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Bia agus Mara
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Food Institutional Research Measure

Final Report

National Cheese Research Programme 2015 – CheeseBoard 2015

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Please place *one* "x" below in the appropriate area on the research continuum where you feel this project fits

Basic/Fundamental → Applied → Pre Commercial

1	2	3	4	5	6 X	7

Please specify priority area(s) of research this project relates to from the National Prioritisation Research Exercise* (NRPE) report;

Priority Area (s)	**H, M**

Key words: *(max 4)*
Cheese, reduced-fat, reduced-salt, diversification, microbiology, cultures, next generation sequencing, marketing, processed cheese, calcium chelating salts,

1. Rationale for Undertaking the Research

Cheddar cheese output in Ireland is expected to reach 175,000 t by 2020. The expansion of cheese required an underpinning programme of research to support the development of cheese varieties other than conventional full-fat Cheddar, for example: reduced-fat, reduced-salt Cheddar variants; continental cheeses, e.g., eye-cheeses, mozzarella-type, e.g. Cagliati. In this diversified cheese portfolio, it is critical to understand the mechanisms that control texture, flavour and functional properties. Much Cheddar cheese is used for the manufacture of process-cheese products (PCPs) for use as a functional ingredient in catering and fast-food applications; an increasing trend in this market is the drive towards reduced-sodium products. Hence, Cheeseboard 2015 also focussed on the role of stabilizers (emulsifying salts) in PCPs and developing methods for the manufacture reduced-sodium PCPs. Moreover, for Ireland's mainstream commodity cheese (e.g., Cheddar and Emmental) to remain competitive in the dairy market, quality of the dairy products will be hugely important to ensure optimum flavour and texture.

2. Research Approach

This large project consisted of 6 TASKS, carried out across 6 institutions i.e., TFRCM, TFRCA, UL, UCC, IFBA and UCD. TFRCM focused on the development of a comprehensive knowledge platform on the interactive effects of fat and salt reduction in Cheddar cheese and ways to improve the texture and flavour of half-fat, half-sat Cheddar, and if Cheddar cheese could be used as a vector for probiotics, vitamins and minerals. TFRCA examined what the needs of the consumer were in relation to cheese and cheese products, and the consumer perception on new reformulated cheeses e.g., cheeses containing probiotics. UCD studied ways to improve low-fat, low-salt processed cheese. Teams based in UL and UCC worked on screening and developing cultures which could be used to improve the flavour of cheese with low fat and salt levels. The research approaches used in the 6 different tasks are listed below:

TASK 1: Reduced fat/salt cheeses with improved texture and flavour
The Task involved a series of sub-tasks, and experimental units aimed at evaluating the effects of:

- (i) varying fat and salt on the cheese matrix (calcium phosphate *para*-casein network, with encased fat globules and entrapped moisture)
- (ii) improving cheese quality by:
    - attenuating the structure of the calcium phosphate *para*-casein network via reduction in the degree of calcium-induced cross linking of the casein through the addition of a food grade acid to the cheesemilk
    - increasing the extent of casein hydrolysis by increasing the levels of added bovine chymosin added to the cheesemilk
    - the use of adjunct-culture as a means of improving low-fat, low-salt cheese flavour

- compartmentalization of salt into regions of low and high concentration
- screening of cultures that improve flavour in half-fat cheese, reduced-sodium cheese using Pacific Biosciences single molecule real time sequencing

TASK 2: Application of cheese curd in processed/other applications for cheese

Task 2. The approach taken in task 2 involved:

- Comparing the calcium-chelating properties of commonly-used emulsifying salts (DSP, TSC) in model aqueous systems comprising varying concentrations of calcium chloride (up tom 50 mM) with different emulsifying salts (up to 100 mM) separately and in combination, or aqueous systems comprising of rennet casein with varying levels of DSP and TSC separately and in combination.
- Development of low-fat, low-salt processed cheese to optimise the physical and sensory properties

TASK 3: Cheese as a vector for bioactives/probiotics/vitamins/minerals

· A designer cheese (low fat, high Ca and Vit D) will be used to investigate whether increasing the calcium and vitamin D content in the cheese could reduce adiposity by influencing lipogenesis and lipolysis in the adipose tissue through a reduction in the plasma the levels of parathyroid and calcitrol hormones, as well as by increasing fat excretion, thus reducing the amount of dietary fat available for absorption through the intestine. The task involved:

- Assessing the health benefits of ingestion of low-fat, high calcium and vitamin D cheese by feeding mice a standard diet or pellets that differed primarily in the contents of fat (ranged from 10.3 to 28.6 %) and vitamin D (ranged from 0.0075 to 0.186 ug/g).
- Once the animal trials are complete, analyse the serum, tissue and faecal samples.
- Study gene expression, in particular levels of mRNA transcript of Fatty Acid Synthase (FASN), an important regular of fat storage.
- Analyse the fat tissue from the mice for the size and number of adipocyte by looking for indicators of adipocyte size (MEST) and adiposity (leptin) in subcutaneous and visceral tissue.

TASK 4: Combination of advanced molecular biology analytical techniques with technological innovation to support development of new cheese varieties and diversification of existing product portfolio

The research approach involves:

- Application of new molecular techniques to determine the role of and to control the non-starter lactic acid bacteria microflora in relation to defects in low or reduced salt Cheddar-variants and Continental type cheeses using next generation molecular techniques i.e., DNA Pyrosequencing
- Determination of the influence of varying manufacturing processes, altered compositional parameters and ripening temperature profile on the profile of dominant and subdominant NSLAB leading to the establishment of control points during manufacture and ripening to minimise the growth of particular NSLAB by:
  - Isolating NSLAB and grow on selective media (MRS, LBS, PCA, LM17 and SLA)to assess ability to produce CO_2
  - Determining the evolution of selected NSLAB from dominant to subdominant microflora
  - Ability of selected NSLAB to inhibit or stimulate propionic acid and produce biogenic amines in a whey medium.
- Manipulation of cheese compositional, physicochemical and processing matrices to diversify the range of textures in dry salted Cheddar- variants and brine salted continental cheese-types by:

- Investigating the effect of adding EDTA a two different levels (i.e., 2.5 or 5 g EDTA/L of whey) during cheesemaking on the textural properties of Gouda-type cheese
- Examine if adding EDTA in different types of micro-encapsulated liposomes (i.e., ProLipo Duo and ProLipo C) to the cheesemilk will minimise the compositional variation that occurs when adding EDTA directly the curds and whey mixture

- Research into the potential capacity of commercial cultures, exploitation of speciality cultures for conventional cheddar variant manufacture processes and utilization of metabolic profiling to modulate and intensify the flavour profile of novel dry-salted Cheddar variant cheeses by:
  - Evaluating the potential of commercial culture strains, mainly *Steptococcus thermophillus* and *Lactobacillus helveticus*, to enhance flavour profiles of dry salted Cheddar variants
  - Assess the metabolic potential of a range of cheese cultures including yeast and bacteria using the cell envelope protease (CEP) assay and by measuring concentrations of Pep X, Pep N, AMC and GDH produced by these cultures.
  - Developing a process to produced stabilised cheese inocula of cultures associated with artisanal cheeses using model curd systems containing a cheese curd base, tryptic peptone f casein, yeast extract and mineral preparation.
  - Expanding the biodiversity of starter strains available for the production of dry salt cheeses using metabolic profiling

TASK 5: Analytical techniques capable of differentiating between natural and industrial trans fats

· The aim of this task was to develop methodology to determine and quantify the levels of fatty acids in Cheddar cheese, butter and dairy spreads by:

- Using the multivariate statistical tools FT-IR and NIR was to develop predictive models which improves sampling of trans fats in milk and to take into accounts the variation with seasonality.
- Investigating the benefits of using Raman spectra data which is a more sensitive and definitive chemical technique, which can be used for confirmatory analysis which will focus on trans-isomers of 16:1, 18:1, 18:2, 18:3, 20:1 and 22:1 fatty acid methyl esters found in milk. Confirmatory and absolute quantification of the TFAs will be achieved using either LC-MS/MS and/or LC-NMR spectroscopic methods

TASK 6: Knowledge of the drivers of consumer choice/perception/acceptance with respect to cheese

 In order to determine what drives consumers to purchase cheese and new cheese products, the following approaches were used:

- An in-depth review of the Irish, UK and International cheese market to identify trends, market size and cheese categories. Using library sources within Bord Bia, Teagasc and UCC.
- A review of product launches, identifying successes and failures as well as types of innovations will be conducted which will enable identification of gaps and/or opportunities of relevance to the Irish cheese industry.
- An extensive scientific research literature review will be conducted on consumer knowledge, understanding, beliefs, perceptions and needs of consumers regarding alternative cheese products and cheese/dairy products as a carrier for functional bioactive compounds. This review will also serve to inform the discussion guide and composition of the focus groups as well as the questionnaire in the data collection Sub-Task 6.2
- Organise a focus group and conduct interviews to collect data about what cheese products have market potential.
- Determine consumer acceptance of cheese with health and/or novel ingredients (e.g., bioactive peptides) claims.

- The data from the study will be collated to recommend areas that would benefit from further development in the dairy industry.

3. Research Achievements/Results

TASK 1: Reduced fat/salt cheeses with improved texture and flavour

Task 1 (Sub-task 1.1). Reduced fat/salt cheeses with improved texture and flavour. ***Subtask 1.1: Optimising the matrix of half-fat, low Na cheese to give desired texture and flavour production***

An extensive database on the interactive effects of fat and salt reduction on Cheddar cheese composition and changes in lactose metabolism, microbiology, proteolysis, rheology and sensory properties during maturation has been established. Fat reduction in Cheddar cheese resulted in several changes including: increases in pH, protein, lactic acid production, free amino acids (FAA) level, firmness and fracture stress; reductions in moisture-in-non-fat substances (MNFS), rate α_{s1}-casein hydrolysis, concentrations of free fatty acids (FFA) and flowability on heating. Sensory analysis of the 270 day-old cheeses indicated that full-fat (FF) cheeses had higher concetrations and a greater array of volatile compounds, including short-chain fatty acids (butanoic, pentanoic, octanoic, hexanoic), ketones (acetone, 2-heptanone, 2-nonanone), ester (ethyl caproate, ethyl butanoate), alcohols (ethanol, 2-heptanol), acetic acid, and carbon disulphide. Fewer compounds were identified in the reduced-fat (RF) and half-fat (HF) cheeses and the volatile compounds differed, comprising these included ketones (2,3 butanedione, 2-butanone, 3-methyl-2-butanone), ester (acetoine, ethyl acetate), alcohols (isopropyl alcohol, 2-butanol), and dimethyl sulphide. The greater array of compounds in the FF cheese is consistent with its higher level of fat, from which most of the volatiles are most likely derived. Reducing salt content from 1.9 to 1.2 or 0.9 % in the FF (33%), RF (21%) and HF (16%) cheese did not significantly affect the total concentration of volatile compounds but influenced the volatile profile e.g., reducing salt from 1.9 to 0.9 % in the FF cheese increased the concentration of short-chain fatty acids. Sensory evaluation of the 270 day-old cheeses showed that FF cheeses (which were identified with the attributes sweet taste, cream flavour and Cheddar flavour, scored highest for overall acceptability) were generally more acceptable than the RF cheeses and significantly more acceptable than the HF cheeses which lacked the latter attributes were excessively firm and lacked pastiness in the mouth. The impact of fat was influenced by salt to an extent dependent on fat content. Sensory analysis of the 270 day-old cooked (95 °C) cheeses showed that the RF cheeses had highest acceptability, out-performing the full-fat cheese which was positively associated included oiling-off, fluidity and succulence, and negatively associated with included firmness in mouth, chewy texture, and rubber texture.

Using the above database, studies were deigned to improve the quality of half-fat, half-salt Cheddar cheese. The texture and cooking properties of half-fat, half-salt Cheddar-style cheese were significantly improved by (i) reducing the calcium-to-casein ratio, and (ii) increasing the level of primary and secondary proteolysis by increasing the level of added bovine chymosin and using an adjunct-containing starter culture. These improvements were verified by reductions in (i) the fracture stress and firmness of unheated cheese, (ii) an increase the flowability, and a reduction in the work to extend, the molten cheeses, and (iii) a more favourable grading, as undertaken by a commercial cheese grader.

Task 1. Reduced fat/salt cheeses with improved texture and flavour. Subtask 1.2: Selection of starter/adjunct cultures for optimum flavour production in half-fat, low-salt cheese.

The objectives of Sub-Task 1.2 are to optimize flavour development in half-fat reduced salt cheese through culture manipulation and selection. This work was undertaken by University of Limerick and

University College Cork. The approach taken involved evaluation of selected cultures in media varying in salt content and characterization for viability, autolysis, permeability, intracellular peptidase activity. Selected strains from both UL and UCC were then evaluated in pilot-scale cheese manufacture for their effect on the quality of half-fat, half-salt cheese using a procedure designed to optimize the cheese matrix (as established in Task 1, sub-task 1). Additionally, the impact of fat and salt reduction on the growth, viability, die-off, autolysis, and populations of live, permeabilised and dead cells in full-fat (33%)-, reduced-fat (21%)- and half-fat (16%) Cheddar cheeses, each with full-salt (1.9%)-, reduced-salt (1.2%)- or half-salt (0.9%).

The salt sensitivity of several defined strain cheese starter cultures (e.g., L. *lactis* subsp. *lactis* 303, 227, 223, R745) and also commercial mixed starters of R604Y (consisting of one *L.lactis* subsp. *lactis* and one subsp. *cremoris* strain) and RSF-742 starter (consisting of one *L. lactis* subsp. *lactis* strain and one S. Thermophilus strain) was assessed. Strains were grown in 10 % reconstituted skim milk (RSM) and subjected to the Pearce activity test, where salt at various levels was added at the end of the process (5.5 h). Salt addition rates of 0.0, 2.0, 2.9 and 4.0% (w/v) were applied for analysis of all strains. Growth was monitored using standard plate count over a 2week period at 8 C, and that of the commercial mixed strain starters over 4 weeks so as determine differences in strain viability. L. *lactis* subsp. *lactis* strains (227, 223, R745, 303) and indicated a high salt tolerance as evidenced by higher viable counts, slower rate of cell permeabilisation, and low levels of cell autolysis. Also these strains were shown to have high acidification rates during initial stages of growth and storage. The commercial mixed strain starter cultures R604Y and RSF-742 had a high viability over a prolonged time period, but yet showed an increase in cell permeabilisation accompanied by the higher levels of cell autolysis in response to increasing salt addition levels. This may be attributed to one strain being salt sensitive within the culture blend.

A bank of Lactococcal strains including AM1, AM2, HP, 303, SK11, 223, 227, LMM81 were screened for their growth in the presence of varying salt levels over the range 0-6% in L-M17 broth using optical density (OD). Samples were analyzed for water activity (aw), culture acidification (decrease in pH), viability (standard plate count methods), cell autolysis (release of intracellular PepX activity), permeabilisation (sub-populations of intact/live and dead/permeabilised cells by Flow Cytometry in combination with SYTO9/PI staining) and cell recovery on L-M17. Salt affected both growth rate and viability. Increasing salt to 6% resulted in extended lag phase and lower final OD values for some strains. Flow cytometry showed that the proportion of intact/live cells was 97% at low salt levels (<2%) and decreased to~70% at higher salt levels (>2%). Moreover, a strain related response to salt was noted. Generally highest salt tolerance was noted for Lc. *lactis* subsp. *lactis* strains e.g., 223, 227, 303 and ML8. The maximum salt level at which growth was possible was 4% for Lc. *lactis* subsp. *lactis* strain 303, a starter used in commercial production of Cheddar in Ireland. Growth of Lc. *lactis* subsp. *cremoris* strains AM2, AM1, SK11, Wg2 was strongly inhibited at salt levels of 3-6% (w/v). Among the Lc. *lactis* subsp. *cremoris* strains tested, lowest cell densities were observed for strains AM2 and SK11 in the presence of 1% added salt indicating that they were the most salt sensitive strains. The Lc. *lactis* subsp. *cremoris* strains R1, Wg2, HP and AM1 were somewhat more salt tolerant with growth noted up to 2-3% NaCl indicating their potential as quick-lysing strains for use low salt Cheddar cheese. Strains of L. *lactis* subsp. *lactis* were the most salt tolerant, with highest tolerance observed for strains 223 and 303. The impact of salt on growth was confirmed by FCM, which showed a progressive decrease in the percentage of intact/live cells as salt was increased, from 98% at 0% salt to ~74% at 2% added salt. In contrast, strain 303 was significantly more salt tolerant and maintained a 70% intact/live sub-population in the presence of 5% salt. Viability, acidification potential, rate of cell permeabilisation and cell autolysis were greatly affected by salt, with the response to salt being strain-dependent. Overall, strains of L. *lactis* subsp. *cremoris* were associated with higher rates of cell permeabilisation and cell autolysis, lower cell viability, poor acidification potential, and lower cell recoveries.

A more detailed study was undertaken on the salt sensitivity of L *lactis* subsp. *cremoris* AM2, as this culture is well recognized as having good autolytic and imparting good flavour in standard Cheddar cheese (full-fat, full-salt). In particular, it was of interest to ascertain if it had these characteristics in low-salt environments, resembling reduced- or half-fat Cheddar cheeses with S/M levels of ~ 1.9 to 4.5%. The modified Pearce activity test, which mimics cheesemaking process (with respect to temperature, time,

and salting), was used to screen AM2 and other starter culture strains for their autolytic and permeabilisation properties in half-fat and reduced- salt Cheddar cheese. This method involves growth of cheese starter cultures in 10 % RSM during the Pearce activity test, where salt was added at the end of the process (5.5 h). Growth of cultures was monitored over a 14-day period at 8 C; salt treatments applied were 0, 0.9, 1.2, 1.8 and 3.0 % NaCl (v/v), corresponding to S/M levels of ~ 0, 1, 2 and 3.3 %, respectively. Viability, autolysis and permeabilisation properties of strains were examined using standard plate count, release of intracellular marker enzyme (lactate dehydrogenase, LDH) and sub-populations of intact/live, dead/permeabilised cells by Flow Cytometry. Lowest viable counts were detected at 1 d. Counts were highest at 0 % salt, reaching log 7 cfu/ml at 4 d and decreasing thereafter to log 6 cfu/ml. A one log reduction in viable counts (log 6 to log 5 cfu/ml) was observed for 1.2 and 1.8 % salt addition levels (S/M levels of 0.86 and 1.62, respectively) after salting and at day 1 post salting. Increasing salt level to 3.0 % resulted in a 3 to 4 log reduction in viable counts of AM2 after salting. Lower percentages of intact/live cells were measured in samples containing higher salt addition levels (1.2, 1.8 and 3.0 % NaCl). Prior to salt addition, percentages of intact/live cells for all samples were between 85-90%. The proportion of intact/live AM2 cells at 0 % salt was ~85- 90% at 1-7 d post salting and decreased to 75 % at 14d. Increasing salt to 0.9% had little effect proportion of intact/live AM2 cells. In contrast, increasing salt from 0 to1.2, 1.8 or 3% resulted in a significant decrease in the percentage of intact/live cells from 80-90 to 70, 60 or 50 %, respectively.

Based on their salt sensitivities, as discussed above, three single strains were selected for laboratory-scale cheesemaking based on their lower proteolytic activity cell recovery, and higher cell permeabilisation and rates of autolysis. These were evaluated for their permeabilisation potential, intracellular enzyme release and also cell autolysis in a lab-scale full-fat (32.0%) full-salt (1.8%) and half-fat (16.0%) half-salt (0.9%) Cheddar-type cheeses. The results indicated that reducing salt content by 50% decreased viability, cell permeabilisation/cell death and cell autolysis of strains. Fat reduction resulted in significantly lower viability of selected starters and also in significant increase in cell permeabilisation and subsequently cell autolysis of all studied strains during cheese ripening.

The impact of fat and salt reduction on the performance of a commercial culture R604Y culture consisting of two L. *lactis* subsp. *lactis* strains was evaluated in pilot-scale cheese manufacture (500 L milk). Nine different cheese types were manufactured in triplicate: full-fat (33 % fat), reduced-fat (22% fat) and half-fat (16 %), each with salts levels of 1.9% (full-salt), 1.2% (reduced-salt) and 0.9% (half-salt). Autolysis, as monitored by release of Pep X and LDH activities released in cheese juice, were affected by both salt and fat content of cheese. Highest autolysis was found in reduced-fat cheeses with 1.9% salt. No major differences in enzyme activity were between full-fat and half-fat cheeses with 1.9% salt. Reducing salt level coincided with lower rates of autolysis in full-fat and reduced-fat cheeses, but not in half-fat cheeses. The FCM data was mirrored by trends found for release of intracellular marker enzymes Pep X and LDH. Highest intact cell populations were found in cheeses with reduced salt levels, e.g., 50-70% of total viable bacteria in full-fat cheese with 1.9% salt compared to 60-90% in full-fat cheese with 0.9% salt. Lowest Intact cell populations (35-59 %) were found reduced-fat cheese with 1.9% salt. No significant differences were found between the intact cell populations in full fat (32%) or half fat (16%) cheeses. Results to date indicate that both fat and salt reduction influenced starter viability and autolysis in cheese and particular salt and fat combinations appeared to result in highest intracellular enzyme release with potential implications for flavour development. Overall, these trials have highlighted the necessity to match starter performance with optimum matrix development and work is on-going to achieve these outcomes.

UL, in conjunction with TMFRC and UCC, half-fat and Half-salt Cheddar cheese was made using the standard procedure developed for matrix optimization (task 1). Three different starter cultures were used: (i) a standard commercial mixed-strain culture (R607), (ii) a *L. lactis* subsp. *cremoris* ((NCIMB Ltd., Aberdeen, Scotland) single strain selected from the prescreening process used in UL , as described above), and (iii) . The resultant cheeses were coded: HFFS-R607, HFHS-UL and *L. lactis* subsp. *cremoris* 158 (UCC), respectively. The cheeses were analysed forcomposition, water activity (a_w), proteolysis, firmness, starter lactic acid bacteria (S-LAB) counts, non-starter lactic acid bacteria (NSLAB) counts, starter culture

permeabilisation (FCM), starter cell autolysis and release of intracellular marker enzymes into cheese matrix, and commercial grading of flavour and texture. Compared to cheese made with the reference culture (HFFS-R607), that made with the selected *L. lactis* subsp. *cremoris* single strain (HFHS-UL): had higher initial starter cell (S-LAB) population; showed a significant decrease in the S-LAB population in the HFHS-UL during ripening (S-LAB count remained constant constant in HFFS-R607 cheese); had higher levels starter cell permeabilisation, starter cell autolysis, and levels of intra-cellular enzymes (Pep X, Pep N and LDH); had lower levels of primary proteolysis and higher firmness. Grading indicated that HFHS-UL cheese was ranked as the most favourable cheese with a slight mealy texture, nutty flavour, good overall flavour and good aftertaste; the reference (HFHS-R607) cheese was less desirable owing to its mealy texture, bitter flavour notes and overall poor flavour attributes.
Overall, the experimental HFHS-UL cheese made with selected starter of *L. lactis* subsp. *cremoris* received better flavour comments than the corresponding control HFHS-R607 cheese made with commercial mixed culture, which is normally recommended for the use in low-fat cheese products for better flavour generation.

In total 16 new strains of L. *lactis* have been sequenced and functional analysis of their cheese making properties carried out. The main outputs from this reporting period were a study accessing the scale of prophage integration within lactococcal genomes and their potential impact on dairy fermentations (manuscript in preparation), and a comparative genomic analysis of all dairy lactococci, assessing the contribution of these strains to dairy fermentations.

Task 1 (sub-Tasks 1.1 and 1.2) resulted in scientific publications 1-9 in section 4b (i) and popular article and/or conference presentation articles 1-3 in section 4b(ii).

Task 2. Application of cheese curd in processed/other applications for cheese

Processed cheese products (PCPs) are conventionally manufactured by blending cheese curd, water, calcium chelating salts (CCS), subjecting the blend to heating while shearing, and cooling the resultant homogenous, emulsified product. Imitation cheese products are made using a similar approach, except that cheese is replaced by casein, typically rennet casein. For products, the CCS used are principally sodium phosphates and/or sodium citrates. Attempts to reduce sodium by reducing or eliminating the CCs, significantly impairs physical functionality (e.g., deformation characteristics of unheated product and cooking characterises of cooked products).
Task 2 focused on developing greater insight into the functionality of calcium chelating salts (CCS) in model systems with a view to assist the development of processed cheese products (PCP) and PCP-like food matrices with reduced sodium content. Key areas of investigation included:

(i) the impact of different CCS types and levels on calcium ion activity and protein solubility in model aqueous-based systems
(ii) Manipulating calcium level provides a new approach for the manufacture of casein-based food structures with different functionalities
(iii) Understanding the dynamics of calcium-ion exchange processes as affected by calcium chelators during manufacture of casein-based food matrices

Disodium phosphate and trisodium citrate at concentrations of 10 and 30 mmol L(-1) and at ratios of 1:0, 2:1, 1:1, 1:2 and 0:1 were added to model CaCl2 solutions (50 mmol/L) or rennet casein dispersions (15 g/100 g). Adding trisodium citrate either alone or as part of a mixed chelating salt (CCS) system resulted in high levels of dispersed "chelated" calcium; conversely, disodium phosphate addition resulted in lower levels, while the calcium ion activity (ACa^{++}) decreased with increasing concentration of both chelating salts. Neither chelating salt produced high levels of soluble protein. Thus calcium chelating salts may play a more subtle role in modulating hydration during manufacture of casein-based matrices than simply solubilising calcium or protein

Casein-based food structures (58% moisture, 33% protein, 4% fat) with different calcium levels (37–1080 mg/100 g) were manufactured by blending rennet casein and acid casein, heating at 80 °C under shear, and cooling. At intermediate calcium levels (358–673 mg/100 g), homogeneous structures were formed without CCS after relatively short processing times. However, homogeneous structures with high (≥775 mg/100g) or low (≤37 mg 100 g/100 g) calcium levels could not be produced without CCS. On cooling, the CCS-free matrices with intermediate calcium levels formed functional cheese-like structures, although with lower hardness, flow on melting and elasticity (storage modulus, G') values at 25 °C than conventionally formulated high-calcium structures made from rennet casein with CS. By reducing total calcium level, it is possible to produce hydrated, functional casein-based structures containing lipid without CS. Omitting CS provides potential to reduce the sodium content of processed cheese type-products by up to 60% and would allow clean label products.

Casein-based emulsion gels prepared with different types of lipid (i.e. milk fat or rapeseed oil) were formulated with high (774 mg Ca per 100 g) or low (357 mg Ca per 100 g) calcium levels by blending acid and rennet casein. Their physicochemical characteristics (i.e. composition, texture, microstructure & water mobility) and in vitro digestibility were compared to conventionally formulated high-calcium (723 mg Ca per 100 g) emulsion gels made from rennet casein with calcium chelating salts (CCS). CCS-free, high-calcium emulsion gels were significantly ($p \leq 0.05$) softer than those with low calcium levels (possibly due to their shorter manufacture time and higher pH) and showed the highest rates of disintegration during simulated gastric digestion. The high-calcium matrices containing CCS had the slowest rate ($p \leq 0.05$) of disintegration in the gastric environment. Gastric resistance was not affected by the type of lipid phase. The results suggest that food matrix physical properties can be modified to alter resistance to gastric degradation which may have consequences for the kinetics of nutrient release and delivery of bioactives sensitive to the gastric environment.

Task 2 resulted in scientific publications 10-13 in section 4b (1).

TASK 3: Cheese as a vector for bioactives/probiotics/vitamins/minerals

Task 3 investigated the potential health benefits of high-calcium, high vitamin-D cheese. Model processed-cheeses with different levels of fat, calcium and vitamin D were formulated: half-vat, low vitamin D (HFLD), half-fat, high vitamin D (HFHD), full-fat low vitamin D (FFLD), full-fat high vitamin D (FFHD); the calcium content of the FF and HF cheeses were 1960 and 2620 mg/100 g respectively. The levels of vitamin D selected to be of relevance to the industry: 30% RDA is what the Irish adult male consumes in their diet (Irish Nutrition Survey). The high vitamin D levels was based on the amount of vitamin D that industry adds to cheese to meet the requirements of a 'high in vitamin D' food label.

The four cheeses were fed to mice under controlled experimental conditions. The results of a 12-week feeding trial showed that feeding HFHD cheese provided positive health benefits to blood, fat cells, body weight and bone. A half fat cheese diet enriched with Vitamin D encouraged fat cells to utilize glucose. Levels of circulating vitamin D were higher in these animals compared to animals consuming the same quantity of vitamin D but in a high fat cheese diet. Calcium % in bone also differed in animals on different cheese and vitamin D diets. There was a 'fat x vitamin D' interaction observed for several of the biomarkers tested including body weight and adipose biomarkers. In a low vitamin D diet, mice consuming full fat cheese were significantly heavier than those consuming half fat cheese even though both diets were isocalorific. Where animals consumed high fat cheese, the group of animals that also received high vitamin D consumed less food. The knowledge gained in this project not only demonstrated the health benefit of half fat cheese high vitamin D diet but also added to the evidence that vitamin D plays a role in energy homeostasis and adipocyte health which is influenced by the level of fat in the diet.

In conclusion, the study demonstrated that consumption of a half fat, high vitamin D cheese product would provide a positive health impact to the consumers in body weight management, circulating vitamin D levels and glucose utilization.

Task 3 resulted in popular article and/or conference presentation 4-5 in section 4b (ii).

Task 4. Combination of advanced molecular techniques with physicochemical and technological innovation to support development of new cheese varieties and diversification of existing product portfolio

Task 4.1. *Application of next generation molecular techniques to determine the role of and to control the NSLAB (non-starter lactic acid bacteria) microflora in relation to defects (gas formation, splits, secondary fermentation, stimulation/inhibition of propionic acid fermentation, biogenic amine formation) in low or reduced salt Cheddar-variants and Continental-type cheeses*./ Research to modulate and intensify the flavour profile of novel dry-salted Cheddar variant cheeses

The main focus of task 3 was to enhance cheese diversification through:

(i) the use of advanced molecular techniques to determine population dynamics in cheese during the course of manufacture and ripening, and detect specific microorganisms associated with defects such as production of biogenic amines or pinking of chees.

(ii) chelation of the calcium of natural cheese (using EDTA) as a means of controlling textural and functional properties of brine-salted cheese.

(iii) modulation and intensify the flavour profile of novel dry-salted Cheddar variant cheeses using bacterial strains isolated from speciality cheeses and pre-screened for metabolic activity

Next generation sequencing (16S rRNA amplicon sequencing) was used to elucidate the microbial community dynamics of brine-salted continental-type cheese in cheeses produced early and late in the production day. Differences in the microbial composition of the core and rind of the cheese were also investigated. Throughout ripening, it was apparent that cheeses produced late in the day had a more diverse microbial population than those produced in the earlier part of the day. Spatial variation between the cheese core and rind was also noted, with the cheese rinds having a more diverse microbial population during the early ripening but thereafter the opposite was the case. Interestingly, the genera *Thermus*, *Pseudoalteromonas*, and *Bifidobacterium*, not routinely associated with a continental-type cheese produced from pasteurized milk, were detected. The significance, if any, of the presence of these genera will require further attention. Ultimately, the use of high-throughput sequencing has facilitated a novel and detailed analysis of the temporal and spatial distribution of microbes in this complex cheese system and established that the period during a production cycle at which a cheese is manufactured can influence its microbial composition, thus potentially impacting on the consistency of ripened cheeses ready for sale.

Next generation sequencing, together with shotgun metagenomic sequencing as well as quantitative PCR (qPCR), was applied to study the microbiology of Continental-type cheeses with a pink discoloration defect. The basis for this phenomenon has remained elusive, despite decades of research. The bacterial composition of cheese containing the defect was compared to that of control cheese. *Thermus*, a carotenoid-producing genus, was present at higher levels in defect-associated cheeses than in control cheeses. Prompted by this finding and data confirming the pink discoloration to be associated with the presence of a carotenoid, a culture-based approach was employed, and *Thermus thermophilus* was successfully cultured from defect-containing cheeses. The link between *Thermus* and the pinking phenomenon was then established when the defect was recreated by the reintroduction of a *T. thermophilus* isolate to a test cheese during the manufacturing process. This finding has the potential to develop new strategies to eliminate this defect.

High-throughput DNA sequencing was to assess the incidence of bacteria with biogenic amine (BA; histamine and tyramine) producing potential from among 10 different cheeses varieties. Cheeses analysed included: dry salted Cheddar-type cheeses with high and low levels of BA: brine-salted continental-type cheeses with varying defects including secondary fermentation and split defects; and a continental cheese. The results showed that that *Lactobacillus curvatus*, *Enterococcus faecium* and *E. faecalis* were the dominant species with tyramine producing potential, while *Lactobacillus buchneri* was found to be the dominant species histaminogenic producing potential. Commonly used cheese starter bacteria, including Streptococcus thermophilus and Lb. *delbreueckii*, were also identified as having biogenic amine producing potential in the cheese studied. Molecular analysis of bacterial communities was then further complemented with HPLC quantification of histamine and tyramine in the sampled cheeses.

Nonstarter lactic acid bacteria are commonly implicated in undesirable gas formation in several varieties, including Cheddar, Dutch-, and Swiss-type cheeses, primarily due to their ability to ferment a wide variety of substrates. This effect can be magnified due to factors that detrimentally affect the composition or activity of starter bacteria, resulting in the presence of greater than normal amounts of fermentable carbohydrates and citrate. The potential for a facultatively heterofermentative *Lactobacillus* (*Lactobacillus casei* DPC6987), a non-starter lactic acid bacterial strain isolated from a cheese plant environment, to promote gas defects in the event of compromised starter activity was determined. A Swiss-type cheese was manufactured using a typical starter culture (*Streptococcus thermophilus* and *Lactobacillus helveticus*) together with propionic acid bacteria. *Lactobacillus helveticus* populations were omitted in certain vats to mimic starter failure of a starter component. *Lactobacillus casei* DPC6987 was added to each experimental vat at 4 log cfu/g. Cheese compositional analysis and X-ray computed tomography revealed that the failure of starter bacteria, in this case *L. helveticus*, coupled with the presence of a facultative heterofermentative *Lactobacillus* (*L. casei*) led to excessive eye formation during ripening. The availability of excess amounts of lactose, galactose, and citrate during the initial ripening stages likely provided the heterofermentative L. casei with sufficient substrates for gas formation. The accrual of these fermentable substrates was notable in cheeses lacking the *L. helveticus* starter population. The results of this study are commercially relevant, as they demonstrate the importance of viability of starter populations and the control of specific nonstarter lactic acid bacteria to ensure appropriate eye formation in Swiss-type cheese.

Task 4.2. *Manipulation of cheese compositional, physicochemical and processing matrices leading to the diversification in the range of textures in dry salted Cheddar- variants and brine salted continental cheese-types*

Calcium in cheese is a major determinant of protein aggregation, which affects many aspects of cheese, including protein hydration, sysneresis, composition, proteolysis, structure, texture and functionality (cooking properties). The influence if calcium is based on its cross-linking of the caseins that form that network of the cheese. Altering calcium in the conventional manufacture of a given cheese is normally achieved by varying pH at rennet addition or at whey drainage. The current worked package investigated a new approach, involving the addition of encapsulated EDTA (ethylenediaminetetraacetic acid), a calcium chelating agent, to the cheese milk. The chelation of calcium by EDTA reduces its ability to cross-link casein.

Liposome-encapsulated ethylenediaminetetraacetic acid (EDTA) was incorporated into a model miniature Gouda-type cheese (20 g) in order to assess its effect on rennet gelation, starter viability, pH, and moisture content. EDTA was encapsulated within 2 different food-grade proliposome preparations, Pro-Lipo Duo and Pro-Lipo C (50% and 40% unsaturated soybean phospholipids and 50% and 60% aqueous medium, respectively), using the following high-shear technologies: Ultra-Turrax (5000 rpm), 2-stage homogenization (345 bar), or microfluidization (690 bar). Liposome size distribution was affected by the high-shear technology employed with the proportion of large vesicles (>100 nm) decreasing in the order microfluidization < 2-stage homogenization < Ultra-Turrax. All EDTA-containing liposomes were stable during 28 d refrigerated storage, with no significant ($P < 0.05$) change in size distribution or EDTA entrapment efficiency (%EE). Liposome composition affected the entrapment of EDTA, with Pro-Lipo C having a significantly greater %EE than Pro-Lipo Duo, 63% and 54%, respectively. For this reason,

Pro-Lipo C EDTA liposomes, with and without EDTA, were incorporated into model miniature Gouda-type cheese. Addition of liposome-encapsulated EDTA to milk during cheese making did not impact pH or rennet gel formation. No differences in composition or pH were evident in liposome-treated cheeses. However, cheese containing liposome-encapsulated EDTA (LE) had reduced levels of insoluble Ca, particularly during the early stages of ripening, compared to control and LC cheeses, most likely attributed to the displacement of Ca from the casein-bound to the aqueous phase caused by the calcium chelating ability of EDTA. Hardness, chewiness and gumminess values for LE cheese were significantly ($P \leq 0.05$) lower at the early stages of ripening when compared to control cheeses. Since there were little differences in proteolysis between C, LC and LE treated cheeses, the lower hardness, chewiness and gumminess values demonstrated by the LE cheese particularly in the first 28 d was most likely due to the difference in the insoluble Ca content. The LE cheese had significantly ($P \leq 0.05$) greater meltability values throughout ripening when compared with the control and LC cheese. As well as this, LE cheese exhibited higher LTmax values than the control and LC cheeses after 28 d ripening, indicating a greater propensity for LE cheese to flow and melt when heated. The results of this study highlight the potential of liposomes to optimise the retention of calcium chelating salts within the cheese curd during the production of brine-salted type cheese. Results also indicate that liposome-encapsulated EDTA incorporated into milk during cheese manufacture alters the distribution of Ca between the insoluble and soluble forms in brine-salted continental-type cheese, thus modifying its structure, texture and functionality, without impacting negatively on the pH or composition of the cheese.

Task 4.3. *Investigation of potential of speciality cheese cultures to enhance the metabolic potential and to diversify and enhance flavour profiles in dry salted Cheddar variants with altered make and ripening processes*

A range of cheese cultures from the Moorepark Culture Collection, both yeast and bacteria, associated with Artisanal cheeses were selected due to their association with diverse flavor profiles. These included *Microbacterium gubbeenense* DPC6288, *Brevibacterium auranticum* DPC6030, *Brevibacterium linens* DPC5699, *Corynebacterium casei* DPC5676, *Corynebacterium variable* DPC5678, *Corynebacterium flavescens* DPC5700, *Yarrowia lipolytica* DPC6266, *Debaromyces hansenii* DPC6261 and *Debaromyces hansenii* DPC6265. They were screened for their potential in cheesemaking based on their production of cell envelope proteinase (CEP), peptidases (post-proline dipeptidyl aminopeptidase-PepX and general aminopeptidase N-PepN), and glutamate dehydrogenase (GLDH). Based on this pre-screening, *M. luteus* DPC6275 and *Y. lipolytica* DPC6265 were chosen on their ability to produce CEP and PepN/PepX, and *Y.lipolytica* DPC6266 and *D. hansenii* DPC6265 on the ability to produce GDH. Cheese trails were undertaken using these cultures in the following combinations: (a) S thermophilus + *L helveticus* (control culture), (b) *S thermophilus* + *L helveticus* + *M. luteus* DPC 6275 (exp. culture 1), (c) *S thermophilus* + *L helveticus* + *M. luteus* DPC 6275 + *Y liploytica* DPC 6266 (exp. Culture 2), and (d) *S thermophilus* + *L helveticus* + *C. variable* DPC 5678 + *Y liploytica* DPC 6266. Cheddar-hybrid cheeses were successfully manufactured with the optimum culture strains selected from model studies and these cheeses are now available for commercial evaluation.

Task 4 (sub-Tasks 4.1 and 4.3) resulted in scientific publications 14-25 in section 4b (i) and popular article and/or conference presentation articles 6-13 in section 4b(ii).

Task 5. Analytical techniques capable of differentiating between natural and industrial trans fats

Methodology was developed for the collection of vibrational spectra (NIR, MIR and Raman) of Cheddar cheese, butter and dairy spreads. A suitable GC method for the determination and quantitation of fatty acids in these product types has been developed. Chemometric models were developed to separately predict naturally-occurring (NT), industrially-induced (IT) and trans-fatty (TT) acids in these three dairy product types. Models based on NIR, FT-IR and Raman spectra predicted NT and TT content in butter with acceptable accuracy; best predictive performance achieved a validation correlation coefficient (R^2V) of ~0.91-0.95, a root mean squared error of prediction (RMSEP) of ~0.07-0.30 for NT; equivalent figures for TT were R^2V~0.92-0.95, RMSEP~0.23-0.29. Prediction of NT, IT and TT in Cheddar cheese was poor due to interference from protein. In the case of dairy spreads and butter, IT prediction was poor due to the limitation of vibrational spectroscopy in predicting very low analyte contents (< 0.2% w/w of trans fatty acids (TFA)).

Given the extremely low values detected, all spectroscopic methods failed to predict IT content of butter; no model could predict trans fatty acids in Cheddar cheese and dairy spreads. Models developed for the individual dairy product types performed better for the prediction of trans fats than those developed using combinations of different dairy product types. Overall, it emerged that NT and TT fatty acid contents in butter could be determined with sufficient accuracy to be useful as a quality control tool in industry, particularly when NIR spectroscopy was used. Opportunities for use of these spectroscopic techniques as quality control tools in dairy spread manufacture seem limited.

Task 5 resulted in scientific publication 26 in section 4b (i).

Task 6. Knowledge of the drivers of consumer choice/perception/acceptance with respect to cheese

Knowledge of the drivers of consumer choice/perception/acceptance with respect to cheese

The overall objective of this work package was to build a data-base on the cheese market, with respect to factors influencing consumer choice, perception and acceptance of cheese. There were three main areas of focus:

1) Review of Irish, UK & international cheese market
2) To conduct qualitative and quantitative research with consumer focus groups to generate consumer insights regarding acceptance of new cheese concepts identified, and to determine the drivers of acceptance of cheese as a functional food
3) To identify cheese consumption patterns in Ireland, UK and EU and model the nutritional impact of modifying the nutrient profile/composition of cheese on population fat and micronutrients intakes

Review of Irish, UK & international cheese market

A thorough analysis of the cheese market was undertaken. The findings for UK and Ireland were similar. Natural cheese has a larger proportion of shelf space than processed cheese and most notably the cheddar variety is the most common retail cheese sold, taking up the largest space in cheese category. However, innovation, especially in the cheddar variety, is low. Cream cheese, mainly under the Philadelphia brand, is the most innovative category especially with cooking and flavored varieties currently on the market. Processed Cheese is a large player in the retail cheese market, with products mainly aimed at the children's market. Private label cheese brand take up a large proportion of the retail cheese category especially in the UK. The European prospective was somewhat different with

Emmental and Camembert being the predominant cheese in France and Edam followed by Emmental being the most popular in Germany.

In conjunction with Bord Bia, a review of product launches was completed. All product launches in the dairy category with a health claim were reviewed. Fromage frais/yogurt and almond/soya milk were the most popular launches that had a bone health type claim. New launches relating to cheese occurred to a much less degree, supporting the earlier finding that there is room for more innovativeness in the cheese category.

Qualitative and quantitative research with consumer focus groups

A range of cheese concepts with market potential were identified from market review. These concepts included, high protein cheese, cheese for brain health, cheese with added novel functional ingredients and cheese for a range of eating occasions and were tested among innovative cheese consumers in a series of five focus groups varying in demographic profile. In general, these consumers were positively disposed to the concepts presented. Some of the general themes that arose were taste, health, perceived need, comfort, family and indulgence. Recommendations arising from this qualitative research include

- Use an established accepted concept from another food domain
- Clear and effective communication of less familiar concepts
- Lower fat products more appealing to the younger female demographic
- Protein cheese has very strong appeal for fitness and younger segments
- Perceived need biggest determinant of health enhanced cheeses
- Health benefits most positively received among those with a carer role and older participants

A questionnaire was designed which incorporated constructs derived from findings in the focus groups as well as constructs measuring food choice motives, cheese consumption patterns, cheese usage situations and innovativeness. In addition, consumers responses to sixteen various cheese concepts (both novel and existing) varying in fat content, benefits and health claims were measured using a conjoint approach. The questionnaire was administered online to 300 respondents from the Rep. of Ireland and 300 UK consumers aged 18 years and over. Data were collected and transferred to SPSS for quality control checking and descriptive analysis. Subsequent data analysis were undertaken to determine acceptance of the various cheese concepts.
In summary the focus groups demonstrated that cheese is considered a traditional healthy staple by most consumers. In addition, there is potential for developing novel cheese products, but success with will be dependent on the perceived needs for the purported benefits in the products. Success may be more likely if new cheese products were modelled on existing formats such as super milk which has been accepted as mainstream. Further quantitative analyses *via* survey showed that with the exception of some attitudes, Irish and British consumers hold similar attitudes regarding the healthiness of cheese and their use of cheese. In particular significant differences were observed in levels of innovativeness, use of specialty cheese with UK consumers more innovative and more positive attitudes to speciality

cheeses. However the Irish consumers were more positively disposed to adding functional ingredients to foods.

Nutritional impact of modifying nutrient composition of cheese

Analyses were undertaken examining cheese consumption and the impact of modifying cheese on population nutrient intakes. The impact of replacing all cheese currently consumed in both the Irish and UK national diets with a cheese concept that can make a claim of 'high' in vitamin D and reduced fat in cheese was analysed. The findings showed that with little change in overall energy consumption chese consumption could be doubled from 12.5 g/day for Ireland and 15g/day for UK with a concomitant 10% increase in mean daily vitamin D from cheese alone. These findings support the beneficial role of cheese as a potential carrier for nutrient fortification and/or bioactive functional ingredients, if the fat content can be sufficiently reduced without sensory compromises.

4. Impact of the Research

How they may be relevant to End Users (Industry, Policy, Farming, Practitioners)
A substantial body of information has been generated across different areas of cheese, using the latest research methodologies and analytical capabilities. Key information/findings include:

- The interactive effects of fat and salt on the composition, biochemical, textural, functional and sensory properties of Cheddar cheese, and two solid hypotheses by which the negative effects of fat and salt reduction can be overcome, i.e. structural attenuation of the calcium phosphate *para*-casein network by reducing the degree of casein cross-linking and/or increasing the extent of primary and secondary proteolysis of the casein.
- A bank of LAB cultures have been screened for their salt sensitivity, autolytic, and intra-cellular peptidase activity in cheeses of varying fat content, and a selected strain (*L. lactis* subsp. *cremoris NCIMB Ltd., Aberdeen, Scotland*) was found to significantly improve the flavour of half-fat half-salt cheese (slight mealy texture, nutty flavour, good overall flavour and good aftertaste) relative to that obtained using a commercial mix-strain culture.
- Sixteen new strains of L. *lactis* were sequenced and functional analysis of their cheese making properties carried out.
- A mechanistic evaluation on the impact of different calcium chelating salts (CCS) on calcium ion activity, protein solubilisation, and properties of model matrices. Interesting outcomes of practical significance include those showing the possibility of producing functional cheese-like structures without the addition of CCS, and how the calcium content of CCS-free matrices can influence their in-vitro digestibility.
- The consumption of a half fat, high vitamin D cheese product provide a positive health impact to the consumers in body weight management, circulating vitamin D levels and glucose utilization.
- The application of molecular techniques (16S rRNA amplicon sequencing, shotgun metagenomic sequencing, quantitative PCR) have been used to identify microorganisms associated with, and elucidating causes of, major defects in cheeses including pinking discolouration, high levels of biogenic amines, excessive formation in Swiss-type cheeses.
- Creating a bank of micro-organisms for potential diversification of cheese flavour. This was based on screening of a diverse range of both yeast and bacteria, associated with Artisanal cheeses, for cell envelope proteinase peptidases and glutamate dehydrogenase, and an evaluation of selected outcomes in cheddar-hybrid cheeses.

- Development of technology for liposome-based encapsulation of a calcium modulator (EDTA) which enables alteration of the level of insoluble (casein-bound) calcium in cheese and provides a means by which physical properties can be engineered.
- Reliable validated methodology, based on vibrational spectroscopy techniques (near infrared, NIR; Fourier-transform mid-infrared, FT-MIR; Raman), has been developed for the measurement of naturally-occurring, industrially-induced and total trans fatty acids in butter, dairy spreads and Cheddar cheese.
- Marketing studies providing information on consumer attitudes and determining consumer attitudes to concepts for innovating cheese.

This body of work is of considerable value at the present time as the cheese sector is under pressure to maintain cheese quality, consolidate the Cheddar market in the UK, and to diversify cheese to cope with the increased Irish milk pool. This comprehensive database and expertise is now available to industry to assist in the development of:

(i) new natural cheeses
   a. reduced-fat, reduced-salt Cheddar variants
   b. continental-style cheeses (e.g., Cheddar-Emmental-type hybrids)
   c. ingredient cheeses with engineered functionality, made possible by controlling the ratio of insoluble/soluble calcium

(ii) Processed cheese products with reduced levels of sodium, and new cheese-like matrices/structures without calcium chelating salts.

How it has added to the research base (skills, leveraging of funding, infrastructure capabilities etc.)
Food chemistry, microbiology, bioscience, and technological development skills are emphasised by this type of cross-disciplinary project

4(a) Summary of Research Outcomes

(i) Collaborative links developed during this research

CheeseBoard 2015 continued to maintain web interlinks with www.pleasure-fp7.com/conference, the EU FP7 project for cross promotion of Dissemination events e.g. La Rochelle, FR conference, June 2014.

An Industry-led, Enterprise Ireland funded 'Dairy Processing Competency Centre (DPTC) formed in 2015. Tow cheese projects feature in the DPTC programme and build on the expertise developed in CheeseBoard 2015.

Teagasc Food Research Centre and Prof Kevin Cashman (UCC), EU expert on Vitamin D, has been strengthened based on results generated from Task 3.

A further collaboration is under development between personnel at Tegasc Food Research Centre Moorepark (Paul Cotter, Diarmuid Sheehan) and Utah State University (Prof. D. J. McMahon) on the application of next generation sequencing of elucidating problems in cheese such as splitting, discolouration, others. Professor McMahon visited the Teagasc in September 2016 and discussions on number potential areas for collaboration.

(ii) Outcomes where new products, technologies and processes were developed and/or adopted

1) Adaption of High-throughput DNA sequencing as a tool to elucidate the role of microbiology and cheese microbes to the pinking defect and occurrence of amines in cheese (Task 4)
2) Process for moisture normalization in cheeses with different levels of salt and calcium (Task 1).
3) The technology for the formation of processed-cheese like matrices without calcium-cheating salts for improved digestibility.
4) A technology for significant improvement in the texture, functionality and flavour of half-fat, half-salt cheese

(iii) Outcomes with economic potential

Many of the outcomes have economic potential (e.g., those described in (iii)), but favourable expression/uptake relies on market opportunities (which react to many factors).

(iv) Outcomes with national/ policy/social/environmental potential

- A very solid database on several aspects of cheese including technology, microbiology, marketing, problem-insights.
- New databases on: platforms reduced-fat, reduced-salt cheese; the impact of salt on LAB cultures, new generation sequencing;
- Peer reviewed scientific publications applying new approaches to solving old- and emerging problems, related to intake of nutrients (e.g. Na, fat, vitamin D, biogenic amines) and physical defects (e.g., malformation of eyes, colour defects).
- New PhDs trained in the fundamental and emerging principles of cheese science; these students are available to apply their skills in the Agri-Food industry.

4 (b) Summary of Research Outputs

(i) Peer-reviewed publications, International Journal/Book chapters.

Scientific publications

1. McCarthy, C. M., Kelly, P. M., Wilkinson, M. G., & Guinee, T. P. (2016). Effect of salt and fat reduction on proteolysis, rheology, and cooking properties of Cheddar cheese. *International Dairy Journal*, 5**6**, 74-86.
2. McCarthy, C. M., Kelly, P. M., Wilkinson, M. G., & Guinee, T. P. (2017). A profile of the variation in compositional, proteolytic, lipolytic and fracture properties of retail Cheddar cheese. *International Journal of Dairy Technology* 70, 469-480.
3. McCarthy, C. M., Kelly, P. M., Wilkinson, M. G., & Guinee, T. P. (2017). Effect of fat and salt reduction on the changes in the concentrations of free amino acids and free fatty acids in Cheddar-style cheeses during maturation. *Journal of Food Composition and Analysis*, **59** 132–140.

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5. McCarthy, C. M., Wilkinson, M. G. and Guinee, T.P. (2017). Effect of coagulant type and level on the properties of half-salt, half-fat Cheddar cheese made with or without adjunct starter: improving texture and functionality Submitted to *International Dairy Journal*, **75**, 30-40.
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10. McIntyre, I., O'Sullivan, M. & O'Riordan, D. (2016). Effects of calcium chelators on calcium distribution and protein solubility in rennet casein dispersions. Food Chemistry, 197, 233-239.
11. McIntyre, I., O'Sullivan, M. & O'Riordan, D. (2017). Manipulating calcium level provides a new approach for the manufacture of casein-based food structures with different functionalities. International Dairy Journal, **70**, 18-25.
12. McIntyre, I., O'Sullivan, M. & O'Riordan, D. (2017). Altering the level of calcium changes the physical properties and digestibility of casein-based emulsion gels. *Food & Function* **8** (2), 1641-1651.
13. McIntyre, I., O'Sullivan, M. & O'Riordan, D. (2017). Monitoring the progression of calcium and protein solubilisation as affected by calcium chelators during small-scale manufacture of casein-based food matrices. Food Chemistry 237, 597-604.
14. McAuliffe, L.N., Kilcawley, K.N., Sheehan, J.J. and McSweeney, P.L.H. (2016). Manufacture and incorporation of liposome-entrapped ethylenediaminetetraacetic acid into model miniature Gouda-type cheese and subsequent effect on starter viability, pH, and moisture content. *Journal of Food Science* **81**, C2708–C2717
15. Kelleher, R. P., Murphy, J., Mahony, J. & van Sinderen, D . (2018). Identification DNA Base Modification by Means of Pacific Biosciences RS Sequencing Technology: *Methods in Molecular Biology*, **1681**, 127-137.
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20. O'Sullivan, D., Cotter, P. D., O' Sullivan, O, Giblin, L., McSweeney, P.L.H. and J.J Sheehan. (2016). Compromised *Lactobacillus helveticus* starter activity in the presence of facultative heterofermentative *Lactobacillus* casei DPC 6987 results in atypical eye formation in Swiss-type cheese. *Journal of Dairy Science*, **99**, 2625-40
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26. Zhao, M, Fearon, A., Beattie, R.J., O'Donnell, C.P. and Downey, G. (2015). "Prediction of naturally-occurring, industrially-induced and total trans fatty acids in butter, dairy spreads and Cheddar cheese using vibrational spectroscopy and multivariate data analysis." *International Dairy Journal*, **51**, 41-51.

(ii) Popular non-scientific publications and abstracts including those presented at conferences

1. Kelly, P.M. and Guinee, T.P. (2012). CheeseBoard 2015. *TResearch* 7(1) 36037.
2. McCarthy, C. M., Wilkinson, M. G., Kelly, P. M., Guinee, T. P. (2014). *The effects of reducing fat and salt on the biochemical, physical and sensory characteristics of Cheddar-style cheeses*. The 9th Annual Cheese Symposium (Clarion Hotel, Co. Cork, Ireland), 12th and 13th of November 2014 – Oral presentation.
3. McCarthy, C. M., Wilkinson, M. G., Kelly, P. M., Guinee, T. P. (2016). *Physical and sensory properties of Cheddar cheese: effects of altering salt and fat*. The IDF Symposia 2016 (DoubleTree Hilton, Co. Dublin, Ireland), 11th and 12th of April 2016– Oral presentation
4. Giblin, L. (2016). *Effects of high vitamin D and half fat cheddar cheese on weight gain*. The IDF Symposia 2016 (DoubleTree Hilton, Co. Dublin, Ireland), 11th and 12th of April 2016– Oral presentation
5. Giblin, L (2016). Time to make up for vitamin D deficiency. Irish Times Article, 30/05/2016: Linda Giblin interviewed by Journalist Barry McCall.
6. L.N. McAuliffe & PLH McSweeney (2013). *The effect of ethylenediamine tetra-acetic acid addition on the textural and rheological properties of a Gouda-type cheese during ripening.* Food Research Conference 2013 (Teagasc, Ashtown).

7. Kelleher P., Mahony J., Bottacini F, Kilcawley K. & van Sinderen D. (2014). *Comparative and functional genomic analysis of dairy lactococci*. The 9th Cheese Symposium (Clarion Hotel, Cork, Ireland). 12-13 November 2014.
8. Kelleher P., Mahony J., Bottacini F, Kilcawley K. & van Sinderen D. (2014). Comparative and functional genomic analysis of dairy lactococci 43rd Annual Food Research Conference (UCD, Dublin, Ireland). 10-11 December 2014
9. Sheehan, J.J. (2015). *Cheese diversification, quality and consistency*. Inlactis 2015- South American cheese symposium, Montevideo, Uruguay. Nov. 17-18th 2015. Invited Oral keynote presentation.
10. Cheeseboard 2015 dissemination seminar, Moorepark, 15/12/15. Presentation of most recent data.
11. Kelleher, P. (2016). Next generation sequencing for the identification of novel lactococcal dairy starter strains. The IDF Symposia 2016 (Double Tree Hilton, Co. Dublin, Ireland), 11-12 April 2016 - Oral Presentation.
12. O'Sullivan, D.J., Fallico V., O'Sullivan, O., McSweeney, P.L.H., Sheehan, J. J. Cotter, D. and Giblin, L. (2016) Next generation sequencing to profile bacterial histidine and tyrosine decarboxylases in raw milk cheeses. IDF Cheese Science and Technology conference, Dublin, 11-13th April, 2016- Oral presentation
13. Sheehan, J.J. (2016). Influence of manufacture parameters on cheese microstructure, microbial localisation and their interactions during ripening. IDF Cheese Science and Technology, Dublin, April 11th – 13th 2016. Oral presentation

(iii) National Report

None

(iv) Workshops/seminars at which results were presented

Poster Presentations:

- The effect of ethylenediamine tetraacetic acid addition on the textural and rheological properties of a Gouda-type cheese during ripening (LN. McAuliffe & PLH McSweeney) Poster presentation at Microbial Diversity conference (Turin, Italy)
- 6th Congress of European Microbiologists (FEMS 2015), Maastricht, NL, June 2015. High-throughput sequencing reveals biogenic amine communities in a variety of artisanal cheeses. (Poster 386: Daniel O Sullivan)
- ASM 2015 115th General Meeting, American Society for Microbiology, May 30-June 2, 2015 New Orleans, Louisiana. Title: 'The Lactococcal Megaplasmidome'(Poster: P. Kelleher, UCC).
- The 42nd Annual Food Research Conference (Ashtown, Co. Dublin, Ireland). '*Reduced fat/salt cheeses with improved texture and flavour*'. McCarthy, C. M., Wilkinson, M. G., Kelly, P. M., Guinee, T. P. Date: 26th and 28th of June 2013 – Poster presentation
- The 9th Annual Cheese Symposium (Clarion Hotel, Co. Cork, Ireland). '*Variation in Commercial Cheddar cheeses*'. McCarthy, C. M., Wilkinson, M. G., Kelly, P. M., Guinee, T. P. Date: 12th and 13th of November 2014 - Poster presentation
- The 44th Annual Food Research Conference (Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland). '*Effects of calcium and salt reduction on the properties of half-fat Cheddar-style cheeses with equal moisture content*'. McCarthy, C. M., Wilkinson, M. G., Kelly, P. M., Guinee, T. P. Date: 14th of December 2015 - Poster presentation

(v) Intellectual Property applications/licences/patents

An International (PCT) Application No. PCT/EP2015/063831, in the name Agriculture and Food Development Authority (Teagasc), and entitled 'Detection of a source of pink discoloration defect in a sample' was been filed in December 23, 2015. Essentially, A method of assaying a sample to determine the presence of a source of pink discoloration defect of a dairy product such as cheese comprises the steps of assaying the sample to detect the presence of a *Thermus* species of bacteria, such as *Thermus thermophilus* bacteria, wherein detection of *Thermus thermophilus* bacteria in the sample indicates that the sample contains a source of pink discoloration of a dairy product such as cheese.

(vi) Other

None

5. Scientists trained by Project

Total Number of PhD theses: 4 (+ 1 being prepared)

O Sullivan. D. (Teagasc/UCC): PhD thesis: entitled: *The Application of Next Generation Sequencing to Profile Microbe Related Cheese Quality Defects*. Supervisors Sheehan, J.J. and Cotter, P. Teagasc, Ph.D. VIVA completed Dec 2nd 2015.

Kelleher, P.R. (UCC): PhD thesis by entitled: *Comparative and Functional Genomic Analysis of Dairy Lactococci*. Supervisor(s): van Sindern, D., UCC. Submitted: Jan 2017.

McCarthy, C.M. (Teagasc/UL): PhD thesis entitled: *Effects of reducing fat and salt on Cheddar cheese composition, biochemistry, texture and sensory properties, and approaches to improve the quality of reduced-fat, reduced-salt Cheddar cheese*. Supervisor Guinee, T.P., Teagasc, Wilkinson, M.G., UL. Submitted: VIVA completed May 26, 2017.

McAulliffe, L.N. (2016). PhD thesis, entitled: *Role of calcium equilibrium in modulating the textural and functional properties of brine-salted cheese*. Supervisor: McSweeney, P.L.H., UCC. Viva completed: 12th December 2016.

Yanachkina, P. (2017). PhD thesis entitled: *The impact of salt of fat salt and on the accessibility and activity of intra-cellular lactocaoccal enzymes*. Supervisor: Wilkinson, M.G., UL (in preparation, to be submitted by Dec 2018).

McIntyre, I. (2017). Control of calcium-protein interactions in designing casein-based food structures with novel functionality. PhD Thesis: submitted on 9/01/2017.

Total Number of Masters theses: 1

Pearce, S. (DIT/Teagasc): MSc Thesis entitled: *Investigation of the potential for speciality cheese cultures to enhance metabolic potential and diversity flavour profiles of cheese*. Supervisor: J.J. Sheehan. Submitted: January 2016.

6. Permanent Researchers

Institution Name	Number of Permanent staff contributing to project	Total Time contribution (person years)
TMFRC	11	5.146
TAFRC	3	1.110
UL	0	0
UCC	2	0.450
UCD	0	0
AFBI	0	0
Total	**16**	**6.706**

7. Researchers Funded by DAFM

Type of Researcher	Number	Total Time contribution (person years)
Post Doctorates/Contract Researchers	1	1.757
PhD students	6	20.022
Masters students	2	7.000
Temporary researchers		
Other		
Total	**9**	**28.779**

8. Involvement in Agri Food Graduate Development Programme

Name of Postgraduate / contract researcher	Names and Dates of modules attended
None	

9. Project Expenditure

Total expenditure of the project: €1,298,009

Total Award by DAFM: €1,298,000

Other sources of funding including benefit in kind and/or cash contribution(specify): €

Breakdown of Expenditure

Category	Name TMFRC	Name TAFRC	Name UL	Name UCC	Name UCD	Name AFBI	TOTAL
Contract staff							
Temporary staff							
Post doctorates	78,929						78,929
Post graduates	165,000	75,833	79,669	159,286	68,584		548,372
Consumables	175,864	7,360	57,248	78,099	10,299		328,870
Travel and subsistence	18,413	3,894	6,295	9,077	875		38,554
Sub total	438,206	87,087	143,212	246,462	79,758	0	994,725
Durable equipment							
Other						9,221	9,221
Overheads	131,461	21,772	42,964	73,939	23,927		294,063
Total	**569,667**	**108,859**	**186,176**	**320,401**	**103,685**	**9,221**	**1,298,009**

10. Leveraging

None currently.

11. Future Strategies

Outline development plans for the results of the research.

Publications are still being prepared or are in the peer review process. These publications will be progressed until publication is complete.

Summaries of the project's findings will continue to be promoted at Teagasc biennial Food Innovation Gateways events which is a major forum for presentation of technological offers to industry