This difference results in a significantly lower extent of casein breakdown in cheese produced using the camel coagulant and thus lower levels of proteolysis, bitter taste as well as intensity of cheese flavour (Bansal *et al.*, 2009).

In this work, the general specificity and hydrolysis kinetics of  $\kappa$ -casein by camel and calf chymosin were studied. Purified  $\kappa$ -casein was obtained by precipitation and anion exchange chromatography. Kinetic constants and proteolytic specificity of camel and calf chymosin were investigated at 30°C, pH 6.5. The hydrolysis of  $\kappa$ -casein was analysed using capillary electrophoresis (CE) for large casein fragments and reversed-phase liquid-chromatography mass-spectrometry (RP-LC-MS) for identification of the pH 4.6 soluble peptides.

Results comparing kinetic parameters relative to the action of camel and calf chymosin on the Phe<sub>105</sub>-Met<sub>106</sub> bond of  $\kappa$ -casein are presented. Furthermore, the general specificity and activity of the two coagulants towards the primary hydrolysis products para- $\kappa$ -casein and caseinomacropeptide are compared.

## **Dynamics of microbial communities in Grana Padano cheese**

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Grana Padano is a protected designation of origin hard cooked cheese. It is one of the most popular Italian raw milk cheeses, produced from semi-skimmed raw milk by using natural whey cultures as starter. The complex microbial community arising from milk and whey starter is selectively favoured by technological and environmental conditions and plays a key role in the achievement of the typical and appreciated sensory characteristics of this cheese. Several studies have been carried out on microbial composition of natural whey starters for Grana Padano, while less is known about the dynamics of such communities during further cheese making and ripening. In particular, to understand the behavior of microbial cells through the different conditions of cheese production, studies on minimal disturbed samples are needed. Therefore, the aim of this study was to study the Grana Padano ecosystem by a culture independent approach, in order to understand the way that viable and non viable cells respectively contribute to cheese ripening. Length heterogeneity PCR (LH-PCR) was used to monitor the microbial dynamics from whey starter during 9 months of Grana Padano ripening for both the whole and lysed cells. Coupling traditional counting methods with culture independent techniques allowed a deeper insight in this ripened cheese complex ecosystem where viable, not viable, and lysed microbial cells are contemporaneously present.

## Real-time PCR typing *Lactococcus lactis* bacteriophages

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Bacteriophages infecting cheese starter culture strains of *Lactococcus lactis* were first scientifically described over 70 years ago, with ten distinct phage species currently recognised. Historically, three phage species (c2, 936 and P335) have been the most widely reported. Multiple genome sequences are available for these species. In our laboratory, PCR methods using primers targeting conserved sequences within genes encoding structural proteins of the c2 and 936 species have been in use for *ad hoc* phage species classification for over a decade. The polythetic P335 species contains both temperate and lytic phages and shows extensive sequence heterogeneity with no universally-conserved sequences, complicating the development of simple PCR assays for this species.

We have adapted similar methods to a 96-well real-time PCR reaction format. With high reaction throughput and no need for analysis of PCR products by gel electrophoresis, this has made it relatively convenient to screen large numbers of phages in parallel. Our archival collection spans 18 years of the Australian cheese industry. At the time of writing, 547 phage isolates have been subjected to c2- and 936-specific PCR, with 274 phages (50%) typed as belonging to the 936 species, and 72 (13%) as c2 species. The 936 species has been consistently predominant throughout the collection period, though fluctuations in relative frequency occur. Analysis of these fluctuations may reveal correlations with environmental changes, cheese manufacturing variables, patterns of culture usage and evolution of industrial phage populations.

## Risk assessment of *Listeria monocytogenes* in ripened ovine Ricotta cheese

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Ricotta cheese, particularly the ovine type, is an Italian typical dairy product obtained by heat-coagulation of whey proteins. It is available in several types as fresh, ripened and smoked and ripened Ricotta. Several UE alerts about *L. monocytogenes* risk in ricotta cheese have recently happened. Surveys for *L. monocytogenes* have shown the ubiquity of the organisms in the food production environment and very often persist in dairy plants despite vigorous sanitation regimes. The aim of this study was to investigate about the ability of *L. monocytogenes* to grow and to survive during the manufacture and ripening of Ricotta cheese. Ovine Ricotta cheese for analytical purpose was obtained from natural whey artificially contaminated with *L. monocytogenes* at high levels, 10<sup>6</sup> CFU/ml in average. A pool of five reference and field strains of *L. monocytogenes* were used. Ricotta cheese composition and target pathogen presence were evaluated in inoculated whey, in Ricotta cheese after 1 day, 7 and 15 days of ripening. The results obtained showed that at 15 days of ripening, long before the commercial ripeness, all cheeses were free from the inoculated pathogen. The physico-chemical parameters of experimental Ricotta cheese (pH, total solids, a<sub>w</sub> and salt content) were within the range of the commercial product. Consequently, the behaviour of the studied pathogen in experimental Ricotta cheese can be extended to the commercial one. In conclusion, the effect of the Ricotta cheese process precludes the possibility of growth and survival of the tested pathogen. Nevertheless a recontamination, principally in the product surface, could be always possible only in the case of a poor hygiene conditions of the ripening environment or packaging materials.

## **A glimpse of Indian soft cheese: the Paneer**

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Paneer, a popular indigenous dairy product of India, is an unripened variety of soft cheese and is used in the preparation of a variety of culinary dishes, snacks and sweetmeats. It is obtained by the heat and acid coagulation of the casein component of milk, entrapping through complex physico-chemical interactions almost all the fat, a part of denatured whey proteins and colloidal salts, as well as a part of denatured whey proteins and colloidal salts, as well as a part of the soluble milk solids. Typically, Paneer is marble white in appearance, having a slightly spongy body and possessing a sweetish-acidic-nutty flavour, firm cohesive and compact body and a close knit-texture. It contains approximately 53-55% moisture, 23-26% fat, 17-18% protein, 2-2.5% carbohydrate and 1.5-2.0% minerals. Preparation of Paneer using different types of milk and varied technique results in wide variation in physico-chemical, microbiological and sensory quality of Paneer. Normally Paneer blocks of required size are packaged in polyethylene pouches, heat sealed and stored under refrigeration conditions. Alternatively Paneer blocks may be vacuum packaged in laminated or co-extruded films. At room temperature Paneer does not keep good for more than one day but at refrigeration temperature its shelf life has been reported to be 6 days though its freshness is lost within 3 days. The spoilage in Paneer occurs mainly due to the surface growth of microorganisms. Hence attempts have been made to curb the surface growth of microorganism and thereby increase the shelf life of Paneer. This review deals with the historical development, method of manufacture, chemical composition, nutritional importance, quality, packaging and shelf life of Paneer.

## **Effect of antioxidants on shelf life of Khoa under refrigerated conditions**

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Khoa is an important milk product in the Indian sub-continent and it is estimated that about 7 per cent of the total milk produced in India and 13.6 per cent of milk used for the manufacturing of milk products is channeled through the production of Khoa. It's a partially heat desiccated Indian dairy product made from buffalo milk or mixed milk with total solids around 65-70 percent. It has a limited shelf-life of 14 days under refrigerated conditions, as reported earlier. One possible method of enhancing the shelf life of Khoa is to add antioxidants. Khoa was mixed with tocopherol acetate at a concentration of 15 ppm, sodium ascorbate at a concentration of 600 ppm individually and combined and stored at  $5 \pm 2^{\circ}\text{C}$ . Samples were taken for sensory, physico-chemical and bacteriological analysis at different storage times. The study revealed that the Khoa sample can be stored for more than 30 days at  $5 \pm 2^{\circ}\text{C}$  without any treatment. It also revealed that addition of sodium ascorbate at 600 ppm reduced changes in TBA followed by tocopherol acetate addition at 15 ppm. Proteolysis and lipolysis were found to increase with increased storage time. Tocopherol acetate, sodium ascorbate and their combination did not significantly influence free fat, free fatty acids, Non Protein Nitrogen and Standard Plate Count of Khoa.

## **Development of various Paneer based spreads**

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Paneer is heat-acid coagulated Indian type of soft cheese, made by heat-acid coagulation of milk, followed by whey drainage. It is used mainly for making culinary dishes. Paneer spread was prepared by adding salt (1.5%) and stabilizer (0.5%) to paneer curd and mixture was subjected to grinding by using mixer-grinder. The moisture % of spread thus obtained was 65%, exhibited excellent spreading property at 7°C.

Dairy spreads are high in fat, saturated fatty acids and cholesterol. Besides, butter exhibits poor spreadability at low temperatures. Objective of this study was to develop a low cost, low fat product, with good spreadability. Incorporation of 10% WPC or 5% sodium caseinate or 5% soy flour resulted in higher yield, better functionality (as determined by instrumental and sensory and statistical analysis) and nutritional value in terms of protein% as compared to control. Replacement of milk fat with vegetable oils (soy, corn or sunflower) at 25% level was acceptable with improved body and texture and spreadability. Incorporation of matured cheddar cheese at 30% level to Paneer curd along with tri-sodium citrate (0.5%) as stabilizer and salt (1.5%). improved acceptability and received higher flavor scores than control. Incorporation of spices such as pepper, clove, cinnamon and their combinations in powder form at 1 per cent level resulted in better flavour scores. Pepper and mixture of pepper and clove-flavored Paneer spreads recorded highest overall acceptance scores. Among various keeping quality studies undertaken, vacuum treatment of paneer spreads resulted in extension of storage up to 35 days at 7±1°C, as against 14 days in control. When product was subjected to vacuum treatment followed by microwave heating the product showed stability up to 77 days at 7±1°C.

## **The effect of substitution of NaCl with KCl on Halloumi cheese during storage: Chemical composition, proteolysis, texture profile, and microstructure**

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The effect of partial substitution of NaCl with KCl on chemical composition, LAB count, organic acids profile, proteolytic pattern, texture profile, and microstructure of Halloumi cheese was investigated. Halloumi cheeses were made and kept in four different brine solutions at 18%: only NaCl (HA), 3NaCl:1KCl (HB), 1NaCl:1KCl (HC), and 1NaCl:3KCl (HD) and then stored at 4°C for 56 days. Chemical composition, proteolysis, texture profile and microstructure were analyzed. No significant ( $p>0.05$ ) effect was observed between control and experimental cheeses in terms of composition and LAB count and pH values at the same storage period. At same salting treatment, moisture content decreased significantly while LAB count significantly ( $p<0.05$ ) increased. There was a significant ( $p<0.05$ ) difference in ash, sodium and potassium contents among cheeses at the same storage period. However, these parameters increased significantly ( $p<0.05$ ) during storage at same salt treatment. There was no significant ( $p>0.05$ ) difference in lactic and citric acid contents among cheeses. In contrary, there was a significant ( $p>0.05$ ) difference in acetic acid concentration among cheeses. There were no significant ( $p>0.05$ ) differences in WSN, TCA-SN, and PTA-SN of experimental cheeses at same storage period. However, these parameters increased ( $p<0.05$ ) during storage at same salt treatment. Peptide pattern and urea-PAGE also showed no significant difference among experimental cheeses. There was no significant difference in TPA parameters between cheeses at same storage period. At same salt treatment, hardness, cohesiveness, and cohesiveness decreased ( $p<0.05$ ) and adhesiveness increased ( $p<0.05$ ) during storage period. ESEM images showed compact and closed texture in all experimental cheeses. Our results concluded that Halloumi cheese can be stored in brine solution consisting of NaCl/KCl mixture without any adverse effect on their quality.

## **Effect of curd washing level on proteolysis and functionality of low moisture mozzarella cheese made with Galactose-fermenting culture**

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The effect of curd washing on functional properties of low moisture mozzarella cheese made with galactose-fermenting culture was investigated. Four curd washing levels (0%; 10%, 25%, 50% wt/wt) were used during Low moisture mozzarella cheese manufacture, and cheeses were stored for 63 days at 4°C and the influence of curd washing on proteolysis and functionality of Low moisture mozzarella cheese were examined. Curd washing had a significant effect on moisture and ash contents. In general, moisture contents increased and ash contents decreased with increased curd washing levels. Low moisture mozzarella cheese made with 10% curd washing levels showed higher proteolysis, meltability, and stretchability during storage than other experimental cheeses. In general, galactose contents decreased during storage; however, cheeses made with 25 and 50% curd washing levels had lower galactose contents than those with control or 10%. L\* -values (browning) decreased and proteolysis increased in low moisture mozzarella cheeses during storage.

## **Effect of fortification of cheesemilk with UF retentate or buttermilk powder on composition and yield of Cheddar-style cheeses**

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Buttermilk contains Milk Fat Globule Membrane (MFGM) components such as proteins, glycoproteins, and phospholipids, all of which can act as emulsifiers. Cream is also subjected to high thermal treatment (e.g. 91 °C x 2 min) during butter manufacture resulting in whey protein fraction denaturation. Previous studies investigated use of ultra-filtered buttermilk to enhance cheese yield, however availability of UF may be facility dependant while buttermilk powder is commercially available, readily transportable and easily stored. The objective of this study was to investigate whether buttermilk powder may be used to increase cheese yield by (1) fortification of cheesemilk protein levels (2) increased moisture retention due to denatured whey proteins and (3) reduced whey fat loss due to emulsification properties of the MFGM components.

Cheddar-style cheeses were manufactured from milks of ~ 3.10 % protein (CTL) or from milks fortified to ~ 3.90 % protein with milk ultra filtration retentate (UFR) or with buttermilk powder (BMP) and standardised to casein: fat ratio of ~ 0.80. Cheese moisture and moisture-in-non-fat –substance levels were significantly higher in the order BMP > CTL > UFR with an inverse relationship for fat contents (P < 0.001). Cheese protein, calcium, salt and salt-in-moisture contents were similar for all cheeses. Fat losses to whey were higher (20-30 %) in BMP compared to CTL cheeses (15-18 %) and significantly higher compared to UFR cheeses (9-12%) with an inverse relationship for fat recovery to cheese (P < 0.05). Moisture adjusted cheese yield with fat and protein contents adjusted to reference levels showed yields of BMP cheeses were significantly lower than those of UFR cheeses.

## **Preliminary investigation of fortification of cheesemilk with UF retentate or buttermilk powder on ripening quality of Cheddar-style cheeses**

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Previous studies have investigated the use of ultra-filtered buttermilk retentate to increase cheese yield due its content of proteins, glycoproteins, and phospholipids, all of which can act as emulsifiers and also due to denatured whey proteins present in buttermilk arising from high thermal treatment (e.g. 91 °C x 2 min) of cream during butter manufacture. However access to UF facilities may be plant specific while buttermilk powder is commercially available, readily transportable and easily stored. The objective of this study was to investigate the effect of fortification of cheesemilk with buttermilk powder on cheese ripening and quality. Cheddar-style cheeses were manufactured from milks of ~ 3.10 % protein (CTL) or from milks fortified to ~ 3.90 % protein with milk ultra filtration retentate (UFR) or with buttermilk powder (BMP) and standardised to casein: fat ratio of ~ 0.80. Cheeses were ripened at 8 °C for 3 months. Preliminary results show significantly lower pH levels ( $P < 0.05$ ) during ripening in BMP in comparison to CTL and UFR cheeses. All cheeses had similar counts of starter and Non Starter Lactic Acid Bacteria (NSLAB) and a similar curd microstructure as shown by confocal laser scanning microscopy. Principal component analysis of volatile compound and descriptive sensory analyses showed UFR and control cheeses to be similar after 3 months of ripening and significantly different to the BMP cheeses which had greater and atypical levels of short chain fatty acid and methyl ketone compounds and a higher score for sour, sulphur, brothy and free fatty acid descriptors in comparison to the CTL and UFR cheeses.

## **Preliminary characterisation of pink discolouration in commercial cheese samples**

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Pink discolouration defect results in a down-grading of ripened cheese manufactured without annatto colourant. It is evident on the surface and to a depth of 1.5 cm in dry salted cheeses manufactured with thermophilic cultures and as a pink border at a depth of 1.5 cm below the cheese surface or in the centre of rindless Emmental cheese blocks. It has also been reported in Cheddar-type cheeses manufactured with and without annatto. Many factors (e.g. cheese redox potential, strains of lactobacilli, cheese pH, oxidation of tyrosine, oxidation of bixin, etc.,) have been linked to the defect but a knowledge gaps exists relating to development of the discolouration. Samples with and without pink discolouration were removed from ripened commercial cheeses manufactured without annatto colourant. The affected samples showed significantly higher redness ( $a^*:0.36$  v  $-3.57$ ) and lower whiteness ( $L^*$ ; 70.79 v 76.41) values in comparison to control samples. The pink discolouration was stable in cheese under storage at 5 °C but faded on storage for 5 days at room temperature. The pink colour partitioned with the water insoluble protein fraction of the cheese and remained stable in a dehydrated sample of that protein fraction stored at ambient and also on heating to 80 °C for 1 hour. The pink colour did not solubilise in water, ethanol or acetone. Varying the pH (3.0- 8.5) and salt concentration (up to 6 %) of pastes manufactured from the cheese samples did not affect the pink colour. Current analysis is focusing on cheese redox potential, oxygen concentration and mass spectrometry analysis.

## Effect of *Lactobacillus helveticus* on reducing the bitterness of Prato cheese during ripening

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Prato – a cheese variety similar to Gouda (Dutch) and Danbo (Danish) - is the most widely ripened cheese consumed in Brazil. This study evaluated the effect of adding 1% (v/v) viable cells of *Lb. helveticus* (**LH**) (B-02, Chr. Hansen) as an adjunct to an aromatic, mesophilic starter culture consisting of *Lactococcus lactis* subsp. *lactis* + *Lactococcus lactis* subsp. *cremoris* + *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuconostoc mesenteroides* subsp. *cremoris* (**LD**) (DVS-CHN-22, Chr. Hansen) in the processing and ripening (60days/13±1°C) of Prato cheese (**C**). During the manufacturing of the cheese made with the LH (**CLD+LH**) adjunct culture, a 4.0 Log UFCmL<sup>-1</sup> decrease in the LD population was observed between pre-pressing and drying as compared to the control cheese (**CLD**). This difference was maintained throughout practically the entire ripening period. The LH population survived and remained in relatively constant numbers under the processing conditions tested, while extensive autolysis was observed at the beginning of ripening (3<sup>rd</sup> and 17<sup>th</sup> day). The proteolysis extension (**PEI**) and proteolysis depth (**PDI**) indices increased significantly in cheese CLD (PEI=155%; PDI=288%) and in cheese CLD+LH (PEI=102%; PDI=282%) during 60 days of ripening. From the 31<sup>st</sup> day onwards, CLD+LH cheese clearly tended to exhibit a higher value PDI as compared to the CLD cheese. This tendency was confirmed in SDS-PAGE by the qualitative difference between the hydrolysis profiles of cheeses CLD and CLD+LH. It was concluded that autolysis of *L. helveticus* at the onset of ripening, with the subsequent release of peptidases, complemented the enzyme system of the LD culture, thereby promoting a positive impact on secondary proteolysis and a reduction in the development of bitterness in Prato cheese.

## **Effect of non-starter lactic acid bacteria (NSLAB) on the ripening of model Prato cheese**

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This study evaluated the impact of the autolytic activity of five NSLABs strains on the proteolysis of an aseptic model cheese made from micro-filtered milk. The model cheese was prepared in a clean room under aseptic conditions from micro-filtered milk obtained from an MS1 microfiltration unit fitted with 0,24m<sup>2</sup> UTP membranes with 1,4 µm average pore size. The curd was divided into 75-g portions which were subsequently inoculated with 1% of the active strains. Ripening was conducted at 13°C for 21 days. The relative percentage of total proteolysis in the cheeses varied from 15 to 24% depending on the strain. Considerable diversity was observed among the phenotypic characteristics of the different NSLABs strains. Premature autolysis was observed for *Lb. rhamnosus* PN 270 and *Pe. pentosaceus* TR 285, and late or tardy autolysis for *Lb. plantarum* PN16. The remaining strains, *Lb. plantarum* TR 260 and *Pe. pentosaceus* TR 570 showed activity after 7 days ripening. Combinations of strains with different lytic and aminopeptidase profiles should be considered to improve the flavor of Prato cheese.

## **Production of cheese using milk fermented by probiotic starter and added of polidextrose**

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The term synbiotics is used to characterize some colonic foods containing probiotic and prebiotic with interesting health and nutritional properties. The manufacturing process of fresh cheese was minimally modified to produce samples of probiotic and prebiotic cheese, which could be named a synbiotic cheese. The probiotic organism used was *Bifidobacterium animalis subsp. lactis*. The pasteurized milk was inoculated with probiotic starter and incubated at 35°C, overnight. The pH of the milk reached 5,7, when CaCl<sub>2</sub> solution and rennet extract were added. After separation of the whey, salt and a water solution of polidextrose (prebiotic) were added to the curd. The probiotic, lactic bacteria and contaminants were counted at the day of the processing and after 8, 15, 21 and 28 days of storage of the cheese samples at 4°C. The probiotic counts of the samples were around 10<sup>7</sup> to 10<sup>8</sup> CFU per gram, after 28 days of refrigerated storage. The composition of cheese samples was, also, established. The portion of 30g of the cheese presented more than 10<sup>8</sup> cells, which agree to the Brazilian legislation requirement related to the functional foods.

## Sensory analysis to estimate the shelf-life of UHT- processed cheese (*requeijão cremoso*)

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Requeijão cremoso is a typical Brazilian processed cheese. Total production of this dairy product increased by 363.64% between 1991 and 2005, a figure that clearly illustrates its great commercial value and potential. UHT *Requeijão cremoso* (RCUHT) was obtained by melting a blend of fresh cheese curd, dairy fat, water and emulsifying salts. The final products were sterilized by UHT processing (143°C/2-5 seconds) before being aseptically filled into 125 mL Tetra Pak packages (RCUHT). Its high fat level (20%) makes this product prone to oxidation, one of the primary causes of spoilage. In this study, quantitative descriptive sensory analysis (ADQ) was used to assess the evolution of quality characteristics of UHT *requeijão cremoso* during storage at 5°C (reference temperature), 10, 25 and 35°C. Shelf life was estimated based on the overall acceptability score for the sensory attribute “loss of quality” as a function of storage time. The main attributes that characterized loss of sensory quality of the product, at the temperatures studied, were: a) onset of oxidized or rancid flavor and bitterness development at 10, 25 and 35°C; b) onset of oxidized/rancid/soapy flavor development at 10°C; c) loss of typical *requeijão* flavor at 25°C; and d) browning, loss of typical *requeijão* flavor and aroma and the onset of oxidized/rancid/soapy flavor development at 35°C. Based on these data, the shelf-life of UHT *requeijão cremoso* was determined to be 6 month when stored under refrigeration at up to 10°C, 5 months at 25°C and 2 months at 35° C.

## **Effect of calcium caseinate and cooking time on the texture, color and functionality of “requeijão cremoso” cheese**

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‘Requeijão cremoso’ is a traditional Brazilian spreadable processed cheese. It is obtained by the fusion of the curd with the optional addition of cream and/or butter and/or butter oil. The product is usually spread over toasts, and also used as a food ingredient for pizza, snacks, pasta, frozen ready-to-eat foods, and savoury foods in general. When the cheese is used as an ingredient, the need to support high oven temperatures and the product handling characteristics demands functional properties, such as a low melting capacity, greater hardness and less thread formation. There are a number of key parameters that can be manipulated to control cheese functionality, such as protein content and cooking time. The impact of calcium caseinate addition (1 to 3%) and cooking time (4 to 12 min) on cheese color, texture, and melting were determined. A central composite rotational design with two variables and three central points was used, totaling 11 experiments. Variation of calcium caseinate and cooking time neither affected color nor melting capacity of cheeses. Cheese springiness was influenced by calcium caseinate addition and by cooking time. Increasing concentration of calcium caseinate decreased cheese fat particle size and increased hardness, adhesiveness and gumminess.

## **Influence of polysaccharide on elongational viscosity, water mobility and microstructure of model processed cheese**

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The impact of microbial polysaccharide on the microstructure, water mobility and rheology of model processed cheese was investigated. The cheeses were formulated for spreads (30.0% fat, 10.0% protein and 50.0% water). The spreadable processed cheese contained 0.0–2.0% high acyl gellan and was manufactured on a small scale (30 g) using a cylindrical mixing vessel with a single stirrer and having control of temperature, stirrer speed and timing (Rapid Visco Analyser). The changes in spreading properties were assessed by measuring the changes in elongational viscosity at a constant force (and temperature) and the changes in the mobility of water by nuclear magnetic resonance (NMR) spin–spin relaxation techniques. The microstructure of the processed cheeses was assessed using light microscopy (LM) and transmission electron microscopy (TEM). Elongational viscosity was influenced by changes in the microstructure and the mobility of water in the matrices. Cheese with polysaccharide had less mobile water and showed a marked increase in elongational viscosity. LM and TEM micrographs showed that the polysaccharide was present in the protein matrix as distinct gel particles and did not interact with fat globules in the structure of the processed cheese. The study revealed that the presence of polysaccharide alters the water mobility and the protein matrix and thus the functionality of processed cheese.

## **Microbiological control of cheese brine with microfiltration**

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Extending the life time of the brine for re-use is now becoming a common practice in cheese manufacturing, to reduce operating costs while minimizing the plant environmental footprint. To prevent brine from becoming a reservoir of unwanted microorganisms, such as gas-producing lactobacilli, pigment-producing micrococcus, yeast and mould, and salt resistant pathogenic bacteria, which can cross-contaminate cheeses and impact their quality, microbial removal needs to be considered.

The purpose of this poster is to present and discuss the microbial retention performance of a hollow fiber microfiltration system for cheese brine recovery, and the cost benefits of its use in the cheese plant.

## **Influence of curd fines on yield of acid-rennet and rennet cheeses**

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The influence of production parameters on yield of industrially produced cottage cheese (acid-rennet coagulation) and Dutch cheese (rennet coagulation) was studied. The obtained results are an average from 12 and 9 production processes for cottage and Dutch cheese, respectively. The curd was cut into cubes of 10x10x10mm at pH 4.65 and 8x8x8mm for cottage and Dutch cheese, respectively. The volume of cheese fines obtained during the production process was evaluated by use of Imhoff funnel. The volume of curd fines was measured in the cheese whey separated from the grains following the thermization process and in first rinsing water for cottage cheese and in cheese whey sampled directly from cheese vat during whey draining before technological water addition and from preliminary pressing vat for Dutch cheese. After 60 minutes of sedimentation a volume of fines was measured and a dry matter was determined. The results showed high amount of curd fines in cottage cheese whey (average 19.6 cm<sup>3</sup>/dm<sup>3</sup>) and slight in rinsing water (average 2.1 cm<sup>3</sup>/dm<sup>3</sup>). Production of cottage cheese from 10 000 L of milk resulted in 19.20 kg and 1.23 kg of dry matter of cheese fines in whey and rinsing water, respectively. For cottage cheese, the volume of the fines was highly correlated with the intensity of mixing, especially at the initial stage of grain treatment. The obtained results showed that from 100 000 L of milk 51.3 kg of Dutch cheese was lost in form of cheese fines. The data showed that the firmness of the curd was a crucial factor for production of curd fines (higher firmness less curd fines).

## **Cattle and global warming**

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Global warming is the increase in the average measured temperature of Earth's near surface air and oceans, specifically since the mid 20th century, and its projected continuation. The average surface temperature on Earth has increased approximately 3 quarters of a degree during the last hundred years, and is predicted to rise another 1.1 degrees during the 21st century. The main cause of global warming is the greenhouse gas effect.

Animal agriculture is responsible for more greenhouse gas (18%) than all of transportation (13%) according to the Food and Agriculture Organization of the United Nations (FAO).

For example a cow does on average release between 70 and 120 kg of Methane per year. Methane is a greenhouse gas like carbon dioxide (CO<sub>2</sub>). But the negative effect on the climate of methane is 23 times higher than the effect of CO<sub>2</sub>. Therefore the release of about 100 kg methane per year for each cow is equivalent to about 2,300 kg CO<sub>2</sub> per year.

Also another study showed that producing a kilogram of beef leads to the emission of greenhouse gases with a global warming potential equivalent to 36.4 kilograms of carbon dioxide (CO<sub>2</sub>). It also releases fertilizing compounds equivalent to 340 grams of sulphur dioxide and 59 grams of phosphate, and consumes 169 mega joules of energy.

The greenhouse gas CO<sub>2</sub> can be reduced, in part, by reducing animal agriculture and increasing plant agriculture. When forest is cleared for pasture, the service which those trees provided as "carbon sinks" is gone. In addition, it takes less arable land and energy inputs to feed a vegan than to feed a non-vegan because food energy (as calories), such as grain, eaten directly by a person is more efficient than cycling that energy through an animal by feeding grain to a "food" animal. Thus, even though both animal and plant agriculture depend on fossil fuel inputs, less is needed for plant agriculture.

Finally, it can be concluded that the great portion of contributions to global warming can be reduced by making improvements in its cattle industry. Grazing animals, such as cows, produce a great deal of methane, one of the leading causes of the greenhouse effect.

## Energy reduction in the dairy industry through advanced water treatment management

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The refrigeration plant is a major power consumer in dairy processing plants. Maintaining clean heat transfer surfaces in the condenser and evaporator is critical in ensuring the refrigeration plant operates at optimum energy efficiency, with the twin benefits of reduced cost of operation and greenhouse gas emissions.

Routine monitoring of the refrigeration plant coefficient of performance (COP) is an effective way of benchmarking energy usage against industry standards. Monitoring methods, and the relationship between water treatment and COP, are presented and discussed, and used to demonstrate the impact of cooling water management on the energy usage in a typical dairy processing plant.

Two novel approaches to water treatment management are presented. The first is a monitoring and control system that responds to corrosion and fouling stresses and ensures cost-effective treatment and optimum refrigeration plant performance. The second is a bio-management approach that minimizes the impact of milk contamination into the chilled water system on refrigeration plant energy usage, a particularly important approach during the production season when rectifying the process leaks which cause the contamination can be difficult.

This poster will present specific examples where energy savings have been achieved by improved cooling and chilled water treatment management in the Dairy Industry.

## **Analysing the carbon footprint (CF) of milk production in New Zealand and Sweden**

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The present study analyses the carbon footprint (CF) of milk produced in New Zealand and Sweden. Several standards on how to calculate the CF are currently being developed, but it is still problematic to compare CF results, due to differences between the standards, but also because the standards can be interpreted differently. The CF of milk in New Zealand and Sweden is dominated by the biogenic emissions methane and nitrous oxide, arising from complex biological processes that are difficult to model, and therefore result in significant uncertainty in the final CF result.

This study highlights how CF results are affected by differences in methodology and data quality, with the aim of providing recommendations on how to improve comparability of CF figures of milk. The CF for one kg milk (including by-products) ex farm-gate is calculated to be 1.0 kg CO<sub>2</sub>-eq in New Zealand and 1.2 kg CO<sub>2</sub>-eq in Sweden. However, due to the sensitivity to methodological choices and variations within the two systems, the obtained difference in CF results cannot be considered significant. To reduce the sensitivity and hereby improve the validity and comparability of CF results, more focus is needed on harmonisation of methodology and obtaining more specific emission factors for calculating biogenic emissions, as these are the most critical aspects in minimising uncertainty in comparison of CF figures of milk.

## **Compressed air energy savings is everybody's responsibility**

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Our poster would consider the following topics:

The cost of electricity generation for all businesses and private users;

Where compressed air is used and the costs associated;

How compressed air is wasted and the generation cost of the wastage;

A Compressed Air Energy Management Plan;

Some likely outcomes and suggestions.

## **Water usage trends in the dairy processing industry**

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For many years, dairy processing facilities have been significant water consumers, although there has been little effort to reduce water usage until recent times. For many processing plants, the easy and obvious projects have generally been addressed. With the cost of water increasing, and a drive to reduce water use and improve water efficiency as part of key reporting metrics, milk processors are increasingly focused on more less proven treatment methods. These efforts have seen water usage dropping from a typical > 3 litres of water per litre of milk intake to < 0.8 litres of water per litre of milk intake over the past 20 years. Opportunities to further reduce water use must increasingly rely on advanced water treatment processes to treat low quality available water, or to reprocess existing site water sources for reuse within the plant. As water usage is highly dependent on the type of processing plant, a thorough understanding of total site water sources and uses must be gained to effectively manage the product quality and environmental risks of each project. This poster will present how advanced water treatment has used to improve dairy processing industry water efficiency, followed by specific detailed examples where water savings have been achieved.

## **Biomass boiler and energy reduction projects**

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Biomass and energy reduction projects can reduce overall energy costs and contribute to more environmentally sustainable production. This presentation reviews several 8MW to 40MW boiler projects involving the combustion of a variety of biomass fuels, and an innovative site wide energy reduction project at a major New Zealand dairy factory. The energy reduction project achieved savings equivalent to 7MW or 10% of total energy consumption per unit of site production.

The drivers which established biomass boilers as the preferred project are described in conjunction with the type of fuels used and available process technology options. The energy reduction project on the Fonterra Co-operative Group, Whareroa site demonstrated that low temperature heat recovery can be implemented to significantly reduce energy consumption and improve plant operation. The project involved integrating widely dispersed hot and cold streams operating to different schedules to generate significant savings in steam, chilled water, cooling tower water and electricity use.

## ***PhenoFinlait*: French research program for adaptative fine milk composition**

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Determination of fine milk composition (proteins and fatty acids, FA) is of major importance with regard to nutritional added value of milk, feeding strategies and animal health that impact milk production and price. For example,  $\omega 6/\omega 3$  ratio in milk is of high interest for consumers' health. Nevertheless, we don't have, in France, cheap and quick methods to analyze fine milk composition and reference database to determine genetic and feeding factors impacting this composition. Then different scientific and economic stakeholders, from milk production to milk processing formed the consortium called *PhenoFinlait* in order to carry out a R&D project on fine milk composition. The aim of this project is to develop a cheap and large scale phenotyping system for individual milk components (FA and proteins) and to apply this procedure on a specific design in commercial farms allowing an analysis of the genetic, the environmental factors (feeding strategy) and their interactions, involved in milk composition. At the end, we will develop dairy farm advice that combines genetic and feeding strategies to improve herd management (sustainability) and to adapt the fine milk composition to the evolving consumer demand in order to give to dairy industry the opportunity to maintain its competitiveness.

This program receives financial support from ANR, Apis-Gène, CASDAR, CNIEL, FranceAgriMer, France Génétique Elevage and Ministry of Agriculture.

## **Cheap and large scale analysis methods to quantify milk fatty acids and proteins content**

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Fine milk composition analysis for proteins and fatty acids (FA) brings information on nutritional value of milk, animal health and herd management. Therefore, it is essential to develop cheap and easy analytical methods to characterize fine milk composition. In France, a national R&D project called *PhenoFinlait* is ongoing. The first objective of this program is to develop a cheap and large scale phenotyping system for determination of milk FA and individual proteins content.

The first year of this project has allowed the development of equations to estimate milk FA composition from MIR (Mid Infra-Red) spectra usually obtained by milk recording laboratory to determinate milk fat and protein content. First results on these equations show that 15 to 20 FA and ratios of FA could be well estimated in the milk of the three ruminant species (cow, sheep and goat). Statistical research is ongoing to improve estimation for other FA and normalize this method.

For protein separation and identification, we have chosen a liquid chromatography system coupled with mass spectrometry. We are looking for improving this method in order to be able to separate and identify genetic and isomeric variants of 12 major proteins. Analysis method is actually setting up and the database to identify these variants is almost ready.

This study receives financial support from ANR, Apis-Gène, CNIEL, France Génétique Elevage and Ministry of Agriculture. Milk samples come from INRA experimental farm (Bourges, La Fage, Le Pin and Mirecourt) and one commercial farm.

## **Applications of mass spectrometry in dairy science**

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Mass spectrometry (MS) has been used at the Fonterra Research Centre (FRC) for over 10 years to analyze milk products and components. The work has encompassed a wide range of dairy systems including milk, cheese, yoghurts and hydrolysates, as well as a range of analytes like peptides, major and minor milk proteins, complex lipids, oligosaccharides, vitamins and amino acids. MS is used as a detector (often in combination with liquid chromatography) because of its selectivity, sensitivity and, applicability to a wide range of analytes and matrices. Historically MS has been used at FRC as a qualitative research tool but we are increasingly using it to identify and quantify dairy components in ingredients and consumer products that cannot be accurately measured using other techniques. We have recently been using mass spectrometry based methods to help demonstrate clinical efficacy in trials utilizing our consumer products and ingredients.

This poster will give an overview of how the analytical power of the mass spectrometer is being utilized in the New Zealand dairy industry in both quantification and characterization studies.

Some examples of work included in the poster will be:

- Quantification of gangliosides in dairy ingredients, consumer products and clinical study samples
- Quantification of several MFGM proteins in commercial lipid fractions
- Quantification of phospholipids in dairy ingredients and clinical study samples
- Micro-nutrient – vitamin D quantification
- Oligosaccharide analysis, characterisation and quantification in milk systems
- Characterisation of peptide molecular weight profiles in hydrolysates to validate a milk factory molecular weight profile method.

## Oilseed supplementations of grass-based diets during two consecutive lactations in Holstein cows: Effects on dairy performances and milk fatty acid composition

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Several short-term studies (1-3 months) have reported that supplementation of cow diets with oilseeds modifies milk fatty acid (FA) composition. As the durations of these experiments were short, putative long-term effects on milk FA composition and dairy performances remain poorly known. The aim of this study is to quantify the effects of supplementation with different oilseeds rich in either c9-18:1 or 18:3 $\omega$ 3 FA during two consecutive lactations on milk yield and composition.

Sixty Holstein cows during a 1<sup>st</sup> year, and 37 during a 2<sup>nd</sup> one, received high-forage diets (60-70% of dry matter intake (DMI)). During indoor period, cows were fed grass silage and hay and during outdoor period cows were grazing pasture. Basal diet was supplemented or not (control diet (C)) with either different forms of rapeseeds, whole seeds (WR), extruded seeds (ER) or a fat rich meal (FRM), or with extruded linseeds (EL). The oilseed supplements provided 2.4-3.2% oil in the DMI.

Compared to C diet, FRM supplementation increased milk yield (+2.9kg/day;  $P<0.05$ ) during indoor period and decreased milk protein content whatever the period (-1.8g/kg;  $P<0.09$ ).

During indoor period, oilseed supplements decreased milk 12:0+14:0+16:0 (-12.7g/100g FA;  $P<0.01$ ) and increased milk c9-18:1 (+6.6,  $P<0.01$ ). ER, FRM and EL supplements increased milk c9,t11-CLA (+0.32,  $P<0.01$ ) as well as total *trans* FA (+3.4,  $P<0.01$ ), whereas only EL increased 18:3 $\omega$ 3 (+0.72,  $P<0.01$ ). The effects observed during grazing period (except for milk c9,t11-CLA) were weaker than those during the 2 indoor periods.

Oilseed supplementation effects were dependent on type and form of oilseeds. Effects on milk FA observed during 2 consecutive lactations were comparable to those observed during short-term studies.

**The most efficient swab for the recovery and release-to-detection of  
*Chronobacter sakazakii* from different surfaces**

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As a consequence of the emergence of *E. sakazakii* as an important infant pathogen, robust and efficient testing protocols for the environmental monitoring for *E. sakazakii* in dairy processing facilities are vital. Such testing protocols generally rely on the sampling of processing and environmental surfaces using a variety of commercially available swabs. The aim of this study was to determine the efficiency of different types of commercially available environmental swabs for both sampling of *Cronobacter sakazakii* from different surfaces, and their subsequent release for detection. Pre-moistened gauze swabs, FlexiSwabs™, 3M™ Hydra-Sponges, and Copan UTM/Nylon flocked swabs were tested. The recovery of *C. sakazakii* cells, inoculated onto stainless steel, linoleum and Sure Shield™-coated surfaces, was most efficient using FlexiSwabs™ at inoculum levels of <2.5 CFU/cm<sup>2</sup>. In addition, the release of *C. sakazakii* cells was most efficient from Copan UTM/Nylon flocked swabs and FlexiSwabs™. However, disadvantages of the Copan UTM/Nylon flocked swabs included the inability to absorb more than a few hundred microlitres of sample at a time, and the cost per swab. Overall, the FlexiSwab™ proved most efficient for both the recovery of *C. sakazakii* from different types of surfaces which may be found in dairy processing environments, and their subsequent release from the swabs for detection.

**Efficacy of ethanol versus isopropanol-containing surface sanitizing sprays against various dairy-related bacteria on stainless steel and linoleum surfaces.**

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Alcohols are known to exhibit an effective antimicrobial activity. In particular, 70% ethanol is found to be the optimum concentration for exerting an antimicrobial effect on bacterial cells, and is widely used as a surface sanitizing spray. However, other ethyl alcohols, such as n-propanol in the range of 60-90%, are becoming more popular for use as surface sanitizers, particularly in Europe. The aim of this study was to determine the efficacies of 70% ethanol versus isopropanol at various concentrations (e.g. 60 or 70%), or a commercially prepared isopropanol spray (59% isopropanol and a mixture of quaternary compounds), against dairy-related bacteria on 2 types of surfaces which may occur in a dairy-processing environment (stainless steel 316 and linoleum). Cultures of various dairy-related bacteria, all previously isolated from dairy processing environments, such as *Staphylococcus aureus*, *Bacillus licheniformis*, *Escherichia coli* and *Pseudomonas*, were spread onto stainless steel or linoleum surfaces and allowed to air dry overnight. Surviving populations were counted after exposure to either ethanol or isopropanol-containing surface sprays for 0, 1 or 5 min, using standard microbiological techniques. Overall, results indicated that within the dairy processing context, isopropanol may not always be as effective an antimicrobial agent, when compared with 70% ethanol, for reducing dairy strains on stainless steel or linoleum surfaces.

## **Control of trichloromethane (TCM) residues in milk**

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Cleaning and disinfection in the milk production process on-farm is critically important to the quality of milk. Chemical solvents containing chlorine are among the most effective and economical, but there is an unintentional side effect: when chlorine comes in contact with milk, trichloromethane (TCM) is formed resulting in residues, particularly in high fat products. There are both legal limits and recommended target levels for TCM in specific dairy products. The objective of this study was to measure TCM in a range of bulk farm milks and identify the causes of high milk TCM on-farm. Approximately 200 milk tanker loads were sampled and milks analysed for TCM by GC methodology. Those of  $\text{TCM} \geq 0.002 \text{ mg/kg}$  were identified. All individual milk suppliers within those tankers were subsequently sampled on-farm and analysed for TCM. Of those with  $\text{TCM} \geq 0.002 \text{ mg/kg}$  a random sample of 45 were investigated as to the factors responsible. These farms were visited and the cleaning procedure of both milking machine and bulk tank investigated. Sixty eight percent of farms used insufficient rinse water to remove milk and chemical solvent from the plant pre- and post- chemical solvent circulation, respectively. Inadequate water usage was influenced by trough size, cost of water and a lack of knowledge on the importance of using sufficient water. Incorrect chemical product type and quantity were used on 39% and 22% of farms, respectively. Thirty one percent of farms re-used the chemical solvent more than once. In conclusion, the factors influencing TCM residue in milk may be due to a single farm practise or a combination of factors. The factors identified may be used as a template when solving TCM residue sources on farm.

## **Differentiation of heat intensity and quality evaluation of pasteurized milk by discriminant function analysis of flavor patterns using electronic nose system**

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In order to investigate differentiation of heat intensity applied to the milk and quality evaluation of milk for four types of pasteurization systems of milk, a total of 1,728 pasteurized milks (LTLT and HTST) and UHT milks (UHT treated and UHT sterilized) produced by six milk factory were used. Samples were purchased directly on arrival of milk transport vehicles to local markets. Milk samples were divided into three refrigerators at 4°C, 7°C, and 10°C for 12 days and analyzed the flavor patterns using SMart nose 300 system equipped with mass spectrometer. Discriminant function analysis (DFA) on flavor patterns milk samples seemed to provide useful criteria for identifying the heat intensity during processing of milk. It was found that a milk factory used incongruent thermal conditions for pasteurization. DFA<sub>1</sub> is highly correlated with the numbers of total bacterial count ( $R^2 = 0.971$  and  $0.935$  for LTLT and HTST milk, respectively) and psychrotrophic bacteria ( $R^2 = 0.961$  and  $0.926$  for LTLT and HTST milk, respectively). However, the correlation was low in UHT milk due to the absence of vegetative cell for proliferation. The results of the DFA were well grouped according to storage temperature and storage time. It may conclude that electronic nose analysis could be used as an appropriate technique for differentiation of heat intensity of milk and as an efficient technique for quality evaluation during storage.

## **New process for detection of frauds in milk**

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Nowadays, equipments and processes used to verify possible addition of water on milk and the corresponding detection of frauds present several problems and limitations. They bring out inconveniences like low portability, trustworthy and efficiency. Moreover, they usually require specialized workforce for its use, as well as constant calibration and elevated costs in some cases. In general they control the freezing point or the density of milk by a cryoscopy technique and a densimeter, respectively. Such techniques are limited to detect adulteration involving only water, and they do not present reliable results in case of frauds when sodium chloride and sodium hydroxide are added. Facing to this situation, it is evident the need of innovation in this area, developing new processes that are practical, efficient, and economically viable. Within this perspective we developed a process to verify the fraud on cow milk, by a combination of electrical conductivity measurements. The developed device is intended to be portable and does not require previous sample preparation. By this simple, efficient, and cheap technique we are able to accuse not only the adulteration with water, with 2% (volume) of sensibility, but even some substances like sodium chloride and sodium hydroxide, that decrease the nutritional value of milk and in some cases can be noxious for health.

## Validation of spectrofluorimetric method for the determination of riboflavin in yogurt

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Storage conditions of yogurt in supermarkets can cause product changes due to the presence of riboflavin (RBF), which under light stimulus and presence of oxygen promotes the oxidation of vitamins, carbohydrates, lipids and proteins, leading to off-flavor formation and loss of nutrients. The main RBF detection method described by AOAC as the fluorimetric method standard involves extraction with organic solvents, autoclaving and sample cleanup prior to fluorimetry, making it time consuming and costly. The use of spectrofluorimetric titration of RBF using a riboflavin-binding protein (RBP) as titrant, based on the method of Zandomenighi, *et al.* (2007) for determination of RBF in milk, was optimized and validated. This optimization offers the advantage of no sample preparation, since the fluorescence is measured directly in the yogurt. The determination of RBF was made in Varian spectrofluorometer, with accessory for measures in front-face, at an angle of 27 °. The selectivity of the method was determined by RBF standard addition in yoghurt and in water. Both curves presented parallel with B = 1.0015 and R = 0.9999 for water and B = 1.0060 and R = 0.9982 for yogurt and the detection and quantification limit was 0.1330 µg/g and 0.4031 µg/g. respectively, showing a method selective and linear. The method was accurate, since the repeatability estimated by the relative standard deviation was 5.12%, and the determinations made on different days presented agreement in the results. The accuracy was 98.53%, and the experiments for robustness showed the method is effective even after 30-days storage of the product. The results proved that the determination of RBF by spectrofluorimetric titration is applicable in yogurt.

## **Phosphates – a functional ingredient for delivering healthy, safe and tasty dairy products**

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Phosphates are functional ingredients in dairy applications – serving as emulsifying salts, protein stabilizers, sources of essential nutrients and synergists in the area of delivering safe food. This poster will review the primary functionality of phosphates in key applications including cheese, milk based beverages, ice creams, dips, puddings, yogurts, yogurt based beverages, meal replacement beverages and soy based dairy alternatives. Research on sodium reduction and fortification with calcium and magnesium using Cal-Sistent® and Mag-nificent® and impact on sensory characteristics is explored.