Technical Session 1 – Monday, 21 May, 15.30-17.00

Chair, Helmut Steinkamp

T1-01 Quantitative Monitoring of the *Campylobacter* Contamination of Broiler Carcasses during Slaughter

SELIWIORSTOW, TOMASZ[1]; Baré, Julie[1]; Uyttendaele, Mieke[2]; De Zutter, Lieven[1]
1. Dept. of Veterinary Public Health and Food Safety, Ghent University, Merelbeke, Belgium.
2. Dept. Food Safety and Food Quality, Ghent University, Ghent, Belgium.

Introduction: *Campylobacter* is the most commonly reported gastrointestinal bacterial pathogen in humans in the European Union. The majority of human infections is attributed to the consumption of contaminated poultry meat. Better knowledge of the source and level of the *Campylobacter* contamination during slaughter might attribute to the identification of risk factors for highly contaminated carcasses and the further implementation of preventive measures at the slaughterhouse level.

Purpose: The study aimed to establish the variation of the *Campylobacter* contamination on broiler carcasses originating from *Campylobacter* positive batches throughout the slaughter process (7 locations sampled 6 times per visit) within four slaughterhouses which were each visited three times.

Methods: Per slaughterhouse (n = 4) three batches were examined in the period from February to July 2011. All collected samples (breast skin, intestinal samples and feathers) were homogenized, serially diluted and directly plated on CampyFood Agar® (bioMérieux SA, France) plates. After incubation under micro-aerobic conditions at 41.5°C for 48 h, *Campylobacter* enumeration was performed. Presumptive colonies were confirmed by Gram staining and PCR.

Results: Results showed that broilers from all visits (n = 12) carried high numbers of campylobacters in their caeca content (≥ 8 log CFU/g). During the slaughter of batches with high initial external contamination of *Campylobacter*, no effect of the plucking and evisceration processes on carcass contamination was observed. However, both process steps increased the contamination load when batches with a low initial external contamination were processed. The combination of a final washing step and cooling decreased the level of *Campylobacter* contamination only in two slaughterhouses. In general, after cooling, 56% of the carcasses contained *Campylobacter* levels lower than the 3 log CFU/g breast skin.

Significance: The results revealed a high variability in *Campylobacter* carcass contamination within batches, between batches in the same slaughterhouse and between slaughterhouses. The initial external contamination level, the cross contamination during plucking and evisceration and the effectiveness of washing and cooling steps have crucial impact on the contamination of the final product. Optimal control of those factors may lead to diminution of the *Campylobacter* levels on broiler carcasses, resulting in a reduction of the number of human campylobacteriosis cases.

T1-02 *Salmonella* Serotyping Using Multiplex PCR and Nanofluidic Electrophoresis

Xie, Yi[1] and WEIMER, BART[2]
1. University of California-Davis, Davis, CA, United States.
2. School of Veterinary Medicine, Population Health and Reproduction, University of California-Davis, Davis, CA, United States.

Introduction: *Salmonella* is one of the most common foodborne pathogens worldwide. It is responsible for diverse diseases ranging from mild, self-limiting gastroenteritis to life-threatening systematic infections such as bacteremia and typhoid fever. The *Salmonella* genus consists of >2,500 serovars that are traditionally serotyped based on its cell surface antigens. The O-antigen type is determined based on the polysaccharides associated with lipopolysaccharide that is very diverse. Traditional serology typing methods are labor-intensive and lengthy, often taking days to weeks to get results. Genome comparison studies increasingly point out the discordance between the genotype and serotype and expose the shortcomings of traditional serotyping method, including the lack of predictability of virulence and ecology of this genus. In spite of this difference detailed genomic analysis enables genotypic differentiation of serotypes.

Purpose: To develop a fast, accurate, and robust *Salmonella* serotyping method that is widely applicable in food industry during salmonella outbreaks using genomic analysis and multiplexed PCR.

Methods: Four PCR primers pairs based on comparative genomic analysis of *rfb* operons from publicly available sequenced *Salmonella* genomes that belong to B, C1, C2, and D1 serogroups.

Additionally, a pair of primers designed from the consensus of *Salmonella invA* gene was used as the positive control for *Salmonella* since it is known to be a genus-specific marker. Using genomic DNA purified using Qiagen DNAasy kit or extracted via boiling bacterial single colony isolates in water, a 5-plex PCR reaction was performed. These amplicons were resolved using nanofluidic electrophororesis using an Agilent 2100BioAnalyzer. The amplicons sizes were used to determine the serogroup of each *Salmonella* isolate.

Results: For each *Salmonella* isolate, the 5-plex PCR produced two bands: 1) the internal positive control for invA (231 bp) for *Salmonella* genus validation and the serogroup-specific amplicon for groups B, C1, C2, and D1 with sizes of 164, 301, 481 and 563 bp, respectively. These amplicons were well resolved during electrophoresis. Using this method, 66 *Salmonella* isolates were typed within one day with 100% accuracy. This method was also successfully used to serotype *Salmonella* serogroups using bacterial total RNA, and it was used in typing *Salmonella* serogroups directly from food matrices.

Significance: Using detailed genomics analysis in conjunction with high-resolution nanofluidic electrophoresis a fast and accurate multiplex colony PCR assay to serotype *Salmonella* was developed. This method resolved all common *Salmonella* serogroup B, C1, C2, and D1 isolates within 1 hour using DNA and RNA directly from colonies on isolation media or a food matrix. The approach can be used with existing isolation workflows or directly from complex matrices, such as food and the feces.

T1-03 Impact of Cold Plasma on *Escherichia coli* O104:H4, *Escherichia coli* O157:H7 and *Escherichia coli* DSM No. 1116 at a Model Surface

BAIER, MATTHIAS[1]; Janssen, Traute[2]; Wieler, Lothar[2]; Ehlbeck, Joerg[3]; Knorr, Dietrich[4]; Schlueter, Oliver[1]

1. Horticultural Engineering, Leibniz Institute for Agricultural Engineering Potsdam-Bornim (ATB), Potsdam, Germany
2. Institute of Microbiology and Epizootics, Freie Universität, Berlin, Germany
3. Leibniz Institute for Plasma Science and Technology (INP Greifswald e.V.), Greifswald, Germany
4. Dept. of Food Biotechnology and Food Process Engineering, Berlin University of Technology, Berlin, Germany.

Introduction: The recent outbreak of multi-resistant *Escherichia coli* O104:H4 in Germany in May, 2011 raises the question of its resistance to inactivation processes. Especially in the case of heat-sensitive materials like fresh produce, to which chemical agents or irradiation are not suitable or not allowed by law, novel gentle processes need to achieve reliable inactivation at low temperatures.

Purpose: In this study the inactivation efficiency of an atmospheric pressure plasma-jet (kINPen®, neoplas GmbH, Greifswald) was tested on *E. coli* O104:H4 in comparison to *E. coli* O157:H7 and non-pathogenic *E. coli* DSM No. 1116 attached to a polysaccharide gel surface. Process parameters were varied to explore the potential and limitations of cold plasma as a gentle inactivation process for heat-sensitive foods.

Methods: The noble gas argon was transformed into cold plasma at a power input of 8 W and a gas flow of 3 and 5 l/min. Bacteria suspensions with an initial count of 10^4 to 10^8 were inoculated on polysaccharide gel discs with an upper surface of about 1 cm^2. Subsequently, the gel discs were treated with plasma varying the treatment times (15s, 30s, 60s, 90s, 120s, 180s, and 240s). Non-thermal conditions of the plasma were controlled by an infrared camera.

Results: Measurements revealed that temperatures on the gel surface did not exceed 25°C after 4 min treatment time. Plasma application for 2 min resulted in inactivation of 1.5 log cycles at an initial bacterial count of $6*10^6$ CFU/cm^2. When decreasing the initial bacterial load and the distance between sample surface and plasma generation zone a 4-log cycle reduction of all strains was observed. Addition of oxygen to the carrier gas enhanced inactivation efficiencies and the detection limit of 10^2 CFU was reached after a treatment time of 30s.

Significance: Inactivation efficiency strongly depended on the initial bacterial load of samples. Following well established GMP and GHP in the post-harvest chain, plasma treatment has the potential to reduce pathogens like *E. coli* O104:H4 below their infective dose. Consequently successful treatment requires a specific adaptation of relevant process parameters.

T1-04 Evaluation of Three Swab-types for Recovery of *Listeria monocytogenes* on Different Food

LAHOU, EVY[1]; Jacxsens, Liesbeth[1]; Uyttendaele, Mieke[1]
1. Food Safety and Food Quality, Ghent University, Ghent, Belgium.

Introduction: The ability of *Listeria monocytogenes* to attach to various food contact surfaces, such as stainless steel, polypropylene and rubber compounds is well documented. Sampling food processing areas and equipment for *Listeria monocytogenes* is necessary and mandatory in many food production systems to avoid product contamination. However, swabbing efficiency is often poor and range from 25% to just 0.1% of the original inoculums.

Purpose: The objective of this study was to compare several swabs in their ability to detect low concentrations of *Listeria monocytogenes* on different food contact surfaces.

Methods: A cocktail of 5 serotypes of *Listeria monocytogenes*, mixed in equivalent concentrations, was inoculated with a concentration of 100 CFU/250 cm^2 onto stainless steel, polypropylene and rubber compounds in a 250 cm^2 area. Immediately after inoculation and after 1h exposure, the surfaces were swabbed with a pre-moistened 3M® Sponge-stick, 3M® Enviroswab and a Copan Foam spatula. The used swab was incubated for 24 h at 30°C in 225 ml of demi-fraser broth. Samples were then plated on ALOA agar, which were incubated for 24 h at 37°C.

Results: The recovery of *Listeria monocytogenes* with the Copan Foam spatula from stainless steel, polypropylene and rubber compounds was possible in respectively 94%, 94% and 100% of the samples (n = 18). Recovery of *L.* monocytogenes was possible on all tested polypropylene and rubber surfaces with the 3M® Enviroswab, while on stainless steel surfaces recovery was possible in 94% of the samples (n = 18). The recovery of *Listeria monocytogenes* with the 3M® Sponge-stick from stainless steel, polypropylene and rubber compounds was possible in respectively 83%, 100% and 100% of the samples (n = 18).

Significance: Only small differences were detected in the performance of the investigated swabs for the different food contact surfaces. Moreover, swabbing efficiency is high and the swabs are suitable to be used for environmental sampling on the different types of materials.

T1-05 Survival of Norovirus, Murine Norovirus 1, MS2 Phage and *Escherichia coli* in Various Types of Water Used for Irrigation of Fresh Produce

DEBELKE, STEFANIE[1]; Baert, Leen[1]; De Keukelaere, Ann[1]; Li, Dan[1]; Uyttendaele, Mieke[1]
1. Food Safety and Food Quality, University Ghent, Ghent, Belgium.

Introduction: Viral foodborne outbreaks, in particular Norovirus, have been associated with the consumption of fresh produce. Crops can be contaminated pre-harvest via irrigation water, (manured) soil or wild life, or post-harvest via contact with infected food handlers, contaminated transport or washing water or equipment. The use of surrogate viruses can be used to model survival of Norovirus in the environment.

Purpose: The present study is focused on the survival and detection of NoV, Murine Norovirus 1 (MNV-1), MS2 phage and *Escherichia coli* in different types of irrigation water (rain water, bore hole water, river water and open well water).

Methods: These types of water were inoculated with human pathogenic NoV GI.3 and GII.4, surrogate viruses MNV-1 and MS2 and *E. coli* as the bacterial fecal indicator and stored at 10°C and 22°C. Monitoring of (non-cultivable) NoV was performed using real-time qPCR, of (cultivable) MNV-1 by RT-qPCR and plaque assay (cell lines), of MS2 phage by plaque assay and *E. coli* by plating on Rapid *E. coli* 2.

Results: All inoculated microorganisms (*E. coli*, MS2, MNV-1 (plaques and RT-qPCR) and NoV) showed a significant faster reduction at 22°C than at 10°C in all different types of water. The type of water had an impact on survival and highest reductions were observed in river water (≥ 2 log after 7, 14, 28, 35 and ≥ 62 days respectively at 10°C) and in open well water (≥ 2 log after 7, 14, 21, 62 and > 35 days respectively at 10°C). As expected, RT-qPCR signals for MNV-1 (and NoV) were detected for a prolonged period time but does not necessarily relate to infectivity, for which plaque assays are needed. At 22°C MNV-1 could be detected up to 35 and 62 days by RT-qPCR unlike only up to 10 days by means of plaque assay in river water and open well water, respectively.

Significance: The stability of the microorganisms depends on the temperature and the type of water. The longest survival was noted at (commonly encountered) low temperature (10°C) and bore

hole water or rain water. In particular NoV and MNV-1 show highest stability when monitoring using RT-qPCR, although this lacks information on the infectivity of micro-organism thus detected.

T1-06 Influence of Starvation Stress on *Escherichia coli* O157 Attachment onto Butterhead Lettuce Leaves

VAN DER LINDEN, INGE [2]; Cottyn, Bart[1]; Vlaemynck, Geertrui[2]; Uyttendaele, Mieke[3]; Maes, Martine[1]; Heyndrickx, Marc[2]

1. Sciences Unit - Crop Protection, Institute for Agricultural and Fisheries Research (ILVO), Merelbeke, Belgium.

2. Technology and Food Science Unit - Food Safety, Institute for Agricultural and Fisheries Research (ILVO), Melle, Belgium.

3. Faculty of Bioscience Engineering, Dept. of Food Safety and Food Quality, Laboratory of Food Microbiology and Food Preservation, Ghent University (UGent), Gent, Belgium.

Introduction: Attachment of enteric pathogens such as *Escherichia coli* O157 onto fresh produce is a crucial first step for contamination to occur. Before contact with the plant surface, *E. coli* O157 can be present for some time in feces, sewage, soil or water. In the case of contaminated water, research has shown that survival of *E. coli* O157 in water could lead to starvation stress for the pathogen. This leads in turn to a rapid adjustment of its physiology by induction of stress responses and up-regulation of virulence expression. Interestingly, these events seem to be involved in bacterial attachment as well.

Purpose: The aim of this study is to better understand the effect of starvation stress on the attachment of *E. coli* O157 onto butterhead lettuce leaves.

Methods: *E. coli* O157 was introduced at 3 x 10^7 log CFU/ml into either sterile distilled water (SDW), phosphate buffered saline (PBS) or irrigation water (IW). The inocula were stored at 4°C and 20°C, and were used at days 0, 2 and 6 for an attachment assay on butterhead lettuce. The pathogen levels in the inocula were determined by the plating method and the live/dead-qPCR technique. By comparing both techniques, starvation stress could be estimated and correlated with the attachment ability of the pathogen.

Results: qPCR-data revealed a die-off of *E. coli* O157 only in IW stored at 20°C although a decrease in culturable cells, an indicator of stress, was observed for most of the treatments. In general, the pathogens were least stressed in PBS and most stressed in IW. None of the treatments revealed an absolute increase in the number of attached *E. coli* O157 after 6 days ($P > 0.05$). However, when comparing plate counts, we observed a relative increase as a function of time for IW and SDW.

Significance: Our study demonstrates that starvation in water could have an effect on *E. coli* O157 attachment onto butterhead lettuce leaves. In future experiments, we will attempt to identify the underlying mechanisms of attachment in order to find more effective practices to prevent contamination. This study was funded by the Federal Public Service of Health, Food Chain Safety and Environment (contract RF 6202).

Technical Session 2 – Tuesday, 22 May, 11.30-12.30

Chair, Christina Harzman

T2-01 Sensitivity of *Bacillus weihenstephanensis* to Acidic Changes of the Medium is Not Dependent on Physiological State

DESRIAC, NOEMIE[1]; Postollec, Florence[1]; Leguerinel, Ivan[2]; Sohier, Danièle[1]; Coroller, Louis[2]

1. ADRIA Development, Quimper, France.
2. LUBEM Quimper, Quimper, France.

Introduction: Exposure to mild stress conditions can activate stress adaptation mechanisms in microorganisms. They might result in a protective effect toward otherwise lethal stresses.

Purpose: This study aims to quantify the effect of salt and acid preexposure on acid-tolerance of vegetative cells of *Bacillus weihenstephanensis*.

Methods: The psychrotolerant strain KBAB4 was cultured until the exponential growth phase (i) in BHI, (ii) in BHI supplemented with 2.5% salt (iii) or BHI acidified at pH 5.5 with HCl. The growing cells were subsequently inactivated in an acidic suspension at a pH from 4.4 to 4.7. Linear and nonlinear microbial survivals models were fitted to the inactivation data. Based on statistical criterion, a Weibullian model was selected and used to describe the acid inactivation and the adaptation of the cells to stress.

Results: The acid-tolerance was enhanced after growth in non lethal acid stress conditions whereas a decrease of the acid-tolerance was observed for cells grown in salt conditions. Both environmental growth and inactivation did not present influence on the shape of the inactivation kinetics. Only the scale parameter, which quantifies the bacterial resistance, depended on acid intensity and growth conditions. The secondary modeling of the bacterial resistance allowed to quantify sensitivity of the cells to acidic change of the medium. This sensitivity was not significantly affected whatever the growth conditions.

Significance: These results highlight that the growth conditions may influence the bacterial acid resistance without affecting the sensitivity to acidic modification. Quantification of such adaptive stress response might be instrumental in understanding adaptation mechanism. And naturally, a tool to link the variation of food composition to its repercussion on the bacterial exposure of consumers.

T2-02 An Open-source Community Resource for Creating, Collecting, Sharing and Applying Predictive Microbial Models (PMM-lab)

FILTER, MATTHIAS[1]; Weiser, Armin A.[1]; Falenski, Alexander[1]; Brandt, Jörgen[1]; Käsbohrer, Annemarie[1]; Appel, Bernd[1]

1. Department 4 - Biological Safety, Federal Institute for Risk Assessment, Berlin, Germany.

Introduction: Quantitative microbiological risk assessments (QMRA) in the farm-to-fork continuum heavily rely on mathematical models for growth, survival and inactivation of microorganisms in different food matrices and processing conditions, collectively subsumed under the heading “predictive microbial models” (PMMs). Unfortunately, the PMMs currently publicly available are characterized by a great heterogeneity with respect to applicability, quality, validity, documentation, application limits and software requirements.

Purpose: The objective of this research was to develop a community resource that facilitates the creation, collection, sharing and application of PMMs extending existing community resources like the ComBase (www.combase.cc) database on experimental microbial data. Such a community software infrastructure could pave the way for collaborative efforts to improve food safety globally.

Methods: On the basis of the open-source software framework Konstanz Information Miner (KNIME, www.knime.org), R (www.r-project.org) and a HSQL database engine (http://hsqldb.org/) the open-source community resource “PMM-lab” was developed.

Results: The PMM-lab plug-in extends the KNIME software framework such that QMRA-specific modeling tasks can easily be accomplished. Because of the available R-software integration, a huge number of model types can be applied in a straightforward way so that experimental data on microorganisms in food can be explored much more easily by interested scientists. Additionally, all information necessary for documentation and model validation can be accessed immediately and in a standardized fashion.

Significance: The PMM-lab KNIME-Plug-in is a full featured community resource in the field of

microbial modeling. This infrastructure will open the path for a high quality community PMM repository. The developed solution has the potential to empower lab scientists to create, document and share PMMs in a standardized fashion which in the end will be beneficial with respect to scientific transparency and model validation opportunities. This work has been funded by BMBF (13N11202) and BLE (07HS019) research grants.

T2-03 Modeling Consumer Exposure and Microbial Growth under Variable Environmental Conditions in the Supply Chain for Microbial Risk Assessment

O' MAHONY, CIAN[1]; Mukherji, Raja[1]
1. Creme Software, Dublin, Ireland.

Introduction: Accurately modeling microbial growth under real environmental conditions in the supply chain requires assessing the impact of variability at each stage in the chain. Assessing microbial risk in a population of consumers requires modeling consumer impact via exposure assessment and/or dose response. In order to sufficiently estimate the distributions of pathogen concentrations in foods and using these distributions in an exposure assessment, millions of simulations can be required, both to estimate variability in both microbial growth and consumer exposure. Rapid risk assessment and scenario analysis can require considerable time and computational effort.

Purpose: To develop a complete web-based cloud computing system for modeling the impact of variability in the food supply chain on microbial growth and consumer exposure, quickly and accurately.

Methods: Predictive models were implemented stochastically for a number of pathogen/commodity pairs (*Escherichia coli* O157:H7 in beef and *Salmonella* Typhimurium in chocolate) to simulate all potential variability at various points in the supply chain (e.g., variable initial conditions, storage times, temperatures, pH, etc.). Extensive experimental data from the ComBase database was used to estimate growth parameters. The calculated distribution of pathogen concentrations in a number of commodities was combined with probabilistic dietary exposure and dose-response models in order to estimate the disease incidence in a population of consumers, using national consumption surveys from the EU and the U.S. (NHANES). Cloud computing was used in order to handle the large data sets and computational effort required.

Results: An assessment involving 100,000 simulated consumers using the U.S. NHANES survey, 40 food beef commodities contaminated with varying levels of *E. coli* O157:H7, under varying multi-stage environmental conditions, can be completed in less than 30 minutes. Complete output for a given assessment was stratified to determine, e.g., the drivers of pathogen growth, sensitivity to different elements of the food chain, consumer exposure, vulnerable subpopulations etc, enabling appropriate risk mitigation strategies to be identified.

Significance: Modeling microbial growth under real environmental conditions in the supply chain can be linked to real consumption patterns in order to assess risk. Multiple scenarios can be examined rapidly to establish optimum food safety strategies for the protection of consumer health.

T2-04 A Bayesian Model for *Bacillus cereus* Contamination in Raw Materials Used for REPFED Production

DAELMAN, JEFF[1]; Membre, Jeanne-Marie[2]; Jacxsens, Liesbeth[1]; Devlieghere, Frank[1]; Uyttendaele, Mieke[1]
1. Food safety and Food Quality, UGhent, Ghent, Belgium.
2. INRA, Nantes, France.

Introduction: Cooked chilled foods, or REPFEDs (refrigerated and processed foods of extended durability), are a heterogeneous group of food products. One of the pathogens of concern is *Bacillus cereus*. To assess the risk related to *B. cereus* in these products, a Quantitative Microbial Risk Assessment (QMRA) has been developed.

Purpose: The first input required in QMRA is the prevalence/level of hazard in the raw materials of which the product is composed. The probability distributions fitted per ingredient will enable to estimate the variability of the *B. cereus* contamination in a crude product composition (e.g. 5% starch, 1% herbs, etc.).

Methods: Microbiological analysis results (n = 541) were collected from multiple REPFED

producing companies. The data, which contained many censored values (below detection limit), was divided into five groups: dry herbs, spices and powders (n = 223, 12.1% positive samples), starch components (n = 64, 6.25%), meat, fish and dairy products (n = 137, 2.9%), fruits and vegetables (n = 89, 1.1%) and ambient stable products (n = 28, 0%). The hierarchical statistical model was set up as follows. It was assumed that (i) the *B. cereus* contamination (in log CFU/g) followed a normal distribution characterized by a mean (μ) and a standard deviation (sd), (ii) the mean of these distributions was product dependent (i.e., one mean for each product group), (iii) while the standard deviation was microorganism dependent (i.e., constant standard deviation whatever the ingredient). This hierarchical model, including censored data, was solved using a Bayesian Inference technique implemented in Winbugs. The Monte Carlo Markov Chain algorithm was run. The model predictions were validated using the actual data by comparing the percentage of samples exceeding the detection limit.

Results: The model resulted in five normal distributions (μ, sd) for the contamination of *B. cereus* (log CFU/g), one for each group. (i) Herbs and spices (-2.7,3.3), (ii) starch components (4.1,3.3), (iii) meat, fish and dairy products (-5.2,3.3), (iv) fruits and vegetables (6.4,3.3) and (v) ambient stable products (-7.2,3.3). Results were satisfactory even if the probability of exceeding the detection limit was slightly higher than the actual "positive" sample percentage.

Significance: The distribution of *B. cereus* concentration in a composite food product at the beginning of production was estimated. The Bayesian inference is a flexible and easy-to-implement technique to assess prevalence/level of hazard in QMRA. It enables to develop a product specific risk assessment.

T2-05 Quantitative Microbial Risk Assessment of *Vibrio parahaemolyticus* in Uncooked Pacific White Shrimp (*Litopenaeus vannamei*) in Thailand

WANASEN, SRI-ANANT[1]; Chokesajjawatee, Nipa[2]; Pornaem, Sarinya[2]; Mahakarnjanakul, Warapa[3]; Suthienkul, Orasa[4]; Tamplin, Mark[1]; Ross, Tom[1]

1. Tasmanian Institute of Agriculture, Food Safety Centre, University of Tasmania, Hobart, TAS, Australia.
2. Food Biotechnology Research Unit, National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani, Thailand.
3. Dept. of Food Science and Technology, Kasetsart University, Jatujak, Bangkok, Thailand.
4. Dept. of Microbiology, Faculty of Public Health, Mahidol University, Bangkok, Thailand.

Introduction: *Vibrio parahaemolyticus* is a marine, Gram-negative bacterium that naturally lives in estuarine water and is one of the leading causes of gastroenteritis disease in Thailand. The Thailand Ministry of Public Health estimated that each year there are approximately 600-1,000 cases of *V. parahaemolyticus* infection associated with the consumption of contaminated seafood. One popular seafood and an important commodity in Thailand is Pacific white shrimp (*Litopenaeus vannamei*), which is sometimes eaten raw. To ensure consumer safety, a risk assessment of *V. parahaemolyticus* in uncooked Pacific white shrimps was performed.

Purpose: The study aimed to estimate the risk of developing illness from the consumption of uncooked Pacific white shrimps contaminated with *V. parahaemolyticus* in Thailand and evaluate existing mitigation strategies.

Methods: Prevalence and concentration of total and pathogenic *V. parahaemolyticus* in shrimps at harvest and processing stages were measured, and potential growth or inactivation during subsequent stages prior to consumption was estimated. Shrimp consumption per capita and behavior of consumers in Thailand was also investigated to enable an estimate of the exposure of Thai consumers to *V. parahaemolyticus* from sashimi-style Whiteleg shrimp. The USFDA's Beta-Poisson dose-response model was used to estimate the probability of illness for different levels of exposure. A stochastic model was developed and a Monte Carlo simulation used to evaluate uncertainty and variability of risk estimates.

Results: The model predicted that the average number of consumers becoming ill from consuming raw shrimps contaminated with *V. parahaemolyticus* per year was 0.27-2.36 per 100,000 population, depending on the levels of *V. parahaemolyticus* at harvest and the storage temperature of prawns throughout the process, distribution and handling chain. For the total Thai population this corresponds to ~180 to 1600 illness per year.

Significance: The risk assessment illustrates that the levels of *V. parahaemolyticus* at harvest and temperature during the supply chain play important roles in levels of *V. parahaemolyticus* and

numbers of illness. The risk assessment can provide a useful tool to facilitate the formulation of guidance on food handling requirements and evaluation of risk mitigation strategies.

T2-06 Farm Food Safety Risk Assessment (FRAMp) Tool – Development and Testing on Fresh Produce Farms

SOON, JAN MEI[1]; Baines, Richard N.[2]

1. Department of Agro-Industry, Universiti Malaysia Kelantan, Pengkalan Chepa, Kelantan, Malaysia.
2. School of Agriculture, Royal Agricultural College, Cirencester, Gloucestershire, United Kingdom.

Introduction: The contamination occurring at the farm level as well as increasing product recalls may be a cause for concern. Hence a farm food safety-risk assessment (FRAMp) tool for fresh produce which identifies potential farm food safety hazards may be timely.

Purpose: The objective of this study is to develop a Farm Food Safety-Risk Assessment Tool (FRAMp) for fresh produce farms. FRAMp serves as a self-assessment risk ranking and educational tool to determine potential farm food safety hazards and level of risk for food contamination.

Methods: FRAMp was developed in Microsoft® Excel spreadsheet software using standard mathematical and logical functions and utilising a qualitative risk assessment approach for farmers to evaluate their food safety practices and to assess potential food safety hazards on their farms. The risk assessment is based on the risk matrix of likelihood × severity, where the farmers will judge the likelihood of the hazards occurring on their farm based on given criteria. Meanwhile, severity scoring is based on literature and expert opinions derived from a separate Delphi study. FRAMp tool was tested on 12 fresh produce farms throughout UK.

Results: FRAMp tool can be described as an illustrative risk ranking tool to facilitate farms to identify potential risk factors during production. Eighty three percent found that it was easy to use, 75% agreed that the tool was practical, suitable for farms to conduct simple risk assessment and farmer-friendly. Refinement of FRAMp was based largely on experimentation with farms. It is best suited for small and medium enterprises (SMEs) to encourage farmers to identify food safety hazards and to develop action plans for improvement.

Significance: An on-farm food safety risk assessment tool may be timely to encourage farms to assess potential hazards and to train both full-time and seasonal farm workers. FRAMp focuses on risk reduction and not risk elimination.

Technical Session 3 – Tuesday, 22 May, 14.00-15.30

Chair, Katie Swanson

T3-01 Oregano and Hibiscus Extracts Inhibit Mold Growth and Extend Shelf-life of Sports Beverage Made with Fish Protein Hydrolysate

LETCHAMO, WUDENEH[1]; Hartman, Thomas G.[1]
1. Center for Advanced Food Technology, Rutgers University, New Brunswick, NJ, United States.

Introduction: Fish protein hydrolysate (FPH), obtained through enzymatic hydrolysis of Atlantic salmon contains indispensable amino acid composition, peptides, minerals and vitamins that could also favor microbial/mold growth. FPH beverages in general are prone to spoilage, formation of gas, malodorous head space and bitter taste during storage.

Purpose: The purpose of this study was to develop natural ingredients to inhibit microbial/mold growth, extend shelf-life and ensure safety while improving the flavor and general appearance of commercially produced FPH based sports beverage.

Methods: A 1:1 oregano leaf hydro-alcoholic extract (OE) and 1:5 hot water hibiscus calyx extract (HE) were prepared from our previous program. FPH was obtained from Zymtech As, Norway. GC analysis of oregano leaf oil showed to contain 60.4% carvacrol, and 1.5 % thymol with other minor components. OE and HE were mixed to a 1:4 ratio. We used 0 (control), 0.5%, 1.0% and 1.5% of the compounded extracts by directly incorporating and mixing in the FPH juice formulated with mango (Mangifera indica), sea berry (Hippophae rhamnoides) and aronia (Aronia melanocarpa) fruit juices. The treatments were replicated three times and stored at 18^{o}C - 20^{o}C in the darkness for 40 days. The FPH juice samples were taken at regular intervals of 2, 6, 10, 14, 20, 30 and 40 days for analysis of visible mold growth, color change, etc, while examining microbial load. The data was subjected to statistical test ANOVA.

Results: We found significant differences between the control and the treatments. Untreated control samples showed visible signs spoilage, mold growth, gas formation, and color changes after 6 to10 days of storage. Treating FPH sports beverage with 1.5% OE and HE extracts inhibited microbial/mold growth, prolonged shelf life, maintained acceptable aroma, flavor and color overhead gas composition compared to the control.

Significance: The data suggest that the 1:1 oregano and hibiscus extracts added to FPH sports beverage at 1.5% concentration significantly improved the quality of the later by inhibiting microbial/mold growth, prolonging the shelf-life and improving palatability. The combination of OE and He can be rationally used in to extend shelf-life in commercial preparation of functional FPH beverage.

T3-02 Hepatitis E Virus: A New Food Safety Issue?

LOISY, FABIENNE[1]; Leturnier, Géraldine[1]; Hattet, Sandrine[1]; Delage, Axelle[1]; Lebeau, Benoit[1]
1. ceeram, La Chapelle sur Erdre, France.

Introduction: In recent years, several autochtonous Hepatitis E cases and a high seroprevalence have been reported in the U.S. and Europe. These data indicate a high prevalence of Hepatitis E virus infections.

Purpose: A potential source of contamination is the consumption of porcine produce or food contaminated by an environmental source. As suggested by the CDC and EFSA, the objective of the study was to evaluate the prevalence of hepatitis E virus (HEV) in food samples, not only evaluating pork produce.

Methods: A global method for HEV detection in environmental or food samples was set up. Based on methods developed for norovirus detection in food samples, standard protocols have been developed and validated. The kit HepatitisE@ceeramTools was used for real time RT-PCR detection. A large prevalence study was then conducted on 440 food samples collected worldwide in food companies in 2011. These samples include pork liver sausages (4), shellfish (36), fruits (77), vegetables (12), herbs and spices (230), process water (62) and ready-to-eat food (20). These samples were also tested for norovirus GI, GII and Hepatitis A virus (HAV).

Results: A limit of quantification for the global method of 500 genome copies was obtained whatever the samples. Below this limit, a sample is considered positive but not quantifiable with reliability. The prevalence levels for norovirus GI, GII and HAV were of 2.95%, 8.6% and 0.45% respectively. The results obtained for HEV demonstrate a prevalence of 0.9% with positive samples

including pork liver sausage, pepper and laurel powder.

Significance: To our knowledge, this is the first large study conducted on HEV prevalence in food samples. Our results demonstrate a prevalence for HEV in food samples, in the same range than hepatitis A virus. These results confirm that Hepatitis E virus should be included in prevalence studies concerning foodborne virus safety issues.

T3-03 Identifying Competencies and Training Needs for Food Safety Regulatory Professionals

BRADSHER, JULIA E.[1]; Kaml, Craig[1]; Wojtala, Gerald[1]
1. International Food Protection Training Institute, Global Food Protection Institute, Battle Creek, MI, United States.

Introduction: In August 2008, The U.S. Food and Drug Administration's Partnership for Food Protection identified the need for a focused food protection training effort to ensure competency and comparability across all levels of government. The reason for this project was to gather underlying data necessary to build an integrated food safety training network.

Purpose: The key purpose of the project was to: 1) convene an expert panel to identify and validate core competencies for food safety regulatory professional and organize into a curriculum framework; 2) survey the universe of food safety training courses available in the United States; 3) catalogue and map existing courses to the curriculum framework; and, 4) identify gaps in training needs, based on existing courses available in the United States.

Methods: The primary methodology was the convening of an expert panel. The information was then validated through a second review by subject matter experts.

Results: The core competencies identified by the expert panel included 134 separate content areas that are organized in a four by three grid. On the vertical axis are four levels of specialty: entry level, journey level, technical level and leadership level. On the horizontal access are three levels of concentration: unprocessed food/raw ingredients, manufactured food and retail food. To date, 818 courses have been identified and catalogued against the IFPTI curriculum framework. Gaps were identified across all levels of the curriculum framework.

Significance: Training is an essential element in an integrated food safety system. The IFPTI Curriculum Framework has created a uniform, competency-based approach for capacity building through standards compliant, science-based, career-spanning professional development for food safety professionals. The Framework also provides a foundation to expand analysis to existing training offerings and gaps on a global level.

T3-04 Efficacy of Yeast Enriched Either with Glutathione (GSH) or with Selenomethionine (SE) to Decrease Ochratoxin A Genotoxicity in Human Renal Cells and in Poultry

Hadjeba-Medjdoub, Kheira[1]; Schrickx, Jan A.[2]; Ballet, Nathalie[3]; Fink-Gremmels, Johanna[2]; PFOHL-LESZKOWICZ, ANNIE[1]
1. Bioprocess & Microbial System, INP/UMR CNRS 5503, Auzeville-Tolosane, France.
2. Faculty Veterinary Medicine, Dept. Veterinary Pharmacology, Utrecht University, Utrecht, The Netherlands.
3. Feed Additives, Lesaffre Feed Additives, Lille, France.

Introduction: Ochratoxin A (OTA) a mycotoxin contaminating mainly cereals but also other crops, can be accumulated in meat, and is not destroyed by food processing. It is nephrotoxic for pig and poultry, and is implicated in Balkan endemic nephropathy and associated urothelial cancer in human. One of the most promising and economical strategy for reducing animal exposure to mycotoxins is the utilization of adsorbents in feed to reduce gastrointestinal absorption of mycotoxins.

Purpose: The aim of this paper is to evaluate the capacity of several yeasts to decrease genotoxicity of OTA and establish if the decrease is only due to absorption of OTA on yeast product.

Methods: Human renal cells were exposed to OTA (10 µM) alone or in presence of yeast enriched with glutathione (GSH) (10 µM) or with selenomethionine (SE) (10 µM). In parallel, 10 poultries per group were fed two days with feed including a yeast product, and then were fed seven days with feed including a yeast product and OTA. Viability of cells was evaluated using MTS test. Genotoxicity was evaluated by detection of DNA-adduct using P32 post labeling method. In addition OTA derivatives formed in human renal cells or in liver and kidney of poultry were analysed after extraction by HPLC coupled to fluorimetric detection.

Results: OTA significantly decreases cell viability (60%; $P < 0.01$) and induces formation of two

OTA-DNA-adducts. Adjunction of pure GSH or GSH-Yeast partially restores cells viability (70% versus 60%; $P < 0.05$) and avoid DNA adduct formation, explained by conversion of OTA into OTB and 4 OH OTA. Pure SE does not restore viability whereas SE-yeast has antagonistic effect (110% versus 60%; $P < 0.01$). SE and SE-yeast increase OTA-DNA adduct formation correlated to the appearance of new OTA metabolites.

Significance: The decrease of OTA toxicity observed with yeast was not only correlated to adsorption but also to biotransformation of OTA which is modulated by yeast. DNA adduct patterns were correlated with OTA derivatives formed in the kidney. GSH-yeast is better to decrease OTA genotoxicity.

T3-05 Evaluation of the Daily Intake of Carcinogenic Mycotoxins – Molecular Evidence of Synergystic Effects

Tozlovanu, Mariana[1]; Hadjeba-Medjdoub, Kheira[1]; Peraica, Maja[1]; Mandervile, Richard A.[1]; PFOHL-LESZKOWICZ, ANNIE[1]
1. Bioprocess & Microbial System, INP/UMR CNRS 5503, Auzeville-Tolosane, France.

Introduction: Crops are susceptible to fungal attack in field or during storage. These fungi may produce mycotoxins which are very stable and could be found in final products such breakfast cereal or meal. Co-occurrence of mycotoxins, even at low doses increases the risk of cancer development.

Purpose: The aim of this paper is the risk assessment of carcinogenic mycotoxin, evaluating the occurrence of the mycotoxins in food and the combined toxic effects in cell culture and *in vivo*.

Methods: Several crops and food have been collected on markets all over the world as consumers do. Ochratoxin A (OTA), citrinine (CIT), fumonisins (FB), aflatoxin (AF) and zearalenone (ZEA) were extracted using partion methods, and analysed by HPLC with fluorimetry detection. Human kidney cells (HK2) were exposed to OTA, CIT, ZEA, FB alone or combined. Cell viability was analysed using MTT test. Genotoxicity was evaluated by DNA adduct detection using 32P-post labelling technique. Rat and pig were fed with OTA and/or FB. DNA adduct formation in kidney of animals were analysed.

Results: We have analysed several cereals (rice, wheat, maize) from different origins (France, Vietnam, Moldavia, Czech Republic, Morocco) but also olives, coffee and breakfast cereals. The contamination of rice by these mycotoxins was at an alarming rate; especially AFB1. OTA, AFB and FB were also detected in maize and wheat to levels over the acceptable EU limit. OTA has been detected in 75% of the breakfast cereal, ranging from LOQ (< 0.2 µg/kg) to 12.7µg/kg. All samples of ground coffee contain OTA, ranging from trace (< LOQ, 5 samples) to 11.9 µg/kg. The amount of OTA passing in the beverage ranged between 20-140%. Based on a typical menu, including some of these ingredients and using the average mycotoxin's amount for calculation, we observed that the tolerable daily intake (TDI) was respectively 39-fold; 7-fold and 3-fold higher than the virtual safety dose (VSD) established for AFB1, OTA and FB.

The simultaneous presence of OTA with either CIT or FB or ZEA, modify human kidney cells (HK2) cell viability. The main covalent OTA DNA-adduct, found in human tumours, identified as C8 dG-OTA is increased by simultaneous presence of CIT and OTA. ZEA also increase OTA genotoxicity in human kidney cells. This is due to modulation of biotransforming enzymes (cytochromes, cyclooxygenase) by the different mycotoxins. In the same way, in in vivo studies on rat and pig fed simultaneously by OTA and FB in feed formation of OTA specific DNA adducts including C-C8dG OTA adduct and the both OTHQ related adduct increase.

Significance: The data indicate that foods are often contaminated simultaneously with several mycotoxins. Estimated intake overpasses the virtual safety dose for almost all mycotoxins analysed. Exposure to low concentration of mycotoxins considered as safe, when they are present together can lead to dramatic effect. Until now, regulation does not take into account co-contamination.

T3-06 Performance Objectives Related to Process Hygiene Criteria: The hPO Concept

BOULAIS, CHRISTOPE[1]; Peladan, Fabrice[1]
1. Danone Research, Palaiseau, France.

Introduction: The Codex Alimentarius provides a structured approach to risk-based food safety management. It relies on the establishment of Food Safety Objectives (FSO) and Performance Objectives (PO) for microbiological hazards. In order to evaluate whether a food safety management system delivers the appropriate level of control and whether lots comply with FSO or PO, within-lot microbiological testing against pre-specified criteria is often used. Published work describes how food

safety criteria can be related to FSO and PO.

Purpose: The EU regulation 2073/2005 specifies process hygiene criteria for *Enterobacteriaceae* in different dehydrated milk-based products. They are applicable at the end of the manufacturing process. The objective was to estimate, for each type of product, the maximum acceptable level of *Enterobacteriaceae* at the end of the manufacturing process that is required to comply with the corresponding criterion.

Methods: The within-lot distribution of *Enterobacteriaceae* in dehydrated milk-based products was described by a bounded log-normal distribution with intermediate variability (0.4 log CFU/g). The probability of lot acceptance was calculated for each criterion as a function of the maximum level of *Enterobacteriaceae* in the lot. The maximum acceptable level corresponding to a probability of acceptance of 95% was called the hygiene performance objective (hPO) so as not to misrepresent the original PO concept related to pathogens.

Results: The maximum acceptable levels (hPO) of *Enterobacteriaceae* at the end of the manufacturing process were estimated at 1.1, -2.2 and -2.5 log cfu/g for milk powder and whey powder, follow-on formulae, and dried infant formulae, respectively.

Significance: Metrics used in risk analysis constitute useful tools not only for pathogens but also for process hygiene indicators. In the case of dehydrated milk-based products, the establishment of hPO at the end of the manufacturing process allows establishing hPO at other points along the food chain by using, for instance, the ICMSF equation in combination with empirical predictive models. They can further be used to establish process hygiene criteria at points of the food chain where regulatory criteria are missing.

Poster Session 1 – Monday, 21 May, 09.00-17.00

Authors will be present at their posters during the conference breaks

P1-01 Antibacterial Activity of Pullulan Films Enriched with Natural Plant Extracts from Sage (*Salvia officinalis* L.)

KRASNIEWSKA, KAROLINA[1]; Gniewosz, Margorzata[1]; Przybyl, Jaroslaw L.[2]; Weglarz, Zenon[2]

1. Faculty of Food Sciences, Warsaw University of Life Sciences-SGGW, Warsaw, Poland.

2. Faculty of Horticulture and Landscape Architecture, Warsaw University of Life Sciences-SGGW, Warsaw, Poland.

Introduction: Traditional packages do not assure completely safe foods. It seems advisable, therefore, to cover surfaces of food products with edible films, which by being additionally enriched with active substances having antimicrobial properties protect food products against the development of detrimental microflora.

Purpose: The aim of this work was to investigate the antibacterial properties of pullulan films containing different concentration of extracts from sage.

Methods: Pullulan films were enriched with water extracts (PWS) and ethanol extract (PES) from sage. The films activity were tested against: *Bacillus subtilis*. ATCC 6633, *Salmonella aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Salmonella* Enteritidis ATCC 13076. Film solution containing pullulan at 8%, demineralized whey powder at 3% and glycerol at 4.8-7%. The final concentration of extracts in dried films were: 10 mg/cm^2, 20 mg/cm^2 and 40 mg/cm^2. The agar diffusion test was used for determining the antibacterial effects of films. The films discs (1 cm^2) placed onto Mueller-Hinton agar plates. These had been previously seeded with inoculums 1×10^8 CFU/ml of tested bacteria and incubated at 37^{o}C for 24 h. Diameter of the inhibitory zone was measured and compared using multifactor ANOVA.

Results: Pullulan films enriched with sage extracts showed antibacterial properties against tested bacteria. PES films shown significant difference ($P < 0.05$) of inhibition against *B. subtilis* and *E. coli* than PWS films. As the concentration of extracts increased the zone of inhibition also increased significantly for all tested bacteria. The greatest zone of inhibition was observed for PES film at 40 mg/cm^2 against *S. aureus* and *B. subtilis* with zone area of 25.46 ± 0.95 mm and 22.33 ± 0.39 mm, respectively. For, PWS films the greatest zone of inhibition was observed for *S. aureus* with zone area of 28.48 ± 1.63 mm.

Significance: Our results suggest that incorporating antimicrobial agents into pullulan films is promising and has good potential in many food applications.

P1-02 Characterization of the Phage Endolysin PlyP825 for Control of *Listeria monocytogenes* in Food

SCHERZINGER, ANNA[1]

1. Hyglos GmbH, Bernried, Germany.

Introduction: The gram-positive bacterium *Listeria monocytogenes* is known as the causative organism in several outbreaks of foodborne disease. *L. monocytogenes* is an opportunistic, intracellular pathogen and leads to a variety of symptoms including diarrhea, abortion and encephalitis. For risk groups like young, old, pregnant, and immunocompromised people Listeriosis is a life-threatening infection with a mortality rate of up to 30%.

To prevent listeriosis, the destruction or inhibition of the bacteria in food is obligatory. A powerful and species specific tool is provided by bacteriophage endolysins. These enzymes hydrolyse bacterial peptidoglycan, the stabilizing polymer of the bacterial cell wall, and lead thus to bacterial cell disruption.

Purpose: Previous described phage endolysins show deficiencies for application like poor enzyme stability or activity in food matrix. Here the novel *Listeria endolysin* PlyP825 is described.

Methods: PlyP825 was characterized for domain structure, serovar specificity, thermal and trypsin stability, pH and salt optimum, tolerance of the common food additive EDTA, Minimum Inhibition Concentration (MIC), and efficacy in milk.

Results: PlyP825 consists of an N-terminal enzymatic active domain with putative L-Ala-D-Glu peptidase specificity and a C-terminal cell binding domain. Most interestingly the purified endolysin

lysed all tested Listeria strains of serovars 1–6 and showed MIC values between 3.6–14.3 pmol/ml. Furthermore PlyP825 destroyed more than 4 log *Listeria* cells in milk exposing considerably better performance compared to hitherto described *Listeria* endolysins.

Significance: The characterization of PlyP825 revealed high potential for the application in food. Most notably, PlyP825 shows superior effectivity in milk and can thus be even applied to such complex food matrices like milk products to prevent listeriosis.

P1-03 *Lactobacillus sakei* as an Alternative for Synthetic Preservatives against Foodborne Pathogens

EIDUKONYTE, DALIA[1]; Juodeikiene, Grazina[1]; Paskevicius, Algimantas[2]
1. Dept. of Food Technology, Kaunas University of Technology, Kaunas, Lithuania.
2. Laboratory of Biodeterioration Research, Institute of Botany of Nature Research Centre, Vilnius, Lithuania.

Introduction: In recent years health-conscious consumers are looking for natural foods without chemical preservatives that will fit in their healthy lifestyles. Bacteriocin-like inhibitory substances producing lactic acid bacteria have gained importance as natural biopreservatives for the control of spoilage and pathogenic organisms in different foods.

Purpose: The aim of this study was to evaluate antimicrobial activity of *Lactobacillus sakeii* KTU05-06 (it was previously found that these isolates produce antimicrobial compounds – bacteriocins (sakacin), that are not identical to any other known) against foodborne pathogens such as *Bacillus*, *Pseudomonas*, *Escherichia*, *Listeria*, *Salmonella* genera and some fungi isolated from various foods.

Methods: Antimicrobial activity determination was performed using the agar well diffusion assay method by measuring the inhibition zones diameter (mm) after cultivation on appropriate medium for 48 h. Antimicrobial activity against indicator organism was evaluated according to the following scale: highly sensitive (15–20 mm), sensitive (10–15 mm), a slightly sensitive (10–7 mm) and resistant (less than 7 mm). The growth rate of *B. subtilis* pre-treated with sakacin was measured using a microplate reader.

Results: *L. sakei* produced sakacin was able to significantly ($P < 0.05$) increase the length of the lag phase during growth of *B. subtilis*. High sensitivity to *L. sakeii* show *B. thuringiensis*, two *B. subtilis* strains, *P. gladioli* pv. aliicola, *P. marginalis*, two *P. cepacia* strains and *E. coli*. As sensitive were determinate *P. faecilis*, *P. aureofaciens*, *P. cichorii* and three *P. fluorescens* strains. Slightly sensitivity to *L. sakei* show *P. marginalis*, *S.* Typhimurium. whereas resistant were *P. pseudoalcaligenes* and two *Listeria monocytogenes strains*. Besides, observed fungistatic activities against *Aspergillus versicolor*, *Penicillium expansum*, *Aspergillus niger*, *Debaryomyces hansenii*, *Candida parapsilosis* and fungicidal against *Fusarium culmorum* (inhibition zone 13.5 ± 1.4 mm).

Significance: The use of *L. sakeii* producing antimicrobial substances may be a good alternative to avoid the microbial spoilage and obtain more safe food products. The research was funded by a grand (project SVE-09/2011 BIOFITAS) from Research Council of Lithuania.

P1-04 Efficacy against *Escherichia coli* and *Staphylococcus aureus* of UV Cured Coatings Containing Quaternary Ammonium Groups

LISANTI, CLARA[1]; Gozzelino, Giuseppe[1]; Beneventi, Sara[2]
1. Scienza Materiali e Ingegneria Chimica, Politecnico di Torino, Torino, Italy.
2. Ecobioqual Laboratory, Environment Park, Torino, Italy.

Introduction: Coatings with polymer films endowed with biocidal properties can represent a way to sanitize surfaces, thus avoiding cross-contamination and increasing safety in food manufacturing. UV curing is a well-established technology for a fast printing or coating of different substrates and, at the same time, is a polymerization technique to insert monomers that can confer different properties to the polymer. Biocide molecules, structurally modified in such a way to participate the polymerization process, can be chemically linked to the polymer network and can generate coatings with antibacterial properties without the drawback of biocide release into the environment.

Purpose: The aim of the research is to obtain functionalized polymers, coated on different substrates, able to develop biocidal activity by contact with a bacteria suspension. The coating activity should be conferred through the co-polymerization of a commercial UV curable resin with monomers

containing biocide moieties, such as quaternary ammonium groups (QAM), obtained by chemical synthesis.

Methods: The QAMs, which are responsible for biocidal activity, were synthesized through the reaction between a tertiary amine, dimethyl aminoethyl methacrylate (DMAEMA) and alkyl bromides, having different alkylic chain length. Different QAMs, mixed in proper proportion with diacrylic resins, were UV photo-polymerized to form a polymeric crosslinked film and the coatings were placed in contact with Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli*) bacterial strains to test the bactericidal activity.

Results: The biological tests, carried out on polymeric coatings with different contents of QAMs having C8, C10, C12 and C16 alkylic chain length, have shown that C12-QAMs and C16-QAM have a significant and similar efficacy, with the action against the *E. coli* being more powerful than the one against the *S. aureus*.

Significance: The obtained results suggest that QAMs are interesting components for UV curable formulations aimed to sanitize surfaces, the length of the alkylic chain being the critical parameter that control the biocide activity.

P1-05 Application of PCR Typing for Investigating *Listeria monocytogenes* Transmission along the Gorgonzola PDO Production Chain

NUCERA, DANIELE M.[1]; Grassi, Maria Ausilia[1]; Morra, Patrizia[1]; Giaccone, Daniele[3]; Meda, Piero M.[2]; Tabacco, Ernesto[2]; Borreani, Giorgio[2]
1. Dipartimento di Patologia Animale, Università degli Studi di Torino, Grugliasco (TO) , Italy.
2. Dipartimento di Agronomia, Selvicoltura e Gestione del Territorio, Università degli Studi di Torino, Grugliasco (TO) , Italy.
3. Associazione Regionale Allevatori del Piemonte (ARAP), Torino, Italy.

Introduction: Literature shows silage as a likely source of *Listeria monocytogenes* for milking cows, which shed the pathogen in farm environments (Nightingale, 2004). Presence of *L. monocytogenes* in farms could lead to its introduction and persistence in processing plants (Lomonaco et al. 2008), as happens in the production chain of Gorgonzola Protected Designation of Origin (PDO). In fact, its peculiar production processes allow survival and growth of *Listeria monocytogenes*, which may contaminate production environments as well as cheese rinds.

Purpose: This study was aimed at evaluating the presence of the pathogen in dairy farms (N = 20), and in the plant of Gorgonzola PDO cheese they give milk to. In addition, genetic typing was performed in order to detect possible transmission links.

Methods: A survey was carried out over 2 years in Piedmont (Italy); samples were collected from primary production (feed, N = 240; feces, N = 40; milk filters, N = 40, and milk, N = 80) and from manufacturer processing environments/equipments (N = 108). *L. monocytogenes* detection was performed using ISO 11290-1:1996/Amd 1:2004 (2004). For each positive sample, 1 to 5 colonies were confirmed by specie-specific PCR (D'Agostino et al., 2004), and selected for PCR typing (Jersek et al., 1999). Results were analyzed by software Bionumerics (Applied Math - Belgium), producing a dendrorgam generated by UPGMA algorithm based on Dice similarity coefficient. Strains were considered identical if their similarity was above 95%.

Results: *L. monocytogenes* was detected in: 10 feed, 3 feces, 1 milk and 8 dairy plant environments. Typing results, showed that all isolates were highly similar (74.5%): a set of 17 isolates (5 PCR profiles) retrieved from milk, feed, and dairy environments were genetically homogeneous (84.4%). Moreover, 2 strains were distributed across farms and 1 was persistent in processing environments.

Significance: *L. monocytogenes* isolated from the selected production chain were genetically homogeneous, indicating a putative transmission. The absence of identity between genotypes retrieved from primary production and from processing environments, may be related to environment adaptation of the strains (Verghese et al., 2011). Milk may not be considered the primary source of *L. monocytogenes*. contamination of the producing plant, considering the low isolation frequency in milk. Thus the introduction of the pathogen may be due to non-compliances with Good Hygienic Practices (GHP) (i.e., unloading truck tanks). These findings strengthen the need for strict application of GHP in order to prevent contamination. Funds granted by Regione Piemonte - Direzione Sviluppo Agricoltura – 2007.

P1-06 WITHDRAWN

P1-07 Problems in Detection of *Listeria monocytogenes* with Traditional Method

ENGELHARDT, TEKLA[1]; Ágoston, Réka[1]; Kiskó, Gabriella[1]; Mohácsi-Farkas, Csilla[1]
1. Microbiology and Biotechnology, Corvinus University of Budapest, Faculty of Food Science, Budapest, Hungary.

Introduction: *Listeria monocytogenes* is a widely spread ubiquiter pathogen which is liable for several foodborne outbreaks. Therefore, there is an increasing demand for a reliable detection method for *Listeria monocytogenes*. The International Organization for Standardization published the 11290 standards about horizontal methods for enumeration and detection of *L. monocytogenes* in 1996. Numerous scientific literatures discuss the problems of several parts of the above-mentioned method especially the enrichment steps with half Fraser (hFB) and Fraser broth (FB) in presence of *L. innocua*.

Purpose: The aim of our work was to study the potential inhibitory effect of *Listeria* enrichment broths and *L. innocua* on the growth of *L. monocytogenes*.

Methods: Different mixtures of four *L. monocytogenes* strains (C1, L4, L16, T3) and one strain of *L. innocua* (C6) were inoculated into hFB and FB (Merck) for enrichment steps. After enrichment in hFB and FB, samples were inoculated onto Ottaviani-Agosti (ALOA) agar (Merck). Growth curves in hFB and FB of one selected strain (T3) individually and in mixed culture with *L. innocua* were also determined.

Results: Among the *L. monocytogenes* strains selected, L16 was not able to produce halo on ALOA agar. Growth of *L. monocytogenes* C1 and T3 strains was inhibited by *L. innocua* C6 when proportion of C6 was higher than 1:1. When *L. monocytogenes* T3 grew in presence of *L. innocua* C6, the initial one log cycle difference in hFB increased to 1.5 log cycle in FB, although their growth curves showed the same tendency.

Significance: The growth of *L. monocytogenes* T3 was affected by both hFB and FB, however, this inhibition was more pronounced in presence of *L. innocua* C6. This work was supported by the TÁMOP-4.2.1/B-09/1 and TÁMOP-4.2.2/B-10 project.

P1-08 Validation of G12 Sandwich ELISA for the Detection of Gluten

ROGERS, ADRIAN[1]
1. Romer Labs UK, Runcorn, United Kingdom.

Introduction: Coeliac disease is an immune-mediated enteropathy caused by the ingestion of gluten, a protein fraction found in certain cereals. Coeliac disease occurs in genetically predisposed persons and leads to the destruction of the microscopic finger-like projections of the small intestine, called villi. Coeliac Disease currently affects roughly 1% of the world's population, primarily adults. Immunotoxic gluten peptides, such as the fragment called 33-mer, which are resistant to degradation of digestive enzymes appear to trigger Coeliac syndrome. Homologues of this peptide are found in every food grain that is toxic to Coeliac Disease patients.

A monoclonal antibody, called G12, which detects the 33-mer from α2-gliadin (identified as the principal contributor to gluten immunotoxicity), was developed. The sensitivity of the G12 antibody is significantly higher than equivalent methods recognising other gluten epitopes.

Purpose: The new G12 antibody was used by Romer Labs® to create a sandwich format ELISA for the detection of low levels of gluten in foods.

Methods: The Limit of Detection of the AgraQuant® Gluten G12 Test Kit was 2 mg/kg Gluten while the lower Limit of Quantitation was set at 4mg/kg Gluten. A range of samples including grains, nuts, seeds and naturally gluten-free foods were tested using the AgraQuant® Gluten G12 Test Kit.

Results: Sample extractions were performed using a proprietary Extraction Buffer developed to improve gluten extraction from heat-treated and processed samples, no cross reactivity was observed with any of the non-gluten containing commodities. Recovery of low levels of gluten from difficult food matrices was also investigated with recoveries ranging from 90.4 to 144.7%.

Significance: Results indicate that the AgraQuant® Gluten G12 Test Kit from is suitable for use in assessing compliance with the new EU Regulation (No 41/2009) concerning the composition and labelling of foodstuffs suitable for people intolerant to gluten.

P1-09 Evaluating Food Safety Management Performance in a Milk Pasteurising Facility Using a Microbiological Assessment Scheme

KENNEDY, THOMAS G.[1]
1. Department of Agriculture, Food and the Marine, Limerick, Ireland.

Introduction: Milk and milk products are a heterogeneous group of food products. Depending on the heat treatment applied during production, different pathogens pose risks. The pathogens of concern are *Listeria monocytogenes*, *Bacillus cereus*, *Salmonella* spp., *Staphylococcus aureus* and *Escherichia coli* since these may survive pasteurisation treatments.

Purpose: The performance of a food safety management system (FSMS) in a drinking milk pasteurisation establishment was measured using a microbiological assessment scheme (MAS). The MAS consisted of multiple sampling locations along the processing line consisting of high-risk raw materials, the processing environment, process water and end products.

Methods: A total of 1268 samples were analysed over an 18-month-period. Nine microbial parameters (*Salmonella* spp., *Listeria* spp., *Bacillus cereus*, *Staphylococcus aureus*, Total Bacterial Counts (TBC), *Enterobacteriaceae*, *Escherichia coli*, Faecal *enterococci* and coliforms) were enumerated using standard methods. Results were benchmarked against legal, industry and best practice norms.

Results: 100% ($n_0 = 233$) of raw milk samples met the European Union TBC standard of $<10^5$ CFU ml^{-1}, however, *Listeria innocua* was isolated in 3% ($n_1 = 134$) of raw milk samples. *Listeria* spp. ($n_2 = 128$), *Salmonella* spp. ($n_3 = 118$), *Staph. aureus* ($n_4 = 118$), *Enterobacteriaceae* ($n_5 = 114$), *B. cereus* ($n_6 = 38$) and *E. coli* ($n_7 = 23$) were not detected in any end products. *Listeria welshimeri* (a poor hygiene indicator) was identified in 2% ($n_8 = 153$) of environmental samples. *Salmonella* was not isolated in any of the 63 environmental sample. 6% and 1% of operator hand swabs ($n_9 = 100$) had TBC and *Enterobacteriaceae* counts respectively in excess of best practice norms of 10^2 CFU cm^{-2} and 10^1 CFU cm^{-2} respectively. One (2.2%) water sample ($n_{10} = 46$) had a coliform count of 201CFU ml^{-1} whereas five samples (11%) had TBC counts above acceptable norms.

Significance: The results indicate that the FSMS is producing a safe product. The MAS is an effective risk assessment tool that is useful to assess the overall performance of the FSMS and allows a more targeted use of resources to implement improvement. Satisfactory end product microbiological results indicate that cold chain control, post pasteurisation contamination from dry ingredients (e.g., buttermilk cultures), packaging or unsanitary pipe work are not issues for this plant. However, the prerequisites of environmental sanitation, raw material supply and control, water treatment and storage and staff hygiene are the areas within the FSMS that pose the greatest risks.

P1-10 Older Adult Consumers' Attitudes and Risk Perceptions Associated with Food Safety in the Home

Evans, Ellen[1]; REDMOND, ELIZABETH[1]
1. Cardiff School of Health Sciences, Cardiff Metropolitan University, Cardiff, Wales, United Kingdom.

Introduction: Older consumers are more susceptible to acquiring food poisoning due to weakened immune function. Therefore it is particularly important for older adults to implement safe food-handling/storage practices in the home to reduce the risk of illness. To enable design and development of effective risk communication strategies, there is a need to understand why behaviours are implemented in conjunction with attitudes, risk perceptions and behavioural influences.

Purpose: This study aims to determine older adults' perceptions of risk, control and responsibility in the home and assess attitudes held towards key food safety behaviours.

Methods: Structured face-to-face interviews were conducted with 100 older adults (> 60 years) using a computer-assisted-personal-interview (CAPI) approach, enabling collection of qualitative and quantitative data. Attitudes toward key food safety behaviours were assessed using 5-point Likert-type scales and risk perception, control and responsibility were assessed using a variation of a visual analogue scale(VAS).

Results: The majority of older-adults (90%) believed they knew all food safety practices necessary to ensure food they prepared is safe to eat, 89% believed were unlikely to get food poisoning from their own home. However many older adults indicated negative attitudes towards practices required to ensure food safety. For example, 67% believed eating food beyond its 'use-by' date was acceptable. 56% believed the smell/appearance of food were good indicators of food safety and enabled them to "tell that food was safe to eat". Eighty-eight percent believed themselves to have no/low-risk of getting food poisoning, 81% perceived themselves to have total/nearly-total control of preventing food

poisoning and 86% thought themselves to have complete/nearly-complete responsibility for preventing food poisoning. A significant correlation ($P < 0.05$) was identified between perception of personal risk of illness and personal control and responsibility concerning food-handling/storage in the home.

Significance: Attitudes and risk perceptions identified in this study may prevent older adults from implementing safe food-handling/storage practices thus increasing the risk of food poisoning. Perceived personal invulnerability may undermine food-safety education. Interventions are required to raise awareness of food safety risks in the home and improve associated behaviours.

P1-11 Identification of Behavioural Risk Factors Associated with Listeriosis in the Home

Evans, Ellen[1]; REDMOND, ELIZABETH[1]
1. Cardiff School of Health Sciences, Cardiff Metropolitan University, Cardiff, Wales, United Kingdom.

Introduction: Reported European listeriosis bacteraemia incidence has doubled since 2001, almost exclusively among consumers > 60 years. Many cases are sporadic, and foods associated with transmission are principally ready-to-eat (RTE) with extended (usually refrigerated) shelf-life capable of supporting *L. monocytogenes* growth. Such foods are frequently brought into the home for preparation/consumption and therefore, implementation of safe food-handling/storage behaviours is crucial to reduce the risk of listeriosis. Currently there is a lack of data to inform understanding of behavioural risk factors that relate to possible listeria infections in the home.

Purpose: This study aims to identify purchase, storage and consumption behavioural factors associated with the risk of listeriosis among older-adults (> 60 years).

Methods: A quantitative self-complete-questionnaire was self-administered to older-adults (n = 100) to ascertain self-reported shopping habits, purchase preference/frequency, storage behaviours, 'use-by' date adherence and refrigeration temperatures.

Results: All older-adults reported purchasing RTE-foods associated with listeriosis/*L. monocytogenes* prevalence, with 89% reporting doing so every week. Post-purchase food-handling behaviours associated with potential temperature abuse were reported, including failure to always go straight home after completing food shopping (36%), failure to always put food shopping away immediately (12%) and never using a coolbag when shopping for RTE-foods (61%). The majority reported prolonged storage of opened-RTE-foods, with only 28% reporting consumption of sliced cooked ham within the recommended 2 days after opening. Discrepancies were identified between knowledge and reported behaviours in relation to 'use-by' dates. Although the majority (72%) believed 'use-by' dates were the best indicator of food being safe to eat and 62% reporting to 'always' take note of 'use-by' dates, many (57%) reported consumption of foods beyond 'use-by' dates. The majority (84%) did not know the recommended maximum refrigeration temperature for safe food-storage (5°C), thus limiting the ability to control and mange risks associated with microbial hazards.

Significance: Cumulative findings suggest that older-adults may implement food-handling/storage behaviours that may increase risks associated with listeriosis in the home. Findings may be used to develop food-safety education approaches designed to specifically decrease implementation of unsafe food-handling/storage and consumption behaviours, and reduce the risks associated with listeriosis.

P1-12 Hospital Caregiver Risk Perceptions and Attitudes toward Powdered Formula Milk Use, Preparation and Storage – Implications for Microbiological Safety

REDMOND, ELIZABETH C.[1]; Griffith, Chris[1]
1. Food Industry Centre, Cardiff Metropolitan University, Cardiff, United Kingdom.

Introduction: Hospitals are the reported location for most outbreaks of *Cronobacter* spp. associated with powdered-formula-milk (PFM). Qualitative research suggests differences in infant-feeding practices in hospitals in terms of use, perceived acceptability and preparation of PFM. Some hospitals use ready-to-consume (UHT) products, others have PFM feeds prepared in central units or hospital wards.

Purpose: The aim of this study was to obtain quantitative data from hospital nurses (HN) and hospital midwives (HMW) who care for infants < 6 months in hospitals, to identify cognitive factors that may influence implementation of unsafe preparation and storage of PFM in hospitals.

Methods: Postal self-complete questionnaires (n = 2000) were administered to HN and HMW in Maternity, Pediatrics and Neonatal Departments in 10% (n = 47) of UK Hospital Trusts (post-ethics approval). Attitudes towards PFM use, preparation/storage were assessed using 5-point Likert-type

scales; perceptions of risk and control were determined using a variation of a visual-analogue-scale (VAS).

Results: Cumulatively, the majority of HN and HMW perceived recommended practices to reduce the risk of illness from feeding with PFM to be important. However, practices associated with preparing 'one-feed-at-a-time', feeding reconstituted feeds immediately after preparation and reconstitution using boiled water cooled for < 30minutes/at >70^{o}C were not considered to be important by 18% of HN/HMW. The majority (60 – 77%) of HMW/HN believed PFM was a sterile product and 77% were unaware of the association between PFM and *Cronobacter* spp./*Salmonella*. Findings suggested perceptions of 'optimistic-bias' and the 'illusion-of-control' whereby HMW/HN considered the risk of infant illness after consuming PFM made-up by themselves to be less than other HMW/HN/parents; similarly, HMW/HN perceived they had more control and were more conscious about hygiene/safety than other HMW/HN/parents.

Significance: Attitudes and risk perceptions identified in this study may prevent HMW and HN from implementing recommended risk-reducing behaviours when preparing/feeding PFM and therefore increase the potential risk of illness. Risk communication initiatives are required to raise awareness of the microbial hazards in PFM and improve PFM preparation/storage behaviours in hospitals.

P1-13 Evaluation of a Knowledge Transfer Feasibility Project to Improve Technical Operations and Microbiological Safety in Food-sector Small and Medium-sized Enterprises (SMEs) in Wales, UK

REDMOND, ELIZABETH C.[1]; Lloyd, David C.[1]
1. Food Industry Centre, Cardiff Metropolitan University, Cardiff, United Kingdom.

Introduction: The KITE (Knowledge, Innovation, Transfer, Exchange) Project was developed and implemented in response to the reported decline of food technologists in Wales (UK) and the critical need to for food-sector SMEs to meet technical demands required for business sustainability. This feasibility project, based on a collaborative partnership between an industrial (SME) partner, a knowledge-based partner and an affiliate (graduate/individual with industrial-experience), aimed to increase food science/technology knowledge and improve technical compliance with 3rd party accreditation standards in food-sector SMEs.

Purpose: A process and output/outcome evaluation of the KITE project was undertaken to assess procedural development/implementation and determine impact.

Methods: In-depth interviews (n = 30) were conducted with KITE and SME Managers, Affiliates and Technologists; quantitative surveys (n = 43) were administered to collaborative partners. Project approaches/reports were reviewed and media reports (n = 90) relating to SME Partners analysed using content analysis.

Results: During KITE Project implementation (3 years) significant outputs have been delivered and positive primary and secondary impacts reported. Evaluation of project processes found that set-up procedures were time-efficient and a flexible approach was used to meet changing SME business needs. Development of innovative processes accommodated employment requirements of private/public sector KITE partners. Provision of technical expertise to affiliates in 21 SMEs resulted in changes in food safety culture and achievement of 12BRC (5/6) accreditations (77% grade A),10 retail quality standards and 4 Safe-and-Local-Supplier-Approval (SALSA) awards. SME Managers reported increased sales exceeding £10million (€12million) as a result of new customers obtained due to improved technical performance and innovative new-product-development (n ≥ 187products). Food production cycles flattened and seasonal fluctuations in production for some SMEs allowed job retention in off-peak periods. Overall, 550 jobs have been safeguarded; 195manufacturing roles and 28 quality assurance roles created, reversing the migration of technical skills away from Wales.

Significance: Cumulatively, the KITE feasibility Project has facilitated medium and long-term strategic development, technical innovation, safety and maintenance/improvement in food production processes in food manufacturing/processing SMEs in Wales, UK. Furthermore, SME partners experienced increased sales and job retention as a result. Evaluation findings suggest the potential for international application.

P1-14 WITHDRAWN

P1-15 Best-before Date Mass Experiment – Food Storage Temperatures Registered by Swedish School Pupils

MARKLINDER, INGELA[1]; Eriksson, Mattias[2]
1. Dept. of Food, Nutrition and Dietetics, Uppsala University, Uppsala, Sweden.
2. Dept. of Energy and Technology, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Introduction: The fourth Friday in September is Researchers' Night, instituted by the European Commission. In autumn 2011, a mass experiment focusing on refrigeration temperatures was organized through 72 Swedish schools. Food storage temperature has a major impact on bacterial growth. Thus a correct temperature might increase the best-before durability of food, which is positive in an environmental perspective.

Purpose: The objective was to investigate the food storage temperature in Swedish refrigerators and to use best-before-date labeling to determine whether school children considered the food items edible. Another purpose was to see if the mass experiment would increase interest and knowledge of food storage and resource management among school pupils.

Methods: The experiment was performed by 1,812 school pupils attending grades 2 through 12 and 'folk high school,' who registered the temperature on different shelves in their own family's refrigerator. The teachers handed out thermometers (Moller-Therm (+0.5/- 0.1°C) and instructions. The school pupils performed the measuring at home and registered their data online; almost half of them (44%) got some help to accomplish the registration.

Results: The temperature on middle shelves at the back was the coldest (average 4.8°C, SD 3.1). The storage temperatures of dairy products, meatballs and sausages exceeded the recommended 8 °C in almost one quarter of cases. In 75 percent ground beef was stored at higher temperatures than recommended (average 5.5°C (SD = 3.72). Even though the use-by date had been passed in 30 percent of cases, the pupils did not rate them as inedible. Just 3.7 percent of the food items were determined to be inedible although the best-before date were passed in 11.4 percent of cases. The experiments contributed to increased discussions of food hygiene, food storage and resource management. The majority of the teachers also agreed to that pupils' interest and understanding of food labeling, food hygiene, food storage and environmental aspects increased.

Significance: A relatively high proportion of food items were stored at higher temperatures than recommended and the pupils sometimes fail to use the best-before date. But the mass experiment contributed to an increased interest and knowledge of food storage and resource management among pupils. Thanks to Vetenskap & Allmänhet (VA) funding and to Lotta Tomasson at VA for organizing the project. Thanks to all participating pupils and teachers.

P1-16 HACCP for Space Flight or Cucumber Production? A Delphi Study of On-farm HACCP and Food Safety Hazard Assessments

SOON, JAN-MEI[1]; Baines, Richard N.[2]
1. Department of Agro-Industry, Universiti Malaysia Kelantan, Pengkalan Chepa, Kelantan, Malaysia.
2. School of Agriculture, Royal Agricultural College, Cirencester, Gloucestershire, United Kingdom.

Introduction: The Delphi process is a standard procedure for eliciting expert opinion. The Delphi technique is best used to tackle a complex problem that could benefit from subjective opinions to determine or develop a range of possible alternative solutions. It has been proven as a useful method for eliciting expert opinions within the food safety domain.

Purpose: To identify and aggregate the opinions of fresh produce experts regarding the future of on-farm HACCP and food safety risk assessments.

Methods: Eighty-six food safety experts were invited to participate in the Delphi study to determine the requirements of on-farm HACCP and food safety hazard assessments in fresh produce farms. Experts were defined as having met two criteria: (1) currently teaching in a university level food safety, agriculture/horticulture or working in the horticulture/agriculture sector (2) experience in the food safety, microbiology, chemical, or risk assessment. The Delphi study consisted of two rounds.

Results: Based on the panelists' feedback, a farm-specific HACCP plan based on risk reduction points is needed as part of the farm to fork food safety management plan. The panelists however, disagreed with the statement that "by 2015-2020, on-farm HACCP should be made mandatory in the production of fresh produce crops". Training and empowering of farmers to conduct their own food safety risk assessments were supported by all panelists. Most experts agreed that farm specific HACCP-based plans were needed at the farm level but should not be made mandatory.

Significance: The implementation of a full-fledged HACCP plan may be impractical; rather the HACCP-based approach is much more feasible.

P1-17 Systematic Review and Meta-analysis of Food Safety Training on Hand Hygiene Knowledge and Attitudes among Food Handlers

SOON, JAN-MEI[1]; Baines, Richard N.[2]
1. Department of Agro-Industry, Universiti Malaysia Kelantan, Pengkalan Chepa, Kelantan, Malaysia.
2. School of Agriculture, Royal Agricultural College, Cirencester, Gloucestershire, United Kingdom.

Introduction: Analysis of food safety training or hand hygiene interventions (i.e., hand hygiene education) to increase knowledge and improve hand hygiene practices are important, since hand hygiene is considered an important measure to reduce cross-contamination risks. In the context of food safety, meta-analyses have only been recently used to integrate and synthesize food safety information. To our knowledge, a meta-analysis comparing the effectiveness of food safety training or hand hygiene interventions in food handlers has never been conducted.

Purpose: To assess the extent to which food safety training or intervention strategies increase knowledge and attitudes towards hand hygiene.

Methods: A systematic review of food safety and hand hygiene training articles was conducted. Search terms included combination of “food safety”, “food hygiene”, “training”, “education”, “handwashing”, “hand hygiene”, “knowledge”, “attitudes”, “practices”, “behaviour” and “food handlers”. Meta-analyses were conducted using Comprehensive Meta-Analysis (CMA) Software version 2. All pooled analyses were based on random-effects model.

Results: Meta-analysis of 9 food safety training/intervention studies on hand hygiene knowledge among food handlers were significantly higher than control (without training), with an effect size (Hedges’ g) of 1.284 (95% CI = 0.830 – 1.738). Five food safety training/intervention studies monitor hand hygiene attitudes and self-reported practices with a summary effect size of 0.683 (95% CI = 0.523, 0.843). Food safety training increases knowledge and attitudes towards hand hygiene practices. A combination of standard training and social cognitive behavioral interventions resulted in the highest effect sizes for both studies.

Significance: Despite the effectiveness of hand hygiene training in improving knowledge, it may be less effective at motivating people to practice them. Refresher training and long term reinforcement of good food-handling behaviours may also be beneficial in sustaining good hand washing practices.

P1-18 Development of AquaFRAM: An Aquaculture Farm Food Safety Risk Assessment Tool for Salmon Farms

SOON, JAN-MEI[1]; Baines, Richard N.[2]
1. Dept. of Agro-Industry, Universiti Malaysia Kelantan, Pengkalan Chepa, Kelantan, Malaysia.
2. School of Agriculture, Royal Agricultural College, Cirencester, Gloucestershire, United Kingdom.

Introduction: Atlantic salmon (Salmo salar) is the most significant aquaculture species in Europe, both in terms of production and economic value, with Norway, followed by Scotland and Ireland as the three major European producers. Potential hazards to food safety by consumption of farmed salmon includes (i) toxic chemicals and chemical compounds which have been accumulated by the fish from their aquatic environment or from their food or as residues from veterinary medicines and (ii) pathogenic organisms in the fish, such as parasites, viruses and bacteria which may be harmful to humans.

Purpose: To develop an Aquaculture Farm-Food Safety and Diseases Risk Assessment Tool (AquaFRAM) for salmon farms in the UK.

Methods: AquaFRAM has been developed using MS Excel software utilising a qualitative risk assessment approach for farmers to evaluate their food safety practices and to assess potential food safety hazards and diseases on their farms. The risk assessment is based on the risk matrix of frequency of likelihood × severity, where the farmers will judge the likelihood of the hazards occurring on their farm based on given examples or scenarios. The severity scoring was derived from a separate Delphi study. AquaFRAM was tested on 9 UK salmonid companies (8 salmon; 1 trout company). The participating companies represent more than 60% of the total UK’s salmonid production.

Results: All of the farms who tried and tested the AquaFRAM tool reported it being farmer-friendly and practical. All the salmonid farms determined that the Good Aquacultural Practices Assessment (Questions and Answers) were the most useful. Using the developed action plan as proof of

assessment to second or third party audits were useful for eight of the farms while six farms stated that the probability ranking and risk ranking output were relevant. Eight farms rated veterinary medicine, water quality and types of diseases and photos of diseased fishes as the most relevant and useful topics; followed by feed, vaccination, harvesting and post-harvest handling.

Significance: It was highlighted that the current tool focused mainly on risk reduction and not risk elimination. It is also appropriate for educational and training of full-time and seasonal farm workers.

P1-19 Development of a Food Safety Educational Comic Book for Restaurant Food Handlers in English and Spanish

DWORKIN, MARK[1]
1. School of Public Health, University of Illinois at Chicago, Chicago, IL, United States.

Introduction: In the United States, restaurants or delicatessens are the most frequent sources of food eaten in foodborne outbreaks. In the few published studies of restaurant food handler knowledge, substantial knowledge gaps have been recognized.

Purpose: A food safety educational comic book was created for restaurant food handlers to provide them with an entertaining and provocative way to increase their knowledge in order to promote best food safety behavioral practices.

Methods: In northern Illinois, 729 food handlers from 211 participating restaurants were interviewed. The 50-question survey was administered in English or Spanish, based on the preference of each participant. If a knowledge question was answered incorrectly by at least 20% of food handlers, either overall or within the language-specific subgroups, it was included in the comic book. The comic book underwent several drafts to improve clarity, provide visual emphasis of concepts and verify facts. Four local and one state health department reviewed the content and provided advice. Focus group meetings were held with food handlers at restaurants to provide recommendations and test cognition. Spanish translation and back translation was performed.

Results: Food handler knowledge gaps were related to cross-contamination, cooking temperatures, defrosting and storage of food and minimum and maximum temperatures that germs grow most easily. A 20-page comic book (including cover pages) was created. The contents included a page of facts, a quiz, a story based on a chef who instructs food handlers about errors he identifies in a restaurant kitchen, a wrestling match with a germ that illustrates hygiene and the temperature danger zone and illustrations of three actual foodborne outbreaks with emphasis on poultry thawing, working while ill and the potential danger of raw ground meat.

Significance: Educational material that targets the most frequently recognized knowledge gaps and is delivered in a story-based illustrated format may serve to augment other efforts to educate food handlers. Story-based educational material may stimulate interest and memory retention more than straightforward brochures. A study comparing the efficacy of the comic book compared to a brochure is ongoing.

P1-20 Production of Alkaline Metalloprotease by Meat Spoiling *Pseudomonas* Species

BAJCSI, NIKOLETT[1]; Fustos, Zoltan[1]; Belak, Agnes[1]; Maraz, Anna[1]
1. Corvinus University of Budapest, Budapest, Hungary.

Introduction: Raw meat stored at refrigerated temperatures has been spoiled by different *Pseudomonas* species, the most common of which is psychrotrophic bacteria, which become dominating as the spoilage progresses. Extracellular enzyme production of the spoiling bacteria has a crucial role in this process. Meat proteins are hydrolyzed mainly by extracellular alkaline metalloproteases (AprX) of pseudomonads and the enzyme production is regulated by different environmental conditions such as temperature, pH and metal ions.

Purpose: Our aim was to determine the alkaline metalloprotease activity of different *Pseudomonas fluorescens*, *P. fragi* and *P. lundensis* strains under different environmental conditions.

Methods: *Pseudomonas* strains have been isolated during our previous investigation of the raw poultry meat spoilage process and selected as isolates of outstanding protease activity. Influences of different environmental conditions have been determined by the application of optimized semi-quantitative and quantitative protease assays.

Results: The optimized agar diffusion assay used for the determination of the protease activity allowed the semi-quantitative screening of a huge number of enzyme-containing supernatants because the gentamycin containing skim milk agar inhibited the growth of bacterial cells therefore membrane filtration of the supernatants could be skipped. The quantitative measurement of alkaline metalloprotease activity was carried out using the azocasein as a substrate. For the optimal

metalloprotease production, the buffered culture media have to contain an inducer like skim milk, but citric acid applied in the buffer for setting the pH inhibited the protease activity. The neutral or slightly alkaline growth conditions and presence of different metal ions can increase the protease activity. Under the optimized circumstances *P. fluorescens* strains proved to be the best protease producers.

Significance: The optimized conditions for protease production and activity allow the determination of circumstances and techniques that suppress the growth and protease production by pseudomonads during meat spoiling. The work was supported by TÁMOP-4.2.1.B-09/12 and TÁMOP-4.2.2/B-10 projects.

P1-21 Portuguese Food Microbiological Information Network – The Mutual Benefits of Information Share

VIEGAS, SILVIA[1]; Oliveira, Luísa[1]
1. Alimentação e Nutrição, Instituto Nacional Saúde Dr Ricardo Jorge,IP, Lisboa, Portugal.

Introduction: The National Institute of Health launched the Portuguese Food Microbiological Information Network (RPIMA) to collect, standardize, gather and analyse food microbiological and epidemiological data from scientific literature, stakeholders and data producers. All steps of the food chain were considered in order to obtain global information, for the first time at national level and in useful time, to be available for data users.

Purpose: Identification and characterization of benefits of food share information for all food chain stakeholders, from farm to fork (Food production, Universities, Politic, Public health, Education, Consumption, Laboratories and Risk management).

Methods: Meetings of working groups of RPIMA (94 participants).

Results: All information compiled will be standardised, validated, qualified, traceable to its source and compatible with other national and international data bases.
The information produced can contribute to:
- Optimize national resource usage
- The control, monitoring and surveillance of microorganisms in food chain
(detection proactive system)
- The detection of emerging organisms (prevention of foodborne diseases (FBD))
- The Integrated Surveillance between Public Health, Veterinary and Food Safety
- The risk management and Food Safety measurement (risk indicators/ strategies)
- The identification of risk assessment information gaps that can be investigated by research
- The calculation of the burden of foodborne diseases

Significance: The implemented system contributes to optimize national resources, identifying weaknesses that require a higher investment. With this work we can gather food microbiological and epidemiological data, presently dispersed in different databases and contexts, about identification, level, frequency, distribution and characterization of pathogens occurrence throughout the food chain, to monitor potential vehicles of human infections and to contribute to the metric optimization of food safety. The global information can serve as scientific evidence, for the first time in useful time, to improve food safety at national and international level, namely for risk assessors/managers.

P1-22 Food-safety Risk Estimation by Catering Professionals: Experts' Cognitive Biases Derive More from Retrieval Deficits Than from Knowledge Deficits

Desaulty, Stéphane[1]; DUFOUR, CHRISTOPHE[2]; Saulais, Laure[4]; Di Martino, Hélène [5]; Terrier, Patrice[3]
1. Silliker SAS - University of Toulouse-CNRS UMR 5263, Toulouse, France.
2. Silliker SAS, Cergy-Pontoise, France.
3. University of Toulouse-CNRS UMR 5263, Toulouse, France.
4. Institut Paul Bocuse Research Center, Ecully, France.
5. EBI - EBInnov laboratory, Cergy-Pontoise, France.

Introduction: Food safety specialists underline the importance of taking into account the psychological aspects of good hygiene practices application. Since the precursor works of Rennie (Rennie, 1995), the use of socio-cognitive models of human behaviors is the most convincing approach to improve the efficiency of food hygiene trainings. In these models, risk perception is a determinant of behaviors. Therefore, understanding how catering professionals perceive risk is a key to improve food safety. Relevant knowledge is necessary but not sufficient to correctly perceive risk.

In addition to having knowledge, professionals must retrieve and use it.

Purpose: This study aims to examine some cognitive aspects of risk perception. Based on an empirically validated model of memory and reasoning, the Fuzzy-Trace Theory (Reyna, 1995; Reyna & Adam, 2003), we test the hypothesis that expertise in sanitary risks could decrease errors linked to knowledge deficit but increase errors linked to the cognitive process of knowledge retrieval.

Methods: One hundred eleven catering sector professionals (38 auditors, 37 managers and 36 operators) completed a questionnaire exploring their perceptions of specific food-related risks, assuming that expertise in risk perception increases from operators to managers, auditors being considered as the experts group.

Results: Overall we show that both knowledge deficit and retrieval failure are sources of errors in risk estimations; and that experts are more sensitive to the latter while novices are more sensitive to the former. Knowledge deficit induces significant underestimation of contamination risk only for operators ($P < .05$). When a higher level of knowledge is required, all groups wrongly estimate risks ($P < .005$) but the error is significantly lower for auditors ($P < .05$). Retrieval failure produces estimation errors (e.g., Underestimation of perceived risk, $P < .05$) for managers but neither for operators nor auditors.

Significance: Experts were not immunized against biases and might even be more sensitive than novices to retrieval failure. Trainings should provide support to knowledge retrieval in addition to knowledge dissemination.

P1-23 Impact of *Staphylococcus aureus* Strain Variability upon Product and Process Design Validation

Johnson, Nick[1]; CHANG, ZHENG[1]
1. Nestlé Research Center, Lausanne, Switzerland.

Introduction: Inter-strain variation in growth response is a potentially important factor to consider in the safe and stable design of foods.

Purpose: This study aimed to determine the importance of inter-strain variability in validating safe product designs by using turbimetric measurements and TTD methods.

Methods: The variability in growth response of 30 strains of *Staphylococcus aureus* was evaluated using a Time-to-Detection (TTD) approach in Tryptone Soya Broth under different environmental conditions of pH (4.0 – 7.0), NaCl (0.5 – 20%) and undissociated lactic acid (0 – 800 ppm). The effects of 20 intervals of each environmental condition were tested independently. Maximum specific growth rate was derived from TTD data under each growth condition using serially diluted cultures and absorbance in a multiple well turbimetric system Bioscreen C.

Results: The inter-strain variability observed between *Staphylococcus aureus* strains was greater than the intra-strain variability observed between replicates ($P < 0.05$). The variability in strain response increased with increasing environmental stress, as indicated by coefficient of variance (%CV) of μmax. Minimum cardinal values (i.e., pHmin, MICHLa and awmin) were also estimated, and show little inter-strain correlation. A product and process design scenario was chosen as the basis to determine through application of the gamma concept and Monte Carlo simulation, those strains most likely to grow fastest under realistic conditions of variable NaCl, Na-lactate, and pH. This study demonstrated only a few strains are best suited to the majority of product and process formulations simulated, and that, of the strains and environmental conditions tested, these strains should comprise the challenge study cocktail in product validation. The inter-strain variability was found to be significant when tested against a realistic product and process design, resulting in large predicted shelf-life differences across the strains tested.

Significance: Apart from aiding the selection of challenge test strains, this study has provided insight into correlations between strains in the pattern and magnitude of individual strain stress responses. The incorporation of this inter-strain variation, and other phenotypic information, into predictive models is seen as an important step in improving the accuracy of internal quantitative risk and exposure assessments.

P1-24 Phenotypic and Toxigenic Profile of Emetic and Non-emetic *Bacillus cereus* Food Isolates

AGNIESZKA BEDNARCZYK-DRAG[1], Elżbieta Daczkowska[1] – Kozon, Alicja Dłubała[1]
Dept. of Applied Microbiology and Biotechnology, Faculty of Food Sciences and Fisheries, West Pomeranian University of Technology, Szczecin, Poland

Introduction: *Bacillus cereus* group bacteria are ubiquitous in the environment and are commonly present in various food products. Pathogenic isolates form a potential risk to consumers when present at sufficiently high numbers and can cause two types of food poisoning. Diarrheal food poisoning is attributed to three enterotoxins produced by *B. cereus* group strains: haemolytic BL enterotoxin (HBL), non-haemolytic enterotoxin (NHE) and cytotoxin K (CytK), while the emetic type of food poisoning is induced by cereulide. Other *B. cereus* virulence factors, such as haemolysins I (cereolysin AB), II and III, and phospholipases, contribute to other types of infections, e.g., post-operative or traumatic wound and eye infections. The data published so far do not give conclusive answers as to whether the emetic strains differ in terms of their phenotype and toxicity from non-emetic *B. cereus*.

Purpose: The aim of the study was to compare the phenotypic and toxigenic profiles of emetic and non-emetic *B. cereus* strains isolated from various food products available at retail in Poland.

Methods: Food analyses were performed according to EN ISO 7932:2005 and EN ISO 21871:2007. Phenotypic characteristics of the isolated strains were based on 72 features (including API 50CHB + API20E tests) and phenotypic similarity of the strains was assessed using the UPGMA method.

DNA from bacterial cultures was extracted with Genomic Mini AX Bacteria (A&A Biotechnology). The strains were confirmed as *B. cereus* by detection of a specific 16S rDNA fragment in real-time PCR analysis. Emetic potential was determined by detection of the *ces* gene fragment. RNA was extracted using Total RNA Mini Kit (A&A Biotechnology). Expression of *cesA* gene was detected at the early stationary phase by quantitative real-time PCR analysis against the four housekeeping genes (*rpsu*, *udp*, *hel*, *gatB*/*Yqey*) using KAPA SYBR FAST One-Step qRT-PCR Kit (KapaBiosystems). Boar sperm motility analysis was performed using Sperm Class Analyzer System (Microptic).

Toxic potential of the strains was determined by testing the presence of 12 gene fragments encoding for several virulence factors, including 3 enterotoxins (*hblD*, *hblA*, *hblC*, *nheA*, *nheB*, *nheC*, *cytK*), phospholipases (*piplC*, *pcplC*, *sph*) and haemolysins (*hlyII*, *hlyIII*).

Results: From 347 food products 1,086 isolates were confirmed as *B. cereus* group strains with 41 determined as emetic on the basis of *cesA* gene expression and boar sperm motility assays. For further analysis 88 strains (both emetic and non-emetic) were selected.
Among the emetic strains tested (41 food isolates and one reference F4810/72) 71.4% (30 strains) carried all three genes (*nheABC*), 19.1% (8 strains) - all three genes encoding for HBL and 7.1% (3 strains) were positive for *cytK* gene fragment. 82.6% (38 strains) of non-emetic representatives were positive for *nheABC* gene fragments, 50% (23 strains) – for *hblDAC* gene fragments and 45.7% (21 strains) carried *cytK* gene fragment. All the enterotoxin-encoding gene fragments were observed in 5 emetic and 10 non-emetic strains.

As much as 97.6% (41/42) emetic and 95.7% (44/46) non-emetic *B. cereus* strains carried at least one of the haemolysins gene fragments, with *hlyIII* being the most abundant. All the genes encoding for two phospholipases and sphingomyelinase were simultaneously detected in 71.4% (30/42) emetic and 52.2% (24/46) non-emetic strains.

On the basis of cluster analysis of all the phenotypic features it was possible to distinguish most emetic isolates from non-emetic ones. Only two emetic strains formed a unique cluster, whereas 39 emetic isolates were grouped together with only one non-emetic strain. Highest differences between the two *B. cereus* groups of strains were observed in degradation of glycogen, salicin, amygdalin, celobiose and starch. Cluster analysis based on the presence of several genes encoding for virulence factors showed that the presence of those genes is not strictly attributed to either emetic or non-emetic *B. cereus* strains.

Significance: The results of the study provide more information on the nature of emetic representatives of *B. cereus* group. Emetic strains differ significantly from non-emetic ones in ability to degrade several carbohydrate compounds and hence can be distinguished phenoptypically. It was shown that emetic *B. cereus* can carry some or all of the genes encoding for enterotoxins. Also the presence of three haemolysins (I, II and III), two phospholipases and sphyngomyelinase – encoding genes is comparable in emetic and non-emetic strains. Therefore, some emetic *B. cereus* group strains isolated from food may have high pathogenic potential and may contribute to other than emetic symptoms of food poisoning.

P1-25 Minimally Processed RTE Food as a Vehicle to Psychrotrophic *Bacillus cereus* with Toxigenic Potential

ALICJA DLUBALA, Agnieszka Bednarczyk – Drąg, Elżbieta Daczkowska – Kozon
Dept. of Applied Microbiology and Biotechnology, Faculty of Food Sciences and Fisheries, West Pomeranian University of Technology, Szczecin, Poland

Introduction: *Bacillus cereus* group comprising of 7 closely related species are presumed frequent but not a numerous contaminant of raw food materials. These spore-forming soil bacteria, including pathogenic species able to produce enterotoxins or emetic toxin when sufficiently numerous in food products, can cause diarrheic foodborne infections or emetic food poisoning, respectively. Increasing interest of consumers in healthy diets with growing popularity of minimally processed RTE food may result in increasing importance of psychrotrophic members of the *B. cereus* group. Whether *B. cereus* is to pose a health risk when in minimally processed RTE food products, is to be a matter of the species present, their initial number, toxigenicity and ability to stay metabolically active when under cold storage.

Purpose: The aim of the surveys was to find out if minimally processed RTE food, when contaminated with *B. cereus*, poses a health risk to consumers based on their prevalence and number in MP-RTE food, toxigenic potential of *B. cereus* isolates and their ability to grow at chill temperatures.

Methods: A total of 165 MP-RTE food products of 5 different food groups (sushi, mayonnaise and green salads, fresh vegetables and fresh juices) were tested for *B. cereus* presence and number according to EN ISO 7932: 2005 and EN ISO 21871: 2007 standards. Presumptive *B. cereus* strains were tested for their ability to grow at chill temperatures (< 10°C) and the presence of *B. cereus* group strains specific 16S rDNA fragment, emetic strains specific *ces* gene fragment and *nheA*, *nheB* and *nheC* gene fragments encoding for non-haemolytic enterotoxin (NHE) with the qualitative real-time PCR method.

Results: Results revealed that 72.3% (125/173) of MP-RTE food items tested to carry *B. cereus* group representatives with the lowest prevalence noted for fresh juices (25/45) and the highest for vegetable salads and peeled vegetables (≦ 90%). In the majority of plant based MP-RTE products tested, *B. cereus* count did not exceed 10^2 CFU g^{-1}, reaching 10^3 CFU g^{-1} in sushi and mayonnaise salad samples.

Of the 452 presumptive *B. cereus* strains, the majority (98%) were confirmed as *B. cereus sensu lato*, with the highest prevalence of potentially emetic strains noted among the sushi (12/24) and mayonnaise vegetable salads (23/100). 44% of 48 emetic strains isolated from MP-RTE food products showed to carry genes encoding for a set of the NHE components, thus being potentially enterotoxigenic. Further to this, all 21 emetic strains with NHE production potential were able to growth under chill storage conditions (<10^0C).

Conclusions: The count of *B. cereus* in MP-RTE food exceeding 10^3 CFU g^{-1}, the emetic strains being over 40% of the isolates. The high percentage of strains with enterotoxic potential, able to grow at chill storage temperatures, make MP-RTE food - such as sushi and mayonnaise vegetable salads in particular - when improperly treated prior to consumption a risk for *B. cereus* foodborne disease to consumers.

P1-26 Antimicrobial Effect of Lactic Acid Bacteria on *Staphylococcus aureus*

Bekpinar, Ece[1]; HEPERKAN, DILEK[1]
1. Faculty of Chemistry and Metallurgical Engineering, Dept. of Food Engineering, Istanbul Technical University, Istanbul, Turkey.

Introduction: Viable lactic acid bacteria and their antimicrobial metabolites are widely used to control the growth of pathogenic bacteria in foods.

Purpose: In this study, the inhibitory effect of antimicrobial substances obtained from lactic acid bacteria (LAB) on *Salmonella aureus* are studied.

Methods: Forty-seven LAB strains isolated from cheese were used. Strains with antibacterial activity were grown in MRS broth for 48h at 30°C. The cultures were centrifuged at 4,000 rpm for 15 minutes and then filtered. The cell-free, non-neutralized supernatants, neutralized supernatants and neutralized-catalase added supernatants were separately used in further antibacterial experiments. Antibacterial characteristics of LAB strains were determined by ELISA microtiter plate method, based on spectrophotometric measurements.

Results: The total antibacterial effect of supernatants were greater than neutralized supernatant

and neutralized-catalase added supernatant in inhibition of *S. aureus*.

Significance: For the inhibition of *S. aureus*, using LAB is a promising result.

P1-27 Anti-Listerial Activity of *Lactobacillus brevis* Isolated From Cheese

Hizarci, Oznur[1]; HEPERKAN, DILEK[1]

1. Faculty of Chemistry and Metallurgical Engineering, Dept. of Food Engineering, Istanbul Technical University, Istanbul, Turkey.

Introduction: *Listeria monocytogenes* is one of the most important human pathogens transmitting via foods. Cheeses are contaminated with *L. monocytogenes* frequently and have been associated with a number of outbreaks.

Purpose: In this study the effects of antibacterial substances obtained from 27 *Lactobacillus brevis* strains are studied.

Methods: Strains with antibacterial activity were grown in MRS broth for 48h at 30°C. The cultures (10^6 CFU/mL) were then centrifuged and filtered. *L. monocytogenes* was grown in Muller-Hinton Broth at 37°C. The cell-free, non-neutralized supernatants, neutralized supernatants and neutralized-catalase added supernatants were separately used in further anti-listerial experiments. Antibacterial characteristics of LAB strains were determined by an ELISA microtiter plate method, based on spectrophotometric measurements.

Results: The results in this study show that non-neutralized supernatants of *L. brevis* were more effective on inhibiting *L. monocytogenes* than neutralized and neutralized-catalase added supernatants.

Significance: The positive effect of non-neutralized supernatants was also confirmed by inoculating them on PALCAM agar.

Poster Session 2 – Tuesday, 22 May, 09.00-17.00

Authors will be present at their posters during the conference breaks

P2-01 Isolation and Characterization of a Bacteriocin with Antilisterial Activity from Shellfish Digestive Gland

Fajardo, P.[1]; Atanassova , M.[1]; Vietes, J.M.[1]; CABADO, A.G.[1]
1. ANFACO-CECOPESCA, Vigo-Po, Spain.

Introduction: The European project "Bio-engineered micro Encapsulation of Active agents Delivered to Shellfish (BEADS)" is focused on the development of micro-encapsulated bacteria able to detoxify shellfish, mainly marine biotoxins, pathogenic bacteria, virus and parasites. To ensure microbiological safety of raw and processed shellfish, it is necessary to remove human pathogens. Bacterial infections produced by consumption of bivalves are mainly caused by species of *Vibrio*, *Salmonella*, *Listeria monocytogenes* and *Aeromonas*. In this context, digestive glands of fresh shellfish were used for isolation of microorganisms with potential antimicrobial activity.

Purpose: The goal of this work was to find mussel, oyster or clam probiotics with possible applications for shellfish decontamination.

Methods: The antimicrobial activity screening was performed by the agar well diffusion assay. Sequencing of the 16S rRNA gene and biochemical characterization using the API 20Strep test were performed to identify the isolate with antimicrobial activity. Zymogram analysis was performed to visualize the proteic antimicrobial compound.

Results: A total of 277 bacteria were isolated and screened for activity against strains of *Listeria monocytogenes*, *Escherichia coli*, *Salmonella enterica*, *Vibrio cholera* and *Vibrio parahaemolyticus*. One isolate obtained from mussel presented anti-pathogen activity against *Listeria monocytogenes*. This isolate, a lactic acid bacteria, produced a proteic compound with a low molecular weight and high thermostability (20 min at 110ºC), resistance to a wide pH range (3–9) and to treatment with certain surfactants (Tween 20, 80 and EDTA). The synthesis of this bacteriocin was associated with the growth of the producer.This strain was also found to be sensitive to chloramphenicol, fusidic acid, ampicillin, vancomycin and gentamicin.

Significance: This bacteria isolated from *Mytilus galloprovincialis* digestive gland synthesized a bacteriocin with activity against different strains of *Listeria monocytogenes*. This work was financed by the project "BEADS", Grant Agreement Number 262649.

P2-02 *Salmonella* spp. and *Listeria monocytogenes* Detection in Food Samples Combining a Single Step Enrichment with Multiplex Real-Time PCR

Garrido, A.[1]; Chapela, M.J.[1]; Vieites, J.M.[1]; CABADO, A.G.[1]
1. ANFACO-CECOPESCA, Vigo-Po, Spain.

Introduction: PCR detection methods have risen as powerful alternative tools for fast and accurate detection of foodborne pathogens. Additionally, multiplex real-time PCR (RT-PCR) allows simultaneous screening of more than one bacterium thus reducing time and costs. However, despite efforts done by industries, some well-known foodborne pathogens, as *Salmonella* spp. and *Listeria monocytogenes*, continue to be a challenge to public health.

Purpose: This study is focused on the enhancement of RT-PCR for the simultaneous detection of *Salmonella* spp. and *Listeria monocytogenes*, even in the presence of a high concentration of interfering bacteria including one single enrichment broth for both pathogens.

Methods: Two different DNA extraction methods were tested. Primers and probe targeting *invA* gene for the detection of *Salmonella* spp., and targeting *hlyA* for detection of *L. monocytogenes* were used. An Internal Amplification Control (IAC) was also included and Ct values were compared.

Results: TA10 medium was selected, optimized and evaluated for the individual and simultaneous recovery and growth of *Salmonella* spp. and *Listeria monocytogenes*. RT-PCR efficiency above 90% was obtained, covering 5 orders of magnitude for both pathogens. Complete method achieved low limit of detection (5 CFU/25 g), and all parameters tested for its evaluation returned values over 90% (relative sensitivity, specificity, accuracy, positive and negative predictive value). The developed method was applied to 95 natural samples including meat, vegetables, molluscs and ready-to-eat products.

Significance: This study provides many advantages, in terms of time and cost savings, and high throughput of multiplex RT-PCR. However, an extra effort is needed to improve and harmonize

molecular techniques and also to encourage its use as screening methods by industries and private laboratories. This work was financed by the Secretary General for the Sea of the Spanish Ministry of Agricultural, Land and Marine Resources (MARM), by order ARM/1193/2009.

P2-03 Microbiological Risk Assessment of Pre-packaged Salad and Salad Bar Ingredients in Singapore

CHAU, MAN LING[1]; Lee, Valarie[2]; Thu, Aung Kyaw[1]; Yuk, Hyun-Gun[2]; Yap, Jennifer[1]; Gutierrez, Ramona[1]; Ng, Lee Ching[1]
1. Environmental Health Institute, National Environment Agency, Singapore, Singapore.
2. Food Science and Technology Programme, Department of Chemistry, Nation University of Singapore, Singapore, Singapore.

Introduction: In the aftermath of the European deadly outbreak associated with the consumption of raw vegetables in 2011 (*Escherichia coli* O104:H4 in bean sprouts), the realization of the lack of published data on the microbial quality of salad in Singapore raised the need to assess the potential food safety issue posed by the consumption of salad - an increasingly popular trend in current Singaporean cuisine habits.

Purpose: The aim of this study was to evaluate the microbial quality of different types of pre-packed salads and salad bar ingredients distributed by local retailers in Singapore, in order to assess the food poisoning risk these may pose to consumers.

Methods: A total of 341 samples were collected, covering four types of pre-packed salads and six types of salad bar ingredients, from different retail food outlets located in four geographical regions of Singapore (Central, West, East, North). All samples went through bacteriological detection protocols adapted from FDA's Bacteriological Analytical Manual.

Results: *Listeria monocytogenes* was detected in 44.1% of seafood salad bar ingredients, including in smoked salmon. Further investigation showed that lower *L. monocytogenes* contamination rates were observed on smoked salmon purchased from supermarkets, as compared to those collected from salad bars, highlighting the importance of food-handling processes implicated in salad bar ingredients preparation. Over 75% of vegetable pre-packed salads had unacceptable Standard Plate Counts (SPC) (Singaporean standards), and 53.5% lettuce tested were shown to persistently have SPCs above the authorized limit, even after undergoing thorough soaking and rinsing processes. The local SPC legal limit (10^5 CFU/g) was found to be surpassed in 43.8% and 36.9% of the pre-packed salads and salad bar ingredients respectively, whereas interestingly, lower percentages would be considered unsatisfactory under other international standards (e.g., 26.0% and 23.8% respectively in Hong Kong SAR).

Significance: Our data provide evidence of critical discrepancies between microbial loads found in salad dishes and legal Singaporean standards (SPC < 10^5 CFU/g). While handling processes may require drastic improvements, with special regard to the preparation of seafood items such as smoked salmon, the definition of local standards may also be questioned when compared to other international requirements.

P2-04 100K Pathogen Genome Project

WEIMER, BART[1]; Xie, Yi[1]
1. School of Veterinary Medicine, Population Health and Reproduction, University of California-Davis, Davis, CA, United States.

Introduction: Despite efforts to reduce foodborne illness, outbreaks from *Salmonella*, *Campylobacter*, enteropathogenic *Escherichia coli*, *Listeria monocytogenes*, *Vibrio* and *Shigella* continue to occur worldwide at high rates. Unfortunately, rapid diagnostics to reduce these zoonotic organisms remain elusive. Concurrently, despite production of genome sequences of these bacteria for application to bacterial pathogenesis studies, this information remains under-utilized in food, animal, and environmental applications. In part, this situation is due to that foodborne pathogen genome sequences do not adequately represent genetic diversity, which is critical for development of robust diagnostic assays. For example, current genome sequencing efforts are focused on outbreak isolates, while other potential pathogenic isolates are often under-represented. In other instances sequencing very closely related serotypes push the genome analysis to incredibly narrow analytic measures that often do not represent the wealth of information in each genome. This bias interferes with our preparedness to defend the food supply, as indicated in recent *E. coli* O104:H4 outbreaks in

Europe, and the lack of elimination from the agriculture and food processing environment in developed countries.

Purpose: This project is sequencing 100,000 genomes from the most prevalent foodborne pathogens for use as a public resource to develop new analysis strategies, more advanced diagnostics, understand genomic plasticity for adaptation, and to provide a richer information to define pathogen ecology.

Methods: The University of California at Davis School of Veterinary Medicine has established an industry, university and government partnership to sequence 100,000 genomes from the most prevalent foodborne pathogens to span the diversity and drill into the depth of individual isolates. The sequencing will be done at BGI@UC Davis, a new public/private partnership, using multiplexed genome libraries and next generation sequencing techniques. The genomes will be assembled, annotated, and released to the public domain via established data hosting services, such as NCBI and EMBL.

Results: To date, the partnership has sequenced a series of isolates, formed a steering committee to participate in the isolate selection and enable international collaboration mechanisms. An affiliates group is forming to provide access to those that do not have the capability of data analysis or desire specific activities that are beyond those of the entire group. The initial isolates will be derived from diverse sources: human outbreaks, animal disease cases, food processing plants, fresh produce and tree nut samples, the environment, wildlife and livestock. Multiple isolates from each bacterial and serogroup will be chosen for sequencing. This systematic strain selection will help us to capture the breadth and depth of genomic diversity of these organisms. We are in the first phase of this project and are actively soliciting partners to contribute isolates from around the world. We are actively seeking partners for advanced bioinformatic tool development that enables easy and informative data display, as well as additional funding partners to ensure the sequencing is maintained and that the culture collection is housed for future use.

Significance: This volume of genome sequences will be the largest single effort undertaken in microbial genomic studies in the world and will enable a new level of genomic science to be accomplished. The results from the effort will enable new diagnostics and provide novel insights into genomic evolution that underlies foodborne pathogen resistance, environmental persistence, host association and food contamination.

P2-05 Performance of the 3M™ Molecular Detection Assay Salmonella and the 3M™ Molecular Detection Assay Listeria in Environmental and Primary Production Samples;

Yang, Julie[1], Zook, Cynthia [1]; Rosauer, Micki[1], MCGOLDRICK, KEVIN[1]
1. Food Safety, 3M, St. Paul, MN, United States.

Introduction: Testing for *Salmonella* and *Listeria* is a critical component of food safety programs as infection by these pathogens can result in significant adverse health conditions and economical losses. The 3M™ Molecular Detection Assay *Salmonella* and the 3M™ Molecular Detection Assay *Listeria* were developed for the rapid and specific detection of these adulterants in samples after enrichment.

Purpose: To evaluate the performance of the novel methods in a variety of environmental and primary production samples.

Methods: More than 550 environmental and primary production samples were collected in duplicates. Two sets of testing were conducted: Set 1) where one of the duplicates was enriched blank and one was artificially contaminated with ~10 CFU of the target organism; and Set 2) where single samples were enriched blank to evaluate for native contamination. Enrichments were tested using the Detection Molecular Assays and selective and differential agar and/or quantitative PCR streaked. A X^2 test was used to compare the results for significant differences.

Results: Compared to agar or qPCR, accuracy, specificity and sensitivity were: 99%, 100%, and 95% for the Molecular Detection Assay *Salmonella*, and 95.3%, 100%, 94.9% for the Molecular Detection Assay *Listeria*.

Significance: The new methods were determined to be reliable and accurate. For all samples evaluated, the 3M Molecular Detection Assay *Salmonella* and the 3M Molecular Detection Assay *Listeria* demonstrated comparable results to the other methods for the rapid, automated detection of these organisms.

P2-06 The Lean Approach to Optimize Quality Indicator Testing in a Food Microbiological Laboratory

Martins, Dra. Ana[1]; Cassorla, Jean-Marc[2]; BONAMICI, TELMA[3]; Deverge, Emeling[3]
1. ControlVet, Tondela, Portugal.
2. Consultant, Montoison, France.
3. bioMérieux, Marcy l'Etoile, France.

Introduction: With the development of innovative and automated solutions for food microbiological laboratories, it becomes increasingly important to integrate them in the best and most efficient way. The main challenge is to manage and combine automated methods with traditional methods in the analysis process in order to optimize the workflow and improve the laboratory's productivity.

Purpose: Lean is a systematic approach to optimize workflow in the laboratory. A Lean design has been completed to be set up for the ControlVet Laboratory in Portugal, a large private food laboratory, to implement the automated quality indicator system, TEMPO® with the objective of eliminating non-added value tasks, reorganizing the current workflow and reducing turn-around-time for results to final customers.

Methods: A laboratory assessment based on the Lean approach was performed over a 3-day period. The first day was dedicated to defining and observing the current workflow (physical layout and sample processing). The second day focused on the analyzes and opportunities to improve laboratory efficiency. The last day led to the establishment of a roadmap giving recommendations, including a new workflow organization for the full integration of traditional and automated methods.

Results: Currently, routine analysis is performed within 540 working hours per week with the existing dedicated resources. The full integration of the automated test system in the heart of the laboratory, coupled to the set-up of an aliquot tube for mix process, has shown the potential to save 224 working hours which are equivalent to either 41% of productivity gained, or, the reallocation of 5.6 FTE (full-time equivalent employees) to high-value activities. The improvements enabled attaining a target of 15 samples per working hour (the former sample management was 9 samples per working hour).

Significance: This implementation of the Lean approach in this laboratory will serve as a template for other laboratories to optimize the layout and microbiology workflow. These improvements will allow the laboratory to increase workloads while decreasing the cost per sample.

P2-07 Plasmidotyping, LPS Determination, Optical Map, PFGE and Whole Genome Sequencing Analyses of *Cronobacter* Isolates Associated with Community-acquired Infantile Disease in the USA

TALL, BEN D.[1]; Franco, Augusto A.[1]; Jarvis, Karen G.[1]; Kotewicz, Micheal L.[1]; Strain, Errol A.[2]; Li, Cong[2]; Sathyamoorthy, Venugopal[1]; Grim, Christopher J.[1]; Gopinath, Gopal[1]; Hu, Lan[1]; Kothary, Mahendra H.[1]; Malais, Tanya E.[3]; Allard, Marc W.[2]; Brown, Eric W.[2]; Silverman, Benson M.[2]; Zink, Donald L.[2]
1. U.S. Food and Drug Administration-CFSAN, Laurel, MD, United States.
2. U.S. Food and Drug Administration-CFSAN, College Park, MD, United States.
3. U.S. Food and Drug Administration-CFSAN, Irvine, CA, United States.

Introduction: *Cronobacter* are opportunistic pathogens associated with many foods, but powdered infant formula (PIF) remains the most commonly linked source of infantile disease. In the USA, 14 cases of infantile community–acquired *Cronobacter* infections were reported between May, 2010 and December, 2011; typically, four to six cases are reported in the U.S. annually.

Purpose: The goal of this study was to resolve any discernible relationships among cases and isolates.

Methods: Plasmidotyping, LPS determination, optical mapping (OM), PFGE, and whole genome sequencing (WGS) analyses were performed on 34 clinical, food and environmental isolates obtained from 12 of the 14 cases.

Results: *Cronobacter* spp. was not recovered from unopened cans of PIF. Thirty-three of the isolates were identified as *C. sakazakii*; the other isolate was *C. malonaticus*. Ten of the twelve cases were caused by *C. sakazakii* possessing the O2 LPS determinant. MLST analysis of sequences obtained from WGS assemblies confirmed that the O2 *C. sakazakii* isolates were of sequence type 4, which has been linked to severe infantile disease. All isolates possessed the common virulence plasmid, pESA3/pCTU1. OM analysis revealed that the *C. sakazakii* isolates were 95% similar to one another; grouping into three clusters. Together with WGS analysis, differences between isolates are

attributed to genomic insertions and deletions and approximately 200 single nucleotide polymorphisms. PFGE analysis confirmed this level of relatedness and demonstrated that for six of the cases, a PIF isolate from opened cans matched the clinical isolate.

Significance: The significance of these findings is that the cases occurred in healthy or recent graduates from hospital care. Though PIF was the vehicle for several cases, the source of contamination was not identified. Lastly, a large number of the isolates possessed the O2 serogroup determinant suggesting that currently within the USA, this serogroup predominates in infant clinical cases.

P2-08 Effect of Irradiation on *Listeria monocytogenes* Inoculated on Fresh-cut Fruits

FEKETE, BRIGITTA[1]; Kiskó, Gabriella[1]; Mohácsi-Farkas, Csilla[1]
1. Microbiology and Biotechnology, Corvinus University of Budapest, Faculty of Food Science, Budapest, Hungary.

Introduction: Fruits and vegetables are increasingly consumed as a part of healthy diets. They are routinely consumed raw, without any further antimicrobial processing. Minimally processed fruits have been involved in several foodborne outbreaks due to the presence of pathogenic microorganisms such as *Listeria monocytogenes*.

Purpose: Consumption of fresh fruits and vegetables is not allowed in low microbial diets, because they can harbor pathogenic organisms. The aim of our studies was to determine radiation doses improving the microbial safety of selected fruits.

Methods: Pre-cut orange, banana and apple (varieties Golden Delicious, Idared, Granny Smith) were inoculated with *Listeria innocua* or *L. monocytogenes* Scott A. The effect of irradiation (1 kGy gamma rays) on *Listeria* cell counts during post-irradiation storage at 5°C for 7 days was determined by traditional culturing techniques.

Results: Inoculated onto apple cubes, both *Listeria* strains survived for up to 8 days at 5°C in untreated control samples. Treatment of apple samples with 1 kGy irradiation dose reduced the numbers of the tested strains by at least 5 log cycles on the day of exposure, and it remained at this level during refrigerated storage. In case of orange and banana samples number of *Listeria* cells was reduced by at least 3 log cycles immediately after irradiation. Furthermore, surviving cells of both strains examined were able to grow on sliced banana during refrigerated storage.

Significance: Low-dose irradiation is able to improve the microbiological safety of the selected fruits. Considering that *L. monocytogenes* occur most probably in low contamination level, under good manufacturing practices this low-dose treatment may practically eliminate these pathogenic bacteria from the pre-cut fruit. Assistance of AGROSTER Co. Ltd Budapest in irradiation of samples is highly acknowledged. This work was supported by the IAEA Nr.16243, the TÁMOP-4.2.1/B-09/1and TÁMOP-4.2.2/B-10 projects.

P2-09 Evaluation of the Merck Duopath® Cereus Enterotoxins and Singlepath® Emetic Tox Mrk Lateral Flow Assays for the Rapid Detection of *Bacillus cereus* in Food

JOHN, LISA[1]; Krause, Nora[2]; Dietrich, Richard[2]; Becker, Heinz[2]; Moravek, Maximilian[2]; Maertlbauer, Erwin [2]; Bubert, Andreas[1]; Slaghuis, Joerg[1]; Lindhardt, Charlotte[1]
1. Merck Millipore, R&D Applications, Merck KGaA, Darmstadt, Hessen, Germany.
2. Institute for Hygiene and Technology of Food of Animal Origin, University of Munich, Munich, Germany.

Introduction: *Bacillus cereus* are becoming increasingly significant in the food industry and may well be one of the most prevalent foodborne pathogens. Almost all strains of *B. cereus* possess the ability to produce one or more toxins. Approximately 95% of isolates produce cytotoxin enterotoxins, >90% produce Nhe toxin, and approximately 55% Hbl. Enterotoxins are associated with the diarrhoeal type of illness. In addition, some *B. cereus* strains (< 5%) produce a heat stable emetic toxin, cereulide, which causes the vomiting type of illness.

Purpose: Merck Millipore have developed two assays for detection of *B. cereus* toxins in food: Duopath® Cereus Enterotoxins and Singlepath® Emetic Tox Mrk (Merck KGaA, Germany) assays. Duopath® Cereus Enterotoxins detects, within one assay, both the enterotoxin complex haemolysin BL (HBL) and the non-haemolytic enterotoxin (Nhe). Singlepath® Emetic Tox Mrk detects the marker protein which is co-expressed during the synthesis of the emetic toxin (cereulide).

Methods: In the Cereus Enterotoxins study artificially (n = 45) and naturally (n = 32) contaminated

samples from two food groups were analyzed: (i) baby formula and (ii) rice and pasta. In the Singlepath® Emetic Tox Mrk study, artificially contaminated food samples were analysed (UHT milk and rice).

Results: The studies show that the Duopath® Cereus Enterotoxins and Singlepath® Emetic Tox Mrk enable detection of all toxigenic *B. cereus* after 24 – 30 hours at contamination levels of 1 CFU/g or less and 1 CFU/10 g or less respectively. Further investigations showed high sensitivity of 6 ng/ml for the Nhe component and 20 ng/ml for the HBL component. Singlepath® Emetic Tox Mrk was able to detect emetic toxigenic *B. cereus* after 24 h with a low spiking level of 1– 10 CFU/10g. Further food & inclusivity studies ongoing.

Significance: The studies show that the Duopath Cereus enterotoxins and Singlepath® Emetic Tox Mrk enables detection of all toxigenic *B. cereus* after 24 – 30 hours at contamination levels of 1 CFU/g or less.

P2-10 From Farm-to-Fork : *Campylobacter* Immunological Rapid Screening Kit For Farm-Based, Pre-Slaughter Screening Of Live Chickens

JOHN, LISA[1]; Slaghuis, Joerg[1]; Wadl, Maria[2]; Poelzler, Thomas[3]; Stessl, Beatrix[4]; Wagner, Martin[4]; Lindhardt, Charlotte[1]
1. Merck Millipore, R&D Applications, Merck KGaA, Darmstadt, Hessen, Germany.
2. Unit for Surveillance, Dept. for Infectious Disease, Robert Koch-Institute, Berlin, Germany.
3. Department for Veterinary Public Health and, University of Veterinary Medicine, Vienna, Austria.
4. University of Veterinary Medicine, Vienna, Austria.

Introduction: The USA and EU are increasing the focus of consumer health and food safety, especially in relation to *Campylobacter* infection. A Dutch study concluded that the risk of contracting campylobacteriosis could be effectively reduced by identifying and eliminating high shedding flocks, pre-slaughter, from fresh poultry meat production.

Purpose: A Lateral Flow-based method suitable for use on the farm by unskilled personnel and capable of delivering results within 2 hours was developed by Merck Millipore in cooperation with researchers in Austria to detect high shedding (>7.0 $\log_{10}$ CFU *Campylobacter* spp./g of faeces) *C. jejuni* and *C. coli* broiler chicken flocks.

Methods: Field studies were conducted to evaluate the suitability and performance of the Lateral Flow method compared to the standard culture ISO 10272 method, and in one study, also to real-time PCR, using a cross-seasonal representative set of broiler chicken faecal samples, collected during the *Campylobacter* baseline study launched by the European Commission in 2008.

Results: From a total of 187 flocks, the Lateral Flow test identified 102 *C. jejuni* and/or *C. coli* positive flocks out of the 103 positive high-shedding flocks detected by culture, indicating a sensitivity of 99.0% for the LFD. Eleven flocks not detected by the LFD were below 7.0 $\log_{10}$ CFU *Campylobacter* spp./g faeces and could be considered as not high-shedding flocks. Real-time PCR revealed 134 *C. jejuni* and/or *C. coli* positive flocks out of 187 (71% prevalence) and so higher than identified by culture and Lateral Flow.

Significance: These studies indicate the Merck Millipore *Campylobacter* Immunological Rapid Screening Kit can reliably identify high shedding *C. jejuni* and / or *C. coli* broiler flocks under field conditions, pre-slaughter, with a time-to-result of 2 hours, and could be used as a tool for a *Campylobacter* Risk Management Program.

P2-11 Evaluation of an Immunological Rapid Test for the Detection of Pathogenic *Listeria* spp. from Foods

JOHN, LISA[1]; Slaghuis, Joerg[1]; Wulff, Heike[1]; Lindhardt, Charlotte[2]
1. Merck Millipore, LB-R&D Applications, Merck KGaA, Darmstadt, Hessen, Germany.
2. Merck Millipore, LB-PM-Pathogens & Applications, Merck KGaA, Darmstadt, Germany.

Introduction: *Listeria* are gram-positive, non-spore-forming, rod-shaped bacteria. Of the six known species of the *Listeria* genus, *Listeria monocytogenes* is the most notable human and animal pathogen, *L. ivanovii* is pathogenic only in animals. *L. innocua*, *L. seeligeri*, *L. grayi* and *L. welshimeri* are considered harmless environmental bacteria.

Listeriosis, the disease caused by *L. monocytogenes*, manifests itself not only as sepsis, but primarily as meningitis or encephalitis. Since *L. monocytogenes* is capable of crossing the placental barrier, an

infection of the pregnant mother with *Listeria* constitutes a special risk for the fetus which can result in spontaneous abortion or stillbirth. Foods constitute one of the main sources of infection, due to the ubiquitous distribution of *Listeria* in the environment and their ability to grow to infectious levels even at refrigerator temperatures (+4°C to + 8°C).

Purpose: In the conduct of risk-related quality controls in foods and in the context of state-of-the-art hygiene-status monitoring procedures, tests should be run not only for *L. monocytogenes*, but also for the *Listeria* genus in general.

The presence of *Listeria,* in particular of *L. innocua,* is an indicator of critical hygienic conditions in the production process. The drastic increase in the incidence of food infection caused by *Listeria* demands reliable and rapid methods of detection. Apart from traditional culture methods, immunological techniques are becoming increasingly popular with users, due to their improved specificity and sensitivity. Singlepath® *Listeria* is an immunological screening test based on the immune (lateral) flow principle and is designed in such a way that time-consuming and personnel intensive working steps are reduced.

Methods: Singlepath® *Listeria* performance was evaluated by i) enrichment of artificially contaminated food samples from dairy, seafood and meat food groups, ii) limit of detection in pure cultures and iii) pure culture inclusivity/exclusivity studies.

Results: Pure culture sensitivity and specificity is 100%. *Listeria* spp. can be detected in artificially contaminated foods from dairy, seafood and meat groups, at the low detection level of 1–10 CFU/25 g food. Pure culture detection limit ranges from 2.15 x 10^5 CFU/ml to 1.1 x 10^6 CFU/ml and is strain and enrichment broth type dependent.

Significance: Singlepath® *Listeria* reliably detects *Listeria* spp. in artificially contaminated foods from dairy, seafood and meat groups at the low detection level of 1-10 CFU/25 g food, within 48 hours.

P2-12 Genedisc Multiplex-PCR and IMS-Chromogenic Media for Detection of VTEC and *Salmonella* in Lettuce, Strawberries and Basil

DELBEKE, STEFANIE[1]; Holvoet, Kevin[1]; Samuels, Eric[2]; Uyttendaele, Mieke[1]
1. Food Safety and Food Quality, University Ghent, Ghent, Belgium.
2. Pall GeneDisc Technologies, Bruz, France.

Introduction: VTEC have been increasingly recognized as foodborne pathogens in fresh produce. Detection methods are primarily focused on *Escherichia coli* O157:H7, but there is increasing attention for non-O157 VTEC. Adequate detection methods are lacking for non-O157 VTEC, in particular for fresh produce, with high levels of competing flora. Multiplex PCR methods are recommended for detection of VTEC virulence factors and have potential to detect multiple enteric pathogens or emerging virulence factors (e.g., aggR) in one run.

Purpose: The aim of the present study is to validate an appropriate approach for high throughput multi-screening for VTEC and *Salmonella* in fresh produce focusing on the case studies of strawberries, lettuce and basil.

Methods: Sample replicates were inoculated (1000, 100, 10 CFU/25 g) and analysed after 1 and 5 days storage at 7°C using in parallel GeneDisc Multiplex PCR (*stx_2*, *stx_1*, *eae*, aggR and *Salmonella*) and IMS-chromogenic media.

Results: Both methods enabled detection of the *E. coli* O157 and O26 and *Salmonella* Thompson strain at all inoculums if analysed after 24 h. For basil, GeneDisc needed ½ dilution of DNA extract to overcome inhibition and competitive flora troubled reading of chromogenic media. After 5 days cold storage, pathogens were recovered for basil (stored at 10°C) whereas lower inoculums levels were more difficult to detect for strawberries (13/18 for VTEC and 10/18 for *Salmonella*) and lettuce (16/18 for VTEC and 18/18 for *Salmonella*).

Significance: GeneDisc Multiplex PCR was shown to be a suitable screening method for simultaneous sensitive detection of VTEC and *Salmonella* in fresh produce, although overcoming PCR inhibition by DNA dilution was needed in particular after 5 days storage and for the leafy vegetables. IMS combined with chromogenic media was shown to be a cumbersome method in particular for VTEC to differentiate from competing flora in leafy vegetables.

P2-13 Incidence of *Campylobacter* spp., *Escherichia coli* and *Salmonella* spp. in Raw and Ready-to-Eat Meat in the Greater Lubumbashi Area

MAFU, AKIER ASSANTA[1]; Kitwa, Mireille Umba[1]; Mobinzo, Paul[1]; Kabwang, Rosette[1]
1. Food science, Agriculture and Agri-Food Canada, St-Hyacinthe, QC, Canada.

Introduction: The official reporting food system in the Province of Katanga (DRC) indicates that the cases of foodborne diseases were partly covered by complete investigation including laboratory analysis of the suspected food products.

Purpose: The work was to investigate the prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* spp. in meat in order to make sure of the safety of products consumed in Lubumbashi.

Methods: In this study, a total of 457 samples of raw meats (chicken, pork, beef, tripe and goat) and ready-to-eat meats (pork and goat) were tested for the presence of *Campylobacter* spp., *Escherichia coli* and *Salmonella* spp. The sample meats were randomly obtained from 6 market chains of six municipalities in the Greater Lubumbashi area from September 2007 to September 2008. *Campylobacter*, *Escherichia coli* and *Salmonella* cells were recovered respectively on *campylobacter* agar with antibiotics supplementation, and XLT and McConkey agars.

Results: The majority of meat samples (56.6%) were contaminated with *Salmonella* spp., and a large percentage of the markets visited had *Salmonella*-contaminated chickens. This revealed that market raw meats are often contaminated with foodborne pathogens. However, there are marked differences in the detection of such pathogen bacteria in different meats products. From results, most large number of isolates of *Campylobacter* (32.5%) and of *Escherichia coli* (30.9%) was obtained from products sold respectively to the market of Kamalondo and Kampemba. Approximately 25.0% of the beef tripes were contaminated by *Campylobacter*, while the samples of ready-to-eat meat of the municipalities of Kamalondo, Kampemba, Katuba and Kenya were free from these bacteria.

Significance: The data suggested that raw meats in market are potential vehicles for the transmitting foodborne diseases and this stresses the need for the implementation of hazard analysis of critical control points (HACCP), good manufacturing practices (GMP) and consumer food safety education efforts.

P2-14 Effect of Probiotic on the Attachment of *Listeria monocytogenes* to Stainless Steel, Glass and Polypropylene Surfaces

MAFU, AKIER ASSANTA[1]; Lemay, Marie-Josée[1]
1. Food Science, Agriculture and Agri-food Canada, St-Hyacinthe, QC, Canada.

Introduction: In the food environment, even with acceptable cleaning and disinfection systems, attached microorganisms can remain and survive on inert surfaces for a long period of time to become a source of food contamination due to contact with inert materials.

Purpose: This study was conducted to evaluate the effect of probiotic on *Listeria monocytogenes* attached to food contact surfaces at different exposure times.

Methods: *Pediococcus acidilactici* and *Listeria monocytogenes* were used. Stock cultures were subcultured in MRS and BHI broths for *P. acicilactici* and *L. monocytogenes*, respectively. For the surfaces attachment, penicylinders in bioreactors were contaminated by 1% culture of *L. monocytogenes* and *P. acidilactici* and/or mixed cultures of both strains at ambient temperature under low agitation (90 rpm) with contact times of 3, 6, 24 and 48 h. After incubation periods, the materials were removed from bioreactor and rinsed with saline water. The surfaces were then placed in a tube containing peptone water and the attached bacterial cells were detached by sonicating before preparation of serial dilutions. The bacterial viable counts were performed by plating *P. acidilatici* on MRS agar and *L. monocytogenes* on Palcam agar, followed by incubation.

Results: Our study indicated that growth of *L. monocytogenes* is affected by the presence of *P. acidilactici*. Elevated contact time directly influenced the population of *L. monocytogenes* in mixed culture of both bacteria. After 24 hours in mixed culture, the population of *L. monocytogenes* was 3.7 log units lower than that of pure culture of *L. monocytogenes*. Furthermore, after 48 hours this difference increased to 6.7 log units. No growth differences were observed between the pure culture of *P. acidilactici* and the mixed culture of *P. acidilactici* and *L. monocytogenes*.

Significance: Probiotics such as *P. acidilactici* could be used on food contact surfaces as a complement for cleaning and disinfection procedures to achieve growth inhibition of *L. monocytogenes*.

P2-15 Occurrence of *Campylobacter jejuni* and *Campylobacter coli* in Latvian Broiler Chicken Production

KOVALENKO, KASPARS[1]; Roasto, Mati[2]
1. Faculty of Veterinary medicine, Latvia University of Agriculture, Jelgava, Latvia.
2. Dept. of Food Hygiene, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu, Estonia.

Introduction: Campylobacteriosis in humans is mostly caused by *Campylobacter jejuni* and *C. coli* and the general source of these bacteria is contaminated broiler chicken meat. In 2009, the European Union averaged 45.6 confirmed campylobacteriosis cases per 100,000 inhabitants. In Latvia the situation is yet unknown, and there is no *Campylobacter* state monitoring program at the broiler chicken production level.

Purpose: The aim of the present study was to determine the occurrence of *Campylobacter* spp. and the most commonly isolated *Campylobacter* species on broiler chicken meat and caecal samples at the slaughterhouse and retail level in Latvia.

Methods: Altogether 2,400 broiler chicken intact intestines, 240 fresh broiler chicken neck skins and 240 fresh broiler chicken carcasses were collected from two of Latvia's biggest poultry meat producing companies in 2010. The isolation of *Campylobacter* was carried out in the Food Hygiene laboratory of the Institute of Food and Environmental Hygiene, Latvia University of Agriculture (Jelgava, Latvia) by using the ISO 10272-1:2006 method. The multiplex PCR assay (Wang et al., 2002) was used to define *Campylobacter* species in 75 randomly selected isolated *Campylobacter* strains.

Results: A total 230 (95.8%) of the pooled broiler chicken caecal samples; 146 (60.8%) of the broiler chicken neck skin samples and 142 (59.2%) of broiler chicken carcasses were positive for *Campylobacter* spp. The most commonly isolated *Campylobacter* species from slaughterhouse 'A' was *C. coli* (87.5%), but from slaughterhouse 'B' *C. jejuni* (89.5%).

The average proportion of *Campylobacter* positive broiler chicken neck skin samples from slaughterhouse 'A' was significantly ($P = 0.03$) higher than in chicken neck skin samples from slaughterhouse 'B'. Similar tendency was observed in caecal samples from slaughterhouse 'A' where the average proportion of *Campylobacter* spp. positive samples was significantly ($P < 0.001$) higher than in pooled caecal samples from slaughterhouse 'B'. There was no significant difference ($P > 0.05$) in broiler chicken carcass contamination level between slaughterhouse 'A' and 'B'.

Significance: In conclusion, high *Campylobacter* occurrence in Latvian broiler chicken production was observed in 2010, much higher than in neighboring Estonia were the occurrence has been less than 10% for many years both for broiler chicken caecal and meat samples. High *Campylobacter* occurrence in Latvian broiler chicken production level may pose direct risks for human health. Therefore, there is need for state *Campylobacter* and campylobacteriosis monitoring as well as for adequate control programs. Academic study and publication is financed by the Project 'Support for doctoral studies in LLU' /2009/0180/1DP/1.1.2.1.2/09/IPIA/VIAA/017/ agreement No.044-08/EF2.D4.01.

P2-16 Inclusivity and Exclusivity Performance of a Scorpion® Probe-based Real-time PCR Assay for *Salmonella*

MOLLER, THOMAS B.[1]
1. DuPont, Kastrup, Denmark.

Introduction: *Salmonella* is a genus of bacteria that are a leading cause of foodborne illness throughout the world. The bacteria are found in a variety of food and environmental sources such as meat, poultry, eggs and milk. Over 2,500 serotypes of *Salmonella* have been identified; therefore, having a rapid, sensitive and specific method for its detection is essential.

Purpose: The purpose of this study was to evaluate the inclusivity and exclusivity capabilities of a Scorpion® probe-based real-time PCR assay for genus *Salmonella* (Sal RT PCR) detection.

Methods: The *Salmonella* inclusivity strains originated from a wide range of food, veterinary diagnostic and non-diagnostic sources. For the inclusivity panel, 409 different strains across the six main subgenera of *Salmonella* were tested. Ninety-four percent of the strains represent subgenera I, while the remaining six percent constitute subgenera II through VI. The exclusivity panel included 44 different non-*Salmonella* species obtained from the DuPont Qualicon culture collection. Most of these isolates originally came from naturally contaminated food samples or animal sources. For inclusivity and exclusivity testing, each pure culture was inoculated into prepared BHI media and incubated

overnight at 37°C. Inclusivity cultures were diluted in culture media to a cell density of one log above the limit of detection for the assay (10^5 CFU/mL). Exclusivity cultures were diluted in culture media to a cell density of approximately 10^8 CFU/mL. The commercial assay for *Salmonella* was tested for comparison.

Results: Sal RT PCR, as well as, the BAX® System assay for *Salmonella* returned positive results for the 409 target strains tested for inclusivity and negative results for the 44 non-*Salmonella* strains tested in the exclusivity panel.

Significance: This study demonstrated that the real-time PCR assay for *Salmonella* is rapid and sensitive and showed no significance difference when compared to the commercial BAX® System assay for *Salmonella*. This was as expected considering that the Scorpion®-based probes were designed from the same target sequences used in the commercial assay.

P2-17 Validation of Commercial Test Kits for the Detection of *Escherichia coli* O157:H7 and Non-O157:H7 STEC

MOLLER, THOMAS B.[1]
1. DuPont, Kastrup, Denmark.

Introduction: Current regulations for *Escherichia coli* O157:H7 and new regulations for non-O157:H7 STEC require proof of effectiveness for commercial tests before they can be used by industry to detect these pathogens.

Purpose: The following studies were conducted to demonstrate the effectiveness of the BAX® System Real-Time PCR Assay for *E. coli* O157:H7 and the BAX® System STEC suite for detecting these pathogens in beef trim with a limit of detection (LOD) at or near 1 colony forming unit (CFU) per analytical portion for each of the target pathogens.

Methods: Twelve experiments were conducted, including four for *E. coli* O157:H7, three for *E. coli* O26, and one for each of the remaining five STEC serotypes of regulatory and public health significance. For each study, twenty 375 g beef trim portions were spiked at a target level of 1.5 CFU per portion. Spiked samples and appropriate negative controls were enriched in the media and tested with each of the appropriate real-time assays for *E. coli* O157:H7 and STEC.

Results: Results obtained were consistent with an LOD of 1 CFU per analytical portion for each of the targeted pathogens.

Significance: USDA policies regarding the new STEC regulations will increase testing for these pathogens in beef trim by the meat industry. As a result, a well validated, rapid method for detecting these organisms will be required. These studies indicated that PCR detection of *E. coli* O157:H7 and the other key non-O157:H7 STEC using the BAX® System method with proprietary enrichment media is rapid and sensitive.

P2-18 Validation Study of Assurance GDS MPX for Top STEC in Raw Beef Products

Feldsine, Philip T.[1]; KRESSNER, ANITA[1]
1. Research, BioControl Systems SpA, Bellevue, WA, United States.

Introduction: Raw beef products have been analyzed for the presence of *Escherichia coli* O157:H7 as defined in the United States Department of Agriculture (USDA) regulations for several years. More recently, the USDA has proposed a new requirement to test certain raw beef products for a broader group of *E. coli* commonly referred to as "the top six shiga toxigenic *E. coli*" or "Top STEC" as adulterants. The USDA considers the following six STEC O serotypes, in addition to O157, to represent the greatest public health risk: O26, O45, O103, O111, O121 and O145. The USDA will require routine testing of all these 7 serotypes for raw beef starting in March 2012. Assurance GDSTop STEC MPX is an assay that allows simultaneous detection of *E. coli* O157:H7 and also the top 6 non-O157 STEC (Top 7 STEC) in a sample.

Purpose: Develop an assay to simultaneously detect *E. coli* O157:H7 and the top 6 non-O157 STEC to fulfill new USDA regulations

Methods: Raw beef (beef trim, ground beef and finely textured beef) samples were inoculated with a 2-day cold stressed target organism. 375 g samples were analyzed with the Assurance GDS Top STEC MPX method for determination of the simultaneous presence of *E. coli* O157:H7 and the top 6 non-O157 STEC. All samples were confirmed via IMS plating on a modified Rainbow agar and BVCC agar.

Results: All seven serotypes were analyzed among all three food types. A total of 198 raw beef samples were inoculated with 0.5 – 0.7 CFU per sample to achieve partial recovery. The enrichments were assayed with Assurance GDS Top STEC MPX and confirmed using IMS onto selective agar

plates. 114 samples were positive by Assurance GDS MPX after 10 hours. There was one sample which was negative by Assurance GDS Top STEC MPX which confirmed positive, for an overall detection rate of 99%.

Significance: The Assurance GDS Top STEC MPX assay is a reliable method for simultaneous detection of both *E. coli* O157:H7 and Top 6 non-O157 STEC in a common sample.

P2-19 Detection of Seven Top STEC *Escherichia coli* Serotypes by the Assurance GDS Top STEC MPX Assay

Feldsine, Philip .[1]; KRESSNER, ANITA[1]
1. Research, BioControl Systems, Inc, Bellevue, WA, United States.

Introduction: The United States Department of Agriculture (USDA) has proposed new regulations applicable to the analysis of certain raw beef products which define six shiga-toxigenic *Escherichia coli* serotypes, in addition to O157, ("Top 7 STEC") as adulterants. The Assurance GDS Top STEC MPX assay has been developed to provide an effective testing solution to satisfy this requirement. System components include top 7 STEC serotype-specific immunomagnetic sample concentration reagents with the PickPen concentration device, amplification and identification reagents defining STEC genes *eaeA* (O157), *eaeA* STEC, *stx1* and *stx2*, with Internal Control in a multiplex format; and microbiological confirmation reagents for Top 7 STEC isolates.

Purpose: To define the specificity (Inclusivity and Exclusivity) of the Assurance GDS Top STEC MPX assay for *E. coli* serotypes O26, O45, O103, O111, O121, O145, and O157.

Methods: Inclusivity was determined by analyzing a panel of 67 STEC strains representing all seven top STEC serotypes. Exclusivity was determined by analyzing a panel of 31 strains representing potential cross-reactive microorganisms.

Results: Sixty-seven (67) *E. coli* top 7 STEC inclusivity isolates were correctly identified and typed for the *eaeA* and *stx* genes by the MPX assay, with correct differentiation of *eaeA* (O157), and *eaeA* (STEC) genes. All 31 Exclusivity panel non-top STEC strains gave negative results.

Significance: These results verify the accuracy of the Assurance GDS Top STEC MPX assay for the identification of top 7 STEC organisms.

P2-20 A Parasite Toxin of *Sarcocystis* in Raw Horse Meat Causes a New Foodborne Disease

SUGITA-KONISHI, YOSHIKO[1]; Irikura, Daisuke[1]; Saito, Morihiro[2]; Yahata, Yuichiro[3]; Kamata, Yoichi[1]
1. Division of Microbiology, National Institue of Health Sciences, Tokyo, Japan.
2. Saitama Meat Inspection Center, Saitama, Japan.
3. Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Tokyo, Japan.

Introduction: Recently, a new foodborne disease associated with raw meat has increased in Japan. This foodborne disease causes nausea, vomiting and diarrhea as the main symptoms within 12 hours after eating. From the epidemiological analysis with odds ratio, raw horse meat was estimated a causative food. From the detailed microbacterial examination of the meat, known pathogenic bacteria and virus have not been detected. Many cases of such foodborne diseases, we found a parasite, *Sarcocystis fayeri*, in the meat and investigated the toxicological and epidemiological properties.

Purpose: Previously, we have reported sarcocystin, which is toxin in *S. cruzi* extracts, showed toxicity such as diarrhea and was lethal in rabbits by intravenous injection. In this study, we studied the relationship between the toxicity of *S. fayeri* and the properties of sarcocystin.

Methods: The ileum loop of the rabbit test and the oral administration test using living cyte of *S. fayeri* were carried out. For cDNA cloning of the sarcocystin of *S. fayeri*, we designed two degenerate primers which were predicted from trypsin-digested two internal peptides of this protein. The full-length cDNA of the sarcocystin was obtained by RACE method.

Results: The ileum loop of rabbit test revealed that the homogenate of raw horse meat containing *S. fayeri* and its bradyzoites evoked the toxicity such as the edema. The oral administration of living cyte of *S. fayeri* to rabbits also caused bloody diarrhea and fluid accumulation in the loop. From these results, we concluded a causative agent of this foodborne disease is *S. fayeri* . Furthermore the intravenous injection of the fraction containing toxin into rabbits caused the same symptoms. The cDNA contained a protein composed with 118 amino-acid residues (Mr = 13,261). The injection of the purified recombinant of "sarcocystin" (200 µg) into a ileum loop of rabbits caused the edema and fluid accumulation (F/A Ratio: 0.5 mL/cm). Our data demonstrated that there might be a possibility that the "sarcocystin" of *S. fayeri* is a causative agent of the new foodborne disease in humans after eating

raw horse meat.

Significance: We have discovered a new causative agent of foodborne diseases associated with fresh raw horse meat. It is a parasite *S. fayeri* and its pathogen would be “sarcocystin” which is a parasite toxin.

P2-21 A Case Study of *Salmonella* Contamination in a Category 3 Fat Rendering Plant

KENNEDY, THOMAS G.[1]

1. Department of Agriculture, Food and the Marine, Limerick, Ireland.

Introduction: Producing safe food and feed is the constant objective of pet food manufacturers. Pet foods and treats are often found in the home kitchen, in food preparation areas and are often handled by children, the elderly and others with immune system deficiencies. Food safety issues involving direct human contact with processed pet foods and animal feeds is becoming a major regulatory focus.

Purpose: This case study describes an intractable case of *Salmonella* contamination in a Category 3 animal by-products melting facility which renders animal fats producing tallow for the oleo-chemical industry and greaves for pet-food manufacture. Over a protracted period of 5 years, 33 out of 305 official greaves samples intermittently revealed the presence of *Salmonella anatum*, *S. kentucky* and *S. newington*. The facility is located adjacent to a beef slaughterhouse, which is the raw material source, and it operates a Hazard Analysis and Critical Control Point (HACCP)-based manufacturing system. The facility is approved under European Union Regulation (EC) No. 1774/2002 and is subject to official inspections, audits and sampling by the Competent Authority.

Methods: The HACCP plan identifies three Critical Control Points (CCP) namely – pre-rendering fat particle size, metal detection, rendering temperature and duration. Analysis of CCP implementation and monitoring did not reveal loss of control. Due to the high rendering temperatures the source of contamination was believed to be post rendering contamination. *Salmonella* was not isolated from any of the environmental samples (n = 62) nor from any products taken within process (n = 88). Analysis of pre-requisite programmes showed deficiencies in pest control e.g. doors being left open, sanitation, zoning e.g., personnel freely moving from low to high risk areas, operator hygienic practices e.g., laying utensils on the ground and structure fabrication e.g., cracks in wall and floors. Deep cleaning and corrections to operational prerequisite programmes resulted in temporary improvements. The establishment was voluntarily decommissioned for 10 months. Prior to re-opening, facility fabrication was improved by laying a smooth surfaced floor, removing roughened welded seams in plant and equipment, smooth plastering the walls and properly sealing and ducting cables and hoses.

Results: Post structural improvement none of the 120 official greaves samples revealed the presence of *Salmonella*. We conclude that the source of contamination was from intermittent shedding from nidi located in the deep recesses of blemishes within the fabric.

Significance: *Salmonella* is capable of surviving for extended periods in a variety of environments and on numerous materials. Complete elimination of pathogens is dependent on the strict adherence to HACCP and GMPs. Some practices are easy to apply, however in this case, restoration of control required significant investment and plant redesign. The maintenance of control at CCPs will not ensure product safety when pre-requisites are grossly deficient.

P2-22 Rapid Detection of Foodborne Pathogens Using Isothermal Nuceic Acid Amplification

NORTON, PAUL [2]; Langley, Rob[1]; Hosking, Edan[2]; Wendorf, Michael[2]; Mozola, Mark[2]; Rice, Jennifer [2]

1. EMEA Division, Neogen Europe Ltd., Ayr, United Kingdom.
2. Research and Development, Neogen Corporation, Lansing, MI, United States.

Introduction: A new family of tests, ANSR™, has been developed for rapid detection of pathogenic bacteria in foods and environmental samples. The tests are amplified, isothermal nucleic acid assays based on the nicking enzyme amplification reaction (NEAR™). It allows users to process sample serially or in parallel form as well as eliminating many of the limitations of antibody-based technologies.

Purpose: The new test platform provides the food industry both the DNA-definitive test results they need, and the much easier and quicker methodology that they require. Recent food recalls have only emphasized the point that the food industry needs easier and quicker precise pathogen tests to lessen the chance that contaminated food products ever reach the consumer.

Methods: Using a molecular beacon probes, the tests generate fluorescent signal which is measured in real-time using a simple incubator/fluorescence reader. Following sample enrichment,

assays are completed in approximately 30 minutes including sample preparation.

Results: The *Salmonella* assay has a limit of detection of 1,000-10,000 CFU per mL and high specificity for serovars of both *S. enterica* and *S. bongori*. Single-step enrichment ranges from 10 to 24 hours in duration depending on the sample type. The method has been validated for use with a variety for use with a variety of sample types including sponge or swab samples from environmental surfaces, poultry and red meats, and pasteurised egg products. In testing of 145 sponge or swab samples taken from five different environmental surfaces, the molecular assay produced 71 positive results (all confirmed) vs. 68 by the U.S. FDA reference culture method. In testing of pasteurised dried egg, chicken carcass rinse, and three types of raw and processed meats, the molecular method produced 57 confirmed positive results vs. 62 by the USDA reference culture procedure. There were no statistically significant differences in the number of positive results by the molecular and reference methods for any of the sample types tested, with the exception of one environmental surface type for which the molecular assay produced significantly more positive results. The *Listeria* assay targets high copy number ribosomal RNA sequences specific to *Listeria* spp. and has a limit of detection of less than 100 CFU per mL. Method validation is focused on environmental samples, processed meats and seafoods, and dairy products. Tests for *Salmonella* spp. and *Listeria* spp. have been developed, and a test for Shiga toxin-producing *Escherichia coli* will follow.

Significance: Considering their accuracy, speed, and simplicity, the new molecular assays represent a significant advancement in diagnostic methodology for food safety management. High assay sensitivity allows the use of abbreviated enrichment protocols, and assays are completed within minutes and require only simple, low-cost instrumentation.

P2-23 *Salmonella* and *Listeria monocytogenes* Detection in Complex Food Matrices Using a Fully Automated Pathogen Purification and Detection System

ARMSTRONG, MARCIA[1]; Fakih, Sarah[1]; Schlappa, Sabine[1]; Kueppers, Corinna[1]; Cornelius, Petra[1]; Luley, Sandra[1]; Engel, Holger[1]
1. QIAGEN GmbH, Hilden, Germany.

Introduction: During the past decades food safety concerns have steadily increased and are presently a major challenge for food authorities. While product approval using traditional culturing methods can take several days, real-time PCR represents a powerful tool for specific and rapid detection of food-borne pathogens with time to results of less than 24 hours. However, the complexity of food matrices can limit the universal adoption of such methods.

Purpose: Difficult food matrices, for instance those with high fat or polyphenol content, can inhibit PCR detection of pathogen DNA. The purpose of the current study was to develop a method for the purification and real-time PCR detection of *Salmonella* spp. and *Listeria monocytogenes* from such matrices.

Methods: We present here results of studies in which *Salmonella enterica* or *Listeria monocytogenes* DNA was purified from enrichment cultures of challenging food matrices. A fully automated purification method for both gram negative and gram positive pathogens was developed on the QIAsymphony Rotor-Gene Q platform. For the purposes of this study, the capacity of the automated DNA extraction and real-time PCR detection system to deal with high inhibitor concentrations was tested by preparation of pathogen enrichment cultures with different food: medium ratios. Starting with the established 1:10 ratio (25 g food in 225 ml enrichment medium) the food matrix background was constantly increased up to a 1:6 ratio (25 g food in 125 ml enrichment medium). For *Salmonella* detection an increase in matrix background was tested with chocolate, peanut butter, whole milk, and ground beef in BPW. The respective matrix was spiked with ~5 – 10 CFU *Salmonella*, homogenized and incubated for 18 hours at 37 C. (n = 4 for each matrix: medium combination). Similarly, 25 g samples of 3.5% fat milk, sliced turkey and smoked salmon were added to decreasing volumes of One Broth medium plus supplement, and spiked with *Listeria monocytogenes* (~5 – 10 CFU). The resulting enrichment cultures for both bacteria types were directly processed without manual pre-treatment protocols. Based on a chemically aided heat lysis, specific depletion of food-derived inhibitors and DNA purification with magnetic silica beads the resulting DNA solution was assayed for the presence of pathogen using real-time PCR based pathogen detection assays.

Results: The efficiency of the automated DNA purification and tolerance of the subsequent real-time PCR assay system was tested against an increasing titration of food matrix per ml enrichment culture. Reliable pathogen DNA recovery and detection for both gram negative and gram positive bacteria was observed over all tested matrix: medium ratios, suggesting an efficient background depletion during DNA isolation. For all tested ratios *Salmonella* detection showed stable Ct values

between 18 (whole milk) and 30 (ground beef). For *Listeria* Ct values ranged between 24 (3.5% fat milk) and 34 (sliced turkey).

Significance: The fully automated pathogen purification and detection system presented here provides a powerful, reproducible, and sensitive method for the detection of *Salmonella* spp. and *Listeria monocytogenes* in difficult food matrices.

P2-24 Microbiological Safety of Fresh Fruit Juices in Pakistan

AKHTAR, SAEED[1]; Riaz, Muhammad[1]; Ismail, Tariq[1]
1. Food Science and Technology, Bahauddin Zakariya University Multan-Pakistan, Multan, Pakistan.

Introduction: Street vended fresh orange, mango, carrot and sugarcane juices in Pakistan are served with ice in improperly cleaned tumblers. The method of extraction, inadequate washing of the fruits before extraction and uncleanness of the machines and utensils are some of the significant indicators posing considerable hazards in fruit juices.

Purpose: Among several determinants, absence of sanitation and hygiene in handling commercial fresh fruit juices in Pakistan have been established as the major cause of many health implications. Therefore, we aimed at exploring the safety of various street vended fresh fruit juices in Multan, the fifth largest city of Pakistan.

Methods: A total of 72 samples of fresh fruit juices were collected from four geographical zones and analyzed for Total Viable Counts (TVC), Total Coliforms (TC), Faecal Coliforms (FC), *Escherichia coli*, and *Salmonella* spp.

Results: The pH of the samples ranged 2.4 - 4.7. TC and *E. coli* were shown to exist in the range of 05-7.2 × 10^4 and 0 - 6.7 × 10^3 CFU/mL respectively, while FC and *Salmonella* were recorded as 33.33 - 66.66 % and 8.33 - 33.33%, respectively. Apple juice was found to contain highest bacterial load (6.7 × 10^7 CFU/mL). Imli aalo bukhara drink and pineapple juice showed minimum contamination for FC and *Salmonella*. Gravity of the prevalence of bacterial contamination in juices among four zones of sampling indicated zone- III and IV to be more precarious, however, the juices collected from zone-I and II were observed to carry least microbiological.

Significance: The pragmatic levels of microbiological contaminants in fresh street vended fruit juices call for immediate measures to be taken to educate the street fruit juice vendors to ensure quality and safety. The authors thank Higher Education Commission of Pakistan for financial support under project No. 1415 to undertake this research.

P2-25 Reduction of *Salmonella* on Harvesting Knives Following Thirty Second Sanitizing Treatments

TAPP, WILLIAM N.[1]; Gragg, Sara E.[1]; Brashears, Mindy M.[1]; Brooks, J. C.[1]; Miller, Markus F.[1]
1. Animal and Food Sciences, Texas Tech University, Lubbock, TX, United States.

Introduction: Pathogenic contamination of the carcass may occur during slaughter if knives used to harvest animals are not sanitized properly, thereby causing cross-contamination of *Salmonella* and other pathogens to subsequent animals throughout harvest. Thermal sanitizing treatments are often used to control this pathogenic contamination. Hot water is commonly used for these treatments, but is not readily accessible worldwide.

Purpose: The purpose of this study aimed to compare room temperature sanitizing treatments to standard water sanitation treatments to determine the treatment most effective at reducing *Salmonella* on harvesting knives.

Methods: Boning knives were inoculated with *Salmonella*, and treated thirty seconds in one of six sanitizing treatments, including: 1.1% sodium metasilicate (SMS), 200 ppm quaternary ammonium compound (QAC), 200 ppm chlorine, 5% lactic acid, hot water (82.2°C), and ambient water (20.6°C). Initial and treated pathogen loads were retrieved using spongesicles hydrated with neutralizing agents specific to each treatment, or buffered peptone water (BPW) in the case of water treatments. Serial dilutions were made in the neutralizing agents or BPW. Samples were plated onto XLD agar before incubation at 37°C for 24–48 hours, prior to enumeration.

Results: Data analysis showed that initial pathogen load means of knives were 4.51 log CFU/cm^2. Treated counts were 4.47, 1.91, 1.76, 1.83, 0.96, and 4.78 log CFU/cm^2 on knives *($P < 0.001$)* after thirty second treatment in 1.1% SMS, 200 ppm QAC, 200 ppm chlorine, 5% lactic acid, 82.2°C water, and room temperature water (20.6°C).

Significance: This study concludes that while thermal sanitation is generally more effective for pathogenic reduction, there are alternatives producing reduction of *Salmonella* if hot water is not available.

P2-26 Milk Sterilization by Pulsed Electric Fields

MENESES, NICOLAS[1],Rineke, Kai[2], Knorr, Dietrich[2]
1. Corporate Technology, Buhler AG, Uzwil, Switzerland.
2. Department of Food Biotechnology and Food Process Engineering, Berlin University of Technology, Berlin, Germany.

Introduction: The intense process needed to inactivate spores in liquid foods produces a high loss of nutrients and sensorial properties. Thus, a novel method to inactive spores may be required. Pulsed Electric Fields (PEF) has already been proposed as an alternative method for killing spores, but so far no proper process conditions have been found and only up to 3.5 inactivation-logs of *Bacillus subtilis* and *Bacillus cereus* have been reported.

Purpose: This study aims to modify a PEF treatment chamber of a co-linear kind to hold temperatures up to 150 °C, and attempts to find proper process parameters, such as electric field strength, treatment time and temperature in order to inactivate up to 5 $\log_{10}$ of spores.

Methods: The treatment chamber was thermally insulated in order to reduce heat transfer between the environment and the liquid product. Low-fat milk was inoculated with *B. subtilis* and *Geobacillus stearothermophilus* spores and then treated by PEF. Process parameters were 22–32 kV/cm, 12–45 µs, and 130–225 kJ/kg, with an initial temperature of 75–110°C and pressure 3–5 bar. The process was simulated by a Finite Element software (Comsol Multiphysics) and validated with temperature measurements.

Results: A thermally insulated treatment chamber showed a heat transfer coefficient of 50 W/m^2K, which allowed a temperature loss of only 5 °C. Average temperatures between 100 and 130 °C were kept up to 30 s after PEF treatment. The F-value was calculated from the temperature–time profile and showed a thermal spore inactivation between 0.2 and 2 $\log_{10}$. The application of PEF under certain process conditions (an electric field strength of 22 kV/cm during 45 µs and 110°C) induced a total spore inactivation up to 5 $\log_{10}$. Thus, a synergistic effect of up to 4.8 inactivation-$\log_{10}$ was found.

Significance: Pulsed Electric Fields (PEF) could be an alternative method for inactivating high thermal resistant bacterial spores in liquid foods at continuous conditions. The proper selection of process parameters allowed up to 5 inactivation-$\log_{10}$ (*G. stearothermophilus*) in low-fat milk in less than 30 s through a synergistic effect between temperature and PEF.

P2-27 Modelling of Microbial and Enzymatic Spoilage of Carrot Juice Treated by Pulsed Electric Fields

MENESES, NICOLAS[1]; Boon, Floor[2]; Martinez, Angie[3], Knorr, Dietrich[3]
1. Corporate Technology, Buhler Ag, Uzwil, Switzerland.
2. Food and Biotechnology Innovations, TNO, Zeist, Netherlands.
3. Department of Food Biotechnology and Food Process Engineering, Berlin University of Technology, Berlin, Germany.

Introduction: The shelf-life of a food product can be defined as the period during which the product is still acceptable for human consumption. The shelf-life is determined by microbial, chemical and physical aspects, which is in most cases a consequence of raw material quality, product formulation, processing, packaging and storage conditions. The combination of processing and storage conditions (package and temperature) determines what microorganisms or enzymes can be regarded as shelf life limiting. Pulsed electric field (PEF) is considered as non-thermal technology, which could improve the quality of carrot juice while increasing or maintaining the period of shelf-life.

Purpose: This work aims to develop experimental and mathematical models in order to predict the microbial and enzymatic inactivation and thus the shelf-life after a continuous PEF treatment.

Methods: Carrot juice was PEF treated and then the shelf-life was analysed during 1 month of storage at 4°C. *Escherichia coli* and yeasts and Pectinmethylesterase (PME) were used as indicators for microbial and enzymatic spoilage respectively during the shelf-life. Kinetics models were experimentally obtained and combined in order to predict the shelf-life of PEF treated carrot juice based upon microbial and enzymatic spoilage.

Results: As a result of the combined inactivation and shelf-life models, it was possible to determine if the food spoilage was caused by microorganisms or enzymes. Depending on the process conditions, either a higher microbial or enzyme inactivation was achieved (i.e., 20 kV/cm, 30°C, 8 µs of treatment time and 5°C storage temperature, the shelf-life was of 3 days and determined by PME, but when the treatment time was increased up to 16 µs, the shelf-life was of 21 days and determined by yeast growth). Root mean square error for kinetics models was under 5%.

Significance: The application of PEF pasteurization technology requires accurately defined treatment intensity, followed by a predictable microbial inactivation, as well as, a minimal impact on heat sensitive compounds. The presented investigation helps to select proper process parameters, in order to guarantee a safe level of enzymatic and microbial inactivation and low growing rate during the shelf-life.

Poster Session 3 – Wednesday, 22 May, 09.00-14.00

Authors will be present at their posters during the conference breaks

P3-01 Determination of Mineral Oil Saturated Hydrocarbons (MOSH) and Mineral Oil Aromatic Hydrocarbons (MOAH) in Food and Packaging

Matteini, Paolo[1]; Rosi, Francesco[1]; Bondi, Chiara[1]; Castellano, Emiliano[1]; Miniati, Alessandro[1]; Bocci, Alessandra[1], ROMANI, MARCO
1. Silliker Italia SpA, Prato, Italy.

Introduction: The wide presence of mineral oils in food and food packaging has been the focus of much research in recent years and generates concern among consumers. Mineral oil mixtures found in food consist of saturated hydrocarbons (MOSH) and/or aromatic hydrocarbons (MOAH). A group of Swiss researchers leaded by Koni Grob demonstrated that MOSH and MOAH in cardboard packaging made from recycled newspaper can migrate into food. Mineral Oils can also contaminate food due to oil leaks, agricultural treatments and frauds.

Purpose: The aim of this project was to get a reliable method for determination of MOSH and MOAH in food and food packaging (paper and cardboard).

Methods: Silliker Italy laboratory chose to validate the HPLC-GC-FID method developed by Koni Grob at the Food Safety Laboratory of the Canton of Zurich.

Results: Complete validation data for dry food (cereals and by-products) and for paper and cardboard have been achieved by Silliker Italy. Spaghetti, rice, bran, paper and cardboard have been studied for MOSH and MOAH at multiple concentration levels.

Statistical data (Trueness 80% to 121%, Repeatability below 14%, Expanded Uncertainty below 30%) have confirmed the possibility of effective on-line-NP-HPLC separation of MOSH, and MOAH, and the effectiveness of IS-GC-FID quantification with good accuracy and precision. Validated LOQs are 0,5 mg/Kg for dry food and 10 mg/Kg for paper and cardboard.

Significance: The analyses conducted on real samples in our laboratory confirm that food contaminations by mineral oil fractions of various origin are widely diffused, and this justify the concern regarding these issues.

Because of the complexity of MOSH and MOAH bands, all the details provided by the chromatograms could also help in localising the origin of contamination, proceeding with additional studies including a comparison between foodstuff and its packaging, or by means of shelf life studies.

P3-02 Study on the Effect of Storage Conditions on the Recovery of *Salmonella* from Bootswabs and Hairnets

RABIE, ANDRE[1]; McLaren, Ian M.[1]; Sayers, Robin[2]; Vaughan, Kelly[1]; Kell, Nick[1]; Davies, Rob[1]
1. Department of Bacteriology, Animal Health and Veterinary Laboratory Agency, Addlestone, United Kingdom.
2. Centre for Epidemiology & Risk Analysis, Animal Health and Veterinary Laboratory Agency, Addlestone, United Kingdom.

Introduction: Bootswabs/hairnets are widely used in the sampling of poultry flocks for *Salmonella* in Europe. This study was designed to investigate the efficiency of detection of *Salmonella* under different conditions.

Purpose: To investigate the effect of moisture level and temperature over time on the ability to recover *Salmonella* Enteritidis or Typhimurium from two types of boot swabs, Tyvek boot swabs and hairnets.

Methods: Testing involved spreading chicken faeces containing ~10 and ~100 CFU/g of *Salmonella* onto the swabs. In the first study, contaminated swabs were maintained in a dry or moist atmosphere at 5.0°C or 20.0°C respectively. After 1, 2, 3, 4 and 7 days four replicates from each test condition were examined individually for *Salmonella*. In a second study, boot swabs were maintained in similar conditions as the first study, but at 4.0°C, 8.2°C and 16.6°C respectively and only one replicate for each.

Results: Logistic regression models were fitted to the proportions of positive samples with categorical factors for day, conditions, concentration and treatment for each round respectively. In the first study, *Salmonella* recovery was better at 5.0°C in moist conditions with a linear regression in time. Boot swabs and hairnets showed similar results predicted means 2.35–2.52 and 2.50–2.66 respectively.

In the second study recovery was best at the higher concentration under moist conditions at 8.2°C with a downward trend in time. Time, conditions (moist/dry) and concentration were all highly significant ($P < 0.001$) at 4.0°C and 8.2°C. Recovery at 4°C was better than at 16.6°C, but not as good as at 8.2°C. Time, concentration and temperature were all significant ($P < 0.001$) at 16.6°C, although conditions were not quite significant ($P = 0.089$).

Significance: To ensure the recovery of low levels of *Salmonella*, samples should be kept cool and moist during the period between collection and examination with a minimal time delay between sampling and processing. Without temperature and moisture control of the samples *Salmonella* recovery will be impaired after 24 hours.

P3-03 Comparison of Applicability Rapid Methods for *Salmonella* Detection in Poultry Meat

ZOLLER, LINDA[1]; Rohonczy, K.[1]; Mráz, B.[1]; Tabajdiné Pintér, V.[1]
1. Wessling Hungary Ltd, Budapest, Hungary.

Introduction: The comparison of three rapid methods for *Salmonella* detection was carried out in order to assess their applicability in poultry meat matrix in a routine diagnostic laboratory.

Purpose: Comparison of two PCR Assay for *Salmonella* (BAX, ABI) and one Vidas diagnostic method for *Salmonella* detection was carried out to obtain the specificity, sensitivity and accuracy of these methods in a poultry meat matrix.

Methods: 141 naturally contaminated poultry meat samples were tested for *Salmonella* using the MSZ EN ISO 6579:2006 method, BAX® System PCR Assay for *Salmonella* (DuPont Qualicon), TaqMan *Salmonella* PCR detection kit (Applied Biosystems) real-time PCR and Vidas ICS2-SLM (bioMérieux) diagnostic method after 24 h enrichment in buffered peptone water.

Results: The agreement percentage with the ISO 6579:2006 method was respectively 97% for TaqMan real-time PCR method and 100% for the Vidas ICS2-SLM and BAX® System real-time PCR methods. Specificity being an indication of the method's ability to discriminate negative samples was respectively 98% for the real-time PCR and 100% for the Vidas ICS2-SLM and TaqMan PCR real-time PCR method. Sensitivity being an indication of the method's ability to detect positive samples was respectively 100% for the BAX® System, and the TaqMan PCR real-time PCR methods and 100% for the Vidas ICS2-SLM method.

Significance: Both of the used real-time PCR methods and the Vidas ICS2-SLM assay provided a rapid and user friendly screening method for detection of *Salmonella* which can be used with confidence for poultry meat matrix in a routine diagnostic laboratory and thus decrease turnaround time at producers due to the fast reporting process.

P3-04 Microbiological Risk Assessment and Mitigation of Deep Fried Ready-to-Eat Foods (Indian Rojak) in Singapore

Thu, Aung Kyaw[1]; Chau, Man Ling[1]; Yuk, Hyun-Gun[2]; Lo, Jerilyn[2]; Yap, Jennifer[1]; GUTIERREZ, RAMONA[1]; Ng, Lee Ching[1]
1. Environmental Health Institute, National Environment Agency, Singapore, Singapore.
2. Food Science and Technology Programe, Department of Chemistry, National University of Singapore, Singapore, Singapore.

Introduction: In 2009 in Singapore, a massive food poisoning outbreak, known as the Geylang Serai incident, saw over 150 persons affected, including 48 hospitalized. Two deaths were reported. The outbreak was linked to the contamination of a local deep-fried dish, Indian rojak, by *Vibrio parahaemolyticus*.

Purpose: Because this incident had a strong impact on Singaporean minds, we aimed at conducting a risk assessment on Indian rojak. Specifically, the aim of this study was to evaluate the effectiveness of traditional reheating of the Indian rojak before serving, on the reduction of its microbial load. We also aimed at understanding the source of the high microbial counts found on specific ingredients of the Indian rojak.

Methods: A total of 455 samples were collected from Indian rojak dishes, covering five types of deep-fried ingredients collected before and after reheating, as well as four types of accompanying ingredients (raw vegetables, sauce), from different retail food outlets located in various regions of Singapore. All samples went through bacteriological detection protocols adapted from FDA's Bacteriological Analytical Manual.

Results: No specific pathogens such as *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* O157, *Salmonella* spp. and *Vibrio parahaemolyticus*/cholera were recovered from any of the reheated

samples, although *E. coli* was detected in a few samples. Traditional reheating practiced by foodhandlers was shown to significantly reduce ($P < 0.001$) the median Standard Plate Counts (SPC) from 7.5×10^4 to 8.3×10^2 CFU/g. A limited number (10%) of reheated Indian rojak dishes collected were found to have overall Standard Plate Counts (SPC) above 10^5 CFU/g. Tofu and fish cake were found to be the ingredients with highest microbial loads, with items purchased from local markets being significantly ($P < 0.001$) more contaminated than those purchased from supermarkets.

Significance: Although the Geylang Serai incident may have had a negative impact on the general perception and consumption of Indian rojak throughout the island, our data provide evidence that traditional reheating of this RTE food dish generally does bring the microbial load under the authorized SPC limit. Even though further reheating recommendations may be needed, current local practices do not make the Indian rojak a particularly hazardous item.

P3-05 Evaluation of the Anti-*Listeria* Activity of Indigenous Lactic Acid Bacteria in Liver Pâté

Romani, Chiara[1]; Granchi, Lisa[2]; ROMANI, MARCO[1]; Dufour, Christophe[3]; Colombo, Stefano[4]
1. Silliker Italia Spa, Prato, Italy.
2. University of Florence, Florence, Italy.
3. Silliker Sas, Cergy, France.
4. Merieux NutriSciences, Lyon, France.

Introduction: Physical and chemical methods are widely employed today to control *Listeria monocytogenes* in foods. Market globalization, growing demand for minimally processed products and the introduction of new manufacturing processes, have led to the development of biopreservation tools, such as organic acids, natural preservatives and cultures of microorganisms which, when duly inoculated, can exert anti-listerial activity without compromising the nutritional and organoleptic quality of product. Scientific studies have shown that the indigenous lactic flora of products can inhibit the growth of *Listeria monocytogenes*, through the production of various anti-listerial substances.

Purpose: The objective of this study was to evaluate the anti-listerial activity of indigenous lactic acid bacteria isolated from liver pâté employing: a) *in vitro* evaluation, b) phenotypic and molecular identification, and c) challenge studies.

Methods: Seven liver pâtés, each different for ingredients, manufacturing process and chemical-physical parameters, were analyzed for the presence of lactic acid bacteria. Four main populations of lactic acid bacteria, indicated as A, E, G and L, and showing the best physiological state (short lag phase, high growth rate) were isolated. A pure culture of each population was tested using the agar well diffusion assay to evaluate the anti-*Listeria* activity against 16 strains of *Listeria monocytogenes*, mainly isolated from meat products. Only G and L isolates showed a consistent inhibition against all 16 strains, and were therefore tested again against the same strains by means of the agar drop test. Their anti-listerial activity was confirmed, likely due to the release of organic acids. Phenotypic (API 50 CH test - bioMérieux) and molecular (PCR - ARDRA) analyses identified the G isolates as *Leuconostoc mesenteroides*, and the L isolates as *Weissella viridescens*. Three challenge tests were subsequently conducted on each of two identical liver pâtés, denoted as B and C, using triplicate samples. The pâté B samples underwent a heat treatment before packaging. Each challenge test consisted of three sets of samples: a non inoculated set, a set inoculated with *Leuconostoc mesenteroides* and *Weissella viridescens* (each at 300 CFU/g), and a set inoculated with *Leuconostoc mesenteroides* and *Weissella viridescens* (each at 3,000 CFU/g) Each sample was also spiked with a mixture of the same 16 strains of *Listeria monocytogenes* (15 CFU/g) previously tested. All samples were incubated at 8°C. Plate counts of *Listeria monocytogenes* (ISO11290-02: 2005) and lactic acid bacteria (ISO15214: 1998) were performed daily until the achievement of the stationary phase, and the data entered in the predictive software ComBase DMfit, thus providing the growth parameters of both microbial populations.

Results: The data generated were statistically assessed using the 1-way ANOVA and the Tukey test. A moderate anti-listerial activity was detected only in the B pâté samples and a significantly lower growth rate of *Listeria monocytogenes* was observed, even though all samples across all sets contained the same level of *Listeria monocytogenes* at the end of the study period. At this stage, a further molecular analysis showed that the inoculated species prevailed over an initial more heterogeneous population which, for instance, consisted of other native species like *Lactobacilllus sakei* and *Lactobacillus curvatus*.

Significance: The selection of lactic acid bacteria capable to exert an anti-listeria activity must be made starting from indigenous lactic acid bacteria, i.e., from the same matrix in which ideally those microbial population will be re-inoculated to control the growth of *Listeria monocytogenes*. The successful bio-preservation produced by a selected lactic flora in a given product results from a

complex interaction based on extrinsic parameters (storage temperature, inoculum level), intrinsic parameters (heterogeneity and initial concentration of lactic acid bacteria), and on its interaction with the matrix, its indigenous flora, and undesired contaminants like *Listeria monocytogenes*. The appropriate synergy of in vitro tests, challenge tests and molecular analysis can help standardize the selection of lactic flora with anti-listerial capability.

P3-06 *Listeria monocytogenes* Growth in Liver Paté Italian Crostini – An Integrated Challenge Test

ROMANI, MARCO[1]; Romani, Chiara[1]; Matteini, Paolo[1]; Crociani, Joel[2]; Dufour, Christophe[2]; Colombo, Stefano[3]
1. Silliker Italia Spa, Prato, Italy.
2. Silliker SAS, Cergy, France.
3. Merieux NutriSciences, Lyon, France.

Introduction: Current models used to study the growth of *Listeria monocytogenes* in the presence of lactic flora are based on the growth potential and the maximum growth rate. They do not take into consideration the lag phase, the growth rate and the stationary phase of the microbial populations, which show mutual interaction. Such data, obtained by means of dedicated challenge tests, are elaborately achieved using the Combase software to define the growth parameters of *Listeria monocytogenes* inoculated in the product and of the natural lactic flora exherting an anti-*Listeria* activity. By simply measuring the concentration of these microbial populations at a given time, the establishment of the so-called "fitting curves" allows us to predict the concentration and the time at which *Listeria monocytogenes* and the lactic flora reach their stationary phase.

Purpose: The study was aimed designed to predict, by means of a calculation method based on the "fitting curves", the concentration of *Listeria monocytogenes* and lactic flora and the time at which they enter into their stationary phase in liver paté crostini.

Methods: The prediction was performed using two different approaches: the former is based on the time difference between the onset of the *Listeria monocytogenes* and the lactic flora stationary phases, while the latter is based on the lactic flora concentration capable to induct the stationary phase of *Listeria monocytogenes*. Predictive data were then compared with challenge test data and statistically assessed for their significance.

Results: Three different strains of *L. monocytogenes* isolated from meat and meat products were used to perform three distinct challenge tests. Triplicate samples from three different batches of finished product (liver paté crostini) were inoculated with a single-strain inoculum of 1.8 Log CFU/g, as recommended by the AFSSA guidelines. Samples were then stored at 4°C, 8°C and 12°C. *Lactobacillus* spp. (ISO15214:1998) and *L. monocytogenes* (ISO11290-02:2005) plate counts were performed daily on each sample until the stationary phase was reached by in both populations. The challenge test results (dates and counts) were input entered in the Combase software to determine the growth parameters later used to calculate the stationary phase time. Calculated data were then assessed against the results of three additional challenge tests using triplicate samples from two different batches, the same strains and the same single-strain inoculum. Samples from the first batch were stored for 5 days at 4°C + 5 days at 8°C + 5 days at 12°C; samples from the second batch were stored for 3 days at 4°C + 3 days at 8°C + 4 days at 12°. The results obtained showed that both approaches used to calculate the onset of the stationary phases of the two microbial populations are equivalent. The stationary phase average concentration of *L. monocytogenes* was 3.73 log CFU/g ("time difference" approach) and 3.92 log CFU/g ("lactic flora concentration" approach) versus a challenge test concentration of 3.74 log CFU/g. While the T-student statistical analysis showed very small statistical differences, the observed standard deviations for all the data set were < 0.2 log CFU/g, a value largely inferior to the uncertainty provided by the count method.

Significance: The use of the Combase prediction integrated with the challenge test experimental data, both combined to calculate the stationary phase onset time for *L. monocytogenes* and the lactic flora in liver paté crostini, can be used to determine a relevant parameter for the safety of such product. This is made possible by simply knowing the concentration of the concerned microbial populations at a given time. This methodology can be applied to other foods by means of specific challenge test results and in presence of a demonstrated interaction between the microbial populations of concern.

P3-07 Assessment of Microbiological Quality of Mechanically Separated Meat Produced Using Techniques That Do Not Alter the Structure of the Bones

POMYKALA, REMIGIUSZ[1]; Michalski, Miroslaw[1]; Osek, Jacek[1]
1. Department of Hygiene of Food of Animal Origin, National Veterinary Research Institute, Pulawy, Poland.

Introduction: Mechanically separated meat (MSM) is a product obtained by removing remaining meat from bones of poultry carcasses using mechanical means, where the normal structure of the muscle fibre is mostly lost or modified in such a way that it is not comparable with regular meat. MSM is an important raw food material used for the manufacturing of meat products and meat preparations. The technology of production of MSM is based on low- and high-pressure techniques, which in different ways influence in the sensitivity of MSM to microbiological contamination during production and further handling. The quality and stability of MSM vary between the techniques used and depend on parameters associated with the machines used.

EU Regulation (EC) No 2074/2005 states that MSM produced using techniques that do not alter the structure of the bones used in the production of MSM should be treated as different from MSM produced using techniques that alter the structure of the bones. Furthermore, MSM of the former type produced under specific condition and of a specified composition should be permitted in meat preparations that are clearly not intended to be consumed without heat treatment.

Microbiological criteria for MSM produced using techniques that do not alter the structure of the bones, contained in the EU Regulation (EC) No 1441/2007 (OJ L 322, 7.12.2007), include *Salmonella* spp. as food safety criterion (n = 5, c = 0, absence in 10 g) , aerobic colony count in 1 g (n = 5, c = 2, m = 5 x 10^5 CFU/g, M = 5 x 10^6 CFU/g) and *Escherichia coli* in 1 g (n = 5, c = 2, m = 50 CFU/g, M = 500 CFU/g), as a process hygiene criteria.

Purpose: The aim of this study was to determine the compliance with microbiological criteria of MSM produced using techniques that do not alter the structure of the bones.

Methods: The samples of MSM tested were obtained by use of a low-pressure technique in Baader type machines. Raw material for the production of MSM was pork bones. MSM was produced immediately after carcasses were cut. The samples of MSM were sampled during the production under the supervision of official control and transported to NVRI at cooling conditions. A total of 52 samples of MSM were tested. The microbiological analyses were performed according to standards: for *Salmonella* spp. – PN-EN ISO 6579:2003, for aerobic total count – PN-EN ISO 4833:2004+Ap1:2005 and for *E. coli* – PN-ISO 16649-2:2004.

Results: The study shows that amongst 52 samples of MSM *Salmonella* spp. were detected in 25 of them (48.1%). The limit of aerobic total count was exceed in 16 samples (30.8%). *E. coli* in numbers higher than 500 CFU/g were found in 23 samples (44.2%).

Significance: In conclusion, MSM produced and stored under not appropriate hygienic conditions may pose a significant risk to public health associated with the consumption of meat preparations and products, when MSM is used as a raw material.

P3-08 STEC Screening – New Real-time PCR Detection Assay Based on Shiga Toxin and *Eae* Genes

SCHOENENBRUECHER, HOLGER[1]; Dueker, Felicitas[2]; Osssmer, Rolf[1]; Slaghuis, Joerg[1]; Buelte, Michael[2]; Lindhardt, Charlotte[1]
1. Merck Millipore, Darmstadt, Germany.
2. Institute of Veterinary Food Science, Justus-Liebig-University, Giessen, Germany.

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) have been identified as an important cause of foodborne infections in humans, involving a range of serious, chronic and potentially fatal diseases. Whilst serotype O157:H7 is the best-recognized strain, other STEC serotypes are emerging, including O111, O26, O45, O103, O121 and O145. Due to the severe illnesses caused by these pathogens, a fast and reliable detection method is required.

Purpose: The purpose of this study was to evaluate the novel Rapidcult™ *Escherichia coli* for the detection of *E. coli* O157 in ground beef and beef trims and extend its applicability to STEC detection by using a novel foodproof™ STEC screening kit.

Methods: An external validation study compared two different methods for the detection of *E. coli* O157:H7 in ground beef and beef trims. The MLG 5.04 “Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat Products” of the Microbiology Laboratory Guidebook from USDA-

FSIS was used as a reference method. Rapidcult™ *E. coli* enrichment broth was used for the detection of *E. coli* O157 in 375 g samples of pooled ground beef and beef trims. Detection was conducted by either applying an immunological based lateral flow test or real-time PCR. In addition, a novel foodproof STEC screening kit was used to detect the required STEC serotypes.

Results: For both matrices the sensitivity rate was 100% and the specificity rate was 75% for ground beef and 81% for beef trims, using Singlepath *E. coli* O157 and the foodproof *E. coli* O157 Detection Kit, after 10–12 h (ground beef) or 8 h (beef trims) incubation time. No false negative results were detected. by using the STEC screening kits, the STEC serotypes of O26, O103, O145, O45, O111 and O121 could be detected in ground beef after 8h incubation.

Significance: In comparison to standard methods, Rapidcult™ *E. coli* shortens enrichment time from 22 h to 8-12 h. The capability to analyze pooled samples dramatically reduces the daily work load and costs. The foodproof STEC screening kit is extending the current product portfolio aiming to comply with US MLG 5.05 and the EU technical specifications.

P3-09 Comparison of Two Commercial Real-time PCR Systems with Culture Methods for the Detection of *Salmonella* spp. in Environmental and Fecal Samples of Poultry

SCHOENENBRUECHER, HOLGER[1]; Lindhardt, Charlotte[1]; Sommer, Dagmar[2]; Slaghuis, Joerg[1]; Lierz, Michael[2]
1. Merck Millipore, Darmstadt, Germany.
2. Justus-Liebig-University, Giessen, Germany.

Introduction: *Salmonella* are an important cause for severe human gastroenteritis after consumption of contaminated poultry products especially eggs and meat. European regulations are aiming to reduce *Salmonella* prevalence in primary production of poultry to less than 1 percent.

Purpose: The purpose of this study was to evaluate the foodproof® *Salmonella* detection kit for the detection of *Salmonella* in sample types being mandatory for monitoring primary production such as boot socks, feces, feed and dust.

Methods: The study compared the efficiency of the detection kit and Bax® PCR Assay *Salmonella* system with standardized culture methods (EN ISO 6579:2002 – Annex D) for the detection of *Salmonella* spp. in poultry samples. For evaluation four sample matrices (feed, dust, boot swabs, feces) directly from poultry flocks as well as artificially spiked samples of the same matrices were used. All samples were tested first for *Salmonella* spp. using culture methods as gold standard. Furthermore all methods were evaluated in an annual ring-trial of the National *Salmonella* Reference Laboratory of Germany.

Results: *Salmonella* detection in the matrices feed, dust and boot swabs led to comparable results of both PCR systems whereas the results from feces differed markedly. Furthermore, the quality especially the freshness of the fecal samples had an influence on the sensitivity of the real-time PCR and culture methods results. In fresh fecal samples an initial spiking level of 100 CFU/25 g *Salmonella* Enteritidis was detected. Dry fecal samples allowed the detection of 14 CFU/25 g. Both real-time PCR protocols appear to be suitable for the detection of *Salmonella* spp. in all four matrices with a sensitivity advantage for the foodproof® system investigating fecal samples.

Significance: RT-PCR reduces time to result to two days only compared to classical culture enabling e.g., faster restocking or product release.

P3-10 WITHDRAWN

P3-11 A Process Capability Study Establishing *Salmonella* as a Process Hygiene Microbiological Criterion in Wild Pheasant

KENNEDY, THOMAS G.[1]
1. Dept. of Agriculture, Food and the Marine, Limerick, Ireland.

Introduction: Foodstuffs should not contain micro-organisms at levels that present an unacceptable risk to human health. Food safety is ensured by a preventative approach such as HACCP. Microbiological criteria can be used to verify HACCP and other hygiene control measures. They also provide guidance on the acceptability of foodstuffs and their manufacturing and handling processes. It is thus appropriate to set criteria defining process acceptability and also food safety criteria setting a limit above which a foodstuff should be considered unacceptably contaminated.

Purpose: Within the European Union, Regulation 2073/2005 establishes the process hygiene criteria (PHC) for carcasses derived from domestic fowl. No such criteria exist for pheasant. Notwithstanding this, pheasant must be processed using HACCP principles. Consequently, PHC have

a role in its verification. This study proposes to establish PHC for pheasant in an Irish establishment using a process capability study.

Methods: The processor selected for the study procures pheasants hunted from protected reserves, which are stocked with 6-week-old pullets from a rearing unit 6–8 months prior to the shooting season. In season 1 on each of 10 sampling days 4 g of the neck skin (NS) were aseptically harvested from 35 pheasants selected at random post-chilling. The NS from 7 carcasses were pooled to create 5 x 25 g final samples. Samples were analysed for the presence for *Salmonella* using ISO method 6579. The process was replicated in season 2 and 3.

Results: One sample each season revealed the presence of *Salmonella*. PHC were determined thus:

Food Category: Pheasant carcasses;
Microorganisms: *Salmonella*;
Sampling Plan: n = number of units comprising the sample = 50 derived from 10 consecutive sessions;
c = number of samples where *Salmonella* is detected = 1;
Limits: m = M = absence in 25 g of a pooled NS sample;
Analytical Reference Method: ISO 6579;
Stage Where Criterion Applies: Post Chilling.

Significance: Ongoing performance exceeding these criteria prompts the establishment to implement timely corrective action to its processing procedures and to review disease control and bio-security measures on the rearing farm. In the absence of legally mandated PHC for pheasant, it is recommended that processors follow the protocols outlined to establish their own PHC.

P3-12 A Relational Study between Ante-mortem Findings and Post-mortem Meat Condemnations in Three Beef Abattoirs

Carroll, Denis[1]; KENNEDY, THOMAS G.[2]; McKevitt, Aideen I.[3]
1. Veterinary Clinic, Urlingford, Kilkenny, Ireland.
2. Department of Agriculture, Food and the Marine, Limerick, Ireland.
3. NICHE, University of Ulster, Coleraine, United Kingdom.

Introduction: Cattle presented for slaughter undergo AnteMortem Examination (AME) and are categorized into 3 groups (1) 'casualties' that receive a veterinary inspection on farm following injury or illness prior to salvage at the slaughterhouse, accompanied with a veterinary casualty certificate (VCC) that is used to focus a more detailed AME and PostMortem Examination (PME), (2) 'remarkable' animals that present without a VCC but have defects identified at AME focusing a more detailed PME and (3) 'apparently healthy animals' (AHA) are those neither presented as 'casualties' or detected as 'remarkable' at AME.

Purpose: PostMortem (PM) condemnation of meat is regarded as the ultimate definition that an animal or part of is unfit for human consumption. This study investigates the relationships between VCC data and AME and PME findings in three beef slaughterhouses.

Methods: VCC, AME and PME data covering a two year period from three slaughterhouses were examined. Results were entered into Microsoft Access® database tables. Filters were applied to the tables to determine relationships between VCC data, AME and PME findings.

Results: 216 certificates were examined. 763 animals had all or part of their carcasses condemned at PM. Casualty animals accounted for 14.5% of all condemnations and 11.3% of full carcass condemnations. AHAs accounted for 69% of full carcass condemnations. Emaciation, oedema and toxic conditions accounted for 80% of full carcass condemnation from AHAs, and therefore went undetected at AM, resulting in high risk animals being slaughtered amongst truly healthy animals and are therefore a potential source of cross-contamination.

Significance: These findings highlight the need for enhanced AM detection of these conditions in the development of a risk based meat inspection system. The present study prompts research into the practical application of body condition scoring and the development of rapid detection methods for acute phase proteins and serum albumen levels to enhance AM.

P3-13 Comparative Detection of *Salmonella enterica* in Meat by 3M Molecular Detection Assay *Salmonella* and the ISO 6579:2002 Method

BONARDI, SILVIA[1]; Alpigiani, Irene[1]; Bacci, Cristina[1]; Brindani, Franco[1]; Pongolini, Stefano[2]

1. Dept. of Animal Health, Faculty of Veterinary Medicine, Parma, Italy.
2. Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Parma, Italy.

Introduction: *Salmonella* spp. is a zoonotic microorganism responsible for the second most common foodborne disease in the European Union.

Purpose: The purpose of this study was the comparative detection of *Salmonella enterica* in meat products by the 3M Molecular Detection Assay *Salmonella* and the ISO 6579:2002 method.

Methods: A total of 200 meat samples (raw meat from chicken, turkey, pork and beef, minced meats, sausages, offal and meat preparations) were tested. After pre-enrichment in BPW (37 ± 1°C for 16–20 h) broth cultures were tested by: i) the 3M method employing a prototype machine; ii) the ISO 6579:2002 cultural method. Samples positive by the 3M method were subjected to a three-tube MPN enumeration method. Twenty-five grams per sample were diluted 1:10 in BPW. The three-tube MPN dilutions represented 1.0, 0.1, and 0.01 g of the samples.

Results: Overall, 22 samples out of 200 (11.0%) were found positive for *Salmonella* spp. Fifteen samples were positive by both methods (68.2%); three more samples were found positive by the 3M method only and four by the ISO 6579 method only. One of the four samples testing positive by the ISO method only was found to be inhibited by the 3M method. Positive and negative results were available after 24 h using the 3M method while the ISO 6579 method required three days for negative results and five-seven days for positive ones. *Salmonella* enumeration ranged from < 0.3 MPN/g to 2.1 MPN/g.

Significance: The Molecular Detection Assay *Salmonella* is a time saving and easy to use method that can be very useful in processing food samples. Discordant results between the two methods could be attributable to the low level of contamination by *Salmonella* spp. in these naturally contaminated samples which implies stochastic randomness in the positive outcome of any low contamination sample regardless of the method used.

P3-14 Comparison of a 3M Molecular Detection System Prototype with the DIN EN ISO-methods for the Detection of *Listeria monocytogenes* and *Salmonella* Enteritidis

PFANNEBECKER, JENS[1]; Becker, Barbara[1]
1. Microbiology, Institut für Lebensmitteltechnologie-NRW (ILT-NRW), Lemgo, NRW, Germany.

Introduction: The 3M Molecular Detection System (3M MDS) is an automated system for the rapid detection of pathogenic organisms, such as *Salmonella*, *Listeria* and *Escherichia coli* O157. The system was developed to detect pathogens in different foods like meat, convenience-food, fruits, vegetables, pet food and surface swabs. Due to the combination of isothermal DNA amplification and bioluminescence detection it is possible to achieve highly sensitive results.

Purpose: In the present study, the results of the automated prototype were compared to the results achieved by using qualitative DIN EN ISO methods for the detection of *Listeria monocytogenes* and *Salmonella* Enteritidis in meat, delicatessen and spices.

Methods: A total of 155 samples (72 naturally contaminated and 83 artificially inoculated) were tested to determine *S.* Enteritidis (DIN EN ISO 6579) and *L. monocytogenes* (DIN EN ISO 11290-1). The enrichment of the samples as well as the testing in 3M MDS was carried out by double testing. The overall testing time with the automated system was 75 minutes after enrichment for 18 hours – with detection of the samples in real-time.

Results: No differences were found between 3M MDS testing and the results of the standard DIN EN ISO methods for *S.* Enteritidis (6579) and *L. monocytogenes* (11290-1) in meat and delicatessen. Differences between the two methods could be observed while testing two mixed spices. In further experiments matrix-inhibiting effects which occurred while testing spices could be eliminated by performing 1:100 dilutions.

Significance: Using the 3M MDS for detection of *L. monocytogenes* and *S.* Enteritidis in food microbiology provides reliable results in reduced time for preparation of samples and analysis of results. The device is easy to use and the time to result can be achieved in less than 20 hours compared to 90–96 hours for DIN EN ISO methods.

P3-15 Novel Multiplex SNP-based Method Allows Identification of the Newly Identified Epidemic Clone V of *Listeria monocytogenes*

LOMONACO, SARA[1]; Knabel, Stephen J.[2]
1. Dipartimento di Patologia Animale, Università degli Studi di Torino, Grugliasco, Italy.
2. Dept. of Food Science, The Pennsylvania State University, University Park, PA, United States.

Introduction: Epidemic clones (ECs) of *Listeria monocytogenes* group genetically related isolates, presumably of a common ancestor that have been implicated in different unrelated outbreaks for the

past 30 years (Kathariou, 2002). For ECs discrimination, six single nucleotide polymorphisms (SNPs) that were able to differentiate a specific EC from unrelated strains were defined as informative (Lomonaco et al., 2008). A novel multiplex SNP-based assay was recently developed, which interrogated these six informative SNPs (Lomonaco et al., 2011). This assay correctly and specifically identified all 4 previously identified ECs, and also identified nine strains as ECs, which had previously been misclassified. All other non-EC strains shared one or more common SNP profiles, different from those of ECs (Lomonaco et al., 2011).

Purpose: Since then, another EC (ECV) has been identified (Knabel et al., 2012). *In silico* determination of the ECV SNP-profile using available whole-genome sequences (Gilmour et al. 2010) showed that the above six selected SNPs would also provide a specific profile for ECV. The objective of this study was to test the novel multiplex SNP-typing assay for identification of ECV.

Methods: SNP-typing was conducted on 49 ECV isolates that had been previously tested with ECV-specific PCR and multi-virulence-locus sequence typing - MVLST (Knabel et al., 2012). One isolate previously found positive by PCR but not confirmed by MVLST was also included (Knabel et al., 2012).

Results: The SNP-based screening revealed a ECV-specific profile for all 50 isolates, including the false-positive ECV sample, thus showing 100% sensibility and 98% specificity.

Significance: Further tests on a wider selection of *L. monocytogenes* isolates from different sources will be needed to confirm these preliminary findings. Notwithstanding, this novel multiplex SNP-typing can be a valuable tool to rapidly screen for all five currently known ECs of *L. monocytogenes*. Detection and differentiation of strains of *L. monocytogenes*, in particular detection of ECs, will be critical for the implementation of more efficient strategies to reduce contamination and thus improving the safety of food products. Funds kindly provided by Prof.ssa Grassi (grant “Quality Milk”, Regione Piemonte, POR-FESR Asse I, Innovazione e transizione produttiva 2010–2013).

P3-16 A Simple Bioluminogenic Detection Method for the Rapid Detection of Bacteria in Foods in 4–7 Hours

EASTER, MARTIN[1]; Meighan, Paul J.[2]; Gamble, Sally[2]; Datta, Shreya[3]
1. Technical, Hygiena International, Watford, Herts, United Kingdom.
2. R&D, Hygiena International, Guildford, Surrey, United Kingdom.
3. R&D, Hygiena LLC, Camarillo, CA, United States.

Introduction: Total Viable Counts (TVC) are used routinely to estimate the bio-burden in raw, cooked and liquid foodstuff. The TVC is used to estimate the quality and safety of raw materials and finished product and give results in 3 days. Simple, rapid, low cost test systems that provide results in the same working day or shift are desirable to monitor the quality and safety of fast moving consumer goods.

Purpose: The study evaluates a new bioluminogenic method that couples the established principle of chromogenic substrates and viability for the detection of nonspecific bacteria with a sensitive light emitting reaction to provide results within 7 hours.

Methods: A bioluminogenic method was used to detect the natural population of bacteria in serial dilutions of raw ground beef within a 7 hour period and compared equivalent to the ISO 4833 method for TVC. Both methods were run at 30 and 37^{o}C. A variety of naturally contaminated raw meat samples and commercially available ready-to-eat foods were also tested by both methods.

Results: The bioluminogenic test detected less than 100 bacteria per gram of ground beef at both 30^{o}C and 37^{o}C in < 7 hours. The result was statistically significant ($P < 0.05$) at 7 hours and 30^{o}C and 37^{o}C; the results were more pronounced at 30^{o}C due to greater proportion of mesophilic bacteria. Contamination levels of <10 CFU / g were detected in 7 hours whereas >10,000 CFU/g were detected in 4 hours. The bioluminogenic test gave results covering a 4-log dynamic range after 6 hours that compared very well with the ISO method; $r^2 = 0.997$ was obtained for diluted ground beef and $r^2 = 0.868$ for different meat samples. Similar results were also obtained for a variety of other ready-to-eat foods.

Significance: The simple rapid bioluminogenic test methods delivers major time savings for the detection and quantitation of bacterial contamination. Results are produced within a same working day or shift, thus permitting the positive release of sensitive raw materials and finished products, as well as facilitating the rapid response to quality and safety issues.

P3-17 Rapid Detection of *Yersinia pestis* in Food Using Pyrosequencing Technology

AMAOKO, KINGSLEY K.[1]; Thomas, Matthew C.[1]; Kong, Fanliang[1]; Shields, Michael J.[1]; Goji, Noriko[1]; Janzen, Timothy W.[1]; Hahn, Kristen R.[1]

1. Canadian Food Inspection Agency, Lethbridge, AB, Canada.

Introduction: When a bioterrorism attack on food is attempted or perpetrated there is considerable risk for public health and large scale socioeconomic consequences. It is imperative that we possess established assays for the rapid identification of biothreat agents with high sensitivity and specificity to ensure emergency response measures can be deployed appropriately. Highly trustworthy information within a relevant timeframe is required to make a rapid and informed decision.

Purpose: Obtaining DNA sequence data from a suspected biothreat agent such as *Yersinia pestis* from food provides an added layer of confidence compared to a presumptive positive PCR amplicon. Sequencing based technologies, such as pyrosequencing, have sufficient discrimination potential to be used for biothreat agent identification.

Methods: This work explores the application of pyrosequencing technology for the rapid sequence based detection of *Y. pestis*. Amplification and sequencing primers specific for *Y. pestis* were designed and tested on a panel of bacteria that may be potentially present in food to determine their specificity. The primers were also tested on ten different *Y. pestis* strains from various sources.

Results: We have shown in this study the power of pyrosequencing in the unambiguous detection and identification of *Yersinia pestis* based on virulence genes. Sequence results ranging from 40-84 bp were generated in about 60 mins following initial PCR amplification and provide a rapid method for detection and identification in food. The absence of sequencing targets from the screening of a panel of potential foodborne pathogens and closely related strains indicates a high specificity of the assay and makes it a novel, rapid detection tool for foodborne bioterrorism application involving *Y. pestis*. In addition, the read lengths of up to 84 bp observed in this study are unprecedented for pyrosequencing using the Pyromark Q24.

Significance: The generation of sequence information in the assay developed is an added layer of confidence to our previously developed assay for *Y. pestis* detection in food (Amoako et al. 2010. *Journal of Food Protection*, 73:18-25).

P3-18 Evaluation of a Bioluminogenic Detection Method for the Rapid Detection of Indicator and Pathogenic Bacteria

EASTER, MARTIN[1]; Meighan, Paul J.[2]; Gamble, Sally [2]; Datta, Shreya[3]
1. Technical, Hygiena International, Watford, Herts, United Kingdom.
2. R&D, Hygiena International , Guildford, Surrey, United Kingdom.
3. R&D, Hygiena LLC, Camarillo, CA, United States.

Introduction: Indicator bacteria such as coliforms and *Escherichia coli* are commonly used for assessing the adequacy of food processing and post-processing contamination in heat-sterilized foods. They are also well established indicators of faecal contamination in water supplies. Test methods for coliform and *E. coli* vary in their sophistication, automation and cost but still require 14–24 hours to produce results. Modern manufacturing procedures and supply chain require rapid test results to verify compliance, quality and safety. Simple, rapid, low cost test systems that provide results in the same working day or shift are desirable to monitor the quality and safety of fast moving consumer goods.

Purpose: The study evaluates a new bioluminogenic method that couples the established principle of chromogenic substrates for the detection of specific bacteria with a sensitive light emitting reaction to provide results within 7 hours.

Methods: Specific bioluminogenic substrates were challenged with a range of target bacteria possessing the beta-Galactosidase and beta-Glucuronidase activity to determine inclusivity and exclusivity. The limit of detection, and time to detection were determined using dilutions of pure culture. Naturally and artificially contaminated foodstuffs were used to compare the rapid method with traditional cultural methods.

Results: The bioluminogenic test detected 95% and 100% for the coliform and *E. coli* strains from a panel of 45 bacteria giving an equivalent performance to traditional methods. Detection criteria was established from Mean Negative values + 3 standard deviation (n = 290.) The test detected 1–5 bacteria in 7 hours and was able to provide a rapid enumeration in the range 100 to 10,000 CFU per ml after 5 hours giving a correlation coefficient of 0.83–0.99. Contamination was detected in 50 foodstuffs from 5 different food groups and there was no significant different between the rapid method and the ISO 16140 method at $P < 0.05$. Similar results were obtained for water and beverage samples, and swabs from solid surfaces.

Significance: Equivalent results to the traditional cultural method were generated within 7 hours

permitting rapid response to quality and safety issues. The use of other specific bioluminogenic substrates will enable other specific organisms to be detected.

P3-19 Verification of the First Automated Sample Preparation and PCR Setup Robotic Workstation for the Food Industry

HARZMAN, CHRISTINA[1]; Dörres, Hans-Henno[1]; Grönewald, Cordt[1]; Berghof-Jäger, Kornelia[1]
1. BIOTECON Diagnostics, Potsdam, Germany.

Introduction: Most *Salmonella* infections are caused by ingestion of contaminated food. For laboratories performing high-throughput routine testing for foodborne pathogens, automation is key to greater efficiency. Advantages of automating sample preparation and PCR setup include traceability, data security, accuracy and reproducibility.

Purpose: Together with an international confectionary manufacturer, BIOTECON Diagnostics designed and performed verification and validation testing on an automated system, the foodproof® RoboPrep+ Series robotic workstation, for sample preparation and PCR setup. This study details the first validation of a robotic instrument for the food industry capable of DNA extraction and PCR setup.

Methods: Automatic DNA isolation was performed on the workstation using the new foodproof® Magnetic Preparation Kit I for automatic DNA isolation. Real-time PCR was then automatically setup by the workstation using the isolated DNA and *Salmonella* Detection Kit for real-time PCR. Real-time PCR results were then evaluated. This verification study of the workstation involved testing specificity, sensitivity, robustness and cross-contamination risk.

Results: The time necessary to process 96 samples on the workstation at one time was 2hr 20 min for DNA isolation and 20 min for real-time PCR setup for a total of 2 h and 40 min. Thirty-one *Salmonella* serovars and strains were tested for specificity with all being detected. Sensitivity of the method with 42 different food samples resulted in a detection limit between 5.0 x 10^3 CFU/ml and 5.0 x 10^4 CFU/ml for all matrices. Robustness testing of abnormally high food sample matrix compared to enrichment broth (1:7) showed no inhibition of *Salmonella* detection for all food samples tested. Studies found no cross-contamination to occur during tests with random placement of 48 *Salmonella* positive and 48 *Salmonella* negative samples.

Significance: The foodproof® RoboPrep+ Series robotic workstation is the first robot able to automatically perform DNA isolation and real-time PCR setup designed and validated specifically for the food industry. These intensive internal validation studies of the foodproof® RoboPrep+ Series robotic workstation and foodproof® Magnetic Preparation Kit I coupled with the foodproof® *Salmonella* Detection Kit demonstrate this is a highly specific, sensitive, and robust system. The effectiveness and capability of the robotic workstation to automatically isolate highly-purified DNA and set up PCR is able to provide laboratories accurate, precise, as well as reproducible results.

P3-20 WITHDRAWN

P3-21 Novel Methods for the Isolation and Detection of Genetic Modifications in Processed Foods

ARMSTRONG, MARCIA[1]; Fakih, Sarah [1]; Kahlau, Sabine[1]; Bogdoll, Annette[1]; Luley, Sandra[1]; Engel, Holger[1]
1. QIAGEN GmbH, Hilden, Germany.

Introduction: There are strict requirements in many regions regarding the import and sale of GMO containing foods. The detection of GMO containing DNA requires first that the techniques for extraction of DNA from foodstuffs are rapid and specific and second, that the downstream real-time PCR detection assay is reliable and sensitive in a reaction containing potential PCR inhibitors.

Purpose: The purpose of this study was to develop a method to isolate DNA from a wide variety of processed foods including honey and to determine the suitability of the DNA for real-time PCR based detection of several widely used GMO constructs.

Methods: Samples of foods potentially containing GMO constructs (corn grits, corn chips, soy crisps, pretzels, cream of wheat and canola honey) were obtained from local grocers. DNA extraction was conducted according to a shortened and optimized version of the well-established CTAB-method – the gold standard for inhibitor-free DNA isolation from food and plant material. A dedicated kit applying this optimized CTAB method was used. In brief, this kit uses a modified CTAB lysis followed by DNA purification with silica membrane based chemistry, which in contrast to the labor-intensive classical CTAB method allows rapid processing of a multiple sample throughput (30 samples in 2.5 h).

Isolating DNA from honey is challenging due to its viscous nature and the low amount of the potentially GMO containing pollen. To isolate pollen DNA, honey was subjected to a mechanical, bead-based pretreatment to ensure efficient lysis of the pollen. This was followed by DNA isolation via the CTAB-based extraction kit.

Three samples of each food type were utilized for isolation of GMO DNA. Subsequently, real-time PCR screening assays for the GMO screening constructs 35S, nos, bar and CTP2-CP4EPSPS were performed. Corn chips were tested with a MON810 corn-specific GMO identification assay. For species identification of the DNA extracted from the isolated pollen of the canola honey, DNA was assayed using literature derived primers and probes specific for canola.

Results: DNA purified from corn based foods (corn chips) was shown to be detected in the real-time assay in a specific and efficient manner with assays for the 35S promoter (Ct = 28), the nos terminator (Ct = 31) and the CTP2-CP4EPSPS (Ct = 30) construct. The bar construct was not detected. For corn grits, similar results were observed, with positive results for 35S (Ct = 27), nos terminator (Ct = 29) and CTP2-CP4EPS (Ct = 29). Corn chips were tested for the presence of the MON810 GMO gene via the mericon MON 810 GMO detection assay. Corn chips were positive for MON 810 with a Ct = 33.5. Cream of wheat and pretzels did not have detectable GMO signals for any of the screening GMO assays. Canola DNA purified from honey was detected with the canola specific real-time PCR system.

Significance: A dedicated DNA extraction method based on the well-established CTAB method (QIAGEN DNeasy mericon Food Kit), in combination with mericon GMO screening and identification kits provides a rapid, sensitive and reliable method for the isolation of DNA from processed foodstuffs and the subsequent detection of GMO constructs in these foods.

P3-22 Water as a Vehicle for Transport of *Salmonella enterica* to Tomato Plants

VAN BRUGGEN, ARIENA H.[1]; Cevallos-Cevallos, Juan M.[2]; Gu, Ganyu[2]; Danyluk, Michelle D.[3]; Wright, Anita C.[3]
1. Plant Pathology, University of Florida, Gainesville, FL, United States.
2. Emerging Pathogens Institute, University of Florida, Gainesville, FL, United States.
3. Food Science and Human Nutrition, University of Florida, Lake Alfred, FL, United States.

Introduction: Several *Salmonella enterica* outbreaks have been associated with tomatoes. Yet, transmission of *Salmonella* via water to plants is poorly understood.

Purpose: To investigate if irrigation ponds could be sources of *Salmonella*, and determine splash dispersal of *Salmonella* onto tomato plants and entry in tomato leaves and fruits.

Methods: *Salmonella* was monitored in 10 irrigation ponds for 1 year. Under controlled conditions, transmission of 2 gfp strains of *S. enterica* Typhimurium to tomato plants was investigated via splash dispersal and aerosolization in a rain simulator or leaf dipping in a suspension. Trichome densities on tomato leaves were modified with salicylic or jasmonic acid. Survival on leaf surfaces and inside inoculated and non-inoculated leaves was monitored for 7–30 days on plants grown in organic or conventional soils. Internalization from leaves via stems in fruits and seeds was checked by confocal laser microscopy.

Results: *Salmonella* was isolated from each pond; there were seasonal differences dependent on oxygen content and temperature. Splash dispersal was enhanced when soil was covered by plastic compared to bare soil or natural mulch. It was greater for strain MAE119 without fimbriae than for MAE110 with fimbriae, especially when trichome density was low; this was reversed for dispersal in aerosol. Aerosolized *Salmonella* was transferred onto tomato fruits. When leaflets were dipped in *Salmonella* suspensions, both strains were internalized, but MAE110 survived longer inside leaves and, in 1 experiment, was the only strain found in the phloem, fruits and seeds. Colonization was less in plants in organic than in conventional soils; fruit and seed infection was not observed for plants in organic soil.

Significance: *Salmonella* is common in irrigation water used for vegetable production. *Salmonella* may get into aerosols and transfer to tomato fruits on plants. *Salmonella* can enter leaves through stomata and move through phloem into fruits and seeds.

P3-23 Prevalence of Major Foodborne Pathogens in Raw Bivalve Molluscs Commercially Available in Poland

POMYKALA, REMIGIUSZ[1]; Lopatek, Magdalena[1]; Michalski, Miroslaw[1]; Osek, Jacek[1]
1. Department of Hygiene of Food Animal Origin, National Veterinary Research Institute, Pulawy, Poland.

Introduction: Seafood has the potential to cause diseases due to bacterial pathogens under certain circumstances. Despite the monitoring programs existing in EU members producing molluscan shellfish, an increasing number of poisonings due to consumption of raw or undercooked shellfish has been observed. In the recent years, a significant increase of seafood consumption is also observed in Poland. With regard to EU food law, each Member State is obliged to supervise microbiological contamination of bivalve molluscs to ensure consumer health protection.

Purpose: The aim of this study was to evaluate the prevalence of selected human pathogenic bacteria in seafood commercially available in Poland.

Methods: A total of 300 samples of raw bivalce molluscs were anlyzed in the years 2009-2011. All kinds of shellfish investigated were from EU countries. The examined material consisted of different species of seafood: oysters (*Crassostrea gigas*), mussels (*Mytilus edulis*), clams (*Mercenaria mercenaria*), Vongola verace (*Tapes semidecussatus*), scallops (*Pecten maximus*), amande (*Glyceria glycymeria*), and palourde (*Tapes philippinarum*). The samples were analyzed for the presence of pathogenic bacteria such as *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter* spp., enteropathogenic *Vibrio*, coagulase-positive *Staphylococcus* and pathogenic anaerobe bacteria. The scope of study also included total plate count, moulds and yeasts count, most probable number of *Enterobacteriaceae* and *Escherichia coli*, which are indicators of microbiological contamination of bivalve molluscs. These examinations were performed according to ISO standard methods with some modifications. All suspected *Salmonella* spp. and *Vibrio* spp. colonies were identified using biochemical panel ID 32 E, whereas isolates of *L. monocytogenes*, coagulase-positive *Staphylococcus* and anaerobes were confirmed by API *Listeria*, ID 32 Staph and ID 32 A, respectively.

Results: The study showed that *Salmonella* spp. was identified in 4 samples (1.3%), whereas *L. monocytogenes* and *Campylobacter* spp. were not found in tested shellfish. *V. parahaemolyticus* was isolated from 55 samples (18.3%). Moreover, 45 samples (15.0%) were confirmed by API system as positive for *S. aureus*. It is surprising that the pathogenic anaerobe bacteria were detected in at least 60.0% of samples tested. The number of *E. coli* established by the MPN method in 27 samples (9.0%) exceed acceptable level 230 MPN/100 g of meat and shell fluid. It was noted that total bacteria count varied between 15 CFU/g and 280000000 CFU/g. In the case of *Enterobacteriaceae* and moulds and yeasts maximum level of contamination was determined as 11000 CFU/g and 110000 CFU/g, respectively.

Significance: The results indicate that seafood constitute the potential risk of human foodborne infections. The microbiological criteria relating to bivalve shellfish destined for consumption are included in the EU Regulation (EC) No 1441/2007 of 5 December 2007. It is seems to be also necessary to develop and implement system for monitoring other pathogenic bacteria, which quite frequently occur in raw shellfish and which may be a source of foodborne diseases.

P3-24 Identification of Fish Using DHPLC: Solution for Processed Food Containing Multiple Species

Carton, Thomas[1]; Le Fresne, Sophie[1]; Popova, Milena[1]; Le Vacon, Françoise[1], CARDOU, ANNE-LAURE[1]

1. Biofortis Research, Biofortis, a Mérieux NutriSciences Company, Saint Herblain, France.

Introduction: Mislabeling can occur in fish industry and substitution of fish species can have economic, environmental, health and product quality consequences.

Purpose: Using specific QPCR or protein electrophoresis, we can only identify single-species fish product and there is a need for new methods that are adapted to heat processed products (ex. Surimi base) containing more than one species.

Methods: Based on a common to all vertebrates region of the cytochrome b gene, we have developed a denaturing HPLC (DHPLC) fingerprinting method which allowed us to identify most of the species in commercial crab sticks (Le Fresne et al., 2011). 17 whole fish and filets were used for the initiation of a library of referent DHPLC profiles and the similarity in the aspect and retention times between a referent peak and a peak in the sample suggests the presence of the referent species. This approach allows only an estimation of the composition of the analyzed sample. In order to be identified with certainty, the peak fractions must be collected, re-amplified and sequenced. The obtained sequences can then be compared to sequences in existing databases and the corresponding species identified.

Results: Crab sticks generated complex DHPLC profiles in which the number of contained fish species have been estimated by the number of major fluorescence peaks. Some of the fish pastes we

analyzed generated complex DHPLC profiles composed of up to six major peaks. This suggests that these pastes are composed of more than one fish species even though they are labeled by the manufacturer as containing only one.

Significance: In conclusion, DHPLC appears to be an interesting qualitative method to analyze multi-species food products. Apart from the evident economical application, the possibility to identify fish species in food products can be useful in environmental issues taking into account information about the habitat or the resources level of the fish species.

P3-25 Hatchery to Harvesting – Farm Food Safety and Biosecurity Practices: Case Study of a Chilean Salmon Farm

SOON, JAN-MEI[1]; Pavez Schulbach, Bruno[2]; Baines, Richard N.[3]
1. Dept. of Agro-Industry, Universiti Malaysia Kelantan, Pengkalan Chepa, Kelantan, Malaysia.
2. Salmones Friosur S. A., Puerto Montt, Region de Los Lagos, Chile.
3. School of Agriculture, Royal Agricultural College, Cirencester, Gloucestershire, United Kingdom.

Introduction: Chile was the second largest producer of farmed salmon in the world but faced a devastating outbreak of Infectious Salmon Anaemia (ISA) between 2007–2009. Biosecurity measures are crucial to fish farms to prevent and reduce disease transmission. Biosecurity in aquaculture is defined as the sum of all procedures (prevention, control and eradication) to protect living organisms from contracting, carrying and spreading diseases and other non-desirable health conditions. When compared to the measures applied to terrestrial animals, biosecurity in aquaculture is still considered a fairly new concept.

Purpose: To explore both the current food safety practices and biosecurity measures of a Chilean salmon farm from hatchery to harvesting process.

Methods: Farm food safety and disease assessment questionnaire was used in the interview and farm observation. The Aquaculture Farm Food Safety and Diseases Risk AssessMent (AquaFRAM) was also tested in the Chilean salmon farm to determine if the tool is customisable to local scenario. AquaFRAM which was tested in Chile was translated into Spanish and back translated by a native speaker into English.

Results: The participating salmon company conducted risk assessments vigilantly to reduce or prevent potential food safety and infectious diseases' risks. The participating farm generally carried out at least 8 types of risk assessments (i.e., health and safety, water, workers' hygiene, site assessment, pesticides/veterinary medicines) on a regular basis. It is also important to note that the farms, when carrying out risk assessment, determined the likelihood of occurrences as low, medium or high, hence the farmers took the responsibility to gauge the significance of a breakdown in their farms.

Significance: Similar to Good Aquacultural Practices, biosecurity measures need to be adopted as a whole package and the reduction of one point may have significant consequences. The present study captured a snapshot of real-time farm food safety and biosecurity practices of a Chilean salmon farm.

P3-26 *Kudoa septempunctata* Causes Novel Food-poisoning Outbreaks in Japan by Consumption of Raw Olive Flounder

OHNISHI, TAKAHIRO[1]; Kawai, Takao[2]; Sekizuka, Tsuyoshi[3]; Yahata, Yuichiro[4]; Kuroda, Makoto[3]; Kumeda, Yuko[2]; Iijima, Yoshio[5]; Kamata, Yoichi[1]; Sugita-Konishi, Yoshiko[1]
1. Microbiology, National Insutitute of Health Sciences, Tokyo, Japan.
2. Bacteriology, Osaka Prefectural Institute of Public Health, Osaka, Japan.
3. Genomics Centre, National Institute of Infectious Diseases, Tokyo, Japan.
4. Infectious Disease Surveillance Centre, National Institute of Infectious Diseases, Tokyo, Japan.
5. Microbiology, Kobe Institute of Health, Kobe, Japan.

Introduction: Outbreaks of an unidentified foodborne disease associated with the ingestion of raw olive flounder (*Paralichthys olivaceus*) have increased in Japan since 2003 and have an averaged more than 100 outbreaks each year, reaching 158 in 2010. The illness occurs within 1-20 hours after a meal including raw olive flounder. Its symptoms are manifested as strong but transient diarrhea and emesis. No known causative agents such as bacteria, viruses, toxins and chemicals have been detected in the foods that were ingested by its victims.

Purpose: The identification of causative agent of foodborne disease associated with the ingestion of raw olive flounder.

Methods: We conducted an epidemiological analysis of outbreaks and analyzed a fish sample by

metagenomic DNA sequencing. The toxicities of possible causative agent were determined by animal models and a cell culture model.

Results: We discovered that the DNA of *Kudoa septempunctata*, a recently described myxosporean spice, is specifically present in the olive flounder ingested by patients. The oral administration of *K. septempunctata* spores induced strong but transient diarrhea and emesis in suckling mice and house musk shrews, respectively. The inoculation of *K. septempunctata* spores rapidly increased the permeability across the cultured human intestinal cell monolayer one hour after inoculation.

Significance: These results identify *K. septempunctata* as the etiological agent of this novel foodborne disease outbreak associated with the consumption of raw olive flounder. As the custom of eating raw fish becomes widespread worldwide, it is possible that this foodborne disease will occur in countries other than Japan.