# ABSTRACTS OF PAPERS PRESENTED AT THE 33<sup>RD</sup> FOOD SCIENCE AND TECHNOLOGY RESEARCH CONFERENCE, UNIVERSITY COLLEGE, CORK, SEPTEMBER 2003

#### Sensory evaluation of and consumer attitudes towards organic meat and other organic foods

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A marked increase in demand for organic foods has occurred over the past decade reflecting consumer concerns about health, nutrition, and food quality. The aim of this work was to compare the sensory character of conventional and organically reared beef, while in parallel, investigate consumer attitudes to organic foods, particularly organic meat. A panel of 10 experienced sensory assessors described the character of conventional and organically reared beef. The factors that influenced the consumption of organic foods in a sample of 100 organic food consumers were determined. ANOVA and Principal Component Analysis (PCA) of the descriptive data failed to show differences between organic and conventionally produced beef. Up to 78% of the consumers surveyed consumed beef, while 50% of them purchased organic beef. Primary reasons for purchasing organic foods included 'animal welfare', 'safety', 'chemical and pesticide free' and 'environment', while 'taste' and 'nutrition' were of less importance, and 'appearance' was of least concern. The majority of consumers surveyed were found to have moderate to high variety seeking tendencies, more than three quarters were from urban areas, while those consumers who ate organic food more regularly were 35- to 44years old. It was concluded that while sensory attributes of organic and conventionally reared beef were similar, taste was not the most important reason for purchasing organic products.

## The influence of hydrocolloids on the perception of flavour in gelatin and pectin gel systems

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Foods require more added flavouring to produce the same flavour intensity when thickeners and gelling agents are added to them. Low, medium and high strength gels of gelatin and pectin were made and matched based on assessment of Young's modulus of elasticity (i.e., log E = 5.2, 5.7, 6.2). A strawberry flavour was added to each and the relationship between gel strength and gel sensory characteristics determined by a 12-person sensory panel. The odour (evaluated by smelling), thickness, strawberry flavour and sweetness (all evaluated during consumption) were rated using magnitude estimation. In addition, to measure flavour binding by the gels, the headspace

concentrations of the strawberry volatile compounds above the gels were measured using static headspace analysis. Significant interactions (P < 0.05) were found between odour, thickness, strawberry flavour and sweetness for both gel types. In general, as gel rigidity increased, perceived odour, strawberry flavour and sweetness intensities decreased. However, the flavour characteristics of the pectin gel were less intense at all gel strengths. Static headspace analysis identified ethyl butyrate and 2-octanone to be the most abundant compounds in the headspace of the gels. Headspace concentrations of these compounds decreased significantly (P < 0.05) as gel strength increased, but not sufficiently to account for the changes to perceived flavour intensity. In conclusion, gel rigidity determined flavour intensity, although the relationship was different for each gel studied and could not be fully explained by flavour compound binding.

#### Relationships between individual oral physiology, odour sensitivity, and perceived intensity and quality of retronasally presented binary odour mixtures

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The influence of oral physiology on the release of volatile compounds from foods and beverages has been widely documented. However, little is known about whether a relationship exists between a person's oral physiology, their odour sensitivity, and their ability to perceive odour intensity and quality. Twenty participants between the ages of 20 and 35 were recruited, and their un-stimulated saliva flow and mastication behaviour were determined. Individual orthonasal odour detection thresholds for diacetyl and ethyl butyrate, presented singly in 5 g of 20% gelatine via 250 ml polypropylene squeeze-bottles, were determined using an ascending three-alternative forced choice test. In a further study, perceived retronasal odour intensity and quality for each compound presented singly, and then in 'equi-intense' binary mixtures that varied in volatile concentration, was measured during consumption of the 5-g gels by each participant. Furthermore, the nose-space concentration of each compound was quantified during consumption of the 5-g gels using Proton Transfer Reaction-Mass Spectrometry. Orthonasal odour thresholds for each compound were found to differ between participants. In addition, significant differences between participants in the nose-space concentration of each odour compound were observed. These differences were significantly correlated with individual participant oral physiological behaviour. Perceived quality and intensity of individual odour compounds and odour mixtures presented retronasally also differed for assessors, and

was explained by both participant oral physiology and odour sensitivity.

#### Consumer responses to novel functional beverages: a study of probiotic orange juice acceptance

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This study examined the sensory impact of probiotic microorganisms and pre-biotic ingredients on the aroma and taste of orange fruit juices. Four commercially available functional orange juices (containing probiotics or prebiotics) were obtained from European fruit juice manufactures. These juices were compared with seven conventional orange juices readily available from a local supermarket. A trained sensory panel measured the aroma and flavour characteristics of the 11 orange juices using descriptive analysis. The functional juices were described as possessing perceptible 'dairy', 'medicinal' and 'dirty' flavour attributes, distinguishing them from the conventional juices. Subsequently, 100 consumers participated in a preference test, whereby five orange juices (three conventional and two functional) were ranked in order of consumer preference. Ranking decisions were based solely on the sensory characteristics of the juices, since product information was not provided. On an overall basis, consumers preferred the sensory characteristics of conventional juices. However, cluster analysis identified a small consumer segment (11%) that significantly preferred the sensory attributes of functional juices. Therefore, a market may exist for novel, non-dairy probiotic juice drinks.

## Using sensory analysis to understand orange juice preferences in younger and older consumers

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Chemosensory acuity decreases with increasing age. However through a deeper understanding of the perceptual capabilities of the residual senses and the relative contribution each makes to an acceptance decision, it is possible to understand the sensory limitations of older consumers and devise compensation strategies to combat chemosensory loss. A commercially available orange juice was modified to provide varied levels of 'sweetness', 'pulpiness' and 'prickle/heat'. Differences for each attribute were established through studying concentration response functions with a panel (n = 20) of objective sensory assessors. These results were used to produce 18 orange juice samples, following a  $3 \times 3 \times 2$  factorial design using variations to the three attributes, which were assessed using descriptive analysis by a panel (n = 6) of trained sensory assessors. The results were used to select eight orange juice samples for acceptability testing with two age groups, a young group (20 to 35 years, n = 48), and an older group (> 65 years, n = 52). The two groups of consumers showed marked differences in their preferences for the samples, which were related to differential sensory sensitivity. Interactions between flavour, texture and irritation were observed that influenced product acceptance in each age group. These interactions were best explained by determining relationships between the descriptive analysis results and participant acceptance using Partial Least Squares Regression. This integrated approach provided new knowledge on cross-modal sensory interactions, and their influence on food acceptance in different age groups.

#### The influence of age and sensitivity to sensory properties on food liking and food consumption

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This study examined the influence of age and sensitivity to food properties on food liking and food habits. A group of 274 consumers belonging to five different age groups (20 to 35 years, 36 to 50 years, 51 to 60 years, 61 to 70 years and > 70 years) participated. Their sensitivity to odour, taste and texture was assessed, followed by a liking test involving soups, flavoured rices and biscuits. The food consumption of these participants was also determined using a food frequency questionnaire. Age was associated with decreased odour and texture abilities and had an impact on the liking of 11 food products, for which the elderly gave higher liking scores. In general, the elderly were less discriminating between foods by liking. Age had an impact on food consumption; however, it was food item specific, showing sometimes a lower or a higher consumption frequency. Overall variety scores did not differ with age, although the elderly had a lower variety in two food categories. Participants over 60 were clustered depending on their sensitivity. It was found that within this age group sensitivity influenced the liking of eight food products, although least sensitive participants tended to give higher preference scores for these products. Regarding food habits, a lower sensitivity was more often associated with a lower consumption frequency, although level of sensitivity did not always influence overall variety scores. Participants with the lowest sensitivity to texture consumed a lower variety in the fruit category.

### Longitudinal differences in vitamin D status of 50- to 70-year-old Irish women

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Vitamin D can be obtained from the diet and through skin synthesis upon exposure to sunlight. Seasonal differences in serum or plasma 25-hydroxyvitamin D<sub>3</sub> [25 (OH) D<sub>3</sub>] the most commonly used index of vitamin D status are well documented. Low levels of 25 (OH) D3 may increase parathyroid hormone concentrations, which, in turn, can lead to bone loss. The objective of this study was to determine the vitamin D status of 50- to 70-year-old Irish women over a 1-year period. Fasting blood samples were collected from 50 healthy free-living Irish women during winter 2002, summer 2002 and during winter 2003. Bloods were processed to serum and analysed for 25 (OH) D, by enzyme-immunoassay. A significant (P < 0.001) seasonal variation was found in serum 25 (OH) D<sub>3</sub> concentrations between winter 2002 and summer 2002 with higher values in summer than winter (mean values 78 nmol/L and 57.3 nmol/L, respectively). However, there was no significant seasonal variation in 25 (OH) D concentrations between summer 2002 and winter 2003 (mean values 78 nmol/L and 73.1 nmol/L, respectively). When serum 25 (OH) D<sub>3</sub> concentrations in both winters were compared, a significant (P < 0.001) variation was found with higher values from the second winter. In conclusion, levels of 25 (OH) D<sub>3</sub> generally tend to be lowest during winter. Factors determining vitamin D status are being investigated such as local sunshine hours during the previous year.

Market-oriented development of functional water beverages A. Murnane and J. Bogue, *Department of Food Business and Development, University College, Cork* 

Functional beverages represent one of the most dynamic and fast growing segments of the functional foods market. The objective of this study was to qualitatively and quantitatively evaluate Irish consumers' attitudes, expectations and preferences towards functional water beverages. Ten in-depth consumer interviews were conducted to gain a greater insight into this innovative product category. Product prompts were introduced to consumers that raised important packaging design issues for product developers. Two hundred 'naive' or untrained end-users were randomly recruited and asked for their preferences for eight bottled functional water samples. A Principal Component Analysis of the end-user data was generated using Guideline v7.5 to give an Internal Preference Map. A number of respondents were unaware of the meaning of the term functional food. The majority of consumers felt that brand name and the length of time the product had been on the market were factors that affected the purchase decision. The majority of respondents were unaware of any nutritionally enhanced waters available on the market. Overall, the most preferred product was a still, non-flavoured, non-fortified water (mean score 6.41 out of 9) and the least preferred product was a retailer's own brand isotonic water (mean score 3.47 out of 9). Hierarchical Cluster Analysis was carried out to identify end-users with similar preferences. This research generated market-oriented information that can be used for the development of functional waters targeted at specific consumer groups.

## Irish consumer perceptions of high-pressure processing V. $Galvin^1$ , A.L. $Kelly^2$ and M. $McCarthy^1$

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The novel technology of high pressure processing (HPP) could help to meet current consumer demands for food safety. The aim of this research was to ascertain Irish consumer attitudes towards the hypothetical introduction of a HPP-treated food product (pork sausage). The Means End Chain Method was used to determine the consequences and values associated with HPP. A consumer-friendly definition for HPP was formulated and, following pilot testing on 50 respondents, was used in 18 1-h interviews. conducted with a representative population sample. Consumers were generally unfamiliar with HPP; however, after presentation of the definition, their reaction was very positive and interviewees felt that the technology would have many benefits for them, including monetary savings, improved taste and increased confidence in the safety of the product. Few concerns about the use of HPP were expressed and interviewees felt that these concerns would be assuaged by further information on the process. Interviewees expressed a willingness to purchase a HPP-treated product even at a premium price; the primary condition placed on purchase was the provision of further information via in-store promotion rather than information on the product label. It was also noted from word association tests in the interviews, that in order to gain consumer acceptance of HPP, the term 'HPP treated' should be replaced with a more 'consumer-friendly' term as the former had negative image association.

An exploratory study of European consumers' attitudes and behaviours towards dietary practices, infant feeding and food choice issues

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There are many differences in both diet and lifestyle characteristics among European consumers. The diversity of these characteristics often lies within the cultural boundaries of these countries. An important research issue today is exploring the effect of such characteristics on consumers' food choices and how infant feeding practices vary across Europe and related issues such as gastrointestinal infection and allergy in infants. This is especially important with the heightened consumer awareness of the links between diet, health and nutrition. The objectives of this study were to: 1) analyse consumers' needs and attitudes to foods and functional food products, processes and labelling; 2) investigate factors influencing food choice and when purchasing for infants; and 3) examine the attitudes and perceived needs of consumers in relation to the uptake of infant feeding guidelines. Three focus groups each were conducted with parents in Scotland, Spain, Sweden, Italy and Germany. This research revealed that parents demonstrated a genuine interest in food for their infants with colourings, additives and preservatives being of greatest concern for mothers when buying prepared infant foods. Although infant feeding guidelines were established to play a major role in infant feeding, parents did not strictly adhere to them when feeding their infants. Market opportunities exist for food manufacturers to develop health-enhancing infant products that meet with greater acceptance by parents of young children.

### Food-related lifestyle segments in Great Britain with a convenience orientation

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Convenience is becoming an ever-more important quality attribute in food products. The main objective of this study was to identify food-related lifestyle segments in Great Britain and to subsequently identify those that are convenience-oriented in their attitudes towards food and the degree to which they use convenience foods. The foodrelated lifestyle tool measures consumer attitudes towards the purchase, preparation and consumption of food products. A nationally representative sample of 1004 consumers completed the survey in Great Britain in 2002. Using the FRL tool, six segments of consumers were identified who differed with respect to their food-related lifestyles. The segments were labelled the snacking food consumers (20% of consumers), the careless food consumers (14%), the uninvolved food consumers (14%), the rational food consumers (26%), the adventurous food consumers (17%) and the conservative food consumers (9%). Attitudes towards convenience and purchase frequencies for convenience foods differed. Three of the segments, viz. the snacking, the careless and the uninvolved consumer segments, were identified as having a convenience orientation. Together, these segments represented 48% of consumers. The study provides an insight into the lifestyles of food consumers in Great Britain, particularly in relation to their attitudes towards convenience food, and provides food manufacturers

with an understanding of what motivates consumers to purchase convenience food products.

## Market-oriented development of new functional drinks: meal replacements and meal complements

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Market-oriented new product development focuses on understanding consumers' changing lifestyles and the resultant changes in consumer needs. Functional beverages such as meal replacement and meal complement products, that incorporate such lifestyles changes, offer new product opportunities for food manufacturers. Consumer interest in meal replacement products is increasing because of time pressures and consumers' desire for healthy, nutritious meals that can be consumed 'on the go'. The objectives of this study were firstly, to understand consumers' lifestyle changes and how these affected their food choices and secondly, to qualitatively evaluate consumers' attitudes and perceptions towards the concept of meal replacement and meal complement drinks. Five focus groups were conducted between April and May 2003. This research revealed that breakfast was the meal that the majority of participants skipped on a regular basis. The main factors for this were time pressure and work-related issues. Different product concepts and attributes were evaluated during the focus groups in order to understand product design issues. Participants rated the product concept 'Complete Meal on the Go Beverage' as being suitable for breakfast and to a lesser extent for lunch. The 'Meal Pal Beverage' concept was perceived, as being suitable for the final meal of the day as by then consumers would know which food group they may have missed during the day. Through understanding consumers' lifestyle changes innovative market-oriented products can be designed and targeted at specific market segments.

## Assessing stakeholder perceptions of soya foods in Ireland M. Heffernan and J. Bogue, Department of Food Business and Development, University College, Cork

Consumers today are more interested in healthy eating and seek out foods that have specific health benefits. Research suggests that the phytoestrogens in soya may be beneficial in aiding the following: the reduction of serum cholesterol; the reduction in the incidence of certain types of cancer; and the slowing of bone loss in post-menopausal women. Soya foods, which are traditionally consumed by Asian populations, are now one of the fastest-growing categories in the food industry. However, the soya foods market in Ireland remains very much under-developed. The objective of this study was to qualitatively evaluate the perceptions of soya foods from a stakeholder perspective. This included industry representatives, special interest groups, university research experts, medical professionals, as well as consumers. Twenty-five indepth interviews were conducted between April and May 2003. This research revealed that most Irish consumers were not aware of the health benefits associated with soya foods. Soya was considered to be a food targeted at vegetarians and those suffering from allergies. Industry respondents reported that the demand for soya foods was increasing each year, particularly from non-genetically modified beans. Most

respondents believed there was a potential for chilled meat analogues that delivered in terms of taste. Respondents believed the greatest barrier to the development of a possible soya foods market was a lack of consumer education. The successful marketing of soya foods in Ireland depends upon an education initiative partnered by manufacturers, retailers, and health education professionals.

#### Public attitude and behaviour towards recycling household food packaging waste

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Waste from domestic sources has been identified as a major barrier to achieving environmental sustainability in the 21st century. Over-consumption and the ethos of a throwaway society, which is further enhanced through marketing and convenience packaging, has lead to a problematic situation. Packaging has become so entrenched in peoples' life that its value is totally overlooked and its role taken for granted. The aim of this study was to identify public attitude and behaviour towards recycling household food packaging waste and the factors that influence such attitudes and behaviours within the Cookstown District Council area. A conceptual model was developed which places three main variables: social factors, individual factors, and external factors as having an equal effect on food packaging recycling attitudes of households, which was subsequently seen to have an effect on behaviour. The study sampled 80 members of the general public who reside within three towns in the Cookstown District. The results found that the following had a significant effect (P < 0.05) on household recycling attitude-social factors (attitude formation, behavioural influence), individual factors (environmental values, education) and external factors (provisions, legislation, price). However attitude was found to have no significant effect (P > 0.05) on current household recycling behaviour but was found to effect future intended household recycling behaviour (P < 0.05). The findings are of value to Government authorities as well as industry groups and have been incorporated into the Cookstown District Environmental Waste Plan.

#### Development of macro-perforated modified atmosphere packaging for mushrooms

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Modified atmosphere packaging has been largely unsuccessful for fresh mushrooms owing to their high respiration rate, the requirement for a high CO<sub>2</sub> concentration, and moisture condensation inside the package. The objective of this work was to explore the use of macro-perforated polymeric packages for controlling the gas composition and relative humidity in packed mushrooms. Experiments were conducted at 4, 8 and 12 °C. Four packages, each containing 100 g of mushrooms, were sealed with a cryovac film which was then perforated with needles of different diameters: 0.3, 0.51, 0.82 and 1.2 mm. The number of holes was such that the effective area of gas diffusion would be the same in all the packages. Results showed that the steady state gas composition and humidity were different in the four packages tested. Microscopic studies suggested

that this is due to imperfectness of the holes, as their area and shape were quite different from those of the needles. The gas composition (8.2 to 14.0% v/v  $O_2$  and 10.0 to 15.2% v/v  $CO_2$ ) was within acceptable limits for mushrooms. Humidity (93 to 95%) was lower when compared to commercial packages, yet slightly above the recommended levels. Thus, the use of macro-perforated polymeric materials appears to show good potential for extending the shelf life of mushrooms.

#### Pretreatment of extracting soybean oligosaccharides by ultrafiltration

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Membrane separation is a new separation technique in the food industry. However, some raw materials should be treated before using the technique. The objective of this study was to investigate pretreatment methods for soybean whey when soybean oligosaccharides were extracted by ultrafiltration. In order to reduce the protein in soybean whey, CaCl<sub>2</sub> was adopted as the sedimentation reagent. Without pretreatment, experiments showed that the retention rate of soybean oligosaccharides was very high (60.0%) in concentrated liquid and the ultrafiltration velocity was very low (6.19  $\times$  10<sup>-3</sup> mm<sup>3</sup>/mm<sup>2</sup>.s) when the soybean whey was ultrafiltrated. In using the sedimentation treatment, the parameters of the treatment were first optimised by using an orthogonal experimental design (four variables at three levels), resulting in the optimum treatment conditions viz. materials should be adjusted to pH 7 by addition of 20% CaCl2, then heated at 100 °C for 15 min. After the pretreatment, a highly pure sovbean oligosaccharides solution was obtained by ultrafiltration. The sugar content of the filtrate was determined to be 17.3 mg/ml, which consisted of 11.8 mg/ml sucrose, 1.2 mg/ml raffinose and 4.3 mg/ml stachyose. The soybean oligosaccharides remained 82.3% and the reduction rate of protein was 72.5%. The ultrafiltration velocity was increased to 8.44 × 10<sup>-3</sup> mm<sup>3</sup>/mm<sup>2</sup>.s. Therefore, sedimentation was a suitable pretreatment method for soybean whey.

## Detection of 3-MCPD, 1,3-DCP and 2,3-DCP in hydrolysed vegetable protein by capillary gas chromatography with electron-capture detection

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Acid hydrolysed vegetable protein (acid-HVP) is a widely used ingredient for savoury foods; however, toxic chloropropanols can be found as contaminants in them. The aim of this study was to develop an efficient and sensitive analytical technique to detect contaminants such as 3-chloropropane-1,2-diol (3-MCPD), 1,3-dichloropropan-2-ol (1,3-DCP) and 2,3-dichloropropan-1-ol (2,3-DCP) in acid-HVP or soy sauce. In this study capillary gas chromatography analysis was carried out on a Hewlett-Packard 5890 gas chromatograph equipped with an electron-capture detector. The results showed that the HP5-MS column and Simplicity-5 column were not suitable for analysis of 3-MCPD, 1,3-DCP and 2,3-DCP. The optimum chromatographic column was HP5. Both 1,3-DCP and 2,3-DCP could be directly

determined; however, a peak for 3-MCPD did not appear. Therefore, in order to detect 3-MCPD, derivation with trifluoroacetic anhydride (TFAA) was investigated. The minimum derivative time of 3-MCPD was 40 min. Therefore, subsequently, the three contaminants were simultaneously measured with applying TFAA. By testing the mass spectrum, the derivatives of 3-MCPD, 1,3-DCP and 2,3-DCP were shown to be present. Recovery rates of 3-MCPD, 1,3-DCP and 2,3-DCP were 94.25 to 105.78%, 98.97 to 101.83% and 99.32% to 102.01%, respectively, showing that 3-MCPD, 1,3-DCP and 2,3-DCP can be simultaneously detected.

#### Characteristics of whey protein concentrate hydrolysates generated at different total solids levels

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Whey protein concentrate hydrolysates were generated at different total solids (TS) levels (5 to 30%) using a foodgrade proteolytic preparation and the physicochemical and sensory properties of the resultant hydrolysates were studied. Quantification of the degree of hydrolysis (DH, %) by the TNBS method showed a faster rate of hydrolysis in reactions performed at lower TS. Hydrolysates generated after 6-h incubation at 5 and 30% TS had DH values of 22.7 and 16.6%, respectively. This finding was confirmed by observing the breakdown of intact whey proteins by SDS-PAGE and gel permeation HPLC. Hydrolysate samples of equivalent DH (~15%) generated at the various TS levels were analysed by reverse-phase HPLC (RP-HPLC). Specific hydrophobic peptide peaks were present at higher levels in hydrolysates generated at lower TS. Hydrolysate samples were presented to a trained sensory panel to determine if bitterness was related to the presence of these hydrophobic peaks in the RP-HPLC chromatograms. Sensory evaluation confirmed that hydrolysates at equivalent DH's generated at 30% TS (mean bitterness score = 20.8%) were significantly (P < 0.005) less bitter than hydrolysates generated at 5% TS (mean bitterness score = 36.1%). Therefore, hydrolysis proceeded at a faster rate at low TS, but hydrolysates with equivalent DH generated at high TS were less bitter than hydrolysates generated at low TS.

#### Detection and quantification of apple adulteration in strawberry purées using PLS regression analysis of SPME-GC data

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Soft fruit purées are used in the production of jams and confectionery products. They are susceptible to adulteration by purées from cheaper fruits such as apples for economic gain. Consumers and reputable manufacturers require a reliable analytical technique to detect such adulteration. The main objective of this work was to assess the potential of combining solid phase microextraction (SPME) and gas chromatography (GC) with chemometric analysis to detect adulteration of authentic strawberry purées by apple purée at levels of 10, 40 and 70% (y/x). This method involved

extraction of aroma volatiles from the headspace of the purée samples using a SPME fibre followed by GC analysis and flame ionisation detection. Partial least squares (PLS) regression using a dummy set of Y-variables (set to 0 for unadulterated and 1 for adulterated samples) resulted in clear discrimination between unadulterated purées and those containing 40 and 70% (v/v) apple. PLS was also performed using a second set of Y-variables, i.e., the actual level of adulterant added. This enabled quantification of apple purée with a standard error of prediction of 11.6%, implying an uncertainty level of  $\pm 23.2\%$  (v/v) apple (P = 0.05). Mass spectroscopic studies identified the principal compounds responsible for discrimination between unadulterated and adulterated samples as 2-hexenal and  $\alpha$ -farnesene, both previously reported to be important components of apple aroma.

#### Formation of thermoreversible gels and films from purified commercial gellan gum

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Thermoreversible edible films with melting or dissolution properties under controlled conditions have many potential food applications. The use of commercial gellan gum as a thermoreversible film matrix is limited by the presence of a high level of contaminant ions (mainly potassium and calcium) which result in melting and gelling temperatures in excess of 100 °C. In this study, purification of commercial gellan involved heating it in a solution of EDTA and subsequent washing of the polymer. This resulted in a 78% reduction in ion content and allowed formation of gels with melting temperatures which could be modified by the readdition of monovalent or divalent chloride salts. Using a Rheometric Scientific rheometer 1% gellan gels with melting temperatures in the range 5 to 60 °C for 30 to 60 mM sodium and 10 to 35 °C for 15 to 55 mM potassium were obtained. When films were prepared by drying 1% gellan solutions containing the monovalent salts, all films were instantly soluble at room temperature. Gellan gels (2%) had melting temperatures in the range 15 to 80 °C for 2.5 to 4.5 mM calcium. Films prepared by drying the 2% gellan solutions containing calcium were soluble at room temperature but dissolution times increased with increasing calcium. The results indicate that monovalent ions affect gel melting properties but not film solubility while divalent ions affect both gel melting and film solubility.

## Assay of angiotensin-I-converting enzyme inhibitory activity using furanacryloyl-L-phenylalanylglycylglycine

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Enzymatic degradation of food proteins can produce potent inhibitors of angiotensin-1-converting enzyme (ACE). Modification of ACE activity is a major target for the control of blood pressure in humans. The objective of this study was to evaluate a spectrophotometric method using furanacryloyl-L-phenylalanylglycylglycine (FAPGG) for the quantification of ACE activity. Hydrolysis of FAPGG by ACE was quantified by measuring the change in absorbency at 340 nm following 30 min incubation at 37 °C. The estimated extinction coefficient for FAPGG at 340 nm was 2270 M<sup>-1</sup>cm<sup>-1</sup>. The influence of the level of ACE activity in the assay on the

inhibitory potency ( $IC_{s0}$ ) for Captopril® and for a whey protein hydrolysate was investigated. The initial rate of FAPGG hydrolysis increased as the level of ACE activity in the reaction increased. Increasing the level of ACE activity (155 to 221  $\pm$  15 U/L) resulted in a corresponding increase in the estimated  $IC_{s0}$  value for Captopril from 9.1 to 39.4 nM. Similarly, increasing the level of ACE activity (117 to 234  $\pm$  15 U/L) resulted in a corresponding increase in the observed  $IC_{s0}$  value for the whey protein hydrolysate from 52.3 to 124.3 mg/L protein. These results demonstrate the importance of carefully controlling the level of ACE activity in the assay system in order to obtain comparable and reproducible  $IC_{s0}$  values for potential ACE inhibitory substances.

## Resolution of the denaturation and aggregation processes during whey protein gelation by differential scanning calorimetery

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It is known that whey protein gelation involves two processes namely denaturation and aggregation, denaturation being an endothermic reaction and aggregation an exothermic reaction. Previous differential scanning calorimetery (DSC) studies have indicated that these two reactions give rise to a single endothermic peak. The aim of this study was to use DSC to resolve the processes of denaturation and aggregation into their respective thermograms and to investigate the optimum whey protein concentration, NaCl concentration and heating rate required to affect resolution. Whey protein isolate solutions (2% to 10% w/w whey protein), prepared in 100 mM NaCl were heated at 1 °C/min in 1 mL stainless steel DSC cells from 25 to 99 °C using the appropriate NaCl solution as a reference. Similar DSC conditions were used to determine the effect of NaCl (0 to 100 mM) at 3% (w/w) whey protein. The effect of heating rate from 0.3 to 1.0 °C/min was determined by heating 4% (w/w) whey protein prepared in 100 mM NaCl. All samples were adjusted to pH 7.0. Whey protein concentrations of 2 to 6%, NaCl concentrations of 75 to 100 mM and heating rates of 0.6 to 1.0 °C were required for resolution of the whey protein gelation process into its respective endothermic and exothermic peaks. Outside of these parameters no exothermic peak was observed. These results suggest that DSC can resolve denaturation and aggregation during whey protein gelation but this resolution is dependent on protein concentration, ionic environment, and heating rate.

#### Application of lacticin 3147 and *Lactococcus lactis* DPC 303-T4 in meat products

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Lacticin 3147 is a novel heat-stable bacteriocin, produced by *Lactococcus lactis* DPC 303-T4, which exhibits a broad-range

inhibition spectrum like nisin. The objective of this study was to determine the impact of lacticin 3147 in whey powder form and nisin, as Nisaplin®, on the microbial safety of cooked meat and L. lactis DPC 303-T4 as a protective culture for fermented whole muscle meat products. Nisin and lacticin 3147 were effective against Listeriae innocua, reducing the initial cell concentrations of  $\sim 10^7$  colony forming units (cfu)/g by 95% and 50%, respectively, over 21 days at 8 °C in vacuum packs. L. lactis DPC 303-T4 (lacticin producer), L. lactis DPC 303 (control) and Lactobacillus sake (commercial culture) were injected at a level of 10<sup>7</sup> cfu/g in Longissimus dorsi pork muscles. Over 7 days at 12 °C, each strain showed similar pH decreases (from ~5.6 to 5.2) and growth increases (from  $\sim 10^6$  to  $10^8$  cfu/g). Lacticin could not be extracted from the whole muscle system. It was concluded that L. lactis DPC 303-T4 can be used as a starter culture for the production of whole muscle fermented hams, but that the production of lacticin 3147 seems to be minimal under the processing conditions used. In contrast, lacticin 3147 powder showed an improvement in the safety of cooked meat products.

#### Effects of high pressure treatment on creaming of bovine milk

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Creaming of raw whole bovine milk at refrigeration temperatures is generally regarded as an undesirable phenomenon; traditionally, creaming is prevented by homogenising the milk. The effects of high pressure (HP) processing on the creaming of raw whole bovine milk were examined in this study. For this purpose, unpressurised or HP-treated milk was incubated at 5 °C for up to 15 days and the level of separated cream was determined. Treatment at pressures ≤ 250 MPa increased the rate and level of creaming in milk, whereas treatment at ≥ 400 MPa reduced both of these parameters. On treatment at 200 MPa, creaming increased with increasing treatment time, whereas at 600 MPa, creaming decreased progressively with the duration of treatment. Treatment at 100 to 600 MPa had little effect on milk fat globule size, whereas the viscosity of the milk serum increased with increasing pressure and treatment time. The amount of milk protein associated with the milk fat globules was increased by HP treatment, the extent of the increase being maximal at 200 MPa. Although increased viscosity and the level of protein associated with the fat globules may partially explain the reduced rate and level of creaming, HPinduced aggregation and denaturation of agglutinins and lipoproteins are likely to have significant effects on HPinduced changes in the creaming characteristics of milk. HP treatment may provide a novel approach to overcome creaming in milk, without the necessity of homogenising.

## Effect of spray-dried milk powder particle size on some properties of chocolate

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Spray-dried milk powders have a smaller median particle size (30 to 80  $\mu$ m) than roller-dried powders (100 to 200  $\mu$ m) which are preferred for chocolate making. Spray-drying parameters were varied in a Niro Tall-Form dryer to produce a range of powders with larger particles for use in chocolate.

The particle size of the spray-dried powders increased with increasing diameter of the spray nozzle and total solids of the concentrate used for drying. The vacuole volume and moisture contents of the powders remained typical for spray-dried powders. However, the free fat content and solubility index of the powders increased above the normal air outlet temperatures used to dry the large powder particles. The particle size of the chocolate mix after refining, the Casson yield value of the chocolate after conching and the hardness of the final chocolate reached minimum values using spray-dried powders with median particle size values of 132 to 162  $\mu$ m. The minimum values in the chocolates were also correlated with the higher contents of free fat in the powders. Greater amounts of free fat were also expressed from the larger powder particles during the refining step. These spray-dried powders produced good quality British-style chocolates, where the particle size after refining should be  $< 26 \mu m$ , but not continental chocolates, where the particle size should be  $< 20 \mu m$ .

#### Use of high-pressure treatment to attenuate starter bacteria for use as adjuncts for Cheddar cheese manufacture

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Attenuated bacteria are microorganisms which are not able to produce acid during cheese manufacture but contain enzymes that contribute to cheese ripening. The aim of this study was to attenuate starter bacteria using high-pressure treatment (HPT), for use in combination with a primary starter for Cheddar cheese manufacture, to determine their effect on secondary proteolysis during ripening. Lc. lactis ssp. cremoris HP and Lc. lactis ssp. cremoris 303 were attenuated by HPT at 200 MPa for 20 min at 20 °C. Cheddar cheese was then manufactured using as primary starters, HP or 303, in combination with their attenuated equivalents (HP+HP, HP+303, 303+HP or 303+303). Attenuated starters did not produce acid during cheese manufacture and the starter count in experimental cheese did not differ from that in the controls throughout ripening. Higher levels of cell lysis were apparent in cheese manufactured using attenuated stains compared with the controls after 30 days of ripening. Little differences were observed in the peptide profiles of cheese when analysed by reversed-phase HPLC; however, cheese manufactured using attenuated starters had higher levels of amino acids than controls at 60 days. Overall, addition of HPT attenuated starter bacteria as secondary starter cultures accelerated secondary proteolysis in Cheddar cheese.

### Effect of starter cultures and growth medium on the flavour profile of fermented whey

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Whey, like milk, can be used as a substrate for producing fermented dairy products; however, there is lack of detailed sensory information concerning the effect of raw material and starter cultures on the flavour of such products. The aim of this study was to compare the sensory characteristics and volatile compound composition, obtained using quantitative sensory analysis and Proton Transfer Reaction Mass Spectrometry (PTR-MS), respectively, of fermented whey and milk. Three strains of commercial starter cultures were used; a yoghurt culture, a probiotic culture and a cheese

culture. The strains were inoculated into skim milk or rennet whey at a level of 0.10%, which decreased the pH to 4.6 in 4 to 6 h. While there was no significant effect (P < 0.05) of medium of growth on any flavour characteristic, the use of different starter cultures had a significant effect on specific flavours. For example, the probiotic culture produced a 'fruity' flavour that was related to ethyl butyrate concentration, the cheese culture produced a 'rancid' odour that was related to free fatty acids concentrations, and the yoghurt culture produced a 'natural yoghurt' flavour that was related to the acetaldehyde concentration. The yoghurt culture also produced an 'acidic' taste. Relationships were established using Partial Least Squares regression. Results suggested that the presence of caseins did not have a significant effect on taste and aroma of fermented dairy products.

## Evaluation of lipolytic activity in Cheddar cheese made with starters of different autolytic properties

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Lipolytic enzymes in Cheddar cheese may originate from the milk or the cheese microflora. The aim of this study was to evaluate the effects of pasteurisation and autolysis of starter cultures on lipolysis in Cheddar cheese during ripening. Cheeses were made with four starter bacteria (Lactococcus lactis AM2, L. lactis HP, L. lactis 303 and Lactobacillus helveticus DPC4571) showing different autolytic properties. Cheese was manufactured in triplicate. ripened at 8 °C and sampled over 224 days. Expressed cheese juice was monitored for autolysis, lipase and esterase activity by measurement of lactate dehydrogenase, p-nitrophenyl palmitate and p-nitrophenyl butyrate/tributyrin, respectively. Loss of starter viability and autolysis in cheese was in the order of AM2>4571>HP > 303. The levels of non-starter lactic acid bacteria were comparable in all cheeses, except in cheeses made with 4571, where they reached lower numbers. Lipase activity was not detected immediately post-pasteurisation but was evident in cheeses at day 1. However, esterase activity was detected post-pasteurisation and was evident in cheeses at day 1. Initially both lipase and esterase activity decreased in all cheeses, but increased slightly after 28 days. This data suggests that esterase activity may originate from milk and/or starter bacteria, with lipase activity originating primarily from starter bacteria.

### The effect of consumption of fish oil and wilted or unwilted silage on the fatty acid profile of bovine muscle

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The fatty acid profile of bovine muscle fat can be influenced by different feedstuffs. Wilting of grass prior to ensiling may alter its fatty acid composition while addition of fish oil in the diet may limit rumen biohydrogenation of dietary lipids. This experiment was designed to investigate the effect of dietary fish oil and wilted or unwilted silage on the fatty acid profile of bovine intramuscular fat. Eighty Friesian steers were blocked on bodyweight and assigned to one of eight dietary treatments (n = 10). Unwilted and wilted silage, and rations with 80 g sunflower oil/kg and either 0, 10, 20 or 40 g fish oil/kg concentrates were used in a 2 × 4 factorial arrangement. Animals were slaughtered after a 108-day feeding period and the fatty acid profile of intramuscular fat extracted from the M. longissimus dorsi was determined by gas-chromatography. Feeding wilted instead of unwilted silage resulted in a higher concentration of conjugated linoleic acid (CLA) (P < 0.01), but had no effect on the n-6:n-3 fatty acid ratio. Increasing oil supply linearly increased CLA concentration in intramuscular fat from 56.9 to 71.6 mg /100 g muscle (P < 0.001), and the n-6:n-3 ratio decreased linearly from 3.76 to 3.12 (P < 0.001). Since increasing CLA and n-3 fatty acid content of meat is viewed as nutritionally beneficial, the results indicate a positive effect of feeding wilted silage and increasing dietary fish oil.

### Manipulation of pre-rigor glycolytic pathways to produce consistent beef tenderness

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Variance in temperature, pH and their relationship with time post mortem is an important aspect in pre-rigor beef as fluctuations in the rate of glycolysis within a carcass, is a root cause in the inconsistency of beef tenderness. The objective of this study was to alter the post-mortem glycolytic potential through temperature regimes to optimise production of consistently tender beef. Bovine M. longissimus dorsi (loin) (n = 12) were hot-boned within 90 min post slaughter and submerged in water baths pre-set at the following temperatures; 0, 5, 10, 15, 20 and 25 °C for 8 h post mortem then stored at 4 °C. Each muscle was divided in 3 giving 6 samples per replicate. Samples tempered between 0 and 10 °C were cold shortened (sarcomere length  $< 1.5 \mu m$ ) and generally tough (WBSF-14 days > 50N). Above 10 °C, samples were generally more tender. Although there was a slight decrease in tenderness at 25 °C, all samples at 20 and 25 °C were tender at 14 days post mortem (WB<50N). There was an increased variability in sarcomere lengths in samples tempered between 10 °C and 20 °C. There was no direct correlation between sarcomere length and tenderness, as shortened sarcomeres did not always render beef tough. The role of proteolysis was evident from comparison of SDS-PAGE of the 30-kDa band from samples of low and high WBSF. Further analysis will determine the suitability of different conditions for inclusion into a model for predicting variability in beef quality.

### Effects of three cooking methods on the quality of large cooked beef joints

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Vacuum cooling is considerably faster than other conventional methods for cooling large cooked meat joints. However, it also has some adverse effects on product quality. The aim of this work was to overcome these disadvantages by improving meat quality at the cooking stage, so that a tender and juicy large meat product can be developed. Three

cooking methods: moist heat (82 °C), dry heat (120 °C) and cooking in water (82 °C) were applied to six topside beef cuts. Muscles (mean weight 4.0 kg) were trimmed, injected with a brine solution at a level of 15% of original sample weight and tumbled (10% vacuum, 4 °C, 3 h) before cooking to a core temperature of 72 °C. All cooked joints were then cooled to a core temperature of 4 °C using air-blast cooling. Beef joints cooked in water had the highest yield (96.7%) compared to the other methods (88.6% for moist heat and 92.2% for dry heat). The overall time per kilogram of sample was similar for joints cooked in water (208 min/kg) and dry heat (207 min/kg), but shorter than moist heat (223 min/kg). Preliminary results showed that cooking in water could result in shorter cooking and cooling times, higher yields and could lead to comparative quality for large cooked beef joints.

#### Principal component analysis to characterize different cooking methods combined with storage

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The control of cooking is a critical procedure for cooked meat. In this project, pork hams of about 4.8 kg were cooked by wet air cooking (82 °C), dry air cooking (120 °C) and water cooking (82 °C) to reach a core temperature of 72 °C, then cooled by air-blast cooling to 4 °C. After cooling, half of the ham was measured immediately while the other half was vacuum-packed and stored at 4 °C for 4 weeks and then tested. The quality attributes measured included water content, springiness, hardness, gumminess, chewiness, cohesion, Warner-Bratzler shear (WBS), and colour (L\*, a\* and b\*). The results were elaborated by the loading plot and score plot of Principal Component Analysis (PCA), indicating 81.4% of the total variance of the quality attributes. The loading plot showed water content had close correlation with springiness. WBS, hardness, gumminess, chewiness, L\* and a\*, and their corresponding correlation coefficients were -0.72, -0.41, -0.75, -0.86, -0.89, 0.53 and -0.53. The score plot indicated that the variance distances PC1 and PC2 caused by storage were 3.45 and 1.05, respectively, for wet air cooking, and were 2.89 and 1.24, for water cooking. However, PC1 and PC2 were 2.74 and 4.59 for dry air cooking. In conclusion, wet air and water cooking had similar effects on the quality variance of pork ham compared with dry air cooking. There was also an interaction between cooking methods and storage.

## Experimental investigation on the effects of shear, as a function of fluid flow and flow geometry, on the break-up of whey protein precipitates

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Substantial breakage of protein precipitates can occur in centrifuge inlet zones and other items of process equipment involved in the recovery process. Whey protein precipitates were produced in a batch stirred-tank, under defined conditions of shear during acid-addition and ageing. Precipitate suspensions were pumped at mass flowrates ranging from 1.5 to 6.5 g/s, through a number of different geometrical configurations including a ball valve, through a straight pipe and through pipes with 45° and 180° bends, each of diameter 0.74 mm and length 60 mm. It was found that at higher flowrates significantly more breakage occurred especially for the more complex geometries. Exposure time was seen to

have a considerable impact on aggregate size. For example, at an average flowrate of 5.63 g/s through a straight pipe, a 54% reduction in average particle size occurred for an exposure time of 0.01 seconds, whereas size reduction was about 77% in 0.1 seconds. A simple model was applied to calculate the energy dissipation rate per unit volume (ERV), and it was found that for the application of a particular ERV, particles were broken down to a certain threshold size (e.g. an ERV of 5 MWm<sup>-3</sup> led to a particle threshold size of about 5  $\mu$ m), and that, in order to break particles down further an increased ERV would be required.

### Evaluating the performance of support vector machine in shape classification of pizza base

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Manually grading pizza base is tedious and costly, and easily induces subjective and inconsistent results. Support Vector Machine (SVM) is a state-of-the-art classification technique, which can be applied to grade pizza base automatically. The objective of this work was to evaluate the performance of SVM in shape classification of pizza base. Two parametric classifiers, the Least Squares (LS) and the Regularised Discriminant Analysis (RDA) classifiers, and two non-parametric classifiers, the K-nearest Neighbour (KNN) and the Radial Basis Function Network (RBFN) classifiers were compared with the SVM classifiers. One hundred and twenty images of pizza base, including 40 standard, 32 flowing base, 24 poor alignment, and 24 poor pressing were captured for shape classification. Sixty pizza base images were randomly selected for training and the remaining 60 images for testing. The classification rates of LS, RDA, KNN and RBFN were 90.0%, 73.3%, 93.3% and 93.3%, respectively. The polynomial SVM and Radial Basis Function (RBF) SVM classifiers performed better than all the other classifiers with a classification accuracy of 95.0% and 98.3%, respectively. For speed, the LS classifier was the best method with classification time of 0.03 s. The classifiers of SVM are roughly comparable to RBFN classifier with 0.38 s for the polynomial SVM and 0.55 s for the RBF SVM. It was concluded that SVM classifiers outperform other classifiers significantly for shape classification of pizza base.

#### Sous vide processing of fish and sauce portions

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The objective was to assess the suitability of *sous vide*/freezing as a technology for processing fish portions ( $ca.250\,g$ ) of seven under-utilised fish species (albacore tuna, cardinal fish, blue ling, orange roughy, redfish, roundnose grenadier and Greenland halibut) were evaluated. Three *sous vide* cook time-temperature regimes (followed by blast freezing at  $-30\,^{\circ}\mathrm{C}$  for  $2\,\mathrm{h}$ ) were tested but none influenced product texture. A *sous vide* cook time-temperature of 20 min at 90  $^{\circ}\mathrm{C}$  followed by blast freezing was used in subsequent tests. *Sous vide* cooked albacore tuna had the firmest texture while samples of roundnose grenadier and Greenland halibut were deemed too soft by a sensory panel (P < 0.001). This was in line with the moisture content of raw fillets of albacore tuna (64%) which was much lower (P < 0.001) than that of the other six species

(range 70.5 to 80.3%). There were also large differences in the cook loss (P < 0.001) (in-pack exudate) and centrifugal drip values (P < 0.01) post sous vide cooking/freezing. Cook losses ranged from 18.9% (albacore tuna) to 40.7% (orange roughy) and centrifugal drip losses from 4.1% (albacore tuna) to a range of 11.6 to 18.9% for the other six species. Correlations between the test variables indicated strong positive coefficients between centrifugal drip and cooking loss (+0.82), and negative coefficients between centrifugal drip and shear value (-0.82), and between moisture content and shear value (-0.93). Further sous vide/freezing tests with five of the species in 11 different sauces indicated that albacore tuna, cardinal fish and blue ling were the preferred species (taste panel tests) (P < 0.001) and tomato + pesto, tikka, arrabiata and hollandaise the preferred sauces (P < 0.001).

#### Classification of pizza sauce spread using computer vision Cheng-Jin Du and Da-Wen Sun, Department of Agricultural and Food Engineering, University College Dublin, Earlsfort Terrace, Dublin 2

Being a signature part of pizza, the pizza sauce spread is still classified manually by trained inspectors in the pizza industry. The aim of this study was to develop an automated classification system for pizza sauce using computer vision. In this research, a hybrid image-processing algorithm was developed to grade the pizzas with tomato sauce spread. The algorithm included image segmentation, colour space transformation and vector quantification. After image processing, a colour histogram was employed to represent the distribution of colour features in the image of pizza sauce spread. For performance evaluation of the classification system, 25 pizza samples with tomato sauce spread were analysed. The samples were evenly categorised into five quality levels before image processing by the inspection personnel, i.e., reject underwipe, acceptable underwipe, evenspread, acceptable overwipe and reject overwipe. The results showed that the histograms differed sequentially from "reject underwipe" to "reject overwipe" with the increase in sauce spread. There were four peaks in the histograms located in the ranges of [1, 13], [17, 27], [33, 39] and [241, 251] for "reject underwipe" and only two peaks located in the ranges of [1, 13] and [242, 251] for "reject overwipe". Meanwhile, there were three peaks for all the other three acceptable levels. The overall accuracy of the system was 88% when the three acceptable levels of quality were considered. The results confirm that the computer vision system developed can effectively classify the images of pizza sauce spread into "reject underwipe", acceptable and "reject overwipe" levels.

#### Modelling the sorption kinetics of soybeans and chickpeas A. Gowen, N. Abu-Ghannam and J. Frias, Faculty of Tourism and Food, Dublin Institute of Technology, Cathal Brugha Street, Dublin 1

Legumes are cheap, highly nutritious, protein rich, cholesterol free, yet under-utilised in Ireland. They require inconveniently long soaking times (up to 16 h) prior to cooking. The sorption process in soybeans and chickpeas after blanching by immersion in boiling water for 1.5 min, before soaking in thermostatically controlled water baths at temperatures of 25, 30, 35, 40, 45, 50, 55 and 60 °C, was examined. Two kinetic models, a first order asymptotic model (I) and a Pelog model (II), were selected as primary models and fitted to the sorption data for both blanched and non-blanched soybean and chickpea samples. The pooled residual standard error obtained from the non-linear regression on nonblanched soybeans was smaller for asymptotic model (rse<sub>1</sub> = 0.0030) than for the Pelog model (rse<sub>ii</sub> = 0.006). Similar results were obtained for blanched soybeans (rse, = 0.0044,  $rse_{ii} = 0.0141$ ), non-blanched chickpeas ( $rse_i = 0.0025$ ,  $rse_{ii}$ = 0.0044) and blanched chickpeas (rse; = 0.0035, rse; = 0.0076). Blanching caused the greatest increase in the asymptotic rate constant, k, at 35 °C for soybeans and at 40 °C for chickpeas. An Arrhenius-type temperature dependence was proposed for k. A discontinuity in the Arrhenius plot was found at 40 ± 5 °C for both blanched soybeans and blanched chickpeas. Subsequently, a mathematical model to predict water intake for chickpeas and soybeans was developed.

#### Quality of pork ham cooked in water as compared with cooking in wet and dry air

Qiaofen Cheng and Da-Wen Sun, Department of Agricultural and Food Engineering, University College Dublin, Earlsfort Terrace, Dublin 2

In order to study the feasibility of water cooking in ham processing, three techniques for ham cooking, i.e. wet air cooking (WAC) at 82 °C, dry air cooking (DAC) at 120 °C and water cooking (WC) at 82 °C, were compared. Pork ham was cooked by the three cooking techniques until the core temperature reached 72 °C, then were cooled by air-blast cooling to 4 °C. Cooking efficiency, yield, textural and nutritional attributes were determined. The results showed that water cooking had the highest yield (99.06%), and cooking time (297 min) was quicker than wet air cooking (363 min) but similar to dry air cooking (292 min). In terms of quality attributes related to texture such as Warner-Bratzler shear (WBS), springiness, hardness, chewiness, and cohesion (24.14 N, 64.62%, 58.16 N, 189.21 N. 0.41 for WAC: 26.57 N. 71.23%, 66.14 N. 199.74 N. 0.44 for DAC; 23.94 N, 63.46%, 49.52 N, 142.23 N, 0.39 for WC), water cooking was similar to wet air but significantly different from dry air cooking except for WBS and springiness (P < 0.05). The water content, dry matter and salt contents (71.74%, 28.26%, 1.79% for WAC; 65.75%, 34.25%, 2.28% for DAC; 71.35%, 28.65%, 1.97% for WC) of water-cooked hams were significantly different from those of dry air cooking (P < 0.05), but were comparable with those of wet air cooking. All the above results suggested that water cooking could be a potential technique for high quality ham processing.

#### The effect of sous vide/freezing technology on the quality of broccoli florets

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Sous vide (sv) has long been used as a method for cooking catering products but is now gaining widespread use in the retail sector. Freezing of sv products may offer greater microbiological safety and shelf-life advantages, but may be detrimental to product quality compared with chilling. The purpose of this study was to investigate the effects of sv cooking followed by freezing on broccoli floret quality. Broccoli florets were given three pre-treatments [(i) full blanch at 90 °C for 2 min; (ii) mild blanch at 50 °C for 15 min + full blanch; (iii) mild blanch at 50 °C for 30 min + full blanch], vacuum packed in sv bags (150 g) and subjected to two mild sv cook treatments (10 or 25 min at 90 °C), followed by two post-cook treatments (blast freezing or chilling). Shear values of sv broccoli were

influenced by pre-treatment (P < 0.01) (1.5, 2.2, 2.3 kN, respectively), length of sv cook (P < 0.001) [3.0 kN (short) v. 1.0 kN (long)] and freezing v. chilling post-cooking (P < 0.001) (1.6 v. 2.4 kN). Taste panel tests on boiled broccoli indicated that the ideal texture was in the range 0.8 to 1.2 kN. The firming effect of the mild blanch pre-treatments was most likely due to the activation of pectin methylesterase which interacts with the methyl ester groups of the pectin chain to produce free carboxyl groups which are then cross linked by either  $Ca^{++}$  or  $Mg^{++}$ . The effects of the various treatments on vitamin C content, product drip loss and colour were minimal. The sv data suggest that sv/freezing gives an acceptable texture and quality in broccoli florets.

### Determination of moisture and fat content of processed cheese using near-infrared spectroscopy

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Near-infrared spectroscopy (NIR) is widely used in the food industry to obtain rapid results with minimal costs but reports of its application to cheese analysis are few. This work aimed to investigate the potential of NIR for measurement of moisture and fat in processed cheeses. Reflectance spectra (400 to 2498 nm) of cheese samples (n = 64) were collected using a NIRSystems 6500 scanning monochromator. Calibrations to predict moisture and fat content were developed by modified partial least squares (MPLS) regression using WINISI software. The performance of the calibration models was compared based on the standard error of cross-validation (SECV), correlation coefficient (R) and number of MPLS loadings. Models were developed using five wavelength ranges; 400 to 2498 nm, 1100 to 2498 nm (near infrared), 750 to 1098 nm (near near infrared), 400 to 750 nm (visible) and 400 to 1100 nm. Spectral data were used (1) without any pre-treatment, (2) after scatter correction (standard normal variate and de-trending) and (3) the latter plus a second derivative step (10 data point gap size). For both moisture and fat, the best models were obtained using option 3. Best fat prediction used spectral data between 1100 to 2498 nm (SECV = 0.45, R = 0.98) with five loadings. For moisture, wavelength ranges of 1100 to 2498 nm and 400 to 2498 nm gave identical results (SECV = 0.48, R = 0.99) using five loadings. These results show that NIR spectroscopy can accurately and rapidly measure moisture and fat content in processed cheeses.

## Comparisons between the Biacore biosensor, HPLC-UV and LC-MS-MS for determination of nicarbazin residues in poultry

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Nicarbazin is widely used as an anti-coccidial feed additive for routine treatment of coccidiosis in broiler production. A range of methods, screening, quantitative and confirmatory, has been developed to determine the occurrence of nicarbazin residues in poultry. Three assays, newly developed biosensor and HPLC-UV assays and an established LC-MS-MS assay, were compared. The performance of the three methods was evaluated based on limit of quantification and rate of false negative and false positive results. The results obtained for paired assays (n = 31) were compared using the regression coefficient (r<sup>2</sup>), while the quantitative comparison between assays was evaluated using the slope of the regression line. Limits of quantification for each assay were as follows: biosen $sor - 25 \mu g/kg$ ; HPLC-UV - 12.5  $\mu g/kg$ ; LC-MS-MS - 1  $\mu g/kg$ . The three-paired assays (biosensor - HPLC-UV, biosensor -LC-MS-MS, and HPLC-UV - LC-MS-MS) compared favourably with each other, giving r<sup>2</sup> values of 0.93, 0.93 and 0.92, respectively. The slope of the regression line, indicating differences in accuracy between the three assays, gave values of 0.58, 0.80 and 0.70, respectively. In this limited study no false positive results were observed with the two newly developed assays. Only one false negative result was observed for the HPLC-UV assay at a relatively low nicarbazin concentration. The results indicate that all three methods are suitable for residue testing for nicarbazin in poultry liver.

### Prevalence of fluoroquinolone resistance in the food-borne pathogens Salmonella and Campylobacter

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Salmonella and Campylobacter are zoonotic pathogens, and are the primary cause of bacterial food poisoning in Ireland. The resistance of these food-borne pathogens, isolated from food animals, food and humans, to fluoroquinolones, which at present are one of the most important classes of antibiotics available for treatment of salmonellosis and campylobacteriosis, was determined. All strains were tested for antimicrobial susceptibility to the quinolone agents, nalidixic acid and cinoxacin, and the fluoroquinolones, ciprofloxacin, ofloxacin, norfloxacin, pefloxacin and enrofloxacin. Two hundred Salmonella strains including 17 serotypes and 153 Campylobacter strains including seven biotypes were collected. All Salmonella strains were susceptible to the fluoroquinolones, however 2.5% expressed resistance to nalidixic acid, which has been associated as an indicator of resistance or reduced susceptibility to the fluoroquinolone agents among Salmonella species. In contrast, 28.3% of Campylobacter strains expressed resistance to the quinolones and 14.5% expressed fluoroquinolone resistance. Increased incidences of resistance to the fluoroquinolones in zoonotic Salmonella and Campylobacter have been associated with their use in animals. Prudent use of the fluoroquinolones in food animals and human medicine must be emphasised.

Incidence of verotoxigenic Escherichia coli O157 and Salmonella species in Cork liquid milk production holdings and microbiological assessment of farm water supplies B.P. Murphyl<sup>1,2</sup>, S. Fanning<sup>2</sup>, H.G. Buckley<sup>1</sup>, J.F. Buckley<sup>1</sup>, R. McKee<sup>3</sup>, D.R. McCleery<sup>3</sup>, M.T. Rowe<sup>3</sup> and D. Gilroy<sup>2</sup> <sup>1</sup>Veterinary Department, Cork County Council, County Hall, Cork, <sup>2</sup>Department of Biological Sciences, Cork Institute of Technology, Bishopstown, Cork and <sup>3</sup>The Queen's University of Belfast, Food Science Division, New Forge Lane, Belfast

In-line milk filters, from 97 dairy farms in the County Cork, were analysed for the presence of *Escherichia coli* serotype

O157 strains and Salmonella spp. over a 2-year period at 4 monthly intervals (cycles A to F). Microbiological quality of water supplies from these farms was also assessed. From 500 filters analysed for the presence of E. coli O157, 16 (3.2%) isolates was detected. Verotoxin (vt) diversity was determined using PCR analysis, two isolates were vt1and 2, and 10 were vt2 producing strains and four remain to be analysed. From 523 filters analysed for the presence of Salmonella spp., eight (1.5%) were positive, four for Salmonella Dublin, three for Salmonella Typhimuruim DT104 and one for Salmonella Agona. None of the above isolates were detected in bulk tank milk samples examined in Cycle C. Water quality was assessed annually in Cycle A and D. Faecal E. coli was detected in water samples in both Cycle A (24%) and D (17%). The results indicate a low incidence of Salmonella spp. and E. coli O157 in a number of these dairy herds, and the need for routine water testing.

#### Quantitative exposure assessment of Eshcherichia coli O157:H7 in Irish retail beef products

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Escherichia coli O157:H7 is a food-borne pathogen that can cause severe human illnesses and death. Minced beef and associated products have been implicated as sources of E. coli O157:H7 poisoning. A Quantitative Exposure Assessment (QEA) model for the spread of E. coli O157:H7 during burger production in Ireland was developed. The QEA model was developed in Microsoft Excel using the @RISK add-in (version 4, Palisade, New York). The QEA models the processing operations (grinding and storage including freezing) which may increase or decrease the counts and prevalence of E. coli O157:H7 in comminuted beef products. The inputs were modelled using probability distributions, taking account of both parameter uncertainty and variability. The model uses predictive microbiology equations taken from existing literature in addition to data gathered from a survey of Irish processing conditions. The output of this model was a distribution for the predicted prevalence of E. coli O157:H7 (approximately 3.1%) and counts of E. coli O157:H7 in uncooked burgers placed on retail shelves in Ireland (counts ranging from 0.5 to 3.5 log<sub>10</sub> colony forming units/g). These results are in good agreement with a recent retail survey conducted in Ireland as part of this project. The risk assessment highlights the potential dangers consumers face if contaminated beef products are not adequately cooked.

#### Factors influencing high pressure-induced inactivation of bacteria in oysters

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Oysters have been widely associated with outbreaks of human gastroenteritis, caused by microorganisms. High pressure (HP) treatment of shellfish is currently of great interest and HP-treated oysters are commercially available. In this study factors influencing HP-induced inactivation of bacteria in oysters were investigated. Broth and oysters, inoculated separately with Vibrio mimicus, Escherichia coli or Listeria innocua were HP treated at 400 to 600 MPa for 5 min at 20 °C.

HP-induced inactivation of all bacteria was greater in broth than in ovsters. The influence of a high salt content, as in ovsters, on HP-induced inactivation of bacteria, was subsequently investigated. Bacteria were considerably more resistant in broth containing 3.5% salt than in broth containing 0.5% salt. In the presence of sufficient salt, bacteria may take up natural osmolytes, present in media and oysters that protect cells from adverse conditions such as high pressures. Preliminary results showed increased barostability in the presence of salt for a Listeria monocytogenes strain with genes for osmolyte uptake, whereas it was not seen for the same strain devoid of these genes. This indicates that the salt-mediated uptake of osmolytes, as by some bacteria in oysters is important in determining the resistance of bacteria to HP. However, the role of osmolytes in HP-induced inactivation of bacteria in broth cannot be extrapolated to oysters and a direct investigation of this phenomenon in ovsters is necessary.

#### Thresholding techniques for measurement of bread crumb features by digital image analysis

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Selection of an optimal grev level value to binarise a breadcrumb image is critical to ensure a successful pores-background partition. The objective of this study was to investigate the suitability of different thresholding methods (six automated methods: isodata, Otsu, minimum error, Pun, moment preserving and fuzzy, and a manual method) compared to a previously used k-means clustering method to consistently segment breadcrumb images. Optimal thresholds and crumb features (cell density, mean cell area, cell uniformity and void fraction) were computed on 135 40 × 40-mm<sup>2</sup> breadcrumb images (scanned at 350 dpi). All the methods were correlated with the k-means and compared using F-test. Two dimensional measures of thresholding performance: uniformity of segments (U) and busyness (B) were estimated on a subset of 30 images. The Pun method produced jagged and non-uniform binary images (B = 0.82). The manual method proved to be inaccurate for quantification of cell uniformity (r = 0.775) and void fraction (r = 0.047). Fuzzy, Otsu, isodata and moment-preserving methods yielded good and consistent binary images (U = 0.59 to 0.60) in comparison to k-means (U = 0.59). Isodata and moment preserving did not differ significantly with k-means on mean crumb features values. Otsu was slightly better correlated to the k-means (r = 0.992) than were isodata and momentpreserving methods (r = 0.989, 0.988). Although the fuzzy method showed relatively high amount of busyness (B = 0.76), it presented the best correlation with k-means (r = 0.995). Overall, the fuzzy, Otsu, isodata and moment-preserving methods were suitable to threshold breadcrumb images.

#### Fundamental comparisons of gluten-free and wheat-based doughs, batters and breads

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Coeliac disease is an autoimmune enteropathy triggered by the ingestion of gluten-containing grains in susceptible individuals. The main objective of this study was the improvement of gluten-free breads. Two new gluten-free recipes were developed, one contained dairy ingredients and the other was purely cereal-based. These breads were subsequently compared to a standard wheat bread and bread made from a commercial gluten-free flour. The quality of breads was evaluated using standard baking tests as well as texture profile analysis (TPA) over 5 days. Batter consistency was measured by extrusion tests. The three-dimensional microscopical structure of the gluten-free batters, wheat dough and all breads was studied by laser-scanning confocal microscopy (LSCM). Results showed that wheat and also the starch-based bread made from commercial gluten-free flour mix yielded the highest loaf volumes (P < 0.01). All the gluten-free breads were brittle at day 2, detectable by the occurrence of fracture and the decrease in springiness (P < 0.01), cohesiveness (P < 0.01) (0.01) and resilience (P < (0.01)) in the TPA. However, these changes were generally less pronounced for the dairy-based gluten-free bread, indicating a better keeping quality of that bread. LSCM showed the dairy-based gluten-free breadcrumb to contain network structures resembling the gluten network in wheat breadcrumb. It was concluded that the two new recipes yielded gluten-free breads of high quality.

## Evaluating the baking potential of organic ingredients using wheat bread as a model system

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The objective of this study was to evaluate the quality and baking potential of organic flours, and to determine the effect of organic fats and improvers on the quality of wheat bread. The control (non-organic) and organic flours were tested initially for the presence of pesticide residues (organochlorine/organophosphorous etc.), and then for moisture content, gluten index, degree of starch damage and rheological properties. The baking characteristics of breads made from these flours were also assessed. No pesticides were found. A correlation was found between flour protein content and dough development time ( $r^2 = 0.79$ ), and between protein content and degree of dough softening ( $r^2 = 0.61$ ). Protein contents of all organic flours were lower than the control (P < 0.05), and the resulting breads had a reduced loaf volume. In a follow-up study, the effects of organic fats and improvers in the bread formulation were studied to give a precise insight as to whether the organic flour, the fat, or the improver affected bread quality most. All breads containing the organic improver had a darker and harder crust compared to bread made with a conventional improver.

#### Malting with buckwheat at different germination temperatures

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Buckwheat, which is a so-called pseudo-cereal, can be regarded as a raw material for the production of functional foods due to its unique composition. The objective of this study was to investigate the impact of germination temperature on the quality of buckwheat malt. Buckwheat with and without hull was used. Malting trials were carried out using a micro-malting facility. Four different germination temperatures (20.2 °C, 16.5 °C, 14.9 °C and 9.5 °C) were evaluated. During malting visual appearance, root lengths and moisture content were

determined. The resulting malt was evaluated using EBC standard methods as  $\alpha$ -amylase activity,  $\beta$ -amylase activity and congress mashing to obtain extract, filterability and FAN. Regarding FAN, the best results were achieved with buck-wheat with hull germinated at 20 °C which reached 100 mg/l, which is close to barley malt values. The extract values showed temperature dependence with best results with buckwheat malt without hull germinated at 16.5 °C, which gave an extract of 76%. Extract results correlated with  $\alpha$ -amylase activities of the samples.  $\beta$ -amylase was only marginally affected by temperature and buckwheat type. Best filterability (125 ml after 30 min) was obtained with buckwheat with hull, germinated at 20 °C. It can be concluded that the best germination temperature for the production of good quality buckwheat malt was 16.5 °C using buckwheat without hull.

#### The indigenous yeast strains involved in traditional Irish cider fermentation

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The evolution dynamics of the different yeasts involved in traditional cider fermentation, using both classical and molecular-based techniques, was studied. Total yeasts were enumerated on a basal apple medium and Walstein Laboratory Nutrient Agar. Isolation of the different yeast species was achieved by supplementing the above media with ethanol and cyclohexamide. Molecular methods involved the use of RFLP analysis of PCR products generated from the regions spanning the internal transcribed spacers (ITS1 and ITS2) and the 5.8S gene, while strains of the genus Saccharomyces were differentiated by RFLP analysis of mitochondrial DNA. With the results obtained we divided the process into three distinct mycological phases based on the predominant yeast species present. In the initial 'fruit yeast phase' Hanseniaspora uvarum type yeasts predominate. They are quickly substituted with strong fermenting Saccharomyces cerevisiae, which dominate the 'fermentation phase', where the alcoholic fermentation takes place. Finally the 'maturation phase' which follows, is dominated by Dekkera and Brettanomyces type yeasts. The Saccharomyces type yeast strains were found to belong to the Saccharomyces sensu stricto group according to analysis of their karyotypes and the RFLP maps of the PCR products from the MET2 gene and the ITS region. These results aided in the selection of yeast strains for use in a more controlled fermentation process.

## An efficient HPLC method for the determination of nicarbazin residues in avian tissue

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Nicarbazin is widely used as an anti-coccidial feed additive for routine treatment of coccidiosis in broiler production. A method, based on high performance liquid chromatography (HPLC), has been developed for the determination of

nicarbazin residues in poultry liver. Liver samples (2 g) were extracted with two portions of acetonitrile (10 and 5 ml). defatted with hexane and evaporated to dryness under nitrogen. Extracts were reconstituted in acetonitrile-water (70/30, v/v, 500 μl), loaded onto C<sub>18</sub> solid phase (SPE) cartridges and eluted with acetonitrile-water (70/30, v/v, 2.5 µl) into clean test tubes. Extracts were evaporated to dryness and reconstituted in acetonitrile-water (80/30, v/v, 500  $\mu$ l). Aliquots of the extracts were determined by HPLC with UV detection at 350 nm. The method was validated according to EC guidelines using liver tissues fortified with 100, 200 and 300 µg nicarbazin/kg while no maximum residue limit (MRL) has been set by EU regulations for nicarbazin in liver, the WHO/FAO JECFA committee have indicated that a MRL of 200 μg/kg is appropriate,  $CC\alpha$  (Decision Limit) values ranged between 213 and 230  $\mu$ g/kg, while CC $\beta$  (Detection Capability) values were between 228 and 281 μg/kg. The mean recovery was typically > 70% and the limit of quantification was 12.5  $\mu$ g/kg (based on the lowest standard on the calibration curve). The accuracy of the method was evaluated using incurred tissue samples, which had been determined by LC-MS-MS.

#### Extraction and determination of the mycotoxin zearalenone and related compounds in wheat flour

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Zearalenone (ZEN) is a mycotoxin produced on cereal grains by Fusarium species. Zearalenone, and  $\alpha$ -zearalenol ( $\alpha$ -ZL) and  $\beta$ -zearalenol ( $\beta$ -ZL), are known to have potential oestrogenic and anabolic effects. The objective of this work was to develop a method for the determination of ZEN,  $\alpha$ -ZL and  $\beta$ -ZL in cereal products. The cereal sample was extracted with methanol:1% sodium chloride (80:20, v/v). The extract was washed with hexane to remove lipids, methanol was partially removed by evaporation, and the extract cleaned-up by solid phase extraction on an Oasis HLB cartridge. Separation of the compounds was carried out on a reversed phase HPLC column with fluorescence detection ( $\lambda$ ex 274 nm,  $\lambda$ em 440 nm), based on a method developed for the quantification of these compounds in porcine kidney. The limit of quantification for the method was 2.5  $\mu$ g/kg ZEN and  $\alpha$ -ZL (25  $\mu$ g/kg  $\beta$ -ZL), which is considerably lower than a proposed EU limit of 10 μg/kg. Mean recovery (± s.d.) for fortified samples of wheat flour were 68 ( $\pm$  6.7)% ZEN, 78 ( $\pm$  2.3)%  $\alpha$ -ZL and 72  $(\pm 1.8)\%$   $\beta$ -ZL. The method provides for the determination of ZEN,  $\alpha$ -ZL and  $\beta$ -ZL in cereal products using a simple extraction and clean-up procedure that can be used to screen multiple samples simultaneously.

#### The genome sequence of the polyvalent anti-Staphylococcus bacteriophage K provides insights into phage biology and intron invasion S. O'Flaherty<sup>1,2,3</sup>, W.J. Meaney<sup>2</sup>, G.F. Fitzgerald<sup>3</sup>, R.P. Ross<sup>1</sup>

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Phage K is a polyvalent phage of the Myoviridae family, which is active against a wide range of staphylococci. In this study we sequenced the entire genome of this lytic antistaphylococcal phage. Genetic analysis revealed a linear DNA genome of 127,395 bp, which encodes 118 putative ORFs. These ORFs were assigned a putative function by sequence homology using the BLAST algorithm. The genome is organised in a modular form encoding modules for lysis, structural proteins, DNA replication and transcription. The structural module shows high homology to the structural module from *Listeria* phage A511. Three introns (lys-I1, pol-I2 and pol-I3) encoding putative endonucleases were located in the genome. Two of these (pol-I2 and pol-I3) were found to interrupt the DNA polymerase gene while another (lys-I1) interrupts the lysin gene. Splicing of the three introns was demonstrated by RT-PCR and insertion sites for each were determined. lys-I1 and pol-I3 encode proteins 167aa and 270aa, respectively, which encode putative HNH endonucleases, pol-12 encodes a 157aa protein which contains two zinc fingers (CX2CX2CX2C). Availability of the genome of this highly virulent phage, which is active against infective staphylococci, should provide new insights for the exploitation of bacteriophage as therapeutic agents.

#### The polyvalent anti-Staphylococcus bacteriophage K exhibits activity against a broad range of clinically relevant Staphylococci including MRSA

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The emergence of drug resistant bacteria including staphylococci has prompted the need for alternate controls to antibiotics. In this study a potent lytic bacteriophage (phage K) was assessed for its ability to inhibit methicillin resistant Staphylococcus aureus (MRSA) strains from Irish hospitals. Inhibition assays with phage K lysed 83% of MRSA strains. A hetro-vancomycin and vancomycin resistant S. aureus (hVRSA and VRSA) and recently emerged teichoplanin resistant isolates were also successfully lysed by this phage. In challenge experiments against a typical MRSA isolate, phage K reduced bacterial counts by 10<sup>7</sup> colony forming units (cfu)/ml within 2 h. Plate counts confirmed no viable bacteria remained in the medium after phage infection. In an effort to make a formulation for topical application  $1 \times 10^9$  cfu/ml of phage K was mixed with 10 g of a bismuth-based cream and stored at room temperature. After 3 days, 1 g of phage cream inhibited 106 cfu/ml of Staphylococcus strain DPC 5246. Given its virulence against drug resistant pathogenic staphylococci we conclude that phage K is a prime candidate as a therapeutic agent.

#### Analysis of the adaptive tolerance response of the foodborne pathogen Campylobacter jejuni using two-dimensional gel electrophoresis

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Campylobacter spp. lack many of the stress response mechanisms commonly found in other food-borne pathogens such as salmonella and E. coli. Yet, they are the most common cause of bacterial food-borne illness. The aim of this study

was to understand the stress mechanisms in Campylobacter spp. A broad range of C. jejuni isolates was examined for their ability to induce an Adaptive Tolerance Response (ATR). The majority of isolates, except CI 120, induced an ATR in midexponential phase but not in stationary phase. In CI 120, induction of an ATR to mild acid (pH 5.5) for 5 h under aerobic conditions resulted in a 100- to 1000-fold increase in survival of a lethal acid challenge at pH 4.5, compared to an unadapted culture. Using chloramphenicol, the ATR was shown to be due to *de novo* protein synthesis. Two-dimensional gel electrophoresis was used to determine protein profile differences between adapted and unadapted cultures. In adapted cultures, de novo synthesis of approximately 25 proteins was observed. Up-regulation and down-regulation of several other proteins was also observed. Identification of 18 of these proteins by MALDI-TOF-MS indicated that the majority of them are involved in several different stress response mechanisms. This induction of stress response mechanisms has important implications for survival of C. jejuni in the environment and during food processing.

## Use of subtractive hybridisation to isolate genes involved in the biosynthesis of the mycotoxin patulin in *Penicillium expansum*

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Patulin is a mycotoxin, produced by several species of Aspergillus and Penicillium, which are found naturally on bruised fruits and can be detected in high levels in fruit juices made from contaminated fruits. The enzymes involved in the biosynthesis of patulin have been well studied but many of the genes involved in the pathway have not been cloned or characterized. The objective of this study was to isolate genes involved in patulin production from Penicillium expansum. RNA was isolated from tissue grown in patulin permissive conditions and from tissue grown in patulin restrictive conditions. This RNA was converted to cDNA and subtractive hybridisation was used to subtract common transcripts and to enrich for genes which were present in the permissive population only. A pool of these genes was cloned and 105 clones were sequenced and compared to sequences in the databanks. Thirty-one percent of the sequenced clones showed significant similarities to characterized genes in the databanks, of which 11 may play a role in patulin biosynthesis. A gene encoding an enzyme which catalyses the production of acyl CoA's which feed into the patulin pathway was idendified. In addition, three clones showing similarity to Cytochrome p450 monooxygenases which are involved in the biosynthesis of several mycotoxins and are likely to catalyse hydroxylation reactions in the production of patulin. These results represent a significant step forward in the study of patulin biosynthesis in this species.

## An investigation into the microbiological quality of dried ingredients for sale in Dublin City ethnic food shops

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Little is known about the microbiological quality of spices and herbs retailed through ethnic food stores in Ireland, and the health risk that they may pose. The aim of this investigation was to determine the microbiological quality of herbs and spices for sale in ethnic food shops situated in Dublin City. Herbs and spices (mixed herbs, oregano, mixed spices, black pepper and curry powder) were purchased from a commercial supplier, a supermarket retailer and five ethnic food shops. Bacterial and yeast and mould numbers were determined using plate count agar and malt extract agar respectively (n = 3). Total viable counts ranged from 2.3 log<sub>10</sub> colony forming units (cfu)/g (oregano) to 5.4 log<sub>10</sub> cfu/g (black pepper) at 37 °C for samples from ethnic food shops. Samples from the commercial supplier gave values from 1.7 log<sub>10</sub> cfu/g (mixed herbs) to 4.1 log<sub>10</sub> cfu/g (black pepper) at 37 °C. Yeast and mould counts ranged from 1.1 log<sub>10</sub> cfu/g (mixed spices), purchased from an ethnic food shop, to 2.0 log<sub>10</sub> cfu/g (mixed herbs), purchased from a major supermarket retailer. No yeast or moulds were detected on the commercial samples. In conclusion, the microbiological quality of herbs and spices from ethnic food shops is variable and is poorer than those obtained from a commercial source.

#### Validation and sensitivity analysis of a simulation model for the transfer of *Escherichia coli* O157:H7 in Irish abattoirs E. Cummins<sup>1</sup>, P. Nally<sup>1</sup>, F. Butler<sup>1</sup>, S. O'Brien<sup>2</sup>, G. Duffy<sup>2</sup>,

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A second-order Monte Carlo simulation model was developed as part of a quantitative risk assessment to assess the prevalence and counts of E. coli O157:H7 on beef trimmings. The model was developed in Microsoft Excel using the @RISK add-in (version 4, Palisade, New York). Distributions were used to model the slaughter operations that may influence the prevalence and counts of E. coli O157:H7 on beef carcasses. The operations modelled included de-hiding, evisceration, carcass washing, chilling and boning out/trimming. The data used in the model was a combination of results from extensive survey work and existing scientific literature. The mean simulated prevalence of E. coli O157:H7 in trimmings was 4.9% and the calculated mean number of pathogens on contaminated trimmings was approximately 0.86 log<sub>10</sub> colony forming units/g. These simulated values were within the range estimated by survey results, which acted as validation steps in the simulation. A sensitivity analysis revealed that E. coli O157:H7 prevalence on trimmings was greatly influenced by the cross contamination factor from hide to carcass (Rank Order Correlation Coefficient (ROCC) = 0.87) and the initial hide prevalence (ROCC = 0.80). The counts of E. coli O157:H7 on contaminated trimmings were influenced by the initial concentration on animal hide (ROCC = 0.86) and bacterial growth (ROCC = 0.14).

### Comparison of methods for the determination of aflatoxins $B_1$ , $B_2$ , $G_3$ , $G_4$ , and $M_1$ in porcine kidney

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The aim of this work was to develop a suitable method for the determination of aflatoxins in animal tissue samples particularly kidney. Methods, which determine the major aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub> and M<sub>1</sub>, are required. Because postcolumn derivatization with iodine quenches the fluorescence of aflatoxin M<sub>1</sub>, pre-column derivatization with trifluoroacetic acid was a preferred procedure to allow for all five aflatoxins to be determined in a single assay. This method gave complete derivatization of  $M_1$  to  $M_{20}$ , of  $B_1$  to  $B_{20}$  and of  $G_1$  to  $G_{20}$  (calibration curve  $r^2 = 0.996$  to 0.999), without any negative effect on B<sub>2</sub> or G<sub>2</sub>. While this method is suitable for all five aflatoxins, a 10-fold dilution step in the derivatization procedure causes the method to lack the sensitivity required for the low levels of aflatoxin expected to occur in animal tissue. Determination of aflatoxins in tissue sample extracts by two methods is required, one without derivatization for aflatoxin M<sub>1</sub> and the other, using post-column derivatization with iodine, for aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>. A solution of iodine in methanol was found to be more stable than an aqueous solution of iodine and wavelengths of 365 nm (excitation) and 430 nm (emission) were suitable. Because of the quenching effect on the fluorescence of some of the aflatoxins at higher iodine concentrations (0.1 to 0.5%), a more dilute solution of 0.02% iodine with heating at 75 °C was found to give the best results. Using these conditions, calibration curves with  $r^2 > 0.999$  were obtained for the four aflatoxins.

### Investigation of the ability of antioxidants to protect against etoposide-induced apoptosis

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Antioxidants may have the ability to protect against apoptosis which is often initiated by the generation of an oxidative stress. Etoposide is a potent inducer of apoptosis in vitro. The pathway of etoposide-induced apoptosis has been well characterised and proceeds via the mitochondrial route which is initiated by the generation of an oxidative stress and depletion of glutathione resulting in perturbation of the mitochondrial membrane. The resultant cytochrome c release activates the caspase cascade which leads to the morphological alterations associated with apoptosis. The objective of the present study was to determine the ability of antioxidants, trolox, ebselen and resveratrol to protect against etoposide-induced apoptosis. U937, a human monocytic blood cell line were exposed to 10  $\mu$ M etoposide in the presence and absence of the antioxidants. Cell viability, apoptosis and glutathione levels were assessed at 24 h. Etoposide decreased cell viability to 50% of the control level after 24 h and the majority of cell death occurred by apoptosis. This was accompanied by a depletion of glutathione. Exposure of cells to etoposide in the presence of the antioxidants did not affect the level of cell death or apoptosis compared to cells treated with etoposide only. In conclusion, under the conditions of the present study, the antioxidants, trolox, ebselen and resveratrol did not protect against etoposide-induced apoptosis.

### Comparison of the pathway of apoptosis induced by $7\beta\text{-OH}$ and $\beta\text{-epoxide}$ in U937 cells

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Oxysterols are the products of cholesterol oxidation and are commonly found in highly processed foods of animal origin. Certain oxysterols have been shown to induce apoptosis in certain cell lines. The objective of the present study was to determine if apoptosis was associated with a depletion of glutathione (GSH) and also to determine the effects of certain

inhibitors of apoptosis on the level of apoptotic cell death, following 24 h, in both 7B-hydroxycholesterol (7B-OH) and β-epoxide (β-epox)-treated U937 cells. Both 7β-OH and  $\beta$ -epox were found to induce apoptosis as assessed by a morphological examination of cell nuclei (10.3%, 12.4%, respectively); however, GSH was depleted in  $7\beta$ -OH-treated cells only. The activation of cellular proteases known as caspases is considered to be central to the apoptotic process. Cells treated with either  $7\beta$ -OH and  $\beta$ -epox in the presence of a broad spectrum inhibitor of caspase activity (1.7%, 3.7%) and a specific inhibitor of caspase-3 activity (4%, 8%) had lower levels of apoptotic cell death compared with the cells treated with oxysterols in the absence of these inhibitors. Cytochrome c release from the mitochondrion to the cytosol has also been suggested to be an event common in certain pathways of apoptosis. In the presence of an inhibitor of cytochrome c release, the levels of apoptosis were reduced in  $7\beta$ -OH but not  $\beta$ -epox-treated cells (3.7%, 13.3%). In conclusion, these results indicate that the pathway of apoptosis differs between  $7\beta$ -OH and  $\beta$ -epox.

## Lactobacillus rossii sp. nov. isolated from wheat sourdough L. Settanni<sup>1,2</sup>, A. Corsetti<sup>1</sup>, D. van Sinderen<sup>2</sup>, G.E. Felis<sup>3</sup>, M. Gobbetti<sup>4</sup> and F. Dellaglio<sup>3</sup>

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Screening of bacteriocin-producing sourdough lactobacilli resulted in the isolation of a strain (CS1) from wheat sourdough sourced from Central Italy, which could not be associated with any previously described Lactobacillus species. The strain was subjected to a polyphasic taxonomic study including phenotypic characterization as well as phylogenetic and genetic methods. Phenotypic characterization showed typical properties found in members of the genus Lactobacillus. Phylogenetic analysis based on 16S rDNA sequences clearly recognized strain CS1 as a distinct member of the genus Lactobacillus. Strain CS1 was shown to be most closely related to Lactobacillus durianis, which belongs to the Lactobacillus casei/Pediococcus rRNA group, and based on 16S rDNA sequence comparison strain CS1 shares 93% similarity with L. durianis. By a species-specific PCR strategy, five additional strains, previously isolated from several wheat sourdoughs, were found to belong to the same species as strain CS1, as determined by 16S rDNA sequence analysis. These six isolates were, however, distinguishable at the intra-species level by RAPD-PCR and biochemical features. It is proposed that the unknown bacterium will be classified as Lactobacillus rossii.

## Simulation of the prevalence of *Escherichia coli* O157:H7 on beef carcasses in Irish abattoirs by means of Event Tree Analysis

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A study of the prevalence of *Escherichia coli* O157:H7 on Irish beef carcasses during the slaughter process was carried out by means of Event Tree Analysis (ETA). ETA is based on binary

logic, in which an event either has or has not happened and works forward from an initiating event identifying all possible combinations of subsequent events. Each event is assigned a probability of occurrence and thus the probability of the various possible outcomes can be calculated. Using a combination of results from extensive survey work and existing scientific literature the ETA modelled the prevalence of E. coli O157:H7 at several crucial steps during the beef slaughter process. These stages included; stunning, sticking/bleeding, dehiding, evisceration, trimming, carcass washing and chilling. The ETA was carried out using Microsoft Excel and the addon software package Precision Tree (Palisade Corporation, New York, USA). The mean calculated prevalence of E. coli O157:H7 on carcasses was 5.04% (95% confidence interval 1.49% to 8.90%), which is in good agreement with survey results. ETA has proved to be a useful tool in identifying and assessing the prevalence of E. coli O157 on beef carcasses following various slaughter processes.

#### Exploitation of lytic bacteriophage for biocontrol of Escherichia coli O157:H7 should not be compromised by bacteriophage insensitive mutant formation

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Escherichia coli O157:H7 is an endemic pathogen leading to a variety of human diseases including mild diarrhea, haemorrhagic colitis, haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura. This study concerns the exploitation of bacteriophages as biocontrol agents to eliminate the pathogen E. coli O157:H7. Three lytic phage (e11/2, e4/1c and pp01), previously isolated against a human isolate of E. coli O157:H7, were evaluated for their ability to lyse the bacterium in vitro. Even though a cocktail of all three highly virulent phage resulted in a 7-log reduction of the pathogen in 1 h at 37 °C, bacteriophage insensitive mutants (BIM) emerged following the challenge. All 10 tested BIM had a growth rate, which approximated (0.448  $\pm$  0.07) that of the parent 0157 strain (0.456). The frequency of BIM formation with different combinations of each phage was determined and ranged from  $3.34 \times 10^{-4}$  (e4/1c) to  $1.11 \times 10^{-6}$  (e11/2 + e4/1c + pp01) colony forming units/ml. In addition, BIM reverted to phage sensitivity within 50 generations. Given that the frequency of BIM formation is low (10<sup>-6</sup>) for two of the phage, allied to the propensity of these mutants to revert to phage sensitivity, we expect that BIM formation should not hinder these phage as therapeutic agents, particularly where low levels of the pathogen are encountered.

## Cloning of a polyketide synthase gene from Aspergillus ochraceus that is essential for the biosynthesis of Ochratoxin A

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Ochratoxin A is an important nephrotoxic and nephrocarcinogenic mycotoxin, produced by *Aspergillus ochraceus* as a secondary metabolite. The presence of ochratoxin A in food and feed is currently a cause of concern. Studies on ochratoxin

production have been hampered by the fact that little is known about the biosynthetic pathway of the toxin. A polyketide synthase gene (pks) involved in the biosynthesis of the mycotoxin ochratoxin A has been identified in Aspergillus ochraceus, using a suppression subtractive hybridisation PCR (SSH-PCR) based approach to select genes expressed during ochratoxin biosynthesis. The 1.4 kb region of the gene, which has been cloned, appears to encode for the highly conserved acyl transferase domain present in a number of other polyketide synthase proteins. The deduced amino acid sequence is 28 to 35% identical to other fungal PKS proteins and approximately 50% similar when positive amino-acid substitutions are counted. Reverse transcription PCR (RT-PCR) studies indicate that the pks gene is expressed only under ochratoxin A permissive conditions and during the early stages in the synthesis of the mycotoxin. A mutant of A. ochraceus in which the pks gene has been interrupted loses its ability to produce the mycotoxin. The pks gene is definitely involved in the biosynthesis of ochratoxin A and is the first ochratoxin A biosynthetic gene to be identified.

#### Effects of cooling methods on cooling efficiency of cooked rice

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Rice is one of the most important components in readyto-eat meals. The current work compared the efficiency of cooked rice using vacuum, air blast, plate and cold room cooling. Effects of final pressure, weight of cooked rice, boiling time during cooking, excessive water in cooked rice and water spraying on cooling efficiency was further studied. About 400 g Silvo USA parboiled long grain rice was soaked for 30 min, and then simmered for 10 min in excess water after boiling. The cooked rice (about 1100 g) was cooled immediately in a stainless steel tray or a plastic bag to less than 4 °C, using the above four cooling methods. It was found that the time (about 4 min) needed by vacuum cooling was much shorter than those by the other three cooling methods; however, the weight loss (about 11.5%) was much higher than for the others (P < 0.005). Further studies indicated that less weight of cooked rice and lower final pressure led to shorter cooling time (P < 0.001). Furthermore, longer boiling time or excessive water resulted in higher moisture contents of cooled rice, and longer cooling times (P < 0.001). Spraying of about 200 ml water during vacuum cooling when the chamber pressure dropped to between 40 and 50 mbar did not prolong the cooling time significantly (P > 0.05), but increased the moisture content of cooled rice. The above results indicated that vacuum cooling is the most efficient method for cooling cooked rice.

### Measurement of apparent volume, shrinkage and porosity in low and intermediate moisture banana slices

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Drying causes changes in the apparent specific volume and porosity of food products and, in general, causes shrinkage at 10 to 30% of the original volume. Different methods have been reported in literature to measure apparent volume, but no study has been conducted on the suitability of these methods to characterize dried products. The main objective of this study was to compare different methods

#### The effect of cutting on the respiration rate of fresh mush-rooms

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The shelf life of fresh and fresh-cut produce depends on the product respiration rate. Cut produce shows higher respiration rates owing to the increased surface area and to the response to stress. The objective of this work was to study the effect of cutting on the respiration rate of fresh mushrooms. Respiration rate was measured at 4 °C with the closed system method. Whole and sliced mushrooms were stored in an airtight container that was flushed with a humidified gas mix (air and a mixture of 5% O<sub>2</sub>, 15% CO<sub>2</sub> and 80% N<sub>2</sub>) until equilibrium was achieved, after which the gas composition was monitored over time. Four replicates were performed for each set of conditions tested. Results showed that the respiration rate of sliced mushrooms was on average 45% higher than that of whole mushrooms (O<sub>2</sub> consumption: 43 ml.kg<sup>-1</sup>.h<sup>-1</sup> in air and 39.5 ml.kg<sup>-1</sup>.h<sup>-1</sup> in the  $O_2 = CO_2$ :N mixture;  $CO_2$ production: 40.3 ml.kg<sup>-1</sup>.h<sup>-1</sup> in air and 32.4 ml.kg<sup>-1</sup>.h<sup>-1</sup> in the mixture). The respiratory quotient did not show any clear dependence on cutting but was lower at the higher CO2 concentrations tested (average 0.99 in air and 0.75 in 5% O<sup>2</sup>, 15% CO<sub>2</sub>). The respiration rate in sliced mushrooms was not as sensitive to gas composition as that in whole mushrooms.

#### Sensory quality of minimally processed Bramley's seedling apples as affected by storage parameters

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The objective of this study was to characterize the sensory quality of modified atmosphere packaged (MAP) apple slices. Changes in the quality of apple slices as affected by MA's and the use of anti-browning agents were described using sensory descriptive analysis combined with instrumental colorimetry and texture analysis. The anti-browning treat-

ment consisted of dipping apple slices for 1 min in a solution containing ascorbic acid, citric acid, and calcium chloride. Apple slices (150 g) were either packaged in high barrier film (TBF4) flushed with 100% N<sub>2</sub> or in a micro-perforated film (PA-90) in air and stored at 4 °C. Storage of treated apple slices in PA-90 film resulted in little atmosphere modification (17% O<sub>2</sub>, 6% CO<sub>2</sub>). Slices had very poor appearance with increased surface dryness and browning, and a slight increase in hardness, crunchiness and juiciness. In contrast, storage for 6 days in TBF4 film generated atmospheres with extremely low  $O_2$  levels (< 1%) and increasing  $CO_2$  (up to 11%) concentrations. Treated apple slices had excellent overall visual quality whereas untreated slices developed a poor brown appearance. Both treatments resulted in a significant loss in hardness, crunchiness and juiciness with an increase in a dry starchy texture and in fermented and plastic notes. Overall, the use of anti-browning agents had significant beneficial effects on visual quality but packaging in a barrier film contributed to poor textural and aroma attributes.

## Investigating the use of micro-perforated film in a low oxygen mother pack storage system for beef cuts M. Beggan<sup>1</sup>, P. Allen<sup>1</sup> and F. Butler<sup>2</sup>

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Shelf life extension of retail beef cuts can be achieved by storage under very low oxygen (O2) conditions followed by aerobic display. A virtually oxygen free environment is maintained during storage using a mother pack system with O2 scavengers. The use of micro-perforated lidding film for MAP packs facilitates O2 ingress during display. In this study, micro-perforated film as a lidding film in this low O<sub>2</sub> mother pack storage system for M. longissiums dorsi (LD) (n = 6) M. semimembranous (SM) (n = 3) and M. psoas major (PM) (n = 3) steaks was examined. Barrier MAP packs (micro-perforations covered), low O<sup>2</sup> mother packs (containing low O2 MAP packs sealed with micro-perforated film with and without O2 scavengers) and controls (vacuum packed primals) were stored for 18 days at 0 °C and aerobically displayed. Spectrophotometric colour measurements (CIELab) were taken after 4 h, 24 h, 48 h, 72 h and 96 h display from which saturation was calculated. Although requiring 24 h for complete blooming, only those LD treatments with scavengers, once bloomed, had similar saturation values to the control. All SM and PM treatments were significantly lower in saturation (P < 0.05), with the exception of PM barrier MAP, due to metmyoglobin formation during storage. Micro-perforated film is thus suitable for mother pack storage using only the LD muscle with O2 scavengers.

## Effect of package volume and geometry on the gas exchange in perforation-mediated modified atmosphere packaging R. Saleiro<sup>1</sup>, F.A.R. Oliveira<sup>1</sup>, J.C. Montanez<sup>1</sup>, P.V. Mahajan<sup>1</sup> and J. Kerry<sup>2</sup>

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Perforation-mediated modified atmosphere packaging (MAP) is an alternative to conventional MAP where gas exchange is regulated by single or multiple tubes that perforate an otherwise impermeable covering. The objective of this work was to study the effect of package volume and

geometry on the gas exchange rate in this type of package. Cylindrical, brick and cubic packages were tested. Tubes with different dimensions (the four combinations of diameters 7 and 17 mm and length 7.5 and 31.5 mm) were inserted across the top surface of the packages, which also contained stoppers for gas sampling and semi-rigid tubes to feed the gas mixture (25 and 100% v/v CO<sub>2</sub> and the balance N<sub>2</sub>). After flushing, the system was closed and the gas composition was monitored over time. Two replicates were performed for each set of conditions tested. The gas exchange was well described by a mathematical model earlier developed that considers a drag effect of CO<sub>2</sub> on O<sub>2</sub> mass transfer and the effect of the tubes dimensions. Packages with the same geometry and smaller volumes (ratio of 80%) showed lower gas exchange rates, down to 70%. Cylindrical packages showed the highest gas exchange rate and cubic packages the lowest. These results show that the package geometry and volume have to be taken into consideration when designing a perforation-mediated MA package.

#### Comparison of mechanical and barrier properties of protein and carbohydrate-based films

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Interest in the manufacture of edible/biodegradable films/coatings has grown in recent years because of the potential they possess to reduce or replace conventional non-biodegradable plastics used for food packaging. In this study, mechanical and barrier properties of films formed from 4% sodium caseinate (4% SC), 2% potato starch (2% PS), 2% carboxymethyl cellulose (2% CMC) and 1% sodium alginate (1% SA) solutions were evaluated. Each solution had 50% (w/w) glycerol added, heated to 80 °C, poured onto casting plates and dried for 24 h at  $50 \pm 3\%$  RH and  $23 \pm 3$ °C prior to peeling and testing. All test films were different in tensile strength, tear resistance and puncture resistance. Puncture resistance and tensile strength in increasing order were: 4% SC, 2% PS, 1% SA, 2% CMC while tear resistance in decreasing order were: 4% SC, 2% PS, 2% CMC, 1% SA. All the test films were significantly (P < 0.05) different from each other in terms of oxygen permeability, with 2% CMC having the highest barrier property (52 cm<sup>3</sup> mkm m<sup>-2</sup> day kPa<sup>-1</sup>). SA (1%) had the lowest oil permeability (0.00067 g) and the lowest water vapour transmission rate (39.91 g.mm/kPa.m<sup>2</sup>), which increased through 2% PS, 2% CMC to 4% SC (62.81 g.mm/kPa.m2). CMC (2%) and SA (1%) dissolved completely in water, acid and alkali solutions, while SC (4%) dissolved partially. PS (2%) had a greater degree of resistance to all solutions.

## Analysis of pre- and post-slaughtering factors affecting beef performance of Chinese Yellow cattle

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Yellow cattle are the principal cattle breed in China having a wide distribution throughout the country. However, this breed of cattle has received little attention in terms of beef production and overall meat quality performance since their primary role within Chinese agriculture is a source of power. The objective of this study was to investigate pre- and

post-slaughter factors affecting beef performance of Yellow cattle. Cattle ranging from 2 to 3 years of age produced more tender (P < 0.05) beef than both younger and older cattle. Dietary composition played a significant (P < 0.05) role on meat quality with diets containing higher energy levels (90% corn and 6% cottonseed cake) producing more marbling and greater cutability and smaller shear force values than lower energy diets. Conditioning beef carcasses at 4 °C for 7 days produced optimum shear force (1.66 kg) (P < 0.05), pH (5.56) (P < 0.05), calcium activated factor (CAF) ( $\mathring{A} = 0.828$ ) (P < 0.01) and Myofibril Fragmentation Index (MFI) ( $\mathring{A} = 18.95$ ) (P < 0.01) values compared to carcasses conditioned for 1- or 3-day periods. Electron micrographs also showed that 7-day ageing produced greater myofibril degradation compared to 1- or 3-day conditioning. Generally, this study demonstrated that Chinese Yellow cattle fed an optimal diet and slaughtered between 2 to 3 years of age, followed by an adequate carcass-conditioning period, could potentially produce high quality beef.

## A simplified HPLC method for the measurement of thiamine and riboflavin in raw meats

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Meat products are important sources of the B-group vitamins such as thiamine and riboflavin. Modern procedures for measuring these vitamins usually require a HPLC assay as the final step. Recent studies have shown that the most accurate method for determining thiamine is by converting it to thiochrome with immediate partitioning into isobutanol. However, HPLC measurement of thiochrome in isobutanol requires column and mobile phase conditions, which are not suitable for riboflavin analysis. The aim of the present study was to devise a simplified HPLC procedure to permit the accurate and reliable analysis of both vitamins under similar chromatographic conditions. Thiamine and riboflavin were liberated from their bound forms in meat samples using acid digestion followed by treatment with a papain/acid phosphatase enzyme mixture of defined activity. Thiamine in aliquots of the clarified extracts was converted to thiochrome using alkaline ferricyanide with simultaneous partitioning into isobutanol. Thiochrome was measured on a  $200 \text{ mm} \times 3 \text{ mm}$  reverse phase C<sup>18</sup> HPLC column with fluorescence detection (excitation, 366 nm; emission, 434 nm) using a methanol-water (80:20) mobile phase. Riboflavin was measured on the same column by fluorescence (excitation, 450 nm; emission, 510 nm) using methanol-water (40:60) as the mobile phase. Thiamine and riboflavin levels measured in raw pork, turkey breast and pig's liver were in good agreement with published values.

#### Effect of catechin isomers on oxymyoglobin oxidation and lipid oxidation in muscle model systems

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The principal catechins found in green tea (Camellia sinensis) are (-)-epicatechin (EC), (-)-epigallocatechin (EGC),

(-)-epicatechin gallate (ECG), and (-)-epigallocatechin gallate (EGCG). The antioxidant activity of individual tea catechins [catechin (C), (EC), (EGC), (ECG) and (EGCG)] in muscle model systems at pH 5.5 and 4 °C was examined. Mixtures containing commercial horse heart oxymyoglobin (1.2 mg/ml in 150 mM KH<sub>2</sub>PO<sub>4</sub>-KOH) and individual catechins (3.13, 6.25, 12.5, 25 and  $50 \mu M$ ) were stored for 7 days. Catechins were added (50, 100 and 250 µM) to 25% longissimus dorsi muscle homogenates stored for 24 h. Lipid oxidation in muscle homogenates was initiated with equimolar (45 μM) ferric chloride:sodium ascorbate. Lipid oxidation was assessed by the 2-thiobarbituric acid assay and oxymyoglobin oxidation by spectrophotometry. Oxymyoglobin oxidation did not significantly increase over time in the presence of C or EC at any concentration, Conversely, oxymyoglobin oxidation increased with increasing concentration of EGC, ECG and EGCG and pro-oxidant activity followed the order: EGCG>ECG>EGC. In muscle homogenates oxyymyoglobin oxidation was unaffected by catechin type or concentration. Lipid oxidation decreased with increasing C and EC concentration and catechins containing gallate groups (ECG, EGCG) exhibited greatest antioxidant activity. Results indicate differing reactivity of catechin isomers with muscle lipids and oxymyoglobin, depending on the test system employed.

#### The effect of frozen storage on the shelf-life of novel surimibased products

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Surimi is extremely functional due to the unique gelling properties of the fish myofibrillar proteins and can be shaped, coloured and flavoured to produce a range of novel products. The purpose of this work was to develop a range of surimibased seafood products and determine their shelf-life. Four different glazes were used to produce four different flavoured products; Balti (B), lemon and pepper (LP), Italian (I) and rosemary and garlic (RG), and shelf life (-18 °C × 50 days) stability was assessed. Hardness and cohesiveness of frozen products did not significantly increase over the 50-day frozen storage period. RG flavoured product had higher (P < 0.05)hardness values in comparison to all other frozen products. TBARS values for raw and cooked products were below 0.8 mg MDA/kg. RG flavoured product had lowest (P < 0.05) microbial counts during storage. pH of frozen products did not significantly change over time with RG flavoured product having the lowest (P < 0.05) pH. Organoleptic analysis showed that consumer acceptability of the products was not significantly affected over the frozen storage period. Textural, colour, organoleptic, microbial and lipid oxidation analysis showed that frozen storage (-18 °C × 50 days) had no overall adverse effect on the quality of these products.

#### A comparison of the compositional content and shelf-life stability of organic v. conventional reared beef

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In recent years the demand for organically grown food has increased. In this study, organic (O, n = 6) and conventional (C, n = 6) reared Angus steers aged between 18 to 24 months were slaughtered during the month of September 2002. Four days post slaughter, the longissimus dorsi muscle was excised from the left side of each carcass, vacuum packed and aged in a chill for 7 days. Steaks were cut from each sample, and from these, lean meat was removed, blended and analysed for composition. C samples were higher (P > 0.05) in levels of protein, ash and carbohydrate content while O samples were higher (P > 0.05) in fat content. C samples were found to have a higher (P > 0.05) content of retinol while O samples were higher (P > 0.05) in  $\alpha$ -tocopherol and  $\beta$ -carotene. The fatty acid profile was also determined and for each of the fatty acids investigated there was no significant difference in levels between O and C samples. Colour stability and fat oxidative stability of samples were also measured, while stored under retail conditions. Samples were packed using both Modified Atmosphere Packaging (MAP) or by overwrapping. The oxidative stability of the O samples was less stable than C samples for both packaging methods. A similar trend was observed for the colour stability of both groups. It was concluded that there was no difference between organically and conventionally reared cattle in terms of meat composition.

#### Investigation into the safety of cooling large cooked meats within public houses

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The catering sector in Ireland has grown substantially in the last few years. One of the main areas of growth is the expansion of public houses providing cooked meat meals to consumers. This is an area, which has the potential to be hazardous, as there are few specific guidelines pertaining to the safe cooling of large cooked meats. Gaining an accurate reflection of actual practices within this sector is an important method of determining the safety of meat served. Cooked and cooled meat samples from 10 public houses were tested. Cooling times were determined using "Testo" data logger temperature probes, with times up to 12.6 h recorded. This exceeded times recommended by Irish guidelines by over 10 h. Hygiene swabbing of public house kitchens' determined general hygiene levels within each premise using the "Biotrace, Unilite-Excel" bioluminescence system. These levels varied substantially, in most cases far exceeding limits set by food production facilities. Bacterial levels within cooked and cooled meat joints were determined using standard plate count methods. Most counts remained within acceptable limits set by the Food Safety Authority of Ireland. The only exception was a Staphylococcus aureus count of  $5.7 \times 10^3$  colony forming unts (cfu)/g, which showed a significant deviation from those limits (< 100 cfu/g). Although only low levels of pathogens have been detected within meat joints tested, a serious potential for contamination and proliferation exists.

#### Changes in porcine proteolytic profiles from tough and tender muscle samples

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Myofibrillar protein degradation plays a predominant role in determining post-mortem tenderness in skeletal muscle. The objective of this study was to identify the contribution of this phenomenon during the ageing of pork muscle in samples

that have been previously deemed to be in one of two categories, tough or tender. While previous studies use 1-day post mortem as the initial sampling time, this study incorporated two additional sampling times (1 h post mortem and 3 h post mortem). From a pool of 40 carcasses, samples were taken from two porcine M. longissimus dorsi (loin) muscle for analysis on SDS-PAGE. These samples had been characterised as either tough or tender based on a combination of quality measurements including; Warner Bratzler Shear Force, sensory analysis, composition analysis and sarcomere length (SL). Samples with the toughest and most tender profiles throughout the ageing period were selected, while controlling for other factors such as SL. The proteolytic profiles generated revealed an earlier disappearance of Troponin T which has preciously been shown to be an indicator of tenderness, and the appearance of the 30-kDa fragment, in the tender sample. New bands, in the 40-kDa region also suggest an increased rate of proteolytic breakdown in the tender sample compared to the tough sample.

#### Edible films from fish by-products

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With dwindling fish stocks in European waters, there is an urgent need to investigate useful applications of fish waste as potential food ingredients. Edible films have the potential to act as novel biopackaging material as well as effective barriers against moisture and gas in food systems. Collagen and gelatin have many uses throughout the food and pharmaceutical industries and alternatives to mammalian sources are constantly sought. The objective of this study was to investigate the mechanical and water vapour permeability properties of films formed from soluble collagen extracted from cold water species including New Zealand hoki (Macruronus novaezelandiae) and ling (Genypterus blacodes), and Irish cod (Gadus morhua) and ling (Molva molva). Edible films were formed from plasticised (glycerol:plasticiser = 0.2) or unplasticised protein solutions (1% w/w) of the extracted collagen, poured (35 g) onto level perspex plates and dried under controlled conditions of humidity and temperature (15  $\pm$  1 °C, 50  $\pm$  2% RH). It was found that films containing collagen derived from NZ species were more extensible, and had greater tensile strength and elasticity than those formed from the Irish species tested. The addition of plasticiser was found to increase extensibility and decrease elasticity in all films but had limiting effects on tensile strength.

#### Improvement of cured pork texture through alternative hanging and holding temperatures

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The objective of this study was to improve processed pork quality, in particular tenderness, by a combination of alternative hanging methods and higher temperature conditioning. The carcass left sides of 32 Large White × Landrace cross pigs were suspended from the Achilles tendon (AT) while the right sides were suspended from the pelvic bone (PB) and then chilled in: (a) air at 1 °C for 24 h; (b) air at 17 °C for ~3 h

followed by air at 1 °C for 21 h. Samples of cured hams, shoulders and loins were stored under MAP (70% N<sub>2</sub>, 30% CO<sub>2</sub>) conditions in visual display units (4 °C, 616 lux) for up to 18 days. Tenderness (Warner Bratzler shear force), TBARS (mg MDA/kg muscle) and CIE L\*, a\*, b\* values were determined. Conditioning of carcasses at 17 °C, for both AT and PB treatment groups, improved tenderness in cured hams and cured loins over 18 days of storage. Both PB suspension and conditioning of carcasses at 17 °C improved loin tenderness up to 12 days storage. In general, PB suspension resulted in lower TBARS values for all primal cuts compared to corresponding AT primal cuts. No significant effects on colour stability were observed. Results indicate potential improvement of cured pork quality through PB carcass suspension and conditioning at 17 °C.

#### Addition of tea catechins through processing and dietary supplementation: effects of catechin level and pH on antioxidant activity in fresh beef

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Tea catechins (TC) have many beneficial pharmacological properties in addition to antioxidant activity. The objective of this study was to investigate the effects of dietary supplementation (0, 1, 4 or 10 g/animal per day for 90 days) of TC and post mortem addition (10 or 50 mg/kg muscle) of pH adjusted TC (pH range 5.5 to 8.0, 60 mM sodium phosphate buffer) on the oxidative stability of beef stored aerobically or in modified atmosphere packages (MAP) (80% O<sub>2</sub>:20% CO<sub>2</sub>). Oxymyoglobin oxidation was monitored by colorimetry and lipid oxidation by the 2-thiobarbituric acid assay. Dietary supplementation with TC did not significantly improve colour and lipid stability in beef steaks stored aerobically or in MAP. Conversely, when TC were added to minced beef, the extent of lipid oxidation decreased with increasing TC level from day 2 up to day 10 at all pH levels. Beneficial effects on colour stability were also observed. Trends indicate lower antioxidant activity of TC above pH 7.0 suggesting pH sensitivity of TC in a pH range representative of the rumen and intestine. These findings may explain the limited potential of dietary TC supplementation, compared to beneficial effects when added directly to beef, as a means of improving the oxidative stability of fresh beef.

## Using a novel intervention technique to reduce variability and improve tenderness of bovine M. longissimus dorsi

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Reducing variability is of primary concern in the beef industry today. Techniques such as electrical stimulation and aitchbone hanging can significantly improve tenderness; however, their effect is not uniform throughout the carcass. If muscles are hot-boned (excised immediately after slaughter) optimum treatment can be established for individual cuts. However, there are two disadvantages of hot boning; increased toughening when chilled quickly and shape distortion. The objective of this study was to reduce variability and improve tenderness of hot-boned bovine M. *longissimus dorsi* 

(LD) muscle using different forms of artificial restraint. Hotboned LD muscles (n = 24) were randomly assigned to the following treatments: 'weights' - 4 kg weight suspended from a hanging muscle, 'Pi-Vac' - which wraps the muscle in a film with a high degree of elasticity to prevent contraction and 'control' - no restraint. Muscles stretched by weights or Pi-Vac had significantly (P < 0.05) lower Warner Bratzler shear force than control muscles after 7 and 14 days ageing. Sensory tenderness scores were also higher for weights and Pi-Vac treated muscles. Sarcomere length was significantly (P < 0.05) shorter for the control muscle (1.34  $\mu$ m) compared to the weighted (1.64  $\mu$ m) and Pi-Vac (1.63  $\mu$ m) muscles. Drip loss was reduced only by the Pi-Vac treatment and the shape of the muscle was not distorted as the pre-rigor meat formed into the shape of its constraining pack. Thus, the novel packaging technique, Pi-Vac, allows hot-boned meat to be chilled quickly without adversely affecting its tenderness and produces a more consistent product.

## Comparison of proteolytic profiles in bovine muscle, generated from three different extraction methods

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The identification of markers that can track post-mortem ageing and tenderisation in beef has been the focus of much research to date. The study of myofibrillar extracts is most commonly used for the identification of products of proteolysis which appear during the ageing period. The objective of this study was compare proteolytic profiles following different extraction methods in bovine M. longissimus dorsi (loin) by SDS-PAGE analysis. Samples were excised from bovine loin at various times post mortem (0 h, 1 h, 3 h, 1 day, 7 days and 14 days) then subjected to one of three methods, namely: myofibrillar, trichloroacetic acid (TCA) and NaCl fractionation. The myofibril method serves to isolate insoluble protein from the muscle whereas the soluble proteins are preferentially extracted into TCA. The addition of NaCl causes increased myofibrillar swelling resulting in a greater extraction of protein from the A-band, hence targeting the higher molecular weight proteins. As anticipated, analysis of the myofibrillar extracts produced a broad protein profile ranging from myosin (205 kDa) down to lower molecular weight components (14 kDa). The TCA method produced prolific banding in the 66 to 29 kDa region with little or no protein visible outside this range. The NaCl fractionation method favoured the isolation of many higher molecular weight proteins including what appears to be Titin, Nebulin and Desmin (>100 kDa). This method was capable of isolating proteins of considerably lower mass down to myoglobin (~14 kDa).

## Objective and subjective characterization of dark coloured beef which has normal ultimate pH

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A recently reported phenomenon of dark coloured beef having a normal pH has proven problematic for some sectors of the Irish beef industry. The main objectives of this trial were to characterize the inherent quality of this 'problematic' beef (dark meat of normal pH). Bovine M. *longissimus dorsi* (loin) (LD), which were classified as normal (n = 10) and 'problematic' (n–10) were selected in the boning hall based

on visual inspection (experienced factory personnel) and pH readings. CIE-L\*a\*b\* measurements were recorded on the surface (TS) of the LD in the boning hall. At 2 days and 7 days post mortem, steaks (2.54 cm) were excised and CIE-L\*a\*b\* recorded immediately after excision (T0), and after 3 h (T3) and 24 h (T24) exposure to air at 4 °C. Subjective colour measurements were conducted at T3 and T24. Conductivity, Warner Bratzler (WB), drip loss, cook loss, compositional analysis, sarcomere length and myoglobin content were also determined. The problematic beef had consistently higher CIE L\* values than normal after different lengths of 'blooming' time and at different times post mortem. CIE L\*a\*b\* values revealed that both quality classes responded in a similar manner to 'blooming' at 2 and 7 days post mortem. Visual differences ( $P \le 0.05$ ) were detected by sensory panellists and preference was expressed for the normal beef over the problematic beef. Significantly higher  $(P \le 0.05)$  myoglobin levels and WB (14 days, 45.2 N v. 70.1 N) were detected in the 'problematic' beef. No differences  $(P \ge 0.05)$  were observed in other quality attributes. Colour pigment and tenderness values may differ between groups.

## The effect of electrical stimulation and a novel wrapping technique on the tenderness of fast chilled hot-boned bovine *M. longissimus dorsi*

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Hot boning (excision of muscles from the carcass early post mortem) offers many economical benefits to the beef industry. However, to be successful one of the major disadvantages of hot boning (i.e. increased toughening due to fast chilling) needs to be overcome. The objective of this study was to examine the effect of different intervention techniques on tenderness and quality of hot-boned beef M. longissimus dorsi (LD). Hot-boned muscles (n = 24) were subjected to four treatments: Control - hot boning alone; Pi-Vac - a novel wrapping system which uses film of a high degree of elasticity to prevent contraction; HVES - high and LVES - low-voltage electrical stimulation. Muscles were fast chilled at 2 °C and aged for 14 days. Electrical stimulation (HVES and LVES v. Control) significantly (P < 0.05) lowered pH, but did not prevent cold shortening during fast chilling. The Pi-Vac treatment resulted in consistently more tender beef than all other treatments [Warner Bratzler shear force and sensory evaluation (P < 0.05)]. Also the sarcomere length was significantly longer and drip loss lower (P < 0.05) for the Pi-Vac samples. The results showed that electrical stimulation accelerates pH decline but does not prevent cold shortening of fast chilled hot-boned beef muscle. A novel wrapping technique (Pi-Vac) allows fast chilling of hotboned beef cuts without the risk of cold shortening, ensuring consistent quality product with improved tenderness.

## Non-destructive measurement of residual oxygen levels in packaged foods using optical oxygen sensing

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An optical oxygen analyser and sensors, based on the principle of luminescence quenching by oxygen, were used in several small-scale industrial trials carried out on Irish food products. Beef lasagne, held at 4 to 9 °C for 4 weeks, packaged and cooked under standard sous vide conditions (70% vacuum) initially had high levels of residual oxygen (~21%) in the majority of packs, as opposed to anticipated levels of 4 to 5%. A decrease in oxygen level to almost zero over 3 weeks was correlated with increased microbial growth from an initial level of 10<sup>4</sup> colony forming units (cfu)/g. No significant change in lipid oxidation from the initial value of 0.28 mg MDA/kg of sample was found. In contrast, Cheddar cheese packaged in a modified atmosphere (70% N<sub>2</sub>:30%CO<sub>2</sub>) and held at 4 °C for 4 to 5 months showed a high degree of packaging efficiency with low levels of residual oxygen (< 1.0%) in the majority of packs. In damaged packs, oxygen levels increased from ~4\% to 8\% over the first 3 weeks and then declined to negligible levels after 7 weeks. This decline correlated with increased microbial growth from  $10^8$  to  $10^{12}$  cfu/g. Despite the slight calibration changes of the sensors, due to contact with food, good correlation between this non-destructive system and standard destructive methods of oxygen determination in food packs were found.

#### Effectiveness of frozen storage on the oxidative stability of the lipid fraction from raw turkey breast and cooked chicken products

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Lipid oxidation is one of the main factors, which negatively affects the storage quality of meat due to the formation of off-flavours and certain potentially toxic components such as cholesterol oxide products (COPs). In this study, the effectiveness of long-term frozen storage (18 months at -18 °C) on the stability of lipids in raw turkey breast and fried minced chicken products was evaluated by GC analysis of COPs. Frozen storage of meat products did not prevent oxidative change of lipid fractions but did delay the rate of such reactions. Both raw turkey breast and cooked chicken products were susceptible to lipid oxidation although the rate at which COPs accumulated in cooked chicken was greater than that of raw turkey breast. After storage for 6 months, the concentration of COPs in cooked chicken products was approximately 7 times greater than that of raw turkey breast. At the end of the storage period, the concentration of total COPs was 4.59 and 5.33 µg/g for raw turkey breast and cooked chicken products, respectively. Such values do not compare favourably with literature figures, which suggest that total consumption of COPs should not exceed 0.1 µg/day. In conclusion, the results of this work indicate that, from a health perspective, a reduction in freezer storage times, particularly for cooked chicken products, is advisable.

## Effects of thermal pasteurisation or high pressure processing on the characteristics of oysters (\it Crassostrea gigas)

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Treatments currently in use for post-harvest treatment of oysters include cryogenic individual quick-freezing, a mild thermal process referred to as cool pasteurisation (CP) and high pressure (HP) processing. CP and HP processing is used for both raw half-shell and shucked oysters.

Preservation of food with HP is of particular interest, as it does not affect the natural appearance, taste and flavour of food. The objective of this study was to determine the effects of commercial heat (CP, 50 °C × 10 min) or HP (260 MPa × 3 min) treatments on compositional parameters of depurated oysters. HP-treated oysters had higher pH than untreated or pasteurised oysters, but differences were not significant (P > 0.05). HP or CP both modified the gross composition of oysters; however, the moisture content of HP-treated whole oyster tissue was higher than that of pasteurized or untreated oyster. HP or CP treatments did not affect the salt content or water activity of oysters. HP treatment and CP both increased Hunter L and b values and decreased a values of oysters; overall, HP-treatment had less negative effects on the colour of ovster tissue than CP. HP-treated and pasteurized oysters had higher shucking yields (15.5 and 12.5%, respectively) than untreated oysters. A significant advantage of HP-treated over CP was that the HP process opened the oyster and separated the muscle of the oyster from the shell.

## Characterisation of the colour, oxidative stability and processing properties of individual fore-quarter beef muscles M.C. Hughes<sup>1</sup>, K. O'Donovan<sup>1</sup>, J.F. Kerry<sup>1</sup>, P. Ward<sup>2</sup>, T. Kenny<sup>2</sup> and E.E. O'Neill<sup>1</sup>

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The development of new convenience consumer products offers opportunities to increase the consumption of beef. The potential to utilize forequarter beef in these products will be determined by the processing characteristics, eating quality and stability of individual muscles from this part of the carcass. The aims of this study were to determine the colour stability (Hunter 'a' values) and susceptibility to lipid oxidation (TBARS) of steaks cut from 10 fore-quarter beef muscles during refrigerated storage at 4 °C for 6 days. Experiments were carried out in quintuplicate. In addition, patties were produced from four of the muscles using different concentrations of salt and phosphate and assessed for cook-loss. For all muscles, the rate of lipid oxidation was slow, while colour deterioration occurred very rapidly, with the greatest decreases in Hunter 'a' values occurring at 2 days. No significant differences (P < 0.05) were observed between muscles for TBARS and Hunter 'a' values throughout the ageing process. Response surface methodology showed differences in optimum salt and phosphate addition rates between muscles for minimum cook-losses. The results of this study showed little variation in the rate of oxidative reactions between the individual fore-quarter beef muscles, while some difference were observed in processing characteristics.

#### Assessment of the usefulness of raw muscle measurements in the analysis of beef tenderness

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Meat tenderness is a complex quality attribute both in terms of factors contributing to it and in terms of its prediction. Current instrumental Warner-Bratzler (WB) methods (INSTRON) of measuring tenderness require cooking of the meat prior to analysis. A method, which enables measurements to be taken on raw muscle, would be useful. The objectives of this study were (1) to develop an instrumental

method for recording measurements on raw beef muscle, (2) to compare readings obtained with established methods using the WB and (3) to monitor changes over a 14-day postmortem ageing period. The development process focused on the sample preparation (cores v. strips of meat) and method of analysis (Texture Profile Analysis  $\nu$ . WB) using bovine M. longissimus dorsi (LD). Cores (1.27 cm diameter, four per steak) taken parallel to the fibre direction and sheared using the WB method for cooked meat was the most accurate and reliable method. This was then employed on raw beef LD (n = 20) at 1, 7 and 14 days post-mortem and compared with analysis on cooked steaks (2, 7 and 14 days post-mortem) to assess the relationship between both meat analyses. Following Pearson's correlation analysis it was evident that no relationship existed between the raw and cooked measurements at any time points. Measurements on raw muscle did not display any significant alterations over the ageing period. The results showed that the method developed for use on raw muscle did not provide any information regarding post-mortem ageing development nor the ultimate tenderness of beef

## Effects of *sous vide* processing and post-processing storage conditions on the survival of *Listeria innocua* in salmon and beef

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The objectives of this study were to examine the effects of sous vide processing and post-processing storage on the survival of L. innocua in beef and salmon. Three heat treatments were applied, a minimal pasteurization process of 70 °C for 2 min and two more severe heat treatments, 85 °C for 11 min and 90 °C for 4.5 min. Both control and acid adapted (pH 5.5) populations of mid-stationary phase L. innocua cells were inoculated into the geometric core of the beef or salmon samples. Samples were analysed for the presence of L. innocua prior to heat treatment, after heat treatment, after 1 week at -40 °C and after 1 week at 8 °C. Initial populations of L. innocua were between 107 and 108 colony forming units (cfu)/ml and 6-log reductions were observed with all heat treatments in both products. L. innocua was detected without enrichment after the 70 °C for 2-min process in both products and also in beef after the 85 °C for 11-min process. Acid adaptation was only a factor for enhanced listerial survival if the heat treatment was minimal (70 °C for 2 min). There were benefits of freezing over refrigerated storage with the two milder heat treatments, with a lower rate of listerial recovery from frozen samples. The 90 °C for 4.5-min process was the most effective regardless of acid adaptation, product or postprocessing storage.

## Effects of electrical stimulation and chilling rate on *post-mortem* biochemical reactions in bovine *M. longissimus dorsi* and *M. semimembranosus* muscles

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Consumers consider tenderness as one of the most important attributes of meat. Many studies have suggested that electrical stimulation (ES) and slow chilling of whole carcasses increase the rate of glycolysis and rate and extent of proteolysis and thus may contribute to an increase in tenderness. The aim of this study was to examine the effects of ES and rate of chilling on post-mortem biochemical changes in hot-boned bovine M. longissimus dorsi and M. semimembranosus muscles. Muscles were either non-electrically stimulated (NES) or low voltage electrically stimulated (LVES) and then subjected to either rapid (2 °C) or slow (10 °C) chilling. Samples were taken for the determination of adenosine triphosphate (ATP), glycogen, L-lactic acid and indices of proteolysis at 0, 4, 8, 24, 48 h and 14 days post-mortem. LVES significantly (P < 0.05) accelerated the rate of ATP breakdown, glycogen breakdown and L-lactic acid production. However, the rate of these biochemical reactions was not influenced (P > 0.05) by chilling rate. Indices of proteolysis (myofibrillar protein breakdown; levels of non-protein nitrogen, small peptides and amino acids) were only affected by ageing and not by either LVES or rate of chilling. Thus, while LVES accelerated the rate of glycolysis, it did not appear to influence post-mortem proteolysis.

#### Electrophoretic analysis of post-mortem protein breakdown in bovine M. longissimus dorsi

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Degradation of myofibrillar protein during post-mortem conditioning of beef results in appearance of proteolytic fragments. While products of proteolysis have been studied in muscle, very little has been reported on the appearance of these products in the exudate (drip loss) from the meat. The objectives of the study were (a) to develop an extraction method for studying proteins in drip loss and (b) to evaluate the protein profile of these extracts generated from aged beef. Drip loss was collected from bovine M. longissimus dorsi (loin) by two methods: gravitational force and centrifugal force, at 2, 7 and 14 days postmortem. After collection, the exudate was centrifuged at 14,000 rpm for 15 min; the pellet was discarded and supernatant was re-suspended in SDS buffer at a 1:15 dilution ratio. The samples were prepared for SDS-PAGE (8%, 12% gels, gradient gels -8 to 18%). Multiple bands in the range of 300 kDa to 12 kDa were found. There were some bands in the following regions: 300 kDa, 160 kDa, 90 kDa, 85 kDa, 21 kDa, 20.5 kDa that differed in staining intensity across the ageing period. There were individual changes between samples during 14-day ageing and one sample revealed a presence of well-defined, extra 205-kDa band only on day 14. This study showed that proteolytic changes in meat can be observed in drip loss using SDS-PAGE.

## Effect of pelvic suspension and cooking method on the processing and sensory properties of hams produced from two porcine muscles

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The influence of pelvic suspension on the processing properties and quality of cooked hams is not well documented.

The objective of this study was to examine the effect of two hanging methods (Achilles and aitch-bone) and two cooking methods (normal and  $\Delta T$ ) on the overall quality of cooked hams produced from M. semimembranosus (topside) and M. biceps femoris (silverside). Pork sides were hung by the Achilles tendon (normal) or the aitch-bone ('hip-hung') 1 h post-slaughter and chilled overnight at 1  $^{\circ}$ C. The topside (n = 6) and silverside muscles (n = 6) were boned out, injected to 120% of green weight with a brine solution and tumbled while curing for 16 h at 2 °C. The hams were steam cooked using a 'normal' cooking cycle (cooked at 85 °C to a core temperature of 72 °C) or a 'ΔT' cycle (maintaining a constant difference of 35 °C, between oven and core temperatures until the oven reached 85 °C, whereupon the core was allowed to come up to 72 °C). Hanging from the aitchbone increased (P ≤ 0.01) the injection rate of silverside hams by 2.6%; the injection rate of topside hams was not affected by the hanging process. The  $\Delta T$  cooking method had a significant effect ( $P \le 0.05$ ) on both muscle types, reducing cook losses by 2.5% in the topside hams and 4% in the silverside hams, compared with the normal cook. Topside hams from aitch-bone hung muscles and using the 'normal' cooking cycle were more tender (P ≤ 0.05) than Achilles hung hams. Hardness values from Texture Profile Analysis also showed that aitchbone hanging gave more tender hams. In this study, a combination of aitch-bone hanging and  $\Delta T$ cooking had a positive effect on the quality of cooked hams.

### Quality characteristics of reduced fat beef burgers formulated with dietary fibres

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Concern about the role diet plays in health and in development of certain diseases has mounted in recent years. Food products that are low in fat, yet retaining full-fat flavour and characteristics are desired. Fibre has been shown to be a suitable replacement for fat in meat products. The objective of this work was to study the effects of pea and potato fibres on the quality characteristics of reduced fat beef burgers. Beef burgers were manufactured containing 10 and 23% fat. Potato and pea fibres were incorporated to the reduced fat (10%) formulation at addition levels of 1.5 and 3%. The products were then tested for composition, cook loss, water-holding capacity (WHC), Kramer shear force and sensory analysis. Increasing the fat level significantly reduced the WHC of the beef burgers, whilst increasing cook loss (P < 0.05). The inclusion of pea fibre at the 3% level resulted in a significant reduction (P < 0.05) in cook loss, however beef burgers with other dietary fibre treatments were not markedly affected. The incorporation of pea and potato fibres (1.5 and 3%) substantially (P < 0.05) reduced Kramer shear force values (31.1 to 36.3) N/g) below that of the reduced and full-fat controls (42.6 to 37.4 N/g). Sensory analysis for tenderness, flavour and acceptability showed no differences (P > 0.05) between any of the treatments; however mean tenderness values were higher in fibre-containing products. The results indicate that the use of dietary fibres in reduced fat beef burgers may improve tenderness and cook yield without producing negative effects on product flavour and acceptability.

Effects of temperature and moisture content on non-enzymatic browning kinetics in a non-crystalline, carbohydratebased, low-moisture food system

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Non-enzymatic browning (Maillard reaction) is one of the most important chemical reactions in foods and affects the nutritional quality and toxicological characteristics of food materials, and also affects flavour, colour and texture. Non-enzymatic browning is known to depend on several factors such as time, temperature, moisture content, water activity, pH, concentration of reactants and reactant type. The objective of present study was to evaluate the effects of temperature and moisture content on browning rate. Lactose/Trehalose (1:1) was dissolved in distilled water. Llysine and D-xylose were added as reactants. Amorphous samples were produced by freeze-drying the solution. Water sorption was determined gravimetrically and data were modelled using the Guggenheim-Anderson-Deboer (GAB) and Brunauer-Emmett-Teller (BET) equations. Samples were exposed to various relative vapour pressure levels (11%, 23% and 33%) to adjust water content. Non-enzymatic browning rates were studied at different temperatures (40, 50, 60, 70, 80 and 90 °C). Glass transition (Tg) was measured by DSC. Non-enzymatic browning was followed spectrophotometrically at 280 nm. The rate of browning increased with water content and temperature. At temperatures above Tg, the Arrhenius model was applicable and linearity was observed. Moisture content seemed to affect the activation energy of non-enzymatic browning and higher moisture contents increased the temperature-dependence of nonenzymatic browning rate. At temperatures close to Tg, the non-enzymatic browning rate was low and less moisture content dependent.

## Effects of multi-waveform ultrasonic irradiation on the growth of Saccharomyces cerevisiae

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Ultrasound parameters and time of exposure must be carefully chosen for its effective application on fermentation engineering. The objective of this study is to investigate the effects of four types of ultrasonic waves on Saccharomyces cerevisiae at different growth phases including lap phase, logarithmic phase and stationary phase. An ultrasonic bioreactor was used which can produce multi-waveform ultrasound with low intensity (3.8  $\times$  10<sup>-3</sup> W/mm<sup>2</sup>) and low frequency (32.5 kHz). The results indicated that when ultrasonic waves were applied to S. cerevisiae during the lag phase, cell number and dry weight increased and the logarithmic phase occurred 4 h earlier than that of the control. With the optimum ultrasonic wave, the lag phase disappeared and a 260% increase in cell number over that of the control was achieved. When S. cerevisiae was irradiated during the logarithmic phase, growth was restrained, and in the most suppressed situation, cell numbers were only 76.5% of the control. During the stationary phase, there was no difference in cell number compared with the control; however, the dry weight was higher than the control. When the optimum wave was applied, the dry weight increased by 14.3% indicating that more metabolites were produced. It was concluded that

ultrasonic irradiation should be performed according to the specific processing requirements needed and applied during the appropriate growth phases of microorganisms.

### Optimization of gluten-free bread using rice flour, potato starch and HPMC

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The properties of gluten become apparent when flour is hydrated giving an extensible dough with good gas-holding properties. The formulation of gluten-free bakery products, therefore, presents a formidable challenge. Following preliminary screening trials, Response Surface Methodology (RSM) was used to develop and optimize a gluten-free bread formulation. The primary ingredients were rice flour and potato starch. A central composite design consisted of two variables: hydroxypropylmethylcellulose (HPMC) (0.5 to 2.5% of flour weight) and water (70 to 95% of flour weight). Five levels of each variable were chosen, with analysis of 13 combinations of these variables being performed. Assessment of error was derived from five replications of one treatment combination. From the data obtained through the experimental design, optimal ingredient levels were revealed. Analyses on the loaves were performed 24 h after baking. Specific volume was most influenced by the level of water added (P < 0.005). Crumb hardness values were reduced as water levels increased (P < 0.005), except when HPMC was at its maximum level of addition. The number of large gas cells increased (P < 0.05) with increasing levels of both water and HPMC. Optimization was based on the generation of the best results for specific volume, crumb hardness and image analysis data.

## Examining the use of biopolymer-degrading lactic acid bacteria as cultures when mashing unmalted barley

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In the brewing process lactic acid bacteria can be applied as bioacidification cultures in mashing during wort production. As well as reducing the pH, these bacteria may also introduce enzymes, which are deficient when mashing with high levels of unmalted barley. The objective of this study was to assess the properties of LAB originally isolated from the cereal and brewing environment and to use selected strains as bioacidification cultures when using 40% unmalted barley grist. The LAB were examined for amylase, protease and  $\beta$ -glucan activity on agar diffusion plates using a variety of substrates ranging from starches to various proteins and complex carbohydrates. Selected enzyme active strains were used to acidify mashes containing 40% barley to pH 5.4, and compared to an unacidified mash and a chemically acidified mash. A modified Congress mashing program was used to evaluate the impact of the strains on wort quality such as extract, fermentability, viscosity, β-glucan, total soluble nitrogen (TSN), free amino nitrogen

(FAN). Of the eight strains examined, four strains displayed both amylase and protease activity on the agar plates. In the mash, a *Lb. amylovorus* strain and a *Lb amylovorus* strain showed significant differences from the chemically acidified controls with respect to extract, fermentability viscosity,  $\beta$ -glucan and FAN. It can be concluded from these studies that enzyme active lactic acid bacteria can be used to improve the quality of wort, especially when working with unmalted barley.

## Brewing with 100% unmalted barley - the application of commercial enzymes in wort production

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Brewing with unmalted barley represents a cheaper alternative to traditional malted barley. In this investigation, laboratory scale trials were carried out to determine the effects of increasing dosage rates of exogenous enzymes when producing a wort from 100% unmalted barley. A range of different exogenous commercial enzymes (proteases, αamylases and  $\beta$ -glucanases) were added during the mashing process at dosage rates ranging from 0 to 1.0% (v/w) of grist. The effect of these enzymes added together and individually was examined. Increasing the amount of bacterial protease gave corresponding increases in free amino nitrogen (40 to 240 mg/L).  $\alpha$ -Amylase addition gave increases in the rate of filtration. However, further increases resulted in negative effects on filtration. By including a heat stable  $\alpha$ amylase, starch negative worts were produced.  $\beta$ -glucanase addition was necessary at low levels to reduce wort viscosity and  $\beta$ -glucan content of the wort. In this investigation, optimal levels of the commercial enzymes were suggested taking into consideration the cost of enzymes to the brewer. Overall it was concluded that, with the aid of commercial enzymes, a wort of a quality similar to a 100% malted barley wort could be produced from a grist comprising 100% unmalted barley.

### Determination of the swelling kinetics of waxy and non-waxy rice starches in excess water

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Pasteurisation/sterilisation processes can damage the mouth feel and consumer acceptance of milk rice. In this study, the effect of such heat processes on the rheological behaviour of dilute (max 8% w/w DB), waxy and non-waxy (11% amylose) rice starch solutions was examined. By measuring small deformation rheological behaviour on a stress rheometer using cone and plate geometry equipped with a Peltier heating plate, samples were rapidly heated (20 °C/min) and changes in sample viscosity due to heat treatment with time were measured. Results for both starches showed a two-step process, highly dependent on temperature. The first step, (at temperatures up to 68 °C and 88 °C for waxy and non-waxy, respectively), corresponded to the irreversible swelling of starch granules and was followed by a second step of granule rupture at higher temperatures. Both steps were kinetically dependent on temperature; the higher the temperature, the faster the process, indicating relatively high activation energies (Arrhenius model). In the case of waxy rice starch, a third step was also observed, which corresponded to a slow increase in viscosity of the sample after granule rupture but which was independent of temperature. It is speculated that this last step is due to retrogradation. This study suggests that rheological techniques are suitable for measuring the gelatinisation kinetics of starches.

#### An examination of the effects of acidification and time on the fundamental rheological properties of wheat sourdough, dough and gluten

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The fundamental rheological characteristics of a biologically acidified, a chemically acidified and a neutral preferment (sourdough) were monitored over the course of a 24-h fermentation period using a split-plot design. Three doughs were subsequently prepared in which 20% of the flour was in the form of the respective preferment. A control dough containing no fermented material was also prepared. The fundamental rheological properties of both the dough and its isolated wet gluten were determined. Laser-scanning confocal microscopy was used to capture images of selected preferments and doughs. Results from the preferment show that there was a significant (P < 0.05) decrease in both elasticity and viscosity with fermentation time for all three preferments, all of which reached similar end values for these parameters. The microscope images illustrated that the gluten strands were dissolved to a more amorphous structure during the fermentation period. Changes in the nature of the preferment were reflected in the rheological characteristics of the respective doughs which were significantly (P < 0.05) less elastic and softer than the control dough. It is concluded that time and the role played by proteolytic degradation is key to the sourdough process.

### Influence of lactic acid bacteria on the quality of gluten-free batter and bread

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Gluten-free breads go stale quickly and have a flat aroma. For wheat bread, both disadvantages can be improved by using sourdough. The objective of this study was to examine the influence of sourdoughs made with two strains of Lactobacillus plantarum and one strain of Lactobacillus san-franciscensis on the quality of a gluten-free bread over a 5-day storage period. Bread volume and height showed no significant differences between the breads, nor did number of cells and mean cell area derived from digital image analysis. Over storage time, breads tended to shrink, as indicated by a significant decrease in height (P < 0.05). At the same time, crumb hardness increased significantly for all breads (P < 0.05). No significant differences in hardness were found between fresh breads and bread after 2 days of

storage. After 5 days of storage, however, Lactobacillus sanfranciscensis and one strain of Lactobacillus plantarum yielded significantly softer bread than the non-acidified control (P < 0.05). This was in distinct contrast to the chemically acidified control, which, at day 5, was significantly firmer than all other breads (P < 0.05). In a triangle test, glutenfree sourdough bread could be discriminated from the control bread (P < 0.05) and was clearly preferred. It is concluded that sourdough improves the quality of gluten-free bread, although the positive effects were lower than those found in wheat bread.

## Influence of thermal treatment on the rheological characteristics, swelling power and granule size of rice starches in excess water

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Rice milk is becoming a key, natural, non-dairy milk replacement beverage and is fully compatible with further taste optimization and enrichment for the production of functional drinks. In order to appreciate the effect of cooking on the mouth feel of rice milk, the impact of heat treatment on solution viscosity, granule size and thermal transitions of dilute rice starch solutions was measured. Waxy and nonwaxy (11% amylose) rice starches were heat-treated in excess water (max 4% w/w DB) from 40 to 121 °C for 30 min. In the case of waxy starch, reaching the peak temperature (Tp) of the endotherm (measured by Differential Scanning Calorimetry) was accompanied by a substantial rise in swelling power, solubility, paste viscosity and granule size as well as a large decrease in granule numbers, indicating complete cooking. In the case of non-waxy starch, this increase was small at Tp, and complete cooking was only achieved when temperature was increased over 30 °C above Tp. This difference in pasting/gelatinization behaviour may be explained by the presence of amylase-fatty acid complexes on the granule surface in the case of non-waxy starch as proven by the swelling and pasting behaviour of defatted non-waxy starch.

## Development and assessment of a new laboratory method to predict lautering performance in the brewhouse

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Malt specifications cover many quality parameters but the prediction of brewhouse lautering behaviour remains elusive. The objective of this research was to develop a laboratory method predicting industrial run-off problems when using different adjunct concentrations in the mash. In comparison to the filter paper based EBC method, the new system incorporates the operating principles of industrial scale lauter tuns. As adjunct, unmalted barley at different concentrations was used. The lautering kinetics (ml/min) in both systems were measured at barley concentrations of 0, 20, 40, 60, 80, 85, 90 and 100%. The highest lautering speed (26 ml/min) was detected at *ca.* 85% barley concentration. Analysis of the filtration profiles of both systems showed

#### Rheological studies simulating the brewery mashing process

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The brewery mashing process is an enzymatic, time and temperature dependent degradation of viscosity creating macromolecules such as starch and  $\beta$ -glucan. The development and assessment of an accurate viscosity method to monitor this degradation process was the objective of this research. This involved a specially designed rotor to keep particles in suspension together coupled to a fundamental rheometer to enable highly sensitive viscosity measurements. Studies were conducted to simulate an industrial mashing process, taking into account temperature/time, grist loads, adjunct amounts and enzyme levels. More fundamental studies using pure barley starch and glucan substrates together with enzyme additions were also carried out. Incremental increases in barley adjunct levels from 0 to 100% resulted in an increase in initial (range, 2.84 to 6.53 Relative Units (RU), peak (range, 9.22 to 156.61 RU), peak-area (range, 82.68 to 1741.66 RU) and end (range, 2.55 to 6.21 RU) viscosity values which could be related back to the endogenopus amylase and glucanase levels of the grain. The fundamental studies showed that the degradation of both the pure starch and glucan substrates by their respective hydrolytic enzymes could clearly be correlated to the added enzymes and the resulting viscosities. This method provides an accurate informative insight into the important mashing degradation processes and can also be used as a screening tool to determine the brewing qualities of the mash raw materials.

#### Comparison of the bread-making potential of different sorghum hybrids

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Grain sorghum [Sorghum bicolor (L.) Moench] is a cereal appropriate for gluten-free diets. Little is known, however, about differences between cultivars and their potential to produce leavened bread. The objective of this study was to investigate the effect of nine food grade sorghum hybrids on bread quality. A standard baking test for sorghum was

developed, in which the flour basis was 70% sorghum and 30% corn starch. Standardisation included the adaptation of the water levels to achieve a constant batter consistency as measured by extrusion, and proofing to a constant height. Loaf specific volume varied little between the breads, whereas digital image analysis of the bread crumb and texture profile analysis revealed clear differences: the ranges found for mean cell area, total number of cells/cm2 and crumb hardness within the nine hybrids were  $1.3 \pm 0.2$  to 3.3 $\pm 0.6 \text{ mm}^2$ ,  $13.5 \pm 1.3 \text{ to } 27.8 \pm 4.4 \text{ 1/cm}^2$ , and  $7.5 \pm 0.7 \text{ to } 21.6$  $\pm$  1.4 N, respectively (average  $\pm$  s.d., n = 3). High quality hybrids yielded breads with a finer crumb structure, characterised by a significantly smaller mean cell area (P < 0.001) and a greater total number of cells (P < 0.001) in comparison to inferior cultivars. Furthermore, the crumb of the high quality breads was comparatively firm. The high quality sorghum hybrids are suitable for the production of superior gluten-free breads.

#### Impact of lactic acid bacteria on malt quality

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Starter cultures are used to control the malt quality, processability and biological safety of beers. In this study, lactic acid bacteria (LAB) were applied during the malting process to evaluate the impact on malt quality. The samples were compared to chemically acidified as well as non-acidified malt. The trials were performed in a micro-malting plant simulating an industrial malting programme. LAB and the chemical, lactic acid were added during the germination process. Microbial growth of bacteria and yeasts, development of pH, enlargement of acrospires and rootlets were monitored. The malt was characterized using extract, pH, colour, viscosity, free amino nitrogen, total soluble nitrogen (TSN), fermentability, friability, and moisture. It was shown that LAB were capable of growing by 2 log cycles on the malt and still survive kilning. Compared to the controls, LAB inhibited the growth of yeasts and moulds by  $20 \pm 6\%$ . Processability was enhanced by lowering the malting loss, the viscosity of the wort, and improving brewhouse yields. The proteinaceous fraction was also improved, providing  $12 \pm 2\%$  more TSN. LAB starter cultures were able to improve the malt parameters indicating a more efficient wort and beer production.

## Comparison of the rheology, meltability and microstructure of imitation cheeses manufactured with different cookers

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Imitation cheese is often made with low-shear (LS) twinscrew cookers while high-shear (HS) single blade cookers are used for making processed cheese. LS and HS cookers differ in both the rate of shearing and the manner of mixing. The objective of this study was to investigate if HS cookers can be used to manufacture imitation cheese so that processed cheese manufacturers who want to develop

imitation cheese may be able to do so with existing equipment. Imitation cheeses were made with an LS and HS cooker to give cheeses of similar compositions (52% moisture, 22% fat and 21% protein). The texture, meltability, dynamic rheology and microstructure of the cheeses were compared. Cheese made with the HS cooker was harder (361 v. 302 N) and had less melt (51 v. 81 mm) than the LS cheese. The storage modulus (G') and the loss modulus (G") crossed over at 54 °C for the LS cheese, but no G'/G" crossover was observed for the HS cheese. Electron micrographs showed that the HS cheese had smaller fat globules and a more extensive honeycomb like structure than the LS cheese. The properties of imitation cheeses made with the two cookers were significantly different. The processing conditions used in the HS cooker need to be modified to obtain imitation cheese with good meltability.

#### Development of probiotic cultures for incorporation into functional drinks

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The functional drinks market is the fastest growing sector of the beverage industry and offers excellent opportunities for the development of novel dairy and fruit-based products. The main objective of this work was to generate a fruit juice with probiotic ingredients; the latter intended to provide health-related properties resulting in functional benefits appropriate to consumer demands. It is recommended that such products sold with health claims should meet the minimum of 10<sup>6</sup> colony forming units (cfu)/ml probiotic bacteria at the expiry date. Survival of a range of probiotic bacteria was monitored in orange juice (OJ), pH 3.66, pineapple juice (PJ), pH 3.40, and MRS broth adjusted to the average pH of OJ, using citric and acetic acids. The results indicate that Lb. casei immunitas and Lb. rhamnosus GG are very robust strains, surviving at levels  $\geq 10^7$  cfu/ml in OJ and PJ for at least 70 days. Therefore they have great potential as ingredients in fruit juice as no manipulation to enhance their survival is required. The more sensitive strain Lb. salivarius UCC 500, which survives approximately 11 days at acceptable levels in OJ, was subjected to sub-lethal pH between 4.4 and 5.0 for 60 min prior to inoculation into OJ, with the aim of inducing an acid tolerance response, thus increasing viability. Exposure to such stresses increased survival of Lb. salivarius UCC 500 by approximately 2 days.

### Effect of fat and lactose content on the cohesiveness of milk powders

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Powder flowability was measured using shear cell techniques. The powder flow-function gives an indication of the cohesive strength developed within the powder under compaction. The flow index was calculated from the flow-function and this gives an index of the cohesiveness of the powder, with lower values representing greater cohesiveness. Whole milk powder (WMP) had a flow index of 1.7, which is classified as being very cohesive, and was much greater than that of skim-milk powder (SMP) at 6.1. Thus, the fat content has a significant effect on increasing the cohesiveness of the milk

powder. Higher temperature increased the cohesiveness of both powders. At 5 °C, the flow index of WMP and SMP were 2.3 and 6.3, while at 25 °C, the respective values had fallen to 1.4 and 4. Thermal plasticity of the lactose may be a factor. In addition, melting of fats in WMP may cause the formation of liquid bridges between the powder particles leading to increased cohesion. Exposing both powders to 46% relative humidity atmosphere for 18 h had little effect on the cohesiveness of the WMP, while the flow index for SMP decreased to 3, making it much more cohesive. A crystalline lactose powder was also subjected to the same atmosphere and showed very little change in cohesiveness. Differential scanning calorimetry studies showed that SMP had a significant amount of amorphous lactose that crystallised during the 18 h exposure time. This causes the formation of solid bridges between the powder particles, which may explain the increase in cohesiveness of the SMP.

#### Analysis of process cheese using texture profile analysis

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Many textural studies employ rheological measurements to imitate the sensory evaluation of cheese. The main objective of this study was to profile a range of process cheeses using a Texture Analyser (model TA. Hdi). The process cheeses were manufactured using generic ingredients of Cheddar cheese, water, emulsifier salt (disodium phosphate) and butter, following a 3\*3 matrix formulation. The variables for one group were moisture and salt while in the second group butter and salt were used. The cheeses were manufactured using a Stephan cooker (model UUM/SK 5) and were made in duplicate. Firmness values obtained for 2% salt at low, medium and high fats were 100.18 N, 160.35 N and 221.51 N, respectively, at 14 days while at 28 days the results were similar. The firmness values for moisture at low, medium and high levels at 3% salt were 155.83 N, 48.85 N and 8.35 N, respectively, at 14 days while the 28-day results generally showed increases across the range. For moisture, the difference in values between the target and actual results for fat and salt variables ranged from 0.19 to 2.00 while the difference for moisture and salt variables ranged from 0.08 to 1.48. These results show that process cheese with different ingredient inputs can be used to produce a range of different textures.

## Formation of heat-induced reactive thiol groups and subsequent acid-induced cold gelation of $\beta$ -lactoglobulin enriched solutions

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Gelation of preheated whey protein solutions can be induced at ambient temperatures by changing the solvent quality (lowering the pH, adding salt). The objective of this study was to evaluate the relevance of the reactive thiol levels in heated  $\beta$ -lactoglobulin-enriched solutions in the formation of acid-induced gels.  $\beta$ -Lactoglobulin-enriched solutions were heated (78 °C, pH 7.0) for up to 360 min. On cooling, reactive thiol groups were assayed by a modified Ellmans procedure. Acid gelation of the denatured whey protein solutions was promoted by the addition of glucono- $\delta$ -lactone (pH 4.6 in 120 min at 40 °C) and monitored in a

Bohlin CVO rheometer. At low protein concentrations (3% w/v), the level of reactive thiol groups (assayed in the absence of urea) decreased as heating time at 78 °C increased (21.5  $\mu$ M SH, 30 min to 15.2  $\mu$ M/g protein, 360 min). At higher protein concentrations (6%) this decrease was not apparent. Acid gel strength ( $G'_{120min}$ ) using small scale deformation of 3% (w/v) preheated protein solutions, was shown to increase with time of heating at pH 7.0 (400 to 1320 Pa). Large-scale deformation of the preformed acid gels (GDL-induced after 120 min) was also determined using a TAXT2 texture analyser. This analysis showed an incremental increase in gel strength. It was concluded that the number of reactive thiol groups, as determined by heating time at pH 7.0, was not responsible for the acid gel strength under our conditions.

#### Alteration of sodium caseinate by incubation of micellar casein with transglutaminase

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Transglutaminase (Tgase) is an aminoacyltransferase that encourages the formation of an  $\varepsilon$ -( $\gamma$ -glutamyl) lysine bond. The objective of the study was to promote micellar casein cross-linking, using Tgase and to evaluate the properties of the resulting sodium caseinate. Micellar casein (2.5% phosphocasein in simulated milk ultrafiltrate, pH 6.7) was incubated with/without 1% microbial Tgase at 40 °C for 5 to 60 min, prior to enzyme inactivation (78 °C for 30 min). It was then acidified to pH 4.6 (using HCl), the precipitate was separated and washed (× 3) prior to re-neutralisation to pH 6.7 (forming sodium caseinate) and adjusted to 5% (w/w) protein. Similar properties in the micellar casein and resulting sodium caseinate were observed with the different Tgase incubation times. Particle size (photo correlation spectroscopy) of micellar casein incubated with Tgase (211  $\pm$  3 nm) was not markedly different from the control micellar casein (195 nm) indicating the absence of inter-micellar crosslinking with added Tgase. In the absence of Tgase, sodium caseinate showed Newtonian behaviour (apparent viscosity of 7.6 mPas). In comparison, sodium caseinate made from micellar casein incubated with Tgase showed significantly increased apparent viscosities ( $80 \pm 17$  mPas) with Herschel Bulkley behaviour. Sodium caseinate (1% w/w) made from micellar casein incubated with Tgase showed increased turbidity (A  $_{\!600},\,1.59\pm0.03$  nm) and ethanol stability (64  $\pm$  1.8%) compared to the control (A<sub>600</sub>, 0.29 and 53.5%), respectively. Incubation of micellar casein with Tgase dramatically increased the viscosity of the resulting sodium caseinate.

### Thermal behaviour of maximally freeze concentrated sugar and sugar-protein food systems

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Glass transition temperature,  $T_g'$ , of maximally freeze-concentrated food systems, determined after annealing samples slightly below the observed temperature of melting,  $T_m'$ , is related to frozen food stability. Sugars and sugar mixtures with proteins can be used as model systems to understand thermal behaviour of various frozen foods. The objectives of

the present research were to determine the T $_{\rm g}$  and T $_{\rm m}$  for disaccharides, their mixtures, and mixtures of disaccharides and protein. Three solute concentrations, 10%, 20% and 30% (w/w) for lactose, sucrose, trehalose, and their 1:1 and 1:1:1 mixtures were analysed using DSC. Egg albumen (1:1) was also mixed with sugars and sugar mixtures. All sugars and sugar mixtures were annealed at T $_{\rm m}-1$ °C for 15 min. Mixtures with protein were annealed for 15 min at T $_{\rm m}-1$ °C. T $_{\rm g}$  and T $_{\rm m}$  values of annealed systems were independent of the initial concentration. Mixtures of sucrose with lactose and trehalose gave lower T $_{\rm g}$  values than lactose and trehalose alone. T $_{\rm g}$  and T $_{\rm m}$  values varied from –51 to –40 °C and –35 to –28  $^{\rm g}$ C, respectively. Protein decreased the T $_{\rm m}$  values of sugars and sugar mixtures but increased the T $_{\rm m}$  values of sugars and sugar mixtures but increased the T $_{\rm m}$  if seems that protein increased the amount of unfrozen water in the maximally freeze concentrated systems.

### Effect of rennet type and storage temperature on the functionality of reduced-fat Mozzarella cheese

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Fat reduction markedly impairs the cooking properties (flow, stretch) of cheese. A study was undertaken to enhance cooking properties of reduced-fat (RF, 10.07% w/w) Mozzarella, using commercially available heat-stable rennets and increasing storage temperature. Cheeses were made in triplicate using the following rennets: fermentationproduced chymosin (FPC), heat stable Rhizomucor miehei proteinase (RMP) and heat stable Rhizomucor pusillus proteinase (RPP). During pilot-scale cheese manufacture, rennets were added at levels sufficient to give a curd firmness of 60 Pa in 45 min at 36 °C for milk of 3.3% protein. The cheeses were stored at 4 or 12 °C for 50 days and evaluated at various periods for heat-induced flowability, by the Schreiber and Olson/Price methods, and stretchability by uniaxial extension of the melted cheese at a speed of 0.066 ms<sup>-1</sup>. Increasing storage temperature from 4 to 12 °C gave significant increases in the mean flowability and stretchability over the 70-day storage period, with the effect being most pronounced at times ≥ 35 days. Overall, rennet-type had a relatively minor influence on the cooking properties. However, at 4 or 12 °C, the mean flowability of RPP cheese was significantly higher than that of FPC cheese and numerically higher than that of RMP cheese. An opposite trend was noted for the effect of rennet type on stretchability; however, the magnitude of the differences between the different rennets was small and non-significant.

### Influence of ingredient formulation on texture of processed cheese, measured by descriptive sensory analysis

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Processed cheese is made by blending natural cheeses with a variety of other ingredients and emulsifying salts, and heating and mixing to make a homogeneous product. To manufacture such products to the highest quality, it is important to understand the influence that ingredient formulation has on texture. This study investigated the effect of formula

manipulation on the sensory texture of processed cheese. Two trials were carried out in which a range of cheeses with varying fat and emulsifier levels (Trial A) and varying moisture and emulsifier levels (Trial B), were manufactured. Descriptive sensory analysis of the cheese texture was carried out following 14 and 28 days storage using an experienced sensory panel and vocabulary of nine texture terms. Analysis of variance (ANOVA) showed significant differences (P < 0.05) between cheeses for all attributes. In Trial A, emulsifier level had the greatest influence on sensory texture. Cheeses manufactured with low emulsifier levels were described as 'creamy', and 'melting', while those with medium to high levels were 'firm', 'rubbery' and 'chewy'. In Trial B, moisture level had the greatest influence on sensory texture. Low moisture cheeses were described as 'firm', 'rubbery', 'chewy' and 'fragmentable', while high moisture cheeses were 'creamy' and 'melting'. Sensory analysis allowed identification of key texture characteristics resulting from changes to the formulation of the cheese.

### Rapid identification of NSLAB isolates from Cheddar cheese using the PCR LightCycler system

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The dominant non-starter lactic acid bacteria (NSLAB) flora throughout most of the ripening period of Cheddar cheese are lactobacilli. Significant evidence has accumulated to indicate that they play an important role in the development of cheese flavour. Identification of the lactobacilli to species level has generally been by biochemical methods, which are laborious and as they often rely on a single metabolic trait may be unreliable. More recently, molecular techniques that are more definitive and decrease the time required for identification have been developed. In this work, species-specific primers in combination with real time PCR (LightCycler) have been used to further reduce the time taken to identify the NSLAB isolates from cheese to species level. Using two well-characterized culture collection strains for each species, confirmation of the species-specific nature of the primers for Lactobacillus paracasei, Lb. casei, Lb. plantarum, Lb. curvatus, Lb. rhamnosus and Lb. pentosus were obtained. Forty-three isolates from Cheddar cheese were tested using this technique. Some of these isolates had been previously identified using RAPD. Where isolates had previously been identified, the results were confirmed in all cases with the LightCycler technique. Isolates not previously identified could be identified to species level, after purification, within the working day.

### New strains of $Lactobacillus\ helveticus\$ for improvement of Cheddar cheese flavour

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Addition of the highly autolytic *Lactobacillus helveticus* DPC 4571, as an adjunct culture to cheesemilk, results in Cheddar cheese with enhanced flavour. The aim of this study was to identify additional *Lb. helveticus* isolates that could improve Cheddar flavour. Cheese was manufactured in independent triplicate trials, using either DPC 4571 (positive control).

DPC 5364 (experimental autolytic culture) or DPC 5353 (experimental non-autolytic culture) as starter adjuncts. The negative control contained no starter adjunct. During ripening, the cheeses were analysed periodically for indicators of autolysis and at 6 months they were analysed for maturity and flavour by a trained panel. In all trials, the indicators of autolvsis were highest for cheese in the positive control and lowest in the negative control. The experimental cheeses were intermediary to these values, cheese made with DPC 5364 having higher levels than DPC 5353. Cheese made with DPC 5364 was rated the best for flavour on all occasions, while analysis of maturity rated cheese made with DPC 5364 the best in two of the three trials. These data support the hypothesis that autolysis is an important characteristic to be considered in Lb. helveticus strains used as starter adjuncts in Cheddar cheese. The work has also identified another autolytic strain, DPC 5364, with similar properties to DPC 4571.

#### Effects of proteins on lactose crystallization in freeze-dried materials

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Lactose is the main carbohydrate in dairy products. In low moisture products, its physical state, i.e., amorphous or crystalline is very important for quality. The objective of the present work was to determine moisture dependence of the state and phase transitions of freeze-dried lactose and to determine the time-dependent crystallization behaviour of amorphous lactose in the presence of various proteins. Glass transition, T and instant crystallization temperature, T<sub>cr</sub> of freeze-dried lactose and lactose/protein mixtures were determined using differential scanning calorimetry (DSC). Time-dependent lactose crystallization was studied by measuring water sorption at room temperature. Whey protein isolate (WPI), casein, albumin and gelatin increased the  $T_g$  and decreased the  $T_{cr}$  of anhydrous materials. Tg and Tcr decreased with increasing water content. In the presence of proteins, a decrease of T<sub>g</sub> at  $a_w \le 0.332$  and increase at  $a_w \ge 0.441$  occurred. Proteins increased the T<sub>cr</sub> at all storage humidities. Crystallization of pure lactose and lactose in lactose/protein mixtures was observed at RVP  $\geq$  44.1%. All proteins decreased the rate of lactose crystallization. This effect was lowest in the presence of WPI and strongest in the presence of gelatin. The results suggest that different proteins interact with lactose differently. This data improve understanding of lactose crystallization behaviour in various food products.

## Rapid analysis of cheese flavour by proton transfer reaction-mass spectrometry

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Proton transfer reaction-mass spectrometry (PTR-MS) is a direct mass spectrometric based on chemical ionisation with  $\rm H_3O^+$ , which reacts in non-dissociative proton transfer reactions with most volatile organic compounds. A previous and time-consuming separation step by gas chromatography can be omitted as little fragmentation occurs. This opens the possibility to use this instrument for online measurements in

food production and quality control. PTR-MS was used for profiling 12 Cheddar cheese samples of three different origins and maturity by static headspace analysis. Resulting spectra from duplicate analysis were averaged over three cycles, respectively. Acetaldehyde, ethanol, acetic acid, dimethyl sulphide, diacetyl, 2-butanone and butvric acid were identified among the most important flavour volatiles in the different cheese samples. The resulting PTR-MS spectra provided a fingerprint for each cheese. Further statistical analysis showed that the cheeses were clearly discriminated by sample, origin and age. Descriptive sensory analysis has been performed on the same samples by three independent sensory panels in Ireland, New Zealand and USA. Relationships were established between sensory descriptors and particular ions by Partial Least Squares Regression, e.g., for the sensory descriptor 'butyric' and the protonated molecular ion 89, which represents butyric acid.

#### Effect of preheating and acidification on the production of heat stable skim milk powders

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Heat stability, the ability to withstand high heat treatment, is an important property of milk, especially in the manufacture of recombined evaporated milk products. The objective of this study was to identify factors affecting the heat stability of reconstituted skim milk powder (SMP). Milk was preheated nominally (72 °C for 15 s) or classically (120 °C for 2 min). In addition, milk was acidified to a similar pH (6.54) using HCl, disodium phosphate (NaH2PO4) or a combination of CaCl<sub>2</sub> and monosodium phosphate (Na<sub>2</sub>HPO<sub>4</sub>) prior to preheating. Each SMP was characterized in terms of protein and mineral content. Heat stability was assessed with heat coagulation time/pH profiles measured using an oil-bath (140 °C for 9% solids reconstituted SMP; 120 °C for 20% solids). Preheating proved beneficial for the heat stability at both 9 and 20% solids, while preheating at a lower pH improved the stability further and over a wider range of pH, with the exception of the phosphate-acidified milk, which was stable over a narrow pH range. At 9% solids there was a shift in maximum stability for all powders to the acid side of the natural pH, with the exception of the HCl-acidified milk. At 20% solids, the maximum stability matched the natural pH except for the HCl and phosphate acidified powders. It was concluded that acidification in association with preheating has a part to play in producing heat stable powder.

#### Effects of moisture content on the sensory and textural properties of imitation cheese containing fibre

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Resistant starch (Novelose240) can be used as a source of fibre to partially replace fat in imitation cheese. However, substitution of fat with fibre tends to increase cheese hardness. The aims of this study were to determine if increasing the moisture content could overcome the hardening effects associated with replacement of 12% fat with Novelose240 and to assess the effect of moisture content on the meltabilty and sensory properties of imitation cheese containing fibre. Cheeses were manufactured with water, rennet casein, Novelose240, vegetable oil and emulsifying salts in a Blentech

cooker, to give moisture contents of 52, 55, 58 or 60%. The meltability was determined empirically and hardness was measured using an Instron testing machine. Sensory panelists conducted a preference test (based on texture and mouthfeel) on the cheese presented as bite-size cold (22 °C) or hot (60 °C) samples. Increasing the moisture content from 52 to 60% decreased the hardness (463 to 222 N) (P < 0.05) but had no effect on the melt of the cheese  $(63 \pm 5.6 \text{ mm})$ . The sensory panelists expressed a preference (P < 0.05) for the 52 or 55% moisture cheeses when consumed hot but did not express a significant preference for the cold samples. Moisture content influences the hardness but does not affect the meltability of imitation cheeses containing fibre. If the latter are consumed hot, a moisture content of 52 to 55% is desirable from a sensory perspective.

#### Influence of moisture and salt content on the dielectric properties of process cheese

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Several studies have demonstrated the potential of using dielectric measurements to determine the quality and composition of food products. However, limited information has been published on the dielectric properties of cheese. The objective of this paper was to investigate the influence of moisture and salt content on the dielectric properties of process cheese. Process cheese samples (n = 30) of varying moisture (39.9% to 50.5%) and emulsifying salt (1% to 3%) were manufactured. Dielectric constant and loss factor measurements were obtained using a HP 85070 Dielectric Probe Measurement System between 300 MHz and 3 GHz. Increases in moisture and salt contents were found to correspond with increases in the dielectric constant and loss factor, respectively. Chemometric models were developed by partial least square regression using Unscrambler software. The ability of the developed models to predict moisture and salt contents were expressed as the root mean square error of prediction (RMSEP), standard error of prediction (SEP) and correlation coefficient (r) between actual and predicted values. For moisture, a model using the dielectric constant data resulted in the lowest error values (RMSEP(Y) = 0.60, SEP = 0.611) and an r of 0.979 [3 principal components (PC)]. The optimum model for salt prediction was developed from the loss factor data (RMSEP(Y) = 0.216,  $\hat{SEP} = 0.22, r = 0.948, 3 PC$ ).

#### A study of the synergistic effect between β-lactoglobulin enriched fractions and sodium tripolyphosphate in a model meat system

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Combining salt (NaCl) with phosphates results in a synergistic enhancement of water and meat binding in meat emulsions.  $\beta$ -lactoglobulin ( $\beta$ -lg) enriched fractions may serve as a healthier replacement ingredient for salt. The objective of this study was to evaluate whether there was a synergistic effect between 2  $\beta$ -lactoglobulin ( $\beta$ -lg) enriched fractions and sodium tripolyphosphate (STPP) to aid the reduction in salt. The two  $\beta$ -lg-enriched fractions contained Ca (655 and 252) mg % (w/w) powder, respectively), Na (450 and 717 mg % (w/w) powder, respectively), and P (160 and 120 mg % (w/w) powder, respectively). Model meat systems were assessed for cook loss, water-holding capacity (WHC) and compositional analysis. A combination of both  $\beta$ -lg fractions and STPP significantly reduced (P < 0.001) the cook loss. The addition of  $\beta$ -lg alone reduced (P < 0.001) the cook loss in comparison to STPP alone. The  $\beta$ -lg fraction with the lower mineral content had the lowest cook loss value. Both the  $\beta$ -lg fractions with added STPP had similar cook losses. The absence of both  $\beta$ lg fractions and STPP had a detrimental effect on the meat system by reducing the WHC (7% v. 32%) (P < 0.001) and also increasing the cook loss by 8 to 10% (P < 0.001). Addition of STPP alone gave similar results. This study shows that combining STPP and  $\beta$ -lg fractions resulted in a synergistic effect by reducing cook loss and increasing WHC in comparison to  $\beta$ -lg fractions and STPP alone and that the mineral content of the  $\beta$ -lg fractions also had an effect.

#### Combination of sensory analysis and proton transfer reaction mass spectrometry for description of the flavour of whey

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Whey is a product of the cheese industry that contains valuable nutrients but its sensory characteristics have not been described, nor has its flavour chemistry been adequately studied. Proton Transfer Reaction-Mass Spectrometry (PTR-MS) is a rapid technique able to detect and quantify volatile compounds at very low levels. This technique was used in parallel with descriptive sensory evaluation in order to study the flavour of whey and to establish an objective model that can be used for whey quality control and product development. Thirteen types of whey were sourced including cheese ('sweet') whey, acid whey from casein production, rennet whey and commercial (unflavoured) fermented whey beverages. Through Analysis of Variance (ANOVA), Principal Component Analysis (PCA) and Partial Least Square Regression (PLS) it was possible to group samples that shared sensory characteristics. Cheese wheys were described by dairy flavours, acid wheys had 'offensive' flavours and the commercial beverages and Mozzarella whey had a natural yoghurt flavour. PTR-MS found 26 masses that discriminated (P < 0.05) between samples. In addition, the first 7 PC's of PCA discriminated between samples. PLS found strong relationships between specific sensory characteristics and volatile composition. PTR-MS proved to be a highly sensitive technique, and it was possible to very rapidly discriminate samples in terms of volatile composition that related to a meaningful sensory description.

#### Effect of hydrolysis of casein by plasmin on the heat stability of milk

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Altering the properties of casein micelles, for example, by the hydrolytic activity of the indigenous milk proteinase, plasmin, may affect the heat stability of milk. In this study, the effect of

hydrolysis of casein by plasmin on the heat stability of reconstituted skim milk powder (RSMP), serum protein-free milk (SPFM) and concentrated milk was examined. Throughout incubation for 24 h at 37 °C, samples of each milk type, with or without 6 mg L-1 added plasmin, were removed and heat stability was determined at 140 °C after adjustment of the pH of milk to values in the range 6.3 to 7.2. Plasmin activity reduced the heat stability of RSMP and SPFM at alkaline pH values (> pH 6.8). During incubation with plasmin the  $\zeta$ potential of casein micelles increased from -19.5 to -15.5 mV over 24 h and it is probable that changes in the surface charge of the micelle contributed to changes in heat stability. Electrophoretic analysis of incubated milk confirmed the presence of increased levels of  $\gamma$ -caseins and proteose-peptones, byproducts of hydrolysis of  $\beta$ -casein by plasmin, which may have altered the charge on casein micelles. There was little effect of hydrolysis of casein by plasmin on the heat stability of concentrated milk. These finding provide further understanding of how hydrolysis of caseins by plasmin influence the properties of casein micelles.

## Growth of psychrotrophic bacteria in refrigerated milk: effect of high pressure treatment and addition of nisin E.P. Black<sup>1,2</sup>, A.L. Kelly<sup>2</sup> and G.F. Fitzgerald<sup>1,2</sup>

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Common psychrotrophic contaminants of milk such as Pseudomonas and Listeria can cause spoilage or serious illness, respectively. High pressure (HP) processing is a novel technology with many potential applications in the dairy industry. As HP products are usually refrigerated postprocess to extend shelf-life, it is important to consider bacterial growth in products such as HP-treated milk at low temperatures. In this study P. fluorescens and L. innocua in sterile skim milk or phosphate buffered saline (PBS) were treated with HP and/or the bacteriocin, nisin (500 i.u./ml) and stored at 4 °C for 10 days. From an initial inoculum of  $2 \times 10^8$  colony forming units (cfu)/ml, numbers of L. innocua in milk, treated at 400 MPa for 5 min in the presence of nisin, were reduced by 3.5 log cycles. A further 2.5 log reduction was obtained after 10 days of storage at 4 °C. P. fluorescens was reduced from initial numbers of  $2 \times 10^8$ cfu/ml to undetectable levels when treated at 250 MPa in the presence of nisin but numbers increased during subsequent refrigerated storage to  $1 \times 10^7$  cfu/ml after 10 days; increasing the treatment pressure to 300 MPa eliminated this recovery. Both organisms were more sensitive to HP in buffer than in milk. These results reinforce the importance of considering the hurdle effect for optimal preservation of milk.

## Effect of exopolysaccharide producing cultures on the composition, cooking properties, rheology, yield and grading scores of half-fat Cheddar cheese

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Exopolysaccharide (EPS)-producing cultures are used in yoghurt manufacture to enhance viscosity and reduce syneresis, and have recently been investigated as a means of enhancing the textural properties of low-fat Mozzarella cheese. The

aim of this study was to determine the effect of EPS-producing cultures on the composition, cooking properties, rheology, yield and grading scores of half-fat Cheddar cheese. Half-fat Cheddar cheese was manufactured using a non-EPS producing starter (Lactococcus lactis 303), either alone (control cheese) or in combination with one of the following EPS-producing cultures, Streptococcus thermophilus MR-1C (STMR-1C), or Lactococcus cremoris 322 (LC322). Cheesemaking trials were undertaken in triplicate. LC322 resulted in significantly higher cheese moisture contents (1.5%, w/w) and yields (3%) than the control but had little effect on the cooking properties or grading scores, except at 360 days where it resulted in markedly lower scores for body/texture and flavour/aroma than the control. The LC322 cheese was less firm, and had a lower fracture stress, than the control cheese, throughout ripening. STMR-1C did not significantly influence the moisture content, yield, rheology or grading scores of the cheese but resulted in a markedly higher stretchability than the control at  $\geq 60$  days. EPS-producing cultures may have the potential to increase the moisture content and yield of cheese without changing its rheological-based functions.

#### Use of exogenous nattokinase to accelerate proteolysis in Cheddar cheese

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Nattokinase, a subtilisin-like serine proteinase produced by Bacillus subtilis, is found in the traditional fermented Japanese soyabean product "natto". The aim of this study was to use a semi-purified preparation of nattokinase, isolated from natto, for the manufacture of Cheddar cheese and to study its effect on proteolysis during ripening. A semi-purified preparation of nattokinase was added to cheesemilk at 80, 160 and 320  $\mu$ g mL<sup>-1</sup> and Cheddar cheese was manufactured therefrom, pH 4.6-Soluble N increased at all ripening times as the levels of nattokinase increased. Increased breakdown of  $\alpha_{s1}$ - and  $\beta$ -caseins was observed in experimental cheese compared to control cheese. In all experimental cheeses,  $\alpha_{c1}$ -casein was hydrolysed faster than  $\beta$ -casein. With increasing levels of addition of nattokinase, increased production of a peptide derived from β-case in similar to the γ-case in swas observed. Principal component analysis of reversed-phase HPLC chromatograms of 70% (v/v) ethanol-soluble fractions of cheeses differentiated cheeses based on the level of addition of nattokinase. Levels of free amino acids were higher in experimental cheeses compared to the control cheese; however, the increase was not proportional to level of addition of nattokinase. The results of this study show that addition of nattokinase to cheesemilk accelerates proteolysis in Cheddar cheese during ripening.

#### Influence of adjunct lactobacilli from sour-dough starters on ripening of Cheddar cheese

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Sourdough is dough fermented by micro-organisms, mainly lactic acid bacteria, especially lactobacilli and yeasts. The objectives of this study were to investigate the usefulness of sourdough lactobacilli as adjuncts to improve the quality of Cheddar cheese. The influence of five adjunct cultures (Lactobacillus alimentarius 52, L. brevis AM2, L. fructivorans DD8, L. plantarum 13 and L. sanfranciscensis E17) on proteolysis and the development of sensory characteristics during ripening of Cheddar cheese was studied. Cheddar cheese was manufactured in duplicate on a 20 L scale. Adjunct lactobacilli were added to the cheesemilk at an initial level of 105 to 106 colony forming units (cfu)/ml. The populations of starter, NSLAB and adjunct in the resulting Cheddar cheese were monitored after 1, 7, 14, 21 days and 2, 4 and 6 months ripening at 8 °C. Adjuncts in the experimental cheeses reached maxima (10<sup>6</sup> to 10<sup>7</sup> cfu/g) after 2 months and then declined somewhat after 4 months. Numbers of starter bacteria declined from ca. 10° cfu/g in all cheeses during ripening reaching 10<sup>6</sup> to 10<sup>7</sup> cfu/g after 6 months. Compositions of all cheeses at 1 month were generally similar and typical of Cheddar cheese made on a pilot-scale. Samples were analysed by PAGE after 1 day and 2, 4 and 6 months of ripening no differences were apparent between the samples. Reverse-phase HPLC chromatograms of the 70% ethanol-soluble fractions showed only minor differences between the cheeses during ripening. Sensory analysis of the cheeses at 6 months showed significant differences between the control and experimental cheeses with respect to some important sensory attributes. The attributes 'bitter', 'strength', 'balanced', 'buttery', 'sweaty' and 'salty' flavour and 'sweaty' odour distinguished between cheeses. The results of this study indicated that sourdough lactobacilli used as adjuncts positively influence flavour development in Cheddar cheese.

Pepstatin as an inhibitor of residual chymosin activity in Cheddar cheese: (A) Effect on composition and proteolysis J.A. O'Mahony and P.L.H. McSweeney, Department of Food and Nutritional Sciences, University College, Cork

Pepstatin, a competitive inhibitor of chymosin, was added at four levels (0.0, 0.1, 1.0 and 10.0  $\mu$ M) to the curds/whey mixture for Cheddar cheese, to obtain different levels of residual chymosin activity in the resultant curd. The moisture content of the cheese with 10  $\mu$ M added pepstatin (38.0%) was significantly (P < 0.05) lower than that of the control (40.1%). Residual chymosin activity (% of control) was 91, 55 and 14% for cheese made with 0.1, 1.0 or 10.0  $\mu$ M added pepstatin, respectively. At 6 months, the level of pH 4.6-soluble nitrogen/total nitrogen in the control cheese (27.75%) was approximately twice that of the cheese made with 10.0  $\mu$ M added pepstatin (13.94%). Densitometric analysis of PAGE electrophoretograms of the pH 4.6-insoluble fractions of control cheese and cheese made with 0.1, 1.0 or 10.0  $\mu$ M added pepstatin, showed the level of intact  $\alpha_1$ -case in to be 11.8, 28.8, 59.2 and 83.8%, respectively. RP-HPLC peptide profiles showed extensive quantitative differences between the control and experimental cheeses. The total free amino acid content of the control cheese increased from 4.68 to 25.46 mg leucine/g cheese during ripening, while the corresponding increase for the cheese made with 10.0  $\mu$ M added pepstatin was from 2.87 to 12.65 mg leucine/g cheese. In conclusion, the pattern of proteolysis was identical in all cheeses. However, its rate decreased with increasing level of pepstatin addition.

#### Pepstatin as an inhibitor of residual chymosin activity in Cheddar cheese: (B) Effect on texture, rheology and microbial evolution

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Pepstatin, a potent competitive inhibitor of chymosin, was added at four levels (0.0, 0.1, 1.0 or 10.0  $\mu$ M) to the curds/whey mixture for Cheddar cheese to obtain residual chymosin activity levels of 100, 91, 55 and 14%, respectively. Texture profile analysis (TPA) showed the hardness of the cheeses made with either 1.0 (127.04 N) or 10.0  $\mu$ M (135.09 N) added pepstatin to be significantly (P < 0.05) greater than that of the control cheese (101.25 N). Dynamic rheological assessment of the cheeses showed storage modulus (G') to decrease in the order 10.0  $\mu$ M > 1.0  $\mu$ M > 0.1  $\mu$ M > control cheese. The differences in G' between each of the treatments were significant (P < 0.05). Tangent of the phase angle  $(\delta)$ , an index of fluidity and flowability of cheese, was measured by subjecting samples to a stress of 10 Pa, at a frequency of 0.1 Hz, while heating from 20 to 60 °C. The maximum tan  $\delta$  value attained for control cheese and cheese made with 0.1, 1.0 or 10.0 uM added pepstatin was 1.15, 1.02, 1.75 and 1.64, respectively. Addition of pepstatin had no effect on the growth or survival of starter or non-starter lactic acid bacteria in the cheeses during ripening. In conclusion, chymosin action on  $\alpha_{s1}$ -casein plays a major role in the development of texture and functionality of Cheddar cheese. of cheeses ripened using seven such treatments. The pH, moisture, fat and protein levels in the cheeses ranged from 5.6 to 5.7%, 41.2 to 42.0%, 32.3 to 32.5% and 19.1 to 19.4%, respectively. At 9 months, NSLAB numbers plateaued at levels dependent on ripening temperature (e.g., 106 and 108 colony forming units (cfu)/g for cheeses ripened at 4 and 12 °C, respectively). Levels of lipolysis, as measured by acid degree value, increased from 2.43, 2.43 and 2.66 mL KOH/g fat at day 1, to 6.72, 7.13 and 9.67 mL KOH/g fat in cheeses ripened for 9 months at 4, 8 or 12 °C, respectively. Quantitative descriptive sensory analysis showed that cheese ripened at 12 °C for 4 months had aroma and flavour characteristics of mature Cheddar cheese, while the aroma and flavour scores of cheese ripened at 4 °C were essentially the same at 9 months as at 4 months of age. Irrespective of maturation time. cheese ripened at 4 °C never attained aroma and flavour characteristics considered typical of mature Cheddar cheese. These results show that it is possible to accelerate Cheddar cheese ripening by increasing temperature, without adversely affecting the sensory properties of the cheese.

#### The effect of lipolysis on the sensory characteristics of Cheddar cheese

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The relationship between lipolysis and Cheddar cheese flavour is poorly understood. One possible reason is the lack of suitable reproducible quantitative methods to assay levels of lipolysis in cheese. In this study, the levels of lipolysis in 10 Cheddar cheeses (full and reduced fat) of known history were evaluated using a number of different methods; copper soaps, acid degree value, GC and HPLC techniques. The compositional, proteolytic and sensory characteristics of these cheeses were also evaluated. The most accurate and reproducible methods to determine lipolysis involved GC of un-derivatised free fatty acids and HPLC of short chain free fatty acids. Compositional data of these cheeses highlighted variations in relation to moisture, salt, fat in dry matter and pH. Levels of proteolysis generally corresponded with age. Sensory properties of the cheeses were found to differ significantly (P < 0.05) for four odour and 12 flavour attributes. Levels of lipolysis in the cheeses positively correlated with age and fat content; however, the ratio of short, medium and long chain free fatty acids appeared to have a greater influence on the sensory characteristics than the extent of lipolysis.

## Influence of ripening temperature on lipolysis, sensory properties and growth of non-starter lactic acid bacteria in Cheddar cheese

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Cheddar cheese ripening is a slow and hence very expensive process. The objective of this study was to determine a temperature-time regime suitable for acceleration of Cheddar cheese ripening by assessing lipolysis and sensory properties

## Partial purification and characterisation of an X-prolyl dipeptidyl aminopeptidase from *Lactobacillus sanfranciscensis* CB1

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X-Prolyl dipeptidyl aminopeptidases (PepX) release X-Pro from the N-terminal extremity of peptide chains. The PepX activities of 12 Lactobacillus sanfranciscensis strains were screened; this organism is a key lactic acid bacterium in sourdoughs. The enzyme was found only in intracellular extracts and L. sanfranciscensis CB1 strain possessed high levels of activity. A monomeric ca. 53 kDa PepX was partially purified from L. sanfranciscensis CB1 by five chromatographic steps. The enzyme was optimally active at pH 6.0 and 30 °C and was strongly inhibited by 10 mM Mn<sup>2+</sup>, Hg<sup>2+</sup> and Zn<sup>2+</sup>. The enzyme showed low activity on Z-Gly-Pro-pNA, Z-Ala-PropNA and L-Glu-pNA. The enzyme lost activity after exposure to 45 °C for 30 min; the D value at 45 °C was ca. 5.46 s. As shown by Central Composite Design applied to study the individual and interactive effects of pH, temperature and NaCl, maximum enzyme activity occurred at a temperature of approximately 32 °C, pH 6.2 and NaCl concentrations less than 2.4% (w/w). The possibility that this PepX can catalyse the breakdown of Pro-rich polypeptides, which are involved in an inappropriate T-cell mediated immune response to gluten in patients suffering from Celiac disease, is under study.

#### Quality assessment of various oils in the manufacture of base white béchamel sauce

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Sauce manufacture is an important aspect of ready-meal manufacture contributing greatly to product quality and stability. In this study, six oils (10%) were assessed including corn oil (CO), grape seed oil (GSO), olive oil (OO), sunflower oil (SFO), soyabean oil (SBO), rapessed oil (RSO) and tallow

(TO). Béchamel sauces were processed (90 °C × 30 min) packaged in PET and held under display (4 °C) for 30 days and tested on 0, 6, 10, 17, 26 and 30 days. Sauces were assessed for oxidative stability (TBARS), pH, viscosity, colour (Hunter lab and CIE), gas analysis of packs, and microbiological analysis. Results showed all sauces prepared using the test oils were microbiologically good over the assessment period. Addition of TO fat resulted in a significant increase in sauce viscosity (3,000 to 3,500 cpoise) compared with the other oils (1000 to 2000 cpoise). RSO (0.7 mg MDA/kg) and OO (0.4 mg MDA/kg) appeared to produce the highest TBAR values with GSO, SFO, SBO and TO producing the lowest values (0.2-mg MDA/kg). Gas analysis of sauces over time showed that % CO<sub>2</sub> decreased from 35% on day 0 to SO (30%), RSO (24%), TO (20%) and OO, SO (15%) on day 30. Per cent oxygen levels of test packs were < 0.2% over the trial storage period. This analysis showed that OO, CO, SFO and GSO were most acceptable for sauce manufacture.

#### Heat induced gels of whey protein and highly cross-linked waxy maize starch

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Gelling mixtures of highly cross-linked waxy maize starch (HCWMS) with whey protein isolate (WPI) and sufficient salt to induce gelation of the protein (100 mM NaCl) have been investigated by differential scanning calorimetry (DSC) and rheological measurements of storage modulus (G'). When HCWMS (2%) was heated in the absence of WPI, gelatinisation caused a sharp increase in G' at ~63 °C, with an accompanying endotherm in DSC. Incorporation of WPI led to a second increase in G' at higher temperature (above ~72 °C) which was again accompanied by a DSC endotherm, arising from salt-induced gelation of protein on thermal denaturation. Moderate concentrations of WPI (up to ~6%) caused a progressive increase in gelatinisation temperature, which can be attributed to segregative interactions with WPI inhibiting granule swelling. At higher concentrations of WPI there was no detectable increase in G' over the temperature range of gelatinisation, but the gelatinisation endotherm was still clearly evident. This suggests segregation into two discrete phases, with HCWMS confined to dispersed regions in a continuous matrix of WPI. However, the presence of HCWMS caused large increases (up to ~500fold) in the strength of the WPI network. These were particularly evident when the network formed in the absence of HCWMS was weak, and can be attributed to transfer of water into the starch granules during swelling, with consequent increases of protein concentrations in the WPI matrix.

#### Effect of long- and short-term frozen storage on the quality of freeze-chilled lasagne

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Freeze chilling involves freezing and frozen storage followed by thawing and chilled retail display. Although work has been carried out on a number of ready-meal components, little work has been carried out on the freeze chilling of complete ready-meals. The objective of this study was to examine the effect of long- and short-term frozen storage prior to thawing on the quality of freeze-chilled commercially made lasagnes. In the short-term trial, four process treatments were used; fresh, chilled, freeze-chilled and frozen; in the long-term trial, the lasagnes were frozen for 3, 6, 9 or 12 months, thawed and analysed (frozen) or chilled at 4 °C for 6 days (freeze-chilled). In the short-term trial, unheated frozen and freeze-chilled lasagne had higher drip loss than fresh or chilled (P < 0.001) and freeze-chilled lasagne had a brighter colour (L/b) than chilled lasagne. No difference was found in firmness or sensory acceptability between the treatments but freeze-chilled and frozen lasagne had higher total viable counts (TVC) than chilled or fresh. In the long-term trial, lasagne stored frozen for 12 months was firmer (higher shear force) than lasagne stored for 3, 6, or 9 months. Frozen storage for 9 or 12 months also led to higher drip loss values than storing for 3 months for unheated lasagnes but, when heated, the opposite effect was seen. Length of time in frozen storage had no effect on TVC values. Lasagne is a suitable product for freeze chilling.

#### Optimization of parameters for successful extrusion of edible/biodegradable films containing pectin

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The use of extrusion technology in the manufacture of edible/biodegradable films has received limited attention. Extrusion technology offers an opportunity towards the commercialization of edible/biodegradable films. The objective of this study was to determine the conditions necessary to produce edible/biodegradable pectin films using twin-screw extrusion technology. Pectin was chosen as substrate and four different extrusion parameter combinations. Combinations 2 (temperature 125 °C; feeder speed 4; glycerol 50%; screw speed 225 rpm) and 4 (temperature 135 °C; feeder speed 4; glycerol 50%; screw speed 150 rpm) had higher (P < 0.05) tensile strength, puncture resistance and lower turbidity values (5.55 MPa and 5.18 MPa; 4.27 kg and 5.24 kg; 0.455 Å and 0.39 Å, respectively) than combinations 1 (temperature 125 °C; feeder speed 6; glycerol 70%; screw speed 150 rpm) and 3 (temperature 135 °C; feeder speed 8; glycerol 70%; screw speed 225 rpm) (3.64 MPa and 3.91 MPa; 3.22 kg and 2.71 kg; 0.880 Å and 0.929 Å, respectively). In addition, combination 3 had lower (P < 0.05) elongation (20.05%) than all other combinations (26.0%, 25.3%, and 24.9%) and higher (P < 0.05) tearing resistance (0.073 kg) than combinations 1 and 4 (0.049 kg and 0.042 kg). Combination 1 had a lower (P < 0.05) Young's modulus (0.28) than combination 3 (0.48). In conclusion, films manufactured by extrusion combination 2 generally had stronger physical properties than those of other combinations and were finally selected as the optimized setting for extruding strong and consistent pectin films.

#### Acceleration of the growth of Saccharomyces cerevisiae by linear ultrasonic wave irradiation

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Low-intensity ultrasonic irradiation can accelerate the growth and metabolic productivity of microbes. This study focuses on the growth changes of Saccharomyces cerevisiae exerted by linear ultrasonic wave during its initial growth stage. An ultrasonic bioreactor was used and S. cerevisiae was immediately irradiated by the linear ultrasonic wave with low intensity and low frequency after inoculation. The results showed that the irradiation time of ultrasound influenced the growth of S. cerevisiae. Irradiation  $(3.8 \times 10^{-3} \text{ W/mm}^2 \text{ in intensity and } 32.5)$ kHz in frequency) for 15 min, 30 min, 45 min and 60 min, shortened the lag phase of S. cerevisiae and increased the cell number and dry weight. Optimum results were obtained on irradiation for 45 min, and the cell number and dry weight were increased by 48.6% and 10.2%, respectively. The effects of pulse-gap (0 s, 2 s, 3 s, 4 s and 5 s) on the growth of S. cerevisiae were also studied. A pulse-gap of 4 s was found to produce the fastest growth, increasing the cell number and dry weight by a further 15.6% and 11.4%, respectively. It was concluded that the growth of S. cerevisiae during the lag phase could be accelerated by linear ultrasound irradiation.

#### Extraction of soybean oligosaccharides by ultrafiltration

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Ultrafiltration is an efficient extraction technique. In this study, soybean oligosaccharides were extracted from pretreated soybean whey by ultrafiltration. A membrane with a molecular cut-off of 3000 Da was used. The results showed that the ultrafiltration velocity decreased with the increase in filtrate volume, and the filtration resistance was linearly related to the filtrate volume. In the regression equation (R = 0.915) the slope and intercept of the linear curve were 0.3659 and 0.0122, respectively. In addition, the percentage of soybean oligosaccharides extracted was 85.2% and the percentage of protein was reduced by 66.5% during the ultrafiltration. The final concentration of soybean oligosaccharides solution was 17.9 mg/ml, consisting of 12.7 mg/ml sucrose, 1.2 mg/ml raffinose and 4.0 mg/ml stachyose. Twelve methods of cleaning ultrafiltration membrane were also investigated, and an efficient cleaning method was developed: first the membrane was washed for 3 min with distilled water in reverse flow, then it was immersed in 0.1 M NaOH for 10 min, washed again with distilled water, and then immersed again in 0.1 M HCl for 10 min and washed again with distilled water. The percentage flux recovery of the ultrafiltration membrane was 96.4% under the optimal condition. Therefore, ultrafiltration can be adopted to extract the soybean oligosaccharides from the soybean whey.

### Selection of ultrafiltration membrane during soybean oligosaccharide extraction

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Soybean oligosaccharide is a potential type of functional food additive, which can be extracted by ultrafiltration from soybean whey. The objective of the study was to select an optimal membrane for extraction of the soybean oligosaccharide. Five membranes with molecular cut-offs of 1.5 kDa, 3 kDa, 4 kDa, 5 kDa and 10 kDa were used. For all these

membranes, it was found that the ultrafiltration velocity increased with pressure and temperature. The velocity was increased by 180%, 167%, 167%, 118% and 168%, respectively, when the pressure was increased from 0.02 MPa to 0.18 MPa at 20 °C. With the increase of temperature from 15 to 45 °C, the velocity was increased by 480%, 327%, 340%. 211% and 140%, respectively, at a pressure of 0.18 MPa. Therefore, the optimal pressure and temperature was determined to be 0.18 MPa and 45 °C, respectively. Under the optimum conditions, the reduction rate of protein was 69.9%, 66.5%, 61.7%, 52.2% and 25.9%, and the extraction rate of soybean oligosaccharide was 51.9%, 85.2%, 59.5%, 69.5% and 88.1%, respectively. Based on the above results, with 3 kDa molecular cut-off, the membrane was chosen as the optimum one; with it the concentration of sovbean oligosaccharide was found to be 17.9 mg/ml and the velocity was  $8.89 \times 10^{-3}$  mm<sup>3</sup>/m<sup>2</sup>.s. An optimum membrane was determined for extracting soybean oligosaccharide.

#### The effect of thawing rates on the quality of freeze-chilled carrots and mashed potato

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Trials were carried out to investigate the effects on quality of different thawing rates on sliced carrots and mashed potato. Both products were blast frozen at -30 °C for 2.5 h, stored at -25 °C for 7 days and then, using air temperatures of 10 °C and 20 °C, thawed at 2 to 4 °C and stored in chilled storage for up to 9 days. Faster thawing had no effect on the colour, drip loss, moisture content or soluble solids of sliced carrot when compared to the traditional method of thawing at 4 °C. Length of time in chilled storage had no effect on firmness, however increasing the thawing rates led to a significant increase in firmness on all test days. After 9 days in chilled storage samples thawed at 4 °C had an average firmness value of 1100 N, compared to 1408 N at 10 °C and 1863 N at 20 °C (P < 0.01). Analysis for potato mash showed that thawing at higher temperatures had no effect on drip loss, moisture content or texture. Significant differences were found in vitamin C content between the test days for the different thawing rates; however, the content was low in all cases. Sensory analysis carried out on sliced carrot and mashed potato, were within the moderately acceptable to very acceptable range. Microbial counts for both products were highest for the 20 °C treatment but all results were within acceptable limits.

## Influence of dietary Vitamin E supplementation and fat sources, on the oxidative stability and volatile production, of cooked duck meat patties

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Volatile compounds derived from lipid oxidation contribute significantly to off-flavour development in cooked, stored meat. There is limited research on the oxidative stability of duck meat. The objective of this study was to determine the effect of dietary vitamin E supplementation ( $\alpha$ -tocopheryl acetate, 20 or 400 mg Ta/kg feed) with different fat sources (tallow, olive oil, sunflower oil or linseed oil, 2.5% of diet) on the oxidative stability of cooked duck meat patties, stored in a retail (4 °C) display cabinet for 10 days. Lipid oxidation

(MDA-TBA complex) and volatile compounds (hexanal, heptanal, octanal, 2-nonanol, 2-pentanol, 2-butanone and diemthyl sulphide) were measured (static headspace gas chromatography). Overall, meat from the sunflower and linseed oil fed ducks resulted in significantly (P < 0.05) higher MDA-TBA complex values and hexanal, heptanal and octanal values compared to meat from the other dietary groups, which may reflect the high polyunsaturated fatty acids content of these oils. Meat from the olive oil fed duck was more stable towards lipid oxidation and aldehyde production. In general, dietary  $\alpha$ -tocopheryl acetate resulted in increased oxidative stability and lower hexanal values. The effect of dietary oils and TA supplementation on the other volatile compounds identified was inconclusive. Overall, the pattern of hexanal values corresponded to the lipid oxidation status of the cooked duck meat.

#### Analysis of the impact of fortified food consumption on overall dietary quality in Irish adults

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The EC has indicated in the White Paper on Food Safety that a directive on the addition of micronutrients to foods will be proposed with a view to harmonising voluntary regulations in the EU member states. This has stimulated a wideranging debate on the benefits and risks of fortified foods including the impact of fortified food consumption on overall diet quality. The objective of this study was to analyse the impact of fortified food consumption on nutrient intakes, compliance with dietary recommendations for macronutrients, fibre, fruit and vegetables and alcohol and adequacy of micronutrient intakes in Irish adults. The analysis was based on data from the North/South Ireland Food Consumption Survey. Almost 3% of the foods consumed were fortified foods, 62% of which were breakfast cereals and 69% of the population consumed fortified foods. Men and women were classified by tertile of fortified food consumption (kcal/day) into low, medium and high consumers. Fortified food consumption was associated with greater compliance with dietary recommendations for total fat, carbohydrate, nonstarch polysaccharide (NSP) and fruit and vegetables. In addition, increased consumption of fortified foods was associated with a more micronutrient dense diet and a reduced level of dietary inadequacy for calcium, iron, riboflavin and folate particularly in women. These results show that fortified food consumption appears to be associated with better overall diet quality.