Technical Session 1 - Microbial Food Spoilage, Pathogens, Food Defense
Wednesday, 7 May 2014: 10.30–12.00

T1-01 Avoiding Interferences of Stx Phages in the Molecular Detection of Pathogenic Shiga Toxin-producing Escherichia coli

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Introduction: Isolation of Shiga toxin-producing *Escherichia coli* (STEC) by culture methods in food is advisable, however, sometimes the strain recovery is not possible. Robust and fast molecular methods for STEC detection are then required, including end-point or real-time PCR (ISO 13136:2012 for STEC) or Next Generation Sequencing analysis.

The presence of *Stx* phages in food samples could interfere with STEC detection by molecular methods, since theoretically bacterial DNA extraction methods could also extract phage DNA. Since *Stx* phages could be everywhere, this would lead to a positive *stx* result, hence a positive STEC detection, even though STEC might not be present.

Purpose: To confirm that DNA extraction methods can extract phage DNA. To avoid interferences of phages in STEC detection by reducing significantly the number of *Stx* phages in food.

Methods: *Stx* phage 933W DNA was extracted with commercial bacterial DNA extraction assays and phage genomes were quantified by qPCR.

Samples of water, minced beef and salad were used as matrices, spiked with known concentrations of *Stx* phages and STEC and homogenized with PBS. A fraction of the homogenate was processed for DNA extraction. To reduce phages, a second fraction of the homogenate was filtered through different membranes. Material retained in the filter was eluted and processed for DNA extraction. Values of STEC and *Stx* phages were compared.

Results: All DNA extraction kits assayed can extract phage DNA efficiently with no significant ($P > 0.05$) differences with phage DNA extraction methods.

Filtration of food homogenates and water samples through 0.45µm polyvinylidene fluoride (PVDF) non-protein-binding-membranes, reduced significantly the densities of Stx phages (2-3 $\log_{10}$-units), without reducing the densities of STEC.

Significance: An additional filtration step will reduce the number of Stx phages enough to keep their numbers below the detection limit of molecular techniques, avoiding the interference in STEC detection.

T1-02 Behaviour of Low Doses of Pathogens in an Artificial GIT-model

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Introduction: Dose response relations are usually based on infection probabilities measured at relatively high doses of pathogens, although such high doses hardly reflect concentrations of pathogens found in food in reality. The effect at low doses is subsequently determined by extrapolation, under the assumption that (1) each microorganism has the same probability of infecting people (single hit theory), and (2) this probability is independent of the actual dose. With an *in vitro* gastrointestinal model, we are able to determine relative virulence of bacterial foodborne pathogens. Based on these data dose-response curves can be derived. Up until now we only investigated high doses (approx. 10^9 CFU/ml) of bacteria.

Purpose: The purpose of our research was to test the generally accepted dose-response assumption that the behavior of pathogens in terms of gastrointestinal tract survival and subsequent invasion of epithelial cells is dose-independent.

Methods: Doses of 9, 7 and 5 log CFU/ml of a strain of *Salmonella* Typhimurium DT104 were fed into an *in vitro* gastrointestinal tract system including simulated gastric fluid, intestinal fluid and interaction (attachment and invasion) with small intestinal epithelial cells (Caco-2). The fraction of cells eventually invaded was calculated (number invaded divided by number entered into the system).

Results: The fraction of *Salmonella* invading Caco-2 cells differed with the starting concentration of *Salmonella*. The probability of one cell resulting in invasion into Caco-2 cells increased by a factor of 100 when lowering the starting concentration from 5 to 9 log CFU/ml.

Significance: The assumption of a dose-independent probability of infection should be questioned, at least for *S*. Typhimurium. These preliminary results indicate that the likelihood of infection increases with lower levels of contamination with *Salmonella.* However, to sustain this hypothesis several factors are still to be investigated (such as: is there a limited number of *Salmonella* that can invade Caco-2 cells).

T1-03 Effect of Cell-Free Culture Extract Containing Autoinducer-2 Signal Molecules on the Growth Kinetic Behavior of Salmonella enterica Individual Cells

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Introduction: Studies on Quorum Sensing (QS) have used large inocula of bacterial mutants deficient in QS, considering *a priori* that the physiological status of cells and their exposure to QS are similar. Individual-cell studies may elucidate the true heterogeneity of a population, e.g., single cells behaving as "outliers" of a larger population may be revealed.

Purpose: *In vitro* evaluation of the effect of autoinducer-2 (AI-2) on the individual-cell growth kinetic behavior of *Salmonella enterica* (*Se*).

Methods: The individual cell growth behavior of *Se* strains (Enteritidis, *Se*E; Typhimurium, *Se*T) was monitored in the absence (0%) and presence (20%) of cell-free culture extract (CFCE), produced by *Se*T ATCC 14028, while a negative control also was used (Heat treated CFCE, HT). The kinetic parameters of maximum specific growth rate (μ_{max}) and lag phase duration (λ) were estimated from optical density (600 nm) measurements

Results: AI-2 had no considerable effect on μ_{max}, while the λ distributions of the estimated values for *Se*E were similar under all conditions tested; for *Se*T, the mean λ values in 0%, 20% and HT CFCE were 2.26, 1.81 and 3.95 h, respectively, and the corresponding coefficient of variation values were 41.6, 69.8 and 29.1%. Thus, depending on the strain, QS may affect the λ variability of *Se*.

Significance: The findings of this study for the first time indicate the role of QS on the kinetic parameters of *Se*, knowledge that maybe used to control this pathogen *in situ*

Acknowledgment: *The action THALIS:* "Biological Investigation of the Forces that Influence the Life of pathogens having as Mission to Survive in various Lifestyles; BIOFILMS", has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: THALES. Investing in knowledge society through the European Social Fund.

T1-04 Ars Alimentaria: An Innovative Tool For Ensuring Food Safety

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Introduction: Ars Alimentaria of the Italian Ministry of Health, is an initiative aimed at ensuring the microbiological safety of foods "Made in Italy." Its central tool is a food safety portal, based on scientific principles and internationally recognized standards. The initiative is a prime example of utilizing the exponentially growing "Big Data" and for the practical application of predictive microbiology techniques.

Purpose: The objectives of the project are to:

- Create a database of food manufacturing technologies and products, with software tools helping utilize the data and making them accessible for Ars Alimentaria partners via the food safey portal;
- Continuously enhance these tools and promote them via training and joint actions with stakeholders;
- Promote international cooperation regarding food safety and quality, including collaboration on areas such as nutrition value and traceability.

Methods: Within Ars Alimentaria, currently 7,640 food companies are surveyed and 54,916 products with the "Made in Italy" brand are catalogued. A database ontology has been developed that will make it possible to generate prompt statistics relevant to food safety and quality.

Statistical analysis of the data will generate input for HACCP recommendations. Furthermore, predictive microbiology techniques will be used to provide intelligent support tools regarding food safety decisions.

Results: Expected results of the project are:

- The portal will become a primary source to develop CCP processes;
- FBO-s will increasingly base food safety decisions on scientifically solid information;
- Food safety information will be made available for Hazard analysis;
- Provide methods to determine shelf life of food based on scientific and objective principles.

Significance: This is the first initiative of its kind in Italy, where advances in computing and predictive food microbiology are used as a direct translation of knowledge to help FBO-s in their efforts to produce, store and distribute safe, good quality and traceable food products.

T1-05 Experiences on Food Suppliers' Audit

Andrea Martin, WESSLING Hungary Ltd., Budapest, Hungary and Katalin Eszesné Tóth, WESSLING Hungary Ltd., Szeged, Hungary

Introduction: Supplier audits have been a permanent feature of retailer's systems and procedures for many years. The WESSLING Hungary Kft. was commissioned by several retailers in 2012 to control suppliers of finish product's manufacturers or traders. Supplier audits are analyses that are done to document the relationship between different companies in order to verify compliance of the supplier's products and processes. In all cases, food safety controls in the featured criteria. The presentation introduces the results of nearly 160 audits, which examined companies selected from micro to medium-size enterprises.

Purpose: The authors aim to present existing inconsistencies between the real operation and the spirit of the standards and to propose requirements based on legal rules and supplier checklist in case of a company having not food safety management system certification.

Methods: In 114 cases, the audits have been implemented according to retailers' checklist, including a 4 level point scheme, disclosing a company not fulfilling any of the critical points. In 44 cases, an expert opinion had to be written in text without any checklist.

Results: If organizations do not have certification of their food safety management system, they may pass the supplier audit on the basis of the operation, although the appropriate documentation according to standards or checklists failed. So some work would need to be done for small scale companies to fulfil the whole checklist.

Significance: Beyond the checklists, the expertise of the auditor has to be taken account as well, and sometimes the conclusion about an audit is not a black and white question**.**

T1-06 Determination of Alternaria Growth and Mycotoxin Boundaries in Tomato Purée

Veronique Huchet[1], Noemie Desriac[1], Anne Lochardet[1], Francesca Valerio[2], **Florence Postollec**[1], Paola Lavermicocca[2], Annalisa De Girolamo[2] and Daniele Sohier[1], (1)ADRIA Développement, Quimper, France, (2)CNR ISPA, Bari, Italy

Introduction: *Alternaria* species were reported to be the most common fungi affecting tomato fruits and plants, causing the so called black mould of tomato. Rapid infection of *Alternaria* in tomato may occur on the crop, or post harvest, yielding high economic loss due to spoilage of industrialized products, such as tomato purée. Under optimal growth conditions, *Alternaria* spp. may also produce various mycotoxins. Alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid (TeA) are mycotoxins commonly found in tomatoes and tomato products, representing a serious risk for human health related to the consumption of these products

Purpose: This study aims at defining boundaries for growth and mycotoxin production in order to optimize product formulation and shelf life.

Methods: A toxigenic isolate of *Alternaria alternata* (ITEM8176) isolated from tomato fruit affected by black mould and deposited at the ISPA collection, Italy (ITEM accession: http://www.ispa.cnr.it/Collection/) was used for growth and mycotoxin production assessment. Growth ability of the strain was determined after inoculating fungal ascospores (7day-old culture) on cold break tomato purée supplemented with agar and followed by regular fungal development observations. A total of 6 levels of pH and 10 levels of temperature were tested for 3 replicates, to define pH and temperature boundaries where fungal development and

mycotoxin production occurred. The pH of tomato purée based medium was fixed at 2, 3, 4, 5, 6 and 7, while plates incubation was performed at 6.5, 10, 15, 20, 25, 30, 35 and 37°C. Analysis of mycotoxins (AOH, AME and TeA) was performed by HPLC with UV/DAD detection according to an adapted protocol.

Results: Stability of pH and water activity of tomato purée based media was checked throughout the experiments. Growth was observed above a pH of 3, whatever the incubation temperature. Growth optimum was determined at pH 5.5 and 24.5°C. Conditions where growth was not observed after 1 month incubation were considered not to allow fungal development as observed for pH lower than 3. Growth/no growth boundaries were compared with mycotoxin production/no mycotoxin production boundaries for similar conditions on tomato purée based medium.

Significance: These results indicate the combination of pH and temperature where *Alternaria* mould development and mycotoxin production occured. Knowing these boundaries will help industrials to optimize tomato product formulation and storage conditions to limit mould and mycotoxin development during shelf life.

Technical Session 2 - Non-microbial Food Safety, Novel Laboratory Methods
Wednesday, 7 May 2014: 13.30–15.00

T2-01 A Method for Prioritizing Chemical Hazards in Food applied to Antibiotics

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Introduction: Part of risk based control is the prioritization of hazard-food combinations for monitoring food safety. There are currently many methods for ranking microbial hazards ranging from quantitative to qualitative methods, but there is hardly any information available for prioritizing chemical hazards. However, ranking chemical hazards may be performed along the same lines.

Purpose: The aim of the current study was to develop a method for risk ranking of chemical food safety hazards using a structured and transparent approach.

Methods: A semi-quantitative method was used, consisting of three steps. First, the case study was defined, determining the food groups/products to be included in the study as well as the (group of) chemical hazards. Then, scores were attributed to severity and probability of the hazard. Finally, these scores were multiplied to determine which food-hazard combinations have the highest priority for monitoring.

Results: The method was tested in a case study on antibiotics. Severity of the hazard was scored using the Acceptable Daily Intake (ADI) as well as a score on the severity of antimicrobial resistance. Probability of the hazard depended on the amount of product consumed and on the amount of antibiotics used in animals as well as evidence of residues found. Based on the scoring of these elements, antibiotics could be ranked for the products studied. The method showed that antibiotics most relevant for monitoring were product specific. Overall, nitrofurans were amongst the most important antibiotics to be included in monitoring.

Significance: The developed method is a transparent and objective method for prioritizing chemical hazards. The method has been applied for antibiotics, but may be applicable for other hazard-food combinations as well.

T2-02 Development of a New Method for the Quantification of Meat Species in Food Samples

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Introduction: The identification of meat species present in food samples is an essential step in order to verify the origin and traceability of raw materials used in product production, as well as a necessary quality control for handling and cleaning processes of production lines. The methods developed to date are primarily based on the qualitative detection of meat species by PCR.

Purpose: The development of a quantitative method based on real-time PCR that allows relative quantification of up to 0.05% of unique animal species compared to total animal material present in the sample.

Methods: Methods have been designed and validated for real-time PCR detection of beef, pork, equine, chicken, turkey and poultry species. Species-specific mitochondrial DNA fragments are amplified using specific primers and TaqMan MGB detection probes. The percentage of each species in the sample can be calculated by performing two absolute quantifications: one to determine the amount of the species specific DNA and the other to determine the total amount of mitochondrial animal DNA present. A synthetic DNA plasmid containing the specific genomic regions of each species was used as a standard for quantitation.

Results: The detection limit for each of the species is set at 0.01%. The relative quantification limit for each species is 0.05%. These limits were calculated using fresh meat. For processed samples, the detection and quantitation limits vary depending on the product processing method. Because the standard plasmid has the genomic target for all the species mentioned above, it is possible to simultaneously quantify multiple different species in the same sample by calculating against the amount of total animal DNA.

Significance: The ability to identify multiple potential contaminants in the same sample greatly reduces the processing time and cost for food producers and distributors to test meat products for origin and content.

T2-03 Impact of Food Safety Supervisor Training on Food Hygiene Practices

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Introduction: Studies have shown that most foodborne disease outbreaks can be attributed to improper food handling from inadequate knowledge in commercial food premises. As such, the Australian States and Territories have developed food safety supervisor certification as a component of food safety programs designed to protect public health and safety. We evaluated knowledge, attitudes and practices of supervisors and food handlers in commercial food premises in a jurisdiction where they were required and one where it was just implemented.

Purpose: To evaluate the effectiveness of the training course in improving knowledge on food safety, and to determine if the training course produce significant impact in food safety handling in food premises.

Methods: A mixed methodology of quantitative and qualitative approach was used with 35 premises selected through convenience sampling. We observed general hygiene and hygiene practices in the kitchen, followed by administration of knowledge questionnaire and semi-structured interview to food handlers. Methods of analysis include T-test for mean score differences, logistic regression used to examine odds of getting a perfect score, and content thematic analysis to explore factors that affect translation of food safety knowledge to proper food handling.

Results: T-test suggested that there was no significant difference in mean knowledge score between those who attended a training course and those who did not ($P = 0.37$, d.f = 48). Logistic regression also agree that the attendance of FSS had no significant effects on obtaining a perfect score (OR = 1.43, CI = 0.19-10.66×10^2, $P = 0.73$).

Qualitative result revealed that while training programs may be helpful in providing knowledge, there are factors such as time-management, attitudes, money and staff supervision that prevent proper food safety practices.

Significance: This study found that the FSS was inadequate in addressing certain issues of food safety. It also identified barriers that prevent proper food handling and demonstrated the need to improve on the practicality of the training course.

Acknowledgment: The authors would like to acknowledge the ACT Health Protection Service and the Queanbeyan City Council for their assistance in the collection of data.

T2-04 An Optical DNA Sensing Method Based on Oligonucleotide-functionalized Gold Nanoparticles for the Detection of Escherichia coli O157:H7

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Introduction: *Escherichia coli* O157:H7 has been a major foodborne pathogen associated with numerous cases of fatal foodborne diseases. Therefore, there is a great need to develop a simple and efficient method to detect this bacterium.

Purpose: A label-free optical sensing method based on DNA sandwich hybridization with oligonucleotide-functionalized gold nanoparticles (AuNPs) was developed in the present study for the detection of *E. coli* O157:H7.

Methods: A pair of specific thiol modified probes [P1, P2; 30mer oligonucleotides] with or without additional 12 deoxythymidine 5'-monophosphate (12-dT) was immobilized onto AuNPs surfaces (AuNPs-P-30-SH and AuNPs-P-30/12T-SH) for the detection of *E. coli* O157:H7 gene *eaeA* (104 bp). The detection was based on aggregation or non-aggregation of probe-functionalized gold nanoparticles controlled by increasing salt concentration and cooling after hybridization and was observed by color reaction.

Results: It was found that the control of salt concentration played an important role in hybridization and detection efficiency. The optimal salt concentration after probe immobilization was 0.1 M and 1 M salt solution added after hybridization brought the best color differentiation among targets, non-targets, and blank ($P < 0.01$). Target DNAs amplified from the concentration of 3.15 x 10^0 CFU/ml of *E. coli* O157:H7 were detectable by the developed assay. AuNPs-P-30/12T-SH showed better color stability and more effective hybridization when compared with AuNPs-P-30-SH, hence was selected for the detection of *E. coli* O157:H7 in food samples. The detection sensitivity for the *E. coli* O157:H7 inoculated blueberry, ground beef and spinach samples was 5.2 x 10^0, 1.8 x 10^1, and 8.6 x 10^0 CFU/g, respectively.

Significance: The label-free optical AuNPs sensing method can be used as a rapid, inexpensive, and highly specific detection method for *E. coli* O157:H7 and the results can be read by naked eyes without needing an optical instrument. This work was supported by USDA AFRI.

T2-05 Application of Binding- and Long Range-RT-Quantitative (Q)PCR to Indicate the Viral Integrities of Noroviruses

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Introduction: Noroviruses (NoVs) are one of the most important foodborne pathogens worldwide. Due to the non-culturablity, the prediction for human NoV infectivity has been attempted from the integrity of viral capsid and RNA molecule, which are the two essential parts for an intact and infectious virus particle.

Purpose: To establish and apply a methodology evaluating both viral capsid and genome integrity of NoVs.

Methods: Firstly, murine norovirus (MNV) in PBS suspensions were treated by heat and UV-light respectively (infectivity reduction > 4-log detected by plaque assay) and detected by RT-qPCR, binding-RT-qPCR, long range-RT-qPCR, and binding-long range-RT-qPCR. Secondly, raspberry samples with naturally occurred human NoVs were also detected by the above methods. In the binding-RT-qPCR, specific cell lines were used as the "capture devices" for viruses with intact viral capsids. In the long range-RT-qPCR, the RT reaction was primed at the poly-A tail so that genomic strand breaks will prevent a successful first strand synthesis.

Results: Firstly, the combination of binding- and long range-RT-qPCR indicated higher viral reductions (> 2-log) than other methods after both heat and UV treatments. Similar reductions (1.2 ± 0.18 and 1.4 ± 0.25-log) were detected by binding-RT-qPCR after heat and UV treatments, however long range-RT-qPCR indicated higher reduction after the UV (1.30 ± 0.11-log) than heat treatment (0.28 ± 0.29-log). It means that heat and UV treatments can cause damage on both viral capsid and RNA, while UV targets on virus genome primarily. Secondly, within the eight raspberry samples (GI and GII detected specifically), RT-qPCR, binding-RT-qPCR, long range-RT-qPCR, and binding-long range-RT-qPCR detected 14/16, 16/16, 9/16, and 16/16 positive signals respectively, indicating the abundant presence of intact NoV particles.

Significance: This study contributes to the understanding of NoV integrities after heat and UV inactivation as well as in the naturally occurred food samples.

T2-06 Rapid Identification of Salmonella Serotypes with Stereo and Hyperspectral Microscope Imaging Methods

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Introduction: The hyperspectral microscope imaging (HMI) method can reduce detection time within 8 hours including incubation process. The early and rapid detection of this concept in conjunction with the high throughput capabilities makes HMI method a prime candidate for implementation for the food industry.

Purpose: This research was conducted to determine if the spectral signature at 24 hours incubation time can be compared to the spectra of earlier time frames from 8 to 12 hours of incubation, and to determine if the five *Salmonella* serotypes could be differentiated by their spectral signatures.

Methods: HMI spectral data from five *Salmonella* serotypes (Enteritidis, Heidelberg, Infantis, Kentucky, and Typhimurium) at various incubation times from 8 to 24 hours were analyzed. Bacterial colonies were picked from agar plates with a stereo microscope. A total of 89 contiguous images were acquired between 450-800 nm. Preprocessing of the spectral data was performed by a global data transformation algorithm, and followed by a principle component analysis (PCA). The Mahalanobis distance (MD) was calculated from PCA score plots for analyzing cluster of serotypes. Partial least squares regression (PLSR) was used for calibration and prediction of the model, while soft independent modeling of class analogy (SIMCA) was used for classification of serotype clusters.

Results: Pearson correlation values indicated spectral patterns for varying incubation times ranging from 0.993 to 0.999. PCA score plots showed cluster separation with average MD for incubation times ranging from 1.116 to 52.937. PLSR had a maximum RMSEC value of 0.084 and RMSEV value of 0.089. SIMCA classification values were above 98.1%, and validation values above 97.7%.

Significance: The early and rapid detection abilities could identify contaminated products before being released to the public marketplace and causing widespread disease. The HMI with analytical methods can be used for quality assurance for in-house product safety assessments.

Technical Session 3 - Meat & Poultry, Risk Assessment
Wednesday, 7 May 2014: 15.30–17.00

T3-01 Development of a Loop-mediated Isothermal Amplification Assay for Commercial Meat Species Identification

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Introduction: To develop a fast and convenient detection method for meat adulteration is required for economic, health, and religious reasons. Both protein and nucleic acid based methods can be used for species identification. Compared to protein-based techniques, DNA-based ones have proved to be more reliable because DNA is more stable in the food products. Loop-mediated isothermal amplification (LAMP) was developed in 2000 which can amplify the target DNA using 2 pairs of primers under isothermal condition. LAMP has been used for pathogen analysis for a period of time, but rare report has indicated the application on food analysis.

Purpose: The objective of this study was to develop a LAMP assay for meat species identification.

Methods: Specific primers targeted on cytochrome b gene of common meat species from the local market including beef, chicken and pork were designed and tested for the sensitivity and specificity. Furthermore, the applicability of the developed method was evaluated for adulterated meat and imitated processed meat samples.

Results: The detection limits of LAMP assay for beef, chicken and pork primers were 10^{-2}, $10^{-2}$and 1 ng of DNA, respectively. The primers were not cross-reactive to other meat species. As low as 1% of beef, chicken and pork in adulterated meat samples could be detected by the developed LAMP assay. After boiling at 100°C or autoclaving for 60 minutes, beef still could be detected.

Significance: A rapid, simple and sensitive LAMP based method was developed for detection of meat adulteration from this study. This method can be used for meat species identification not only in raw but also processed meat products. This work was supported by grant from National Science Council NSC102-2221-E-020-022, Taiwan, ROC.

T3-02 Impact of Chilling Conditions on Chicken Thigh Contamination by Campylobacter jejuni

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Introduction: *Campylobacter* is a major foodborne pathogen in the EU. EFSA estimates that poultry is responsible for up to 80% of cases. Risk assessment studies have indicated that campylobacteriosis associated with consumption of chicken products may be reduced 30 times by a 2 log reduction of *Campylobacter* concentration on carcasses.

Purpose: The objective of this work is to define chilling conditions allowing to reduce *Campylobacter* levels on poultry carcasses. For this purpose, this study was set up to investigate four major parameters in the chilling process (temperature, duration, air velocity and initial concentration of *Campylobacter*) individually and in interaction on the behaviour of *Campylobacter* using the Doehlert shell design.

Methods: Twenty-four tests were performed using a chilling prototype with 3 levels for the initial concentration (from 10^3 to 10^5 CFU/g), 5 levels for the air velocity (from 1 to 3 m/s), 7 levels for the temperature (from 1 to 7°C) and for chilling duration (from 1 to 7 hours). Chicken thighs were artificially contaminated before chilling at the defined concentration. After chilling, *Campylobacter* counts were conducted in accordance with the ISO standard 10272-2.

Results: Contamination reduction ranged from 14 to 43% of initial loads corresponding to a reduction of 0.5 to 1.5 log CFU/g. Duration of chilling ($P = 0.04$) and initial concentration ($P = 0.03$) had significant effects: the reduction rate increased when the duration increased and the initial concentration decreased. An interaction between temperature and initial concentration had also a significant effect ($P = 0.01$) on *Campylobacter* contamination. The maximum of reduction was obtained at lower temperature whatever was the initial concentration. At higher temperature, *Campylobacter* reduction was possible only for low initial concentration.

Significance: The most important result is that carcasses presenting more than 10^3 CFU/g of *Campylobacter* would not be significantly cleared during the chilling process.

T3-03 The Heterogeneity of Campylobacter flaA Types Isolated throughout the Slaughter Process of Campylobacter Positive Batches

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Introduction: Campylobacteriosis is the most commonly reported zoonosis with an estimated nine million cases per year in the EU. About one third of human infections are caused by handling and consumption of broiler meat. Investigation of the variability in *Campylobacter* strains collected throughout the slaughter line might contribute to identify the exact *Campylobacter* transmission routes and consequently to implement effective strategies to reduce carcass contamination.

Purpose: To assess the diversity of *Campylobacter flaA* types isolated throughout the slaughter process of *Campylobacter* positive batches.

Methods: Samples were collected in slaughterhouses during the slaughter of *Campylobacter* positive batches. *Campylobacter* was isolated by direct plating from carcasses at three sampling sites during the slaughter of positive batches and from intestines of both investigated and preceding batch, when possible. Per sampling site, forty isolates were identified on species level and further genotyped (*flaA*-RFLP).

Results: All isolates from the first batch were identified as *C. coli*. In the second and the forth batch only *C. jejuni* was recovered. Both species were detected on samples from the third batch. Results revealed that birds´ intestines can be colonized by different species or different genotypes belonging to the same species. Carcasses were mainly contaminated with the same *Campylobacter* genotype as recovered from their intestines. Genotypes recovered from intestinal samples of the preceding batch were not present on carcasses from the investigated batch. However, additional types were present on carcasses during sluaghter process in investigated slaughterhouses.

Significance: Genotyping of *Campylobacter* isolates throughout the slaughter line indicates lack of cross-contamination between following *Campylobacter* positive batches. Additionally, high variability in *Campylobacter* genotypes at the end of the slaughter process might indicate a difference in the survival capacity of certain genotypes along the slaughter line.

T3-04 - The Development of FAO/WHO Web-based Tools for the Strengthening of Capacities in Food Safety

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Introduction: Decision support tools have been around for many decades. However, only recently have they been used for food safety management applications. FAO, in support of capacity development for countries to manage food safety and quality, provides support on a range of food control issues through online training tools.

Purpose: To provide information and create awareness of FAO/WHO tools developed to support the implementation of specific Codex standards and other food safety management decisions and to highlight the advantages of these tools for the decision maker. This is particularly important for transition economy countries involved in modernizing their food safety systems within a risk based framework.

Methods: Tool development is driven by the needs of FAO member countries and the work of the Codex Alimentarius. Web based platforms are used to minimize the need for specialised software and make them widely accessible and easy to update. Tool development is guided by input from subject matter experts and all tools undergo peer review before their public release.

Results: The decision support tools which cover sampling issues (microbiological hazards, histamine and mycotoxins), management of pathogens in poultry and *Cronobacter* in powdered infant formula are freely accessible at http://www.fstools.org/ together with user guides to support their application. Pilot testing of the tools has highlighted their value in helping countries understand the types of data and information needed for evidence based decision making. Making such tools available in multiple languages has also been identified as important for local uptake and application.

Significance: The tools provide scientifically sound, user-friendly and freely available support to assist countries in applying a scientific and risk based approach to their food safety management systems. They are particularly relevant for countries that have limited expertise and can help them to make optimal use of limited capacities.

T3-05 Probabilistic Model of Escherichia coli O157:H7 Survival on Cucumbers During Distribution and Retailing

Arícia Mara Melo Possas[1], Guiomar Denisse Posada-Izquierdo[2], Fernando Perez-Rodriguez[2] and Gonzalo Zurera[2], (1)State University of Campinas, Campinas, Brazil, (2)University of Cordoba, Cordoba, Spain

Introduction: Predictive microbiology allows estimating, with mathematical models, the behaviour of foodborne pathogens in foods and to assess the risks associated with their consumption. In May 2011, an outbreak caused by a Shiga toxin-producing *Escherichia coli* strain in Germany resulted in 50 deaths. First case-studies suggested the association between disease and the consumption of cucumbers imported from Spain.

Purpose: This work was aimed to assess the risk associated with the survival of *E. coli*O157:H7 on contaminated cucumbers during transportation, through an exposure assessment model.

Methods: To this end, eight inactivation models were adjusted using the GinaFIt, Add-in for Excel, to survival data obtained for *E. coli*O157:H7 on cucumber surfaces. Then, a probabilistic exposure assessment model was built in Excel Software using real data collected from vegetable distribution chain, on temperature and time profiles. Three different scenarios were considered to represent for the initial concentration on contaminated cucumbers, which corresponded to 1, 3, and 6 log cfu/cm², respectively. The exposure model simulation, including the selected survival model, was performed using @Risk Palisade.

Results: The survival model used for the exposure model corresponded to the biphasic model, since the pathogen exhibited two reductions rates along time. Simulation under the indicated conditions showed that distribution time greatly reduced concentration on cucumber with final mean values of -15,-13 and -10 log cfu/cm² at retail. In spite of these low concentrations on average, in the three scenarios, some few iterations resulted in products being positive for the pathogen as demonstrated by the maximum values obtained, corresponding to 0.3, 2.3 and 5.3 log CFU/cm², respectively.

Significance: Results indicated that food distribution chain conditions for cucumber distribution can enable *E. coli* O157:H7 survival along transport and retailing, which lead us to consider that additional control measurements should be implemented to reduce risk by this pathogen in this type of products.

T3-06 Modeling Survival of Salmonella spp. in Lettuce as a Function of Chlorine Concentration

Guiomar Denisse Posada-Izquierdo[1], Arícia Mara Melo Possas[2], Antonio Valero[1], Gonzalo Zurera[1] and Fernando Perez-Rodriguez[1], (1)University of Cordoba, Cordoba, Spain, (2)State University of Campinas, Campinas, Brazil

Introduction: Produce can become contaminated by fecal pathogens during primary production or processing. *Salmonella* spp. has been linked to several outbreaks related to fresh-cut vegetable industry. In this sense, washing with chlorinated water is the only treatment able to reduce microbial risks in processed vegetables.

Purpose: The objective was to develop a mathematical model that describes the reduction of *Salmonella* spp. as a function of chlorine level (ppm).

Methods: Iceberg lettuce pieces of 1 x 1 cm were inoculated with 4 log *CFUSalmonella*. Sodium hypochlorite solutions were prepared in sterilized water to obtain different concentrations of free chlorine (0, 25, 50, 100, 150, and 200 ppm). Inoculated samples were then introduced into 20-ml tubes with different chlorine concentrations and analyzed at different treatment times (0, 10, 30, 60, 150 and 300 s). The surviving *Salmonella* cells were enumerated by using Sorbitol MacConkey Agar (Oxoid). Counts were log-transformed and statistical modeling and analysis was performed by using Matlab™ software (Mathwork).

Results: Results indicated that *Salmonella* was able to survive at all assayed free Cl levels, with a maximum log-decrease corresponding to 2 logarithms observed in replicates treated with 150 and 200 ppm free Cl. The greatest log-decrease rate was found in the first 10-30 seconds, and followed by a gradual reduction until the end of the treatment (5 min). The survival pattern was described by a two-phase log-linear model ($R^2 > 0.87$) which considered the two populations with different sensitivity to chlorine. Levels > 50 ppm free Cl yielded similar initial reduction rates.

Significance: Disinfection models for *Salmonella* on lettuce are valuable tools for the validation of control measures in the fresh-cut vegetable industry and contribute to the improvement of quantitative risk assessments.

Technical Session 4 - Food Defense, Produce
Thursday, 8 May 2014: 8.30–10.00

T4-01 Simulating Compliance Behaviour Using Agent-based Modelling (Fraud and Adulteration Section)
Esther van Asselt, RIKILT - Wageningen UR, Wageningen, Netherlands and Sjoukje Osinga, Wageningen University, Wageningen, Netherlands

Introduction: Recent incidents have shown that food fraud is an increasingly important subject that is difficult to tackle. Reasons why people comply or don't comply with legislation differ. A previous study identified 11 factors that influence people's compliance behaviour, ranging from factors reflecting voluntary compliance (such as cost-benefit considerations) to factors reflecting coercive compliance (such as probability of detection). In order to influence people's compliance behaviour, it is important to gain insight into the most effective strategies for different target groups. As it is too costly to test all strategies in real-life, computer simulations may be useful in this respect.

Purpose: The aim of this study is to determine whether compliance behaviour can be simulated using computer modelling.

Methods: Agent Based Modelling is used for simulating compliance behaviour. The model is applied to the correct use of antibiotics by pig farmers. An agent in this case was either a pig farmer, an inspector (imposing sanctions that will influence coercive compliance) or an educator (influencing voluntary compliance by educating farmers). Several of the 11 factors that influence compliance were incorporated in a user-friendly interface of the model. The model outcome was validated with real-life inspection results.

Results: The model showed that it was capable of predicting trends in compliance behaviour over time. Furthermore, social influence appeared to be an important factor influencing people's compliance behaviour as well as people's acceptance of legislation. This information helps to design effective strategies for improving compliance behaviour. Furthermore, it gives indications of potential transgressors in target groups.

Significance: The developed ABM is a first attempt to simulate compliance behaviour and in its current form is useful in exploring various intervention strategies.

T4-02 The Distribution of Sustainable Development through Agroforestry at Atlantic Rainforest Biome in Southern Brazil
Luiz Henrique Pocai[1], Zilma Isabel Peixer[1] and José Luís Carraro[2], (1)Brazilian, Curitibanos, Brazil, (2)Brazilian, Lages, Brazil

Introduction: The rebuilding of the ecosystem in a mixed ombrophilous forest or araucaria forest, located in an Atlantic rainforest biome of a specific mountain region in the state of Santa Catarina, is at its full restoration and conservation. In this context, the payments for environmental services have stimulated the protection and sustainable use of natural resources and raised the profitability to farm families.

Purpose: This study had the objective to analyze the sustainable development of family farms using agroforestry in properties of family farmers in the mountain region of the state of Santa Catarina.

Methods: In 2011, the Social Network Carbon Project, sponsored by Petrobras through Petrobras Environmental Program, planted 500,000 native plants in areas of permanent preservation and legal reserve, forming agroforests in approximately 1,000 family farm properties in 18 cities. In the properties that joined the project, only native regional species (approximately 100 species) with food and economic potential for family subsistence were planted.

Results: The results were that agroforestry has been accepted by a lot of farmers as a revolution for sustainable production. Farmers had a considerably more economical and productive production while they were dealing with the complexity of agroforestry, integrating animals with plants in areas that should be preserved. Many of the farmers reported that this system enabled more ecological production between their crops and livestock, becoming sovereign with their productions.

Significance: We conclude that the use of agroforestry improved the production of about 1,000 farm families that make part of project, working on a sustainable-basis producing fruits, grains, honey, meat and milk, in a more ecological and productive way compared to the traditional system.

T4-03 Pulsed Light Technology for Sterilization of Fresh Produce
Peter Muranyi, Fraunhofer IVV, Freising, Germany

Introduction: Ready-to-eat convenience products, especially fresh-cut fruits and vegetables, are a rapidly growing market segment in the food sector. Due to their fresh nature, these products are very susceptible to microbial spoilage. This is because of the intrinsic microflora on the product surface and from secondary contamination during manufacturing processes. Pathogenic bacteria (e.g. EHEC, *Listeria monocytogenes*) and especially multiresistant pathogens represent a potential risk to consumers.

Purpose: The collaborative SAFEFRESH project has set out to develop innovative methods for the rapid detection and inactivation of pathogenic microorganisms on the surface of fresh and minimally processed plant-based foods (fresh produce) in industrial production processes. In combination, these methods shall enable customized treatments in order to improve the microbiological safety of the products.

Methods: A pulsed light system equipped with a three xenon tubes reflector was used within this study. For determination of the sterilization efficiency, inactivation tests with artificially inoculated leafy greens and sprouts were performed. The decontamination effect with regard to the intrinsic microflora on the food surface was likewise investigated. A possible impact on the food quality was determined on the basis of storage tests in conjunction with microbiological, chemical and sensorial analytics. Furthermore, the endophytic propagation and colonization behaviour of bacterial pathogens were studied in order to create the basis for optimized cleaning and sterilization processes.

Results: The sterilization experiments have shown that the selected test strains *Listeria innocua* and *Escherichia coli* can be inactivated by up to 2 $\log_{10}$ cycles on the produce surface without any significant quality changes by applying the pulsed light technology (1 flash, 3000 V). The intrinsic microflora was reduced by the same magnitude.

Significance: The outcome of the SAFEFRESH project are novel approaches for controlling foodborne pathogens in fresh cut industry.

T4-04 Relative Humidity Conditions before Harvest Influence Survival of* Salmonella *Typhimurium in Leafy Greens
Francisco López-Gálvez, Mabel Gil and Ana Allende, CEBAS-CSIC, Murcia, Spain

Introduction: Pre-harvest contamination of fresh produce by pathogenic microorganisms has been attributed to different vectors such as irrigation water, wildlife and workers. Survival of pathogenic bacteria in leafy greens can be affected by changes in weather conditions which include, among others, fluctuations in relative humidity (RH) due to the presence and absence of free moisture on the leaf surface from rain, mist and sprinkler irrigation.

Purpose: In the present study, the effect of RH on the survival of *Salmonella enterica* ser. Typhimurium on growing 'baby' romaine lettuce was assessed.

Methods: Plants were spray inoculated with a level of $\approx 10^6$ CFU/g of *S.* Typhimurium. Two inoculum carriers, distilled water and diluted buffered peptone water, were compared. Half of the plants were grown in an environmental chamber with a constant RH of around 85% (high RH), while the other plants were kept in a chamber with a RH of 60% (low RH). Temperature and photoperiod were controlled during 12 h of darkness at 18°C and 12 h of light conditions (280 µmol m^{-2} s^{-1}) at 23 °C in both chambers.

Results: In all cases, *S.* Typhimurium numbers declined during the growing period. However, the effect of RH on the survival of *S.* Typhimurium was affected by the composition of the inoculum carrier. When distilled water was used, no significant differences in the levels of *S.* Typhimurium were observed between plants kept at different RH. Thus, survival of *S.* Typhimurium was only significantly higher under high RH when buffered peptone water was used as carrier.

Significance: These results suggest higher risk of pathogen survival and persistence under weather conditions that support high RH on leaves surfaces combined with a high availability of nutrients such as organic matter from different sources.

T4-05 Impact of Irrigation with Reclaimed Water on the Microbiological Safety of Greenhouse Hydroponic Tomatoes

Francisco López-Gálvez, Ana Sanz-Pérez, Ana Allende, Francisco Pedrero-Salcedo, Juan José Alarcón and **Mabel Gil**, CEBAS-CSIC, Murcia, Spain

Introduction: The presence of *Salmonella* spp. in tomatoes has been identified as one of the five top specific food/pathogen combinations most often linked to foodborne human illness cases in fresh produce in the EU. Several factors might contribute to tomato contamination with *Salmonella*, among them, irrigation with contaminated water has been described as a source of pathogenic bacteria, and therefore, the use of untreated or improperly treated wastewater is of high concern.

Purpose: The purpose of this study was to assess the impact of irrigation with reclaimed water on the microbial safety of greenhouse hydroponic tomatoes.

Methods: Greenhouse hydroponic tomatoes were grown with two different types of irrigation water (surface water and reclaimed water) and on two different substrates (rock wool and coconut fiber). Irrigation water, drainage water and tomatoes were analyzed periodically for presence of generic *Escherichia coli* and pathogens during the tomato harvest period. A total of 208 water samples and 72 tomato samples were analyzed during the study. The prevalence of *Salmonella* and Shiga-toxigenic *Escherichia coli* (STEC) was studied by using real time PCR after enrichment. *E. coli* detection was carried out in chromogenic media (Chromocult agar).

Results: *E. coli* was not detected in tomato with a detection limit of 1.5 log CFU/tomato. However, *E. coli* counts were higher in reclaimed water than in surface water. The two pathogens were absent in tomato samples, and there were no positives for STEC in water. However, 8 water samples were positive for *Salmonella* spp. Of the positive samples, 5 corresponded to reclaimed water and 3 to surface water.

Significance: Although no presence of pathogens in tomatoes were detected, positive water samples demonstrated that *Salmonella* spp. was present in the environment and could potentially have contaminated the plants, for instance by internalization through the roots or by application of pesticide solutions prepared using contaminated water.

T4-06 Effect of Disinfection Technologies on Quality and Nutritional Properties of Lettuce, Strawberries and Cherry Tomatoes

Angeliki Birmpa, Michalis Leotsinidis, Eleni Sazakli, Gina Tsichlia and Apostolos Vantarakis, University of Patras, Patras, Greece

Introduction: Disinfection remains one of the most important steps in the processing line, for the safety of fresh ready-to-eat vegetables and fruits. It is known that lettuce, strawberry fruits and cherry tomatoes are rich in natural antioxidants, and phenolic compounds which protect human health.

Purpose: The purpose of the present study was to investigate the effects of alternative, conventional and combined disinfection technologies on lettuce, strawberry and cherry tomatoes' antioxidant capacity (TAC), total phenolic content (TPC) and Vitamin C (VitC) concentration as well as color.

Methods: Commercially available lettuce, strawberries and cherry tomatoes were bought from a supermarket. Foods were treated with different disinfection technologies for various treatment times (0-60 minutes). Ultraviolet Light (UV), Ultrasound (US), Sodium Hypochlorite (SH) and combined technologies (UV-US, UV-SH and US-SH) were used for food disinfection. TAC was measured according to FRAP method. The TPC were measured using Folin-Ciocalteau Reagent and determination of VitC was made by titration against 2,6 Dichloroindophenol. A colorimeter was employed for color measurements.

Results: UV and US increased TAC (for UV, 731, 222 and 155 μmol Fe^{2+}•g^{-1} and for US 273, 316 and 115 μmol Fe^{2+}•g^{-1}) and TPC (3.76, 2.16, 2.31 mg gallic acid•g^{-1} and 3.01, 2.06, 3.07 mg gallic acid•g^{-1}) for lettuce, strawberry, cherry tomatoes respectively. SH did not alter them significantly. On the contrary, VitC remained constant in conventional technologies, or was slightly decreased when alternative disinfection technologies were used. Color did not change significantly at treatment times (< 30 min).

Significance: Disinfection technologies play an important role in commercial practice and prevent the survival of pathogens. However, nutritional properties are enhanced by non thermal technologies thus must be taken under consideration for the selection of disinfection process parameters.

Technical Session 5 - Antimicrobials, Seafood
Thursday, 8 May 2014: 10.30–12:00

T5-01 Effect of Desinfectia on Pathogens in Processing Water for Fresh Produce

Hermien Bokhorst-Van de Veen[1], Masja Nierop Groot[1], Leo Van Overbeek[2], Cees Waalwijk[2], H.J. (Ine) Van der Fels-Klerx[3] and **Jennifer Banach**[4], (1)FBR Wageningen UR, Wageningen, Netherlands, (2)PRI Wageningen UR, Wageningen, Netherlands, (3)RIKILT - Wageningen UR, Wageningen, Netherlands, (4)RIKILT, Wageningen, Netherlands

Introduction: Fresh produce are vulnerable to contamination with human pathogens as they only undergo a washing step, but no further processing steps, to eliminate potential contaminations with pathogens. Leafy greens are among the most frequently found fresh produce involved in outbreak incidents (EFSA 2013). Most incidents have been related to *Salmonella* ssp. or *Escherichia coli* O157:H7. Washing with water of suitable quality removes contamination to some extent, but may also pose a risk for cross-contamination in the washing. Adding desinfectia to processing water can possibly reduce the cross-contamination and, as such, limited the impact.

Purpose: The aim of this study was to evaluate the effects of two alternative types of desinfectia, Ag/Cu solvent and hypochlorite, on reducing pathogen presence during washing of fresh produce. The desinfectia were compared with those from using chloor dioxide (which is used in many European countries) and normal drinking water.

Methods: *Salmonella* Typhimurium 1638 (an isolate from lettuce) and an *Escherichia coli* isolate were cultivated in an overnight culture at 37C. For exposure to dieinfectia, cells were either directly exposed or allowed to adjust at 5°C for 24 h before exposure to simulate conditions in practice Concentrations of the pathogens were determined after 0 to 20 minutes, representing the efficiency of the desinfectia.

Results: Concentrations for both the *Salmonella* Typhimurium and the *Escherichia coli* strains showed a log 4 reduction after a relative short contact time with 10 ppm active chloor from hypochlorite (seconds) or chloordioxide (1 minute). This result was found for both the overnight culture and the stressed culture. The Ag/Cu solvent resulted in a log 4 reduction within 10 minutes contact for the *E. coli* and the *Salmonella* strain. For *Salmonella*, no difference was found between the culture to cold drinking water (stressed cultures) and the overnight culture. However, for *E. coli*, a log 2 reduction was found for the stressed culture. Concentrations of the pathogens on fresh produce were also determined, and will be presented at the conference as well.

Significance: Results of this study showed that all three desinfectia tested are able to reduce quickly pathogens that enter the processing water. They can therefore help prevent cross-contamination during washing of fresh produce.

T5-02 Comparison of Two Scale Plants Processed Pangasius Hypophthalmus Fish: Dynamics of Microbiological Quality and Safety

Anh Ngoc Tong Thi, Ghent University, Ghent, Belgium

Introduction: Vietnamese Tra fish (*Pangasius hypophthalmus)* have become highly appreciated by consumers in the European Union, USA, Canada, etc. and are therefore of worldwide economic importance. The availability of data in microbiological quality and safety of this fish species is however limited.

Purpose: The dynamics of microbiological performance of Vietnamese processing companies between large and small scale plants were evaluated from raw material until final product by microbial assessment scheme.

Methods: A total of 279 samples (144 samples in large scale plant) were taken for monitoring: overall microbial quality (psychrotrophic aerobic count), hygiene indicators (*Eschericha coli* and *Staphylococcus aureus*), and relevant pathogens (*Listeria monocytogenes* and *Vibrio cholerae*).

Results: The low levels of total psychrotrophic bacteria and *E. coli* on final products sampled from large scale planst were ca. 3 log CFU/g and below detection limit, respectively. In addition, the pathogen of *Listeria monocytogenes* and *Vibrio cholerae* was absent in all samples analysed. On the contrary, high numbers of total psychrotrophic bacteria (*ca*. 6 log CFU/g on fish and *ca*. 6 log CFU/ 100 cm^2 on food contact surface) were found on the small scale plant during processing. Additionally, the foodborne pathogen was present in water, hands and fish; especially the presence of *L. monocytogenes* on a final *Pangasius* product.

Significance: These data are of major importance in order to provide valuable information for the local and international trade point of view in general and for the intended customers in particular.

T5-03 Fishery Product Quality: Assessment of Mercury Concentration of the Western Mediterranean Fished

Vincenzo Ferrantelli[1], Andrea Macaluso[2], **Gaetano Cammilleri**[1], Gianluigi Maria Lo Dico[3], Stefania Graci[1] and Maria Drussilla Buscemi[3], (1)Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italy, (2)Istituto Zooprofilattico della Sicilia, Palermo, Italy, (3)Zooprophylactic Institute of Sicily, Palermo, Italy

Introduction: Mercury is the 62nd most abundant element in the earth's crust, but it's application in several industrial activities determines a production of about 10,000 tonnes per annum. The methyl mercury formed by aquatic microorganisms enters in the food chain via filter-feeding bottom invertebrates up to the fish fauna.The level of total mercury in animals, excluding fish, varies from a few micrograms to 50 μg/Kg. Fish can exceed these levels to a concentration of 10 mg/Kg in highly polluted water. This occurred in Sicily where the activity of the Syracusan petrochemical pole determined a high mercury concentration in the fish of east Sicilian coasts. Consumption of contaminated fishes caused a peak of 5.6% of births with malformation in 2000.

Purpose: In this work the concentration of mercury was calculated in the fish of the western Mediterranean Sea in order to ensure the fishery product quality and to assurance consumer's health.

Methods: 17 fish species, for a total of 140 samples, were examined by a direct mercury analyser (Milestone_DMA-80) through these steps: Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry.

Results: The analyzed samples detected an average mercury concentration of 0,165 ± 0,22 ppm (mg/Kg) with a maximum value in *Lepidopus caudatus* (1,71768 ppm) that exceed the limits provided by Reg.UE 1881/2006 and the minimum in *Sparus aurata* (0,00006 ppm). Results were subdivided by species, ecologic distribution (benthonic or pelagic) and length classes of the fish.

Significance: Fish food constitutes the main route of Hg uptake for humans. Only 4 to 130 of analysed samples exceed the mercury concentration limit given by the European Commission. These results demonstrate that there's no substantial influence of the major Sicilian industrial activities on the uptake of mercury in the western Mediterranean fish.

T5-04 Public Health Risks of Histamine and Other Biogenic Amines from Fish and Fishery Products

Vittorio Fattori and **Sarah Cahill**, Food and Agriculture Organization of the United Nations, Rome, Italy

Introduction: Scombrotoxin fish poisoning (SFP), often called "histamine poisoning", is caused by ingestion of certain species of marine fish that contain high levels of histamine and possibly other biogenic amines.

Purpose: Review and assess the available information on histamine and other biogenic amines in fish and provide a scientific basis for the harmonization of histamine limits in Codex standards and guidance on the relevant sampling plans.

Methods: To examine the issue of histamine and other biogenic amines in fish and fishery products, a risk assessment process was followed by FAO/WHO involving internationally recognized experts on the matter.

Results: A hazard identification concluded that there is compelling evidence that histamine is the most significant causative agent for SFP. Using the no-observed-adverse-effect level (NOAEL) for histamine of 50 mg as the appropriate hazard level and considering a serving size of 250 g, the maximum concentration of histamine in that serving was calculated to be 200 mg/kg. However, a review of data from food business operators indicated that through the application of GHPs and HACCP, the achievable level of histamine in fish products was lower than 15 mg/kg.

Significance: Histamine formation and SFP can be easily controlled by applying good hygienic practices (GHP) and hazard analysis critical control point (HACCP). Appropriate sampling plans should be used to validate the HACCP systems, verify the effectiveness of control measures, and detect failures in the system. FAO and WHO have subsequently developed a publicly available tool (www.fstools.org/histamine) to provide support in both the design and analysis of sampling plans for histamine and thus facilitate discussions around the establishment of appropriate and feasible sampling plans to ensure that product does not exceed the established limits from a food safety perspective.

T5-05 - Anisakids in the Mediterranean Sea: Statistical and Health-Related Risks Assessment

Vincenzo Ferrantelli[1], Angela Alongi[1], Simone Platania[2], Antonio Vella[1] and Gaetano Felice Caldara[1], (1)Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italy, (2)ASP Catania, Catania, Italy

Introduction: Anisakid nematodes are widespread as a natural event and may cause anthropozoonosis as a result of eating infested raw, undercooked or improperly processed food. The geolocalization of the anisakid species benefits preventive actions because it allows us to make appropriate health decisions.

Purpose: This work is a part of a wider mapping scheme of the parasite in the Mediterranean sea, led by Italian Centro di Referenza Nazionale per le Anisakiasi, aiming to indentify risk factors and subsequently come up with prevention measures.

Methods: Samples from five different commercial fish species caught in GFCM sub-area 16 were screened for Anisakid nematodes by means of morphological and genetic methods. Using NC5/NC2 primers, ribosomal genomic regions ITS1, 5.8 SrRNA and ITS2 of DNA were amplified and PCR products were sequenced. Sequences were analysed using a NCB online Blast tool. Anisakis species were detected by comparing obtained sequences with those in the GenBank and by phylogenetic analysis.

Results: Parasites collected from the sampled fish amount to 6,318. Anisakid nematodes were found in 18.32% of fish. Within this percentage range, the distribution of larvae for each species varied. Scabbard fish was heavily infested with 100% prevalence. Conversely, prevalence percentage was 15.3%, 25%, 5%, 4.2% among European anchovies, hakes, European pilchards and red mullets, respectively. The only anisakid species found in this study was *Anisakis pegreffii*.

Significance: The data suggest that *Anisakis pegreffii* infestation in fish in GFCM sub-area 16 is dominant. Visual inspection of fish should be carried out by qualified operators to remove the parasite and prevent it from reaching consumers. Control strategies of anisakid worm infestation to achieve reductions of pathogenicity were discussed both by the FDA and the EFSA.

T5-06 Development of a Microbial Time Temperature Indicator Prototype for Monitoring the Quality of Chilled Grouper Fillets

Hsin-I Hsiao, National Taiwan Ocean University, Keelung, Taiwan and R. N. Chang, Department of Food Science, Keelung, Taiwan

Introduction: Temperature control is important during transportation, storage and distribution, especially for perishable products with short shelf life. Time temperature indicators (TTIs) are used as cost-effective devices to monitor the effect of temperature history on food quality in the chilled chain. The microbial TTIs response is directly related to microbial food spoilage as it reflects the bacterial growth and metabolism. The TTIs has been extensively applied in frozen vegetables, meat and fresh seafood. However, development of both total aerobic plate count and volatile basic nitrogen as quality indicators for seafood has received less attention. Furthermore, design of the color change point is complicated since it can be influenced by of specific spoilage organism, chemical chromatic indicators etc.

Purpose: This study aims to develop a microbial TTI prototype to monitor grouper fillets quality change through its cold chain distribution by using both total aerobic plate count and volatile basic nitrogen as quality indicators.

Methods: Our design system considers following factors: selection of specific spoilage organism (*Lactobacillus sakei, Carnobacterium maltaromaticum, Pseudomonas fluorescen),*selection of chemical chromatic indicators (Chlorophenol red, Bromocresol green), selection of inoculum level of bacteria.

Results: Results indicated *Lactobacillus sakei*is the major spoilage bacteria. Chlorophenol red at 0.1 mg/ml is used as an appropriate chemical chromatic indicator. 3 log CFU/ml is the inoculum level. Under such conditions, the system successfully shows the color change occurs when dE is 20 and pH is 5.8. Further studies will be taken to calculate and compare activation energy of TTI system, microbial growth reaction of grouper fillet, and volatile basic nitrogen reaction.

Significance: The findings offer a novel view of developing TTIs when using multiple quality indicators for seafood. Moreover, our results suggest that it is necessary to develope such TTIs since more than one quality indicator is important for an effective quality assurance system.

Technical Session 6 – Pathogens
Thursday, 8 May 2014: 13.30–15.00

T6-01 Survival of Listeria monocytogenes in Cheese Brines

Bjørn C.T. Schirmer[1], Even Heir[2], Trond Møretrø[2] and Solveig Langsrud[2], (1)Nofima, Ås, Norway, (2)Nofima, Norwegian Institute of Food, Ås, Norway

Introduction: Brines are commonly used for salting of cheeses, and are for quality reasons not exchanged frequently. Outbreak investigations have shown that brines may serve as harborage sites for *Listeria monocytogenes*.

Purpose: To investigate the effect of in-use vs. fresh brines and pH and NaCl concentrations on the survival of various *Listeria monocytogenes*strains in cheese brines.

Methods: Five different *L. monocytogenes* strains (two clinical isolates, two food isolates and one type strain) were each added to three in-use ([NaCl] = 20.0 - 25.5 %, pH 4.54 - 4.95) and one fresh ([NaCl] = 29.8 %, pH 5.80) cheese brine, and survival was studied for 200 days. One of the human outbreak *L. monocytogenes* strains was selected for studies of combined effects of pH (4.5, 5.25 and 6.0) and NaCl (15, 20 and 25 %) in fresh, filter sterilized brines.

Results: Results showed that pathogen populations decreased over time in all brines, but there were significant differences in survival, both depending on the strains and the brines. Strains of human outbreak listeriosis cases showed greater ability to survive in the brines compared to food isolates, and a *L. monocytogenes* type strain (1–2 $\log_{10}$difference after 200 days). All strains showed higher survival in the freshly prepared brine compared to the in-use brines. Survival was generally lowest at low pH (4.5) and low NaCl concentrations (15 %).

Significance: This study showed that *L. monocytogenes* survived longer in fresh brines compared to in-use brines. Furthermore, in addition to low pH, lower NaCl concentrations may be more suitable to reduce *Listeria* survival than high NaCl concentrations.

T6-02 Pathogenic Growth and Toxin Production under Temperature Abuse Resembling Consumer Handling of Cold Cuts in the Domestic Environment

Elin Rössvoll[1], Helene Thorsen Rönning[2], Per Einar Granum[2], Trond Möretrö[1], Marianne Röine Hjerpekjön[1] and Solveig Langsrud[1], (1)Nofima, Norwegian Institute of Food, Ås, Norway, (2)Norwegian University of Life Sciences, Oslo, Norway

Introduction: For the quality and safety of ready-to-eat (RTE) foods it is crucial to maintain an unbroken cold chain from production to consumption. Although temperature abuse may occur in every stage in the food chain, the least controllable part is at the consumer stage.

Purpose: The objectives in this study were to i) measure the temperatures cold cuts are exposed to in the domestic environment during vacations, and ii) investigate both pathogenic growth and toxin production under such temperature abuse.

Methods: A case study with temperature loggings in the domestic environment during vacations was performed to find relevant time and temperature courses. The effect of such temperature abuse related to daily meals and elevated refrigerator temperatures on the growth and toxin production of *Bacillus cereus, B. weihenstephanensis* and *Staphylococcus aureus* and the growth of

Listeria monocytogenes and *Yersinia enterocolitica* was studied using nutrient agar plates as a food model. The results were compared with predicted growth using the modeling tool ComBase Predictor.

Results: The consumers in the case study exposed their cold cuts to room temperatures as high as 26.5°C for periods up to an average of 116 minutes daily for breakfast/brunch during the vacations. Short (≤ 2 h) daily intervals at 25°C nearly halved the time the different pathogens needed to reach levels corresponding to the levels associated with human infection or intoxication, compared with the controls continuously stored at refrigerator temperature.

Significance: *B. weihenstephanensis* showed toxin production at a temperature as low as 8°C, however the growth of *L. monocytogenes* and *Y. enterocolitica* was found to be the limiting factor for safety. In combination with data on temperature abuse in the domestic environment, modeling programs such as ComBase Predictor can be efficient tools to predict growth of some pathogens but cannot predict toxin production.

T6-03 Hepatitis A Virus (HAV) Outbreak in Italy: Correlation Between Clinical Cases and Foodstuffs

Enrico Pavoni[1], Marina Nadia Losio[1], Chiara Chiapponi[2], Caterina Rizzo[3], Anna Rita Ciccaglione[3], Roberto Bruni[3], Simona Di Pasquale[3], Sarah Guizzardi[4] and Benedetta Cappelletti[4], (1)Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy, (2)Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Parma, Italy, (3)Istituto Superiore di Sanità, Rome, Italy, (4)Ministero della Salute, Rome, Italy

Introduction: In Italy, from January to September 2013, 1,125 cases of hepatitis A were reported, corresponding to a 2.4 fold increase of notifications compared to the same period in 2012. Northern regions accounted for 59% of total cases. The case-control study conducted for the identification of risk factors suggested a strong association of the disease with the consumption of mixed frozen berries. The sequencing of HAV genome in mixed frozen berries and clinical cases isolates showed 100% similarity, corresponding to HAV1A strain. As a consequence, Italy notified through the RASFF the HAV findings. Moreover, the Ministry of Health started the tracing back of the food item. The investigation identified many dealers that received consignments of berries from different foreign countries. Following the RASFF notification, different regions recalled the positive lots and advised the population regarding the use of the leftover frozen mixed berries.

Purpose: To find a correlation between clinical cases and foodstuffs and trace back the contaminated batches to the source.

Methods: From May to December 2013, 1,889 food samples (including 1,140 berries) were tested for HAV. Analyses were performed according to an in house accredited method. Virus genotyping was performed on the VP1/2A region of the viral genome, and by Next Generation Sequencing on the whole genome.

Results: HAV sequences (454-458 nt) from 2 berries samples showed 100% identity to the outbreak strain; a shorter sequence (349 nt) obtained from a third sample showed 99.7% identity, due to 1 nt difference.

Significance: Analysis of the case interviews on risk factors identified consumption of frozen mixed berries. This assumption was supported by the detection of HAV in these. The surveillance on berries and other vegetables potentially carrier of the HAV has been intensified, to provide a picture of the distribution of the contaminated items and the risk of exposure.

T6-04 Decontamination of Lettuce and Survival of Pathogenic Bacteria

Lucas Wijnands, El Bouw, Angela van Hoek and Eelco Franz, RIVM - Centre for Infectious Disease Control, Bilthoven, Netherlands

Introduction: In the Netherlands there is discussion on the use of disinfectants in washing water for fresh produce to be used for raw consumption, which is normal practice in most EU member states. The EU project SUSCLEAN tries to optimize the use of chlorine and to find alternatives for chlorine as disinfectant in the fresh produce industry.

Purpose: The purpose of our research was i) to investigate the influence of disinfectants in the washing water on the selection of pathogenic bacteria, and ii) to test the hypothesis that disinfectants in the washing water reduces the native leaf microflora (decontamination) and subsequently increases the growth potential of pathogenic bacteria.

Methods: For the first purpose, *Escherichia coli* O157 strains with and without a mutated general stress-response *rpoS*-operon were inoculated on lettuce leaves treated with 40 ppm chlorine Their subsequent growth dynamics was monitored (21 days, room temperature). For the second purpose, lettuce leaves were either not-treated at all, washed with water, or washed with water

containing a high concentration of chlorine dioxide. Subsequently, non-STEC *E. coli* and *Salmonella* Typhimurium were inoculated on the lettuce leaves at 3 log CFU/g, and their persistence/growth on the leaves was monitored.

Results: *E. coli* O157 strains with mutations in the *rpoS*-operon and an impaired *rpoS* functioning (as measured by acid shock survival) showed reduced persistence on lettuce leaves compared to strains with mutations and fully function *rpoS*. The growth rate of pathogenic bacteria was significantly increased on leaves with reduced levels of native microflora compared to on non-treated lettuce leaves.

Significance: The plant environment may select for stress-resistant strains that additionally pose a higher risk for humans. Reduction in the numbers of native microflora as a result of disinfection may pose a food safety risk by increasing the growth-rate of pathogenic bacteria that have survived the process or in case of re-contamination.

T6-05 Soil Survival of Enteroaggregative* Escherichia coli *O104:H4 Strains

Lucas Wijnands, El Bouw, Angela van Hoek and Eelco Franz, RIVM - Centre for Infectious Disease Control, Bilthoven, Netherlands

Introduction: In 2011, an extensive outbreak with an unusual Shiga toxin-producing enteroaggregative *Escherichia coli* (stx-EAEC) O104:H4 occurred. In contrast to classical Shiga toxin-producing *E. coli* (STEC), which have a reservoir in ruminants, enteroaggregative *E. coli* are considered to be restricted to humans. The source of the German outbreak was traced to fenugreek seeds, which likely became contaminated during primary production. While considerable attention has been given to the environmental persistence of STEC, virtually nothing is known on the fate of (*stx*)-EAEC in the environment.

Purpose: The purpose of our investigations was to investigate the persistence of EAEC O104:H4 strains in soil, and to relate that to the persistence of *Stx*-O157 and commensal ESBL-producing *E. coli*.

Methods: Soil, checked for the absence of *E. coli*, was set to 60% of the water holding capacity and divided into several 250 g portions . Soil portions were inoculated with several strains of *Stx*-producing EAEC O104. In addition, the ancestral non-*Stx*-producing EAEC O104:H4 55989, and two *Stx*-O157 strains with known soil survival patterns were included for comparison. At regular intervals a sample was tested for the number of surviving *E. coli* by means of dilution plating.

Results: The O104 outbreak strains and their ancestral strain persisted for the longest time (70 days, resp. 63 days). Both France strains only showed 35 days survival. The two *Stx*-O157 strain survived respectively 21 and 38 days; both commensal ESBL-producing strains on average 42 days. A strong relation was observed between the levels of persistence and the presence of mutations in the general stress response operon *rpoS*.

Significance: The soil environment may be a temporary source for virulent *E. coli* O104 strains. From the soil these strains can be transferred to vegetables or animals, and from there via the food chain to humans. In addition, humans can be infected directly through contact with contaminated soil.

T6-06 - Pathatrix Auto™ - the First AFNOR-Approved Real-time PCR Method for Detecting Salmonella in Pooled Food Samples

Jason Wall[1], Daniele Sohier[2] and Rick Conrad[1], (1)Life Technologies, Inc., Austin, TX, (2)ADRIA Développement, Quimper, France

Introduction: The Pathatrix Auto™ pathogen isolation platform provides a workflow that is able to process as many as ten individual food enrichments in the same sample pool. This sampling format has never been approved in the EU market, and would require extensive validation efforts by an expert testing lab to evidence that the approach is not only possible, but practical.

Purpose: In order to validate this product for food safety testing in the EU, this workflow would need to demonstrate a relevant relative detection limit, show statistical similarity to the ISO 16140 reference through accuracy, sensitivity, and specificity; and prove its robustness and practicability in the field.

Methods: Adria Developpement was selected to perform the evaluation to ascertain the Pathatrix Auto's ability to detect *Salmonella* in pooled food sample types by Real-time PCR and selective agar plating. A Ring Trial proficiency study with

15 independent food safety testing labs was also conducted to verify that the workflow was functional and accurate with minimal training.

Results: In both the Adria Developpement study and the Ring Trial, the candidate and reference methods were found to be statistically similar. Of the 202 different food sample types tested during this evaluation, a relative accuracy of 93.1%, a relative sensitivity of 87.7%, and a relative specificity of 96.7% was attained. The relative detection limit was determined to be 0.4-1.5 log CFU/25g of sample, which was statistically similar to the reference. The selected Ring Trial labs demonstrated 100% proficiency and accuracy in performing the workflow.

Significance: This is the first validated method for sample pooling in the EU. The demonstrated robustness, accuracy, and ease of use of this workflow enables the user to rapidly screen for rare contamination events with high confidence, with up to a 90% cost savings over other PCR-based platforms.

Technical Session 7 - Applied Laboratory Methods, Communication Outreach and Education, Epidemiology
Thursday, 8 May 2014: 15.30–17.00

T7-01 Growth of Pure Cultures of Stressed non-O157 Shigatoxin-producing Escherichia Coli in Five Enrichment Broths
Bavo Verhaegen, Institute for Agricultural and Fisheries Research, Melle, Belgium

Introduction: In the last decades, the non-O157 serotypes of STEC have been frequently associated with serious foodborne diseases in humans. An important issue concerning the bacterial isolation of STEC is the presence of low numbers and stressed or injured state of the organisms in contaminated food. Therefore, a suitable enrichment medium is needed to facilitate later detection, isolation and confirmation.

Purpose: The aim of this study was to assess the growth capability of stressed STEC O26, O103, O111 and O145 cells in five enrichment media.

Methods: Two strains of each serotype were used for each stress type. Acid, cold or freeze stress cells(10^2 CFU/ml) were enriched in the different enrichment broths. During incubation at 37°C, counts were determined at several timepoints. The growth capability was defined by four parameters: the lag fase (λ), the maximal growth rate (μ_m), the maximal growth (A) and the area under the curve (AUC).

Results: Enrichment in BPW showed the most efficient resuscitation for all types of stress and the performance was not altered by the supplementation of sodium pyruvate, except during enrichment of cold stressed cells.

The other enrichment media, mbTSA, Brila and SEB demonstrated less efficient resuscitation capabilities, especially during enrichment of freeze stressed cells. Nevertheless, after an incubation period of 24 hours at 37°C all enrichment broths contained approximately the same amount of viable cells (10^9cfu/ml). No differences in growth were found between the different serotypes.

Significance: Acid and cold stress appears to have little effect on the growth during enrichment, while freeze stress has a more significant impact. By using the non-selective enrichment medium BPW a more efficient recovery of pure stressed non-O157 STEC strains was observed.

T7-02 No Effect of Aging on Bacillus licheniformis Spore Heat Resistance
Veronique Huchet[1], Lisa Berriet[1], Anne Lochardet[1], Daniele Sohier[1], Noemie Desriac[1], Anne-Gabrielle Mathot[2] and **Florence Postollec**[1], (1)ADRIA Développement, Quimper, France, (2)Université de Brest, Quimper, France

Introduction: *Bacillus licheniformis*is a ubiquitous sporeforming bacteria showing high enzymatic activities responsible for food spoilage and associated huge economical losses. While it is well known that environmental conditions encountered during sporulation will strongly impact spore resistance, the impact of storage time on spore resistance is not reported.

Purpose: This study aims at quantifying the impact of storage time on subsequent *B. licheniformis*spore heat resistance.

Methods: Spore suspensions of *Bacillus licheniformis* 115L14 were produced on milk agar medium and stored in sterile water at 4°C for 6 years, 9 months, 6 months or 8 days. Thermal inactivation kinetics were performed in nutrient broth using the capillary method and exposure to 4 temperatures (94, 96, 98 and 100°C). Survivors were counted after plating on nutrient agar to determine the inactivation kinetics that were then fitted using a Weibull model. Based on statistical criterion, applied mathematical model was reduced and used to describe the impact of time of storage on the thermal inactivation, i.e., heat resistance.

Results: Observed heat resistances were not statistically different for all storage time tested. Indeed, δ value, i.e., the time necessary to lose 90% of the spore suspension, was estimated at 19.09 ± 1.16 min for 6-year-old-spores treated at 98°C whereas it was estimated at 20.14 ± 1.25 min for 8-day-old-spores stored in water at 4°C. Spore sensitivity was assessed using a Bigelow model and estimated at 8.54 ± 0.61 °C. This secondary modeling step allowed the quantification of the variation of temperature allowing ten fold variation of the spore resistance (Z_T value). In other words, a treatment with a temperature increase of 8-9°C will yield a 10 fold decrease of spore resistance.

Significance: These results highlight that although the condition of sporulation has a great impact on spore heat resistance, the time of storage has no impact on the subsequent resistance. Thus, characterizing δ values for spore suspensions produced in different laboratory conditions maximizes the risks associated with spore contamination. This study further confirms actual knowledge and know-how associated with food artificial spore inoculation and heat treatment optimization.

T7-03 A Meat and Poultry Food Safety Survey Designed to Determine Educational Targets for African Americans of Low Socioeconomic Status

Mark Dworkin, Apurba Chakraborty and Preethi Pratap, University of Illinois at Chicago School of Public Health, Chicago, IL

Introduction: Foodborne illness disproportionately affects the African-American community due to a large percentage living below the poverty level and a holiday food preference (pork chitterlings [intestines]) associated with yersiniosis.

Purpose: To determine meat and poultry food safety knowledge and behavior among African Americans of low socioeconomic status in Chicago.

Methods: A food safety questionnaire was administered to low socioeconomic status meat eating African Americans. A food safety score was calculated out of 14 weighted questions.

Results: Among the 200 African American consumers interviewed, the most commonly prepared meat dishes were chicken, hamburger and pork chops. Half (108, 54%) had heard of at least one of these food safety programs, Fight BAC (Partnership for Food Safety Education [PFSE], 14%), Be Food Safe (USDA, FDA, CDC, PFSE, 49%), and Thermy (USDA, 3%). The mean food safety score was 12.2 (72%). Concerning food handling behavior, 56 (28%) thought it was ok to thaw ground meat on the counter, 63 (32%) would use color of beef to indicate doneness, 31 (16%) would eat store-bought hot dogs without heating, and 122 (61%) would rinse vegetables for salad (splashed with raw chicken juice) rather than throw it away. Although 172 (86%) knew where to place a meat thermometer, only 59 (29%) owned one. Fifty-six (28%) consumers cooked or prepared pork chitterlings. Among these chitterling handlers, 34 (61%) did not boil for 5 minutes before cleaning (a recommendation of the USDA), only 17 (30%) had heard of this recommendation, and 32 (57%) knew to lather their hands with soap during hand washing for at least 15 seconds. Ninety-one percent of chitterling handlers who did not boil chitterlings did not know the USDA recommendation.

Significance: These data demonstrate significant food safety gaps to emphasize in educational interventions and they have been used to design an educational culturally acceptable photonovella.

T7-04 Toxicity and Memory—Consumer Reactions to Foods from Japan, A Year Later

Aurora Saulo[1], Nadejda Livshits[2], Howard Moskowitz[2] and Janna Kaminskaia[3], (1)University of Hawaii at Manoa, Honolulu, HI, (2)Moskowitz Jacobs Inc., New York, NY, (3)Queen's College, New York, NY

Introduction: Rule Developing Experimentation (RDE) is a systematic exploration of ideas that has not been used to link elements that drive people to buy foods with the emotions they feel when they buy those foods. RDE reveals clear, quantitative links between emotions and the person's criteria for making a purchase decision. RDE is particularly relevant here because it cannot be 'gamed' by the respondent, i.e., answered in a politically correct way.

Purpose: To study and understand differences in thinking, behavior, and feelings toward foods from Japan after the Fukushima disaster. To develop coherent messages and explore if over time these messages could reduce anxiety.

Methods: Rule Developing Experimentation (RDE) was used instead of traditional surveys, creating a six by six matrix of 36 messages, realizations of the different consumer response dimensions involved in consumer reactions to radioactivity in food. Additional demographic and self-profiling questions were asked to identify consumer mindsets.

Results: A year of outreach using truthful, science-based statements did not seem to alter people's behavior. In many cases, they became more nervous and suspicious.

Significance: Food safety RDE, first employed by the authors, was used to link elements that drive decisions to buy food from Japan to emotions of people who buy the foods. Results indicate that emotional state changes purchase decision criteria. Using the messages has to be done in a structured way, taking into account the emotions that accompany the messages.

T7-05 Multi-Provincial Outbreak of Escherichia coli O157:H7 Infections in Canada Sourced to Gouda Cheese Made from Unpasteurized Milk

Regan Murray[1], Davendra Sharma[2], Lynn Wilcott[3], Robert Parker[4], Pedro Chacon[2], Sion Shyng[3], Paul Kirkby[2], Lance Honish[5], Eleni Galanis[3], Victor Mah[6], Ana Paccagnella[3], Linda Hoang[3], Linda Chui[7], Roger Pannett[8], Enrico Buenaventura[9], Lorelee Tschetter[10], Sujani Sivanantharajah[1], Andrea Currie[1] and For The Investigative Team[11], (1)Public Health Agency of Canada, Guelph, ON, Canada, (2)Canadian Food Inspection Agency, Ottawa, ON, Canada, (3)British Columbia Centre for Disease Control, Vancouver, BC, Canada, (4)Interior Health Authority, Kelowna, BC, Canada, (5)Alberta Health Services-Environmental Public Health, Edmonton, AB, Canada, (6)Alberta Health, Edmonton, AB, Canada, (7)Provincial Laboratory for Public Health - Alberta, Calgary and Edmonton, AB, Canada, (8)British Columbia Ministry of Agriculture, Vancouver, BC, Canada, (9)Health Canada Bureau of Microbial Hazards, Ottawa, ON, Canada, (10)Public Health Agency of Canada, Winnipeg, MB, Canada, (11)Health Canada and PHAC, Across Canada, BC, Canada

Introduction: An outbreak of *Escherichia coli* O157:H7 infections was identified in Canada in early September 2013. Based on initial case interviews, the suspected source was cheese from a dairy plant in British Columbia (BC).

Purpose: A multi-agency investigation was conducted to characterize the outbreak, identify and remove contaminated product from the market and understand contributing causes.

Methods: Public health investigators defined and interviewed cases using a focused questionnaire, and collected food specimens from case homes. Federal and BC food safety, public health and agriculture authorities conducted an investigation of the dairy plant, the on-site dairy farm and retail store, including inspections, records and process review, and collection of food and environmental specimens. *E. coli* O157:H7 isolates were identified using routine culture methods and were characterized by pulsed field gel electrophoresis and multi-locus variable number tandem repeat analysis.

Results: This outbreak resulted in 29 illnesses in five Canadian provinces, including five (17%) hospitalizations and one death. Symptom onsets ranged from July 12 to September 29, 2013. Twenty-six cases (90%) consumed Gouda cheese originating from the BC dairy plant. All 22 cases with sufficient product details available consumed Gouda cheese made with unpasteurized milk. The outbreak strain was isolated from seven samples of the Gouda cheese, including one core sample from an intact cheese wheel that had been aged for more than 60 days. The investigation of the dairy farm, plant and retail areas identified minor deficiencies in processing, sanitation and documentation. Opportunities for cross-contamination were noted in the cutting and packaging room and retail store.

Significance: Unpasteurized milk was the likely source of *E. coli* O157:H7 which persisted through production and the aging period (i.e., $\geq$ 60 days) to finished product. This is the third outbreak of *E. coli* O157:H7 sourced to Gouda cheese made from unpasteurized milk in North America.

T7-06 Whole Genome Sequencing of Escherichia coli O157 Isolates (Clinical, Ruminant and Food) from Scotland

Norval Strachan[1], Bruno Lopes[1], Marion Macrae[1], Chad Laing[2], Vic Gannon[2], Lesley Allison[3], Mary Hanson[3] and Ken Forbes[1], (1)University of Aberdeen, Aberdeen, United Kingdom, (2)Public Health Agency of Canada, Lethbridge, AB, Canada, (3)Scottish E. coli O157/VTEC reference laboratory, Edinburgh, United Kingdom

Introduction: Scotland has consistently one of the highest rates of *Escherichia coli* O157 infection in the world. Human infection is acquired by foodborne, environmental (e.g., contact with farm animals), waterborne and person to person transmission pathways. Typing has traditionally been carried out by phage typing and pulsed field gel electrophoresis. The developments in next generation sequencing (NGS) have now made it possible to readily sequence isolates at a reasonable cost.

Purpose: This pilot project was conducted to investigate the utility of NGS to determine the phylogeny of isolates (clinical, foodstuffs and ruminants) and their shigatoxin profiles in relation to source and phage type.

Methods: Whole genome sequencing of 148 isolates from food, veterinary, environmental and human clinical sources was conducted using an Illumina HiSeq sequencer with 100 nt paired-end sequencing. Raw paired end reads were assembled by SPAdes. Shigatoxin typing was performed by in-silico PCR. Core genome SNP analyses was performed using Panseq and the neighbour joining tree was generated in BioNumerics.

Results: More than 100 isolates carried either or both of *stx2a* and *stx2c* shigatoxins. Twenty-six of the isolates carried a combination of *stx1a* with *stx2a* (4) or *stx2c* (22). The majority of the food isolates carried the *stx2a* shigatoxin. The different shigatoxin genotypes and phage types were clustered in the phylogenetic tree. In particular, the supershedding PT21/28 generally carries the most potent shigatoxin (*stx2a*) which is human disease associated. Isolates from humans, cattle and sheep appear to be distributed throughout the phylogeny of *E. coli* O157.

Significance: The high prevalence of the potent *stx2a* shigatoxin may be part of the reason for the high incidence of human disease in Scotland. Also, the overlapping phylogeny of the cattle and sheep isolates indicates that both of these reservoirs are likely to be important for maintenance of this organism in the farm environment.

Technical Session 8 Microbial Food Spoilage
Friday, 9 May 2014: 8.30–10.00

T8-01 Multispectral Imaging vs. Fourier Transform InfraRed (FTIR) Spectroscopy for Monitoring Meat Spoilage
Dimitris Pavlidis, Athina Ropodi, Dimos Loukas, Efstathios Panagou and George-John Nychas, Agricultural University of Athens, Athens, Greece

Introduction: Numerous analytical methods are used to assess meat spoilage, nevertheless they are time-consuming, invasive and require trained staff. However, emerging, fast, non-invasive technologies based on hyper/multi-spectral imaging and vibrational spectroscopy in tandem with chemometrics are a promising tool to predict meat spoilage.

Purpose: The aim of this work was to correlate the changes occurring in multispectral imaging and FTIR with the spoilage of minced meat, regardless of packaging and storage conditions.

Methods: Portions of minced beef (75-80 g) were stored at 5^{o}C and 10^{o}C under aerobic and modified atmosphere (80%O_2/ 20%CO_2) packaging conditions. Multispectral images and FTIR spectra were acquired in parallel with the enumeration of total viable counts (TVC).In total, 51 TVC measurements were collected, along with the results of multispectral image analysis and FTIR acquisition. After preprocessing, Partial Least Squares Regression (PLS-R) was applied for the bacterial loads. For the FTIR data, a moving-window PLS (mwPLS) method was also used to pinpoint significant wavenumbers. Mean values for bias, accuracy and RMSE after 100 random partitions were used for model validation

Results: The bias and accuracy factors were close to 1 whilemean RMSE was 0.67 and 0.49 for multispectral imaging and FTIR, respectively. Lastly, the mwPLS contributed in defining important FTIR wavenumbers, especially outside the fingerprint area.

Significance: This study showed the potential of rapid techniques in the monitoring of spoilage independently of the product's history. Between the two methods FTIR analysis was found to describe spoilage more accurately.

This work has been supported by the project "Intelligent multi-sensor system for meat analysis - iMeatSense _550" co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: ARISTEIA-I.

T8-02 Inter-strain Interactions among Bacteria Isolated from Australian Vacuum-Packaged Refrigerated Beef

Peipei Zhang[1], Jozsef Baranyi[2] and **Mark Tamplin**[1], (1)University of Tasmania, Hobart, Australia, (2)Institute of Food Research, Norwich, United Kingdom

Introduction: Australian vacuum-packaged beef is recognized for exceptionally long shelf life, accompanied by low levels of microbial growth. Although factors responsible for these observations are poorly understood, previous research implicates bacterial interactions may have a role.

Purpose: The objective of this research was to detect and measure the kinetics of growth-inhibiting and growth-promoting interactions among a complex bacterial consortium derived from meat.

Methods: One hundred and eighty isolates were pre-screened pairwise for interacting effects on bacterial growth, identifying 39 and 20 effector and target isolates, respectively, which constituted 10 different species. The influence of effectors on the growth of target isolates was investigated with live cells and cell-free supernatants of effector cultures in both agar- and broth-based assays.

Results: Thirty-three percent (260/774) of the pairings interacted; 29% of these produced growth-inhibition and 4% growth-promotion. The majority of *Pseudomonas* spp. isolates displayed antagonistic properties against ~50% of target isolates, whereas two isolates of *Bacillus* spp. inhibited 80% of all targets. *Carnobacterium maltaromaticum* showed the highest inhibition spectrum compared to other lactic acid bacteria, whereas most growth-enhancing effector isolates were Gram-negative bacteria, including *Pseudomonas* and *Enterobacteriaceae*.

Significance: These findings provide a comprehensive and quantitative description of interactions among spoilage bacteria in a complex bacterial community. The study can potentially lead to a mechanistic model of meat spoilage under environments which changes dynamically, depending on the interactions within the food microbiota in question.

Acknowledgments: We gratefully acknowledge support by Meat and Livestock Australia, the Chinese Scholarship Council, and Zhejiang University.

T8-03 TiO_2-UVC Photocatalysis for Inactivation of Escherichia coli O157:H7 on Orange Surface

Sungyul Yoo, Sanghun Kim, Sunghyun Lee, Jinho Cho and Jiyong Park, Yonsei University, Seoul, South Korea

Introduction: Commercial non-pasteurized orange juice products are normally manufactured without a peeling process and directly squeezed to extract the juice from the orange, which leaves possibility of cross-contamination of foodborne pathogens on orange surface. Residual pesticide on orange surface can also contaminate the juice during the extraction process. TiO_2-UVC photocatalysis (TUVP) is a non-thermal technique that inactivates microorganisms and removes pesticide residues under aqueous conditions by generation of hydroxyl radicals.

Purpose: The aim of this study was to evaluate the effectiveness of the TUVP system to reduce pathogenic bacteria (*Escherichia coli* O157:H7) and pesticide (carbaryl) residues on orange surface.

Methods: Oranges were immersed in *E. coli* O157:H7 inoculum and dried. Samples were put into the reactor and treated with tap water, chlorine, UVC and TUVP. Surface microbial loads were determined by peeling spotted areas of the orange and washing with peptone water. Squeezed orange juice samples were used to determine the cross-contaminated microorganisms and pesticide carbaryl residue.

Results: Surface inoculated *E. coli* O157:H7 was reduced by 4.3 log CFU/mL when treated with TUVP for 20 min, whereas 1.5, 3.9 and 3.6 log CFU/mL reduction was achieved by treatment of tap water, chlorine 200 ppm and UVC alone, respectively. Orange juices extracted from *E. coli* O157:H7 inoculated orange were reduced to non-detectable levels (inactivation of 2.5 log CFU/mL) when treated with TUVP for 20 min; however, only 1.5 log CFU/mL inactivation was achieved when treated with UVC alone. Carbaryl reduction was observed when treated with TUVP.

Significance: TUVP treatment proved to be an effective surface disinfection method. Combining TUVP treatment with other non-thermal technologies such as high hydrostatic pressure will inactivate microorganisms synergistically for the non-pasteurized orange juice products.

T8-04 Growth Limits as a Single Set of Parameters to Predict Sporulation Boundaries, Heat Resistance and Outgrowth of Spores

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Introduction: *Geobacillus stearothermophilus* is a well-known spore forming bacteria in the canning industry, due to the high heat resistance of its spores. To control the spore's high heat resistance, the heat treatment intensity can be associated with environmental conditions (before and after heat treatment).

Purpose: The purpose of this study was to model the effect of temperature and pH on the growth, the sporulation and the heat resistance, using only the growth limits parameters.

Methods: The bacterial growth rate of *G. stearothermophilus* was estimated on nutrient broth at different temperatures and pH. Otherwise, spores of *G. stearothermophilus* were produced at different temperatures and pH, and their heat resistance was evaluated at 115°C, following a recovery at different temperature and pH.

Results: Based on experimental results, growth limits were estimated at 38.52°C, 68.02°C, 5.27 and 8.91 respectively for *Tmin, Tmax, pHmin* and *pHmax.* Decimal reduction values obtained from spores produced and recovered at different temperatures and pH showed that the highest heat resistances ($D_{115°C}$) was obtained in conditions allowing optimal growth ($D_{115°C}$ = 12.27 min). The current observations revealed also that sporulation boundaries correspond to growth limits and the outgrowth after a heat treatment occurred only in the range of temperature and pH allowing the growth.

Significance: Growth limits could be the unique set required to model and predict heat resistance, heat recovery and sporulation boundaries. These results have been transposed in a mathematical model.

T8-05 Genetic and Phenotypic Biodiversity of Bacillus licheniformis from the Dairy Industry

Anne-Gabrielle Mathot[1], Emeline Cozien[2], Anne Lochardet[2], Louis Coroller[1], Noemie Desriac[2], Veronique Huchet[2], Daniele Sohier[2] and **Florence Postollec**[2], (1)Université de Brest, Quimper, France, (2)ADRIA Développement, Quimper, France

Introduction: Among the Gram-positive aerobic spore-forming food spoiling bacteria, *Bacillus licheniformis* has a high prevalence in raw materials, ingredient and food, particularly in dairy products. Moreover, its properties of resistance to treatments, adhesion to surfaces and degradation of various substrates underlines potential abilities as a "super spoiler".

Purpose: The objective of this work is to evaluate genotypic and phenotypic biodiversity of this species by working on strains mainly isolated in the dairy industry but also foodborne strains from outside the dairy ecosystem as well as collections or epidemic strains. A better understanding of this diversity will allow better assessment of the spoilage risks linked to this microorganism.

Methods: More than sixty strains were studied by molecular methods: mainly PFGE and PCR based clustering analysis (REP PCR, M13 RAPD). Among these strains, about thirty were studied for their ability to produce biofilm, surfactant and different enzymes (gelatinase, caseinase, amylase and lipoprotease). All manipulations were performed at least in duplicate and the average result is used for further processing. Clusters of strains were made primarily by hierarchical clustering (Bionumerics, Minitab and JMP)

Results: Composite molecular fingerprint analysis generated by Bionumerix underlined a major group composed of the type strain, raw materials, environment and most dairy product isolates. In order to complete the picture of the potential risks, the combination of molecular and phenotypic diversity yields to a more diverse clustering. Even though in minority, a few strains were distinguished by their high potential for spoilage and / or biofilm formation.

Significance: These results underline the large diversity of strains of *B. licheniformis* encountered in dairy ecosystem as compared to strains from other origins. The study highlights the existence of clusters with similar behaviors or characteristics but also of more atypical strains that could be considered as "super spoilers".

T8-06 Genetic Diversity of Clostridium spp. Isolated from Spoiled Hard-cooked and Semi-hard Types of Cheese

Sebastien Fraud[1], Nadine Henaff[2], Marie Odile Perron[1], Noemie Desriac[2], Veronique Huchet[2], Anne-Gabrielle Mathot[3], Florence

Postollec[2] and **Daniele Sohier**[2], (1)ACTALIA, La Roche sur Foron, France, (2)ADRIA Développement, Quimper, France, (3)Université de Brest, Quimper, France

Introduction: Butyric acid fermentation, the late-blowing defect in cheese, caused by the outgrowth of Clostridia spores present in raw milk, can lead to considerable loss of product, especially in the production of semi-hard and Gruyère cheeses. Although *Clostridium tyrobutyricum* is the most frequently isolated strain from late-blown cheeses, spores of other clostridia, particularly *C. sporogenes, C. beijerinckii*, and *C. butyricum*, have also been isolated from natural and processed cheeses and raw milk. Conventional methods for the isolation of *Clostridium spp.* from cheeses with late-blowing defects are tedious and the identification of isolates is often complicated.

Purpose: The aim of this work was to develop and evaluate the use of molecular typing tools to detect and differentiate major species involved in late-blown cheeses.

Methods: A collection of over 300 Clostridia isolates was analysed using various molecular typing methods to perform clusters (REP-PCR) and assess genetic relatedness with a multi-locus sequence typing (MSLT) method targeting 5 different genes (recA, groEL, tpi, rpoB, 16S rDNA). Specific PCR methods were developed i) to detect the major species involved in late-blown cheeses and ii) differentiate the major targeted species in particular distinguish closely related *C. sporogenes* and *C. botulinum*.

Results: 274 isolates were analysed using the REP-PCR method combined to the 16S rDNA sequencing (> 600 bp), providing more than 60 clearly differentiated groups. Several primer sets were designed to specifically detect *C. sporogenes, C. butyricum* and differentiate *C. botulinum* from other dairy-related Clostridia. Primer pairs were designed in the recA gene and the tpi gene for specific PCR detection of *C. sporogenes* and *C. butyricum* respectively. A different primer pair was designed in the recA gene to specifically detect group A *C. botulinum*. The optimised protocols can distinguish the three target Clostridia species and no specific amplifications were obtained among other *Clostridium spp.* or non-target species (*Streptococcus thermophilus and thermophilic Lactobacilli*). The MLST of 16 *C. sporogenes*isolated revealed important intra-species diversity and locus frequencies that ranged from 4 to 8 alleles per locus. 11 unique profile patterns or STs were identified. The 16-23S rDNA PCR method yielded discriminative inter-species genomic fingerprint.

Significance: Clostridia strains isolated from raw milks and spoiled dairy products have been analyzed, leading to the set up of a characterized collection. Moreover, several methods have been developed to type and identify the isolates, respectively a REP-PCR fingerprinting method, as well as a MLST and species specific PCR methods.

Poster Session 1 - Non-Microbial Food Safety, Novel Laboratory Methods, Pathogens, Sanitation Wednesday, 7 May 2014: 10.00–18.30

P1-01 Pre-Drying Effects on the Quality of Frying Oil

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Introduction: Deep-fat frying causes desirable as well as undesirable changes in oil and potato, and changes the quality of the oil by hydrolysis, oxidation and polymerization.

Purpose: The main objective of the present study was to investigate pre-drying effects on the quality of both frying oil and potatoes.

Methods: Prior to frying, potato slices (10 mm x 10 mm x 30 mm) were air-dried at 60°C for 15, 30, 45, 60, 90 and 120 min, respectively. Potato slices without pre-drying treatment were considered the control variable. Potato slices were fried in sunflower oil at 180°C for 5, 10 and 13 min. The deep-frying experiments were repeated five times using the new potato slices in the same oil without oil replenishment. Samples of the fresh oil, together with those sampled at the end of successive frying operations (1th, 3th and 5th) were removed and analyzed.

Moisture content, color and oil intake of the potato and color, peroxide value (PV), free fatty acid (FFA), fatty acid composition and viscosity of the used oil were evaluated. The effect of frying time was also examined.

Results: Results show that the pre-drying treatment had a significant effect on physicochemical properties and color parameters of potato slices and frying oil. Pre-drying considerably decreased the oil absorption. The lowest oil absorption was found for potatoes that were pre-dried for 120, and fried for 5 min. The FFA levels decreased permanently for each pre-treatment

throughout the frying period. All the pre-drying treatments had reached their maximum levels of FFA by the end of the frying procedures. The PV of the control and 60 min pre-dried sample decreased after the third frying. However, the PV of other samples increased constantly throughout the frying periods. Lastly, pre-drying did not affect the fatty acid composition of frying oil considerably when compared against previously unused oil.

Significance: Pre-drying dramatically decreased the oil absorption of potato slices and improved the oil quality.

P1-02 Seroprevalence of Salmonella spp., Yersinia spp., Trichinella spp. and Toxoplasma gondii in Finnish Finishing Pigs

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Introduction: The European Food Safety Authority (EFSA) considers *Salmonella* spp., *Yersinia enterocolitica*, *Toxoplasma gondii* and *Trichinella* spp. as the most important biological hazards in the context of meat inspection of pigs.

Purpose: This report aimed to assess the seroprevalence of these important zoonoses in finishing pigs from different farming types in Finland.

Methods: Meat samples from 1,353 fattening pigs from 259 farms across Finland were collected at slaughter and analyzed with commercial ELISA kits. Farms had different farming types: large fattening farms (> 1000 pigs per farm), small fattening farms (< 1000 pigs per farm) and farrow-to-finish farms.

Results: Anti-*Salmonella* antibodies were rare (3% of pigs and 14% of farms) especially considering methodological issues as cut-off OD 0.2. Antibodies to pathogenic *Yersinia* spp. were detected in 57% of pigs and in 85% of farms (OD $\geq$ 0.30). Seroprevalence of *T. gondii* was 3% of pigs and 9% of farms (OD $\geq$ 0.15) and it was significantly higher in pigs originating from small scale fattening farms ($P < 0.05$, ANOVA, Tukey HSD). Antibodies to *Trichinella* spp. was not detected (OD $\geq$ 0.3).

Significance: The seroprevalence of these important zoonotic pathogens was small in Finnish finishing pigs, except that of *Yersinia*. Anti-*Toxoplasma*–antibodies were significantly more prevalent in pigs originating from small scale fattening farms, supposedly reflecting ineffective biosecurity measures on these farms.

Acknowledgment of support: The study was supported by research funding from the Ministry of Agriculture and Forestry, Finland (1933/312/2011). The official veterinarians, auxiliaries and the slaughterhouses are gratefully acknowledged for their cooperation.

P1-03 Impact of Climate Variability and Future Climate Change on Shellfish Poisoning in South Korea

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Introduction: Shellfish poisoning toxin blooms in April and May in South Korea, causing severe oceanic environmental problems. This study provided a regression model, which explained the relationship between the toxin and environmental conditions. Also, based on RCP 8.5 scenarios, we constructed the future toxin-distribution maps.

Purpose: To access the effects of environmental conditions and shellfish species on the amount of shellfish poisoning toxin collected in the coastlines of South Korea and to predict the seasonal pattern of occurrence of shellfish poisoning within the next 100 years based on future climate RCP scenarios.

Methods: A total of 5,258 observations of shellfish poisoning toxin from 4 shellfish species were analyzed after some exclusion criteria were applied. In order to account for not-detected (ND) observations below the limit of detection, a censored regression model and an ordinary regression model with ND observations being replaced with the half of the limit of detection were fitted to the data. In the model fitting, a list of environmental conditions in addition to shellfish species was considered as covariates; seawater temperature, weekly change of seawater temperature, salinity, precipitation, insolation and areas prone to red tide. For future prediction, we used values for the list of environmental conditions predicted under future climate RCP 8.5 scenario.

Results: It was observed that there was a seasonal variation in the amount of shellfish poisoning toxin; higher in the spring season than all other seasons. All the continuous environmental covariates showed a quadratic relationship with the amount of shellfish poisoning toxin. With quadratic effects of environmental conditions accounted for, the censored regression model showed more realistic results than the ordinary regression model. From the future prediction, it is expected that the timing of the

highest outbreaks of shellfish poisoning will move toward earlier months of year like February and March, while the highest outbreaks of shellfish poisoning in the current years were observed in April and May.

Significance: This is the first study to examine the association between climate variability and shellfish poisoning using a predictive models considered regional variations in South Korea. The results play an essential tool for developing food safety programs and climate change adaptation in Korea.

P1-04 Integrated Impact Assessment on Food Safety Due to Climate Change Using Indicator-based Models in South Korea

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Introduction: Climate change may have both direct and indirect impact on food safety in South Korea. Therefore, the corresponding studies have been actively conducted under local/regional/sectoral levels. But, few studies have been conducted in terms of integrated impact assessments on food safety due to climate change.

Purpose: To identify the overall impact of climate change on food safety in South Korea, integrated impact assessment, including food safety index and its host environmental factors, was conducted using indicator based models (CC-FS IIAS) developed in this study. Also, future trends of food safety due to climate change in Korea were predicted by the models combining new climate change scenarios produced by KMA (Korean Meterological Administration) based on the RCP scenarios.

Methods: Indicators for food safety were obtained according to 9 steps developed in this study. Expert AHP analysis were conducted to obtain the weighting of each indicator. Simulated scenario developed in previous study were used to evaluate the impact of environmental factors of food safety. Indicator based models (CC-FS IIAS) were developed with food safety index, simulation scenarios, and predictive models (developed in previous studies, Poisson GLM models). For the future trends, the climate change scenario produced in KMA based on the RCP (RCP 4.5 and 8.5) scenarios were used.

Results: The results obtained with indicator based model suggested food industry (3.0%, 0.5%) and social and economy (2.1%, 3.9%) will be reduced in 2030 and 2050, and negative impact may be affected food safety in South Korea. But, science and technology (13.5%, 26.9%), government and politics (10.1%, 22.7%), consumer behaviour (16.35%, 33.4%), and information (7.7%, 12.2%) will build up adaptation capacity on food safety. The overall level of food safety in South Korea will be down from 2.3% in 2030 and 9.5% in 2050.

Significance: This is the first study to evaluate integrated impact of food safety due to climate change using an indicator based model. The results are an essential tool for developing food safety programs and climate change adaptation in South Korea.

P1-05 Analysis For Genes Related to Biofilm Formation In Staphylococcus spp. Strains Isolated From Milk Samples

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Introduction: Biofilm communities have been associated with the persistence of environmental contamination and recurrent infections. The bacteria in biofilm are more resistant to antimicrobial treatments and to decontamination procedures. The persistent circulation of pathogenic bacteria in food premises and in livestock can be risk factors for food transmitted diseases. *Staphylococcus aureus* causes mammary infections, such as mastitis, resulting in a reduction in milk production and quality. Coagulase-negative staphylococci (CoNS) are considered less pathogenic but they are involved in sub-clinical mastitis and are frequently isolated from milk samples.

Purpose: The capability to organize biofilm communities of *Staphylococcus* spp. strains isolated from milk samples was analyzed for the presence of biofilm related genes. A comparison of *S. aureus* and CoNS isolates was performed for the presence of biofilm-associated protein *bap* gene and the intercellular adhesion locus ica, involved in biofilm formation. Some strains were tested for biofilm capability in relation to the presence of the genetic loci.

Methods: CoNS (83) and *S. aureus* (95) strains were isolated from bulk milk samples. The isolated colonies were typed by API strip (BioMerieux). PCR was performed for *ica* and *bap* genes. The analysis for biofilm formation was performed by safranin method in microtiter plates.

Results: The results showed that (*ica*) operon was present in *S. aureus* isolates and *CoNS* in equal proportion (almost 88%) whereas *bap* gene was present at higher percentage (45%) in CoNS compared to *S. aureus* isolates (8%).The biofilm capability of different *S. aureus* isolates showed no correlation with the presence or absence of the two genetic loci but in "vitro" assay may depend on culture media.

Significance: A better understanding of the biofilm of *Staphylococcus*spp. contaminating milk samples may be useful for their control throughout the various stages of the food chain, from farm to fork. Grant RCSI 2011 to C.P. is acknowledged.

P1-06 Penetration of Salmonella Enteritidis in Chicken Breasts Stored under Refrigeration

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Introduction: Contamination of carcasses and poultry cuts with *Salmonella* initially occurs on the surface, due to the natural autolytic processes and decomposition caused by inadequate preservation. These processes may favor the migration of bacterial cells to deeper layers of muscles, and may be difficult to eliminate by usual food preparation processes, increasing the risk of transmission of this important foodborne pathogen.

Purpose: The objective of the study was to assess the ability of *Salmonella*Enteritidis in penetrating poultry breasts at refrigeration temperatures.

Methods: Small cubes (30 x 30 x 30 mm, height, width, length) were cut in deboned, skinless chicken breasts. Only one side of the surface was contaminated with *S.* Enteritidis resistant to nalidixic acid (3 log CFU). Cubes were stored at 2, 7 and 12^{o}C, and analyzed after 24, 48 and 72 h of inoculation. After storage, cubes were cut in three segments, each 10 mm high, making up a superficial segment (near the site of inoculation), a medium and final segment. The three segments were used to be analyzed for the presence of *Salmonella*. The analysis were carried out in duplicate for each time and temperature, and the experiment was performed in six repetitions.

Results: *Salmonella*was detected in all segments, no matter the time and temperature analyzed. It is noteworthy to observe that all segments were positive even at 2°C for 24 h, demonstrating the fast migration of the pathogen from the surface of the cut to the deeper muscle layers.

Significance: Due to the ability of *Salmonella* in quickly penetrating the chicken breasts even at low temperatures, it is essential that preventative tools are well established in poultry processing plants to minimize contamination of the carcasses and poultry cuts.

P1-07 Food Safety and Scrapie Control through Genetic Selection. Prion Gene Analysis in "Girgentana": A Sicilian Goat Breed

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Introduction: The prion bovine strain responsible for BSE (Bovine Spongiform Encephalopathy) in cattle and Creutzfeldt-Jakob variant in humans, represents a food safety problem in the last two decades. The discovery of BSE in two goats apparently affected by scrapie, the sheep prion disease and the recent discovery of TSE (Transmissible Spongiform Encephalopathies) infectivity in milk raised new concerns over risks related to TSE exposure with dairy products. The genetic selection for scrapie resistances in sheep is being implemented with success in Europe. In goats, the analysis on prion gene polymorphisms associated to resistance is still ongoing and the genetic selection cannot be planned until polymorphisms conferring resistance are confirmed.

Purpose: A polymorphism that seems related to scrapie resistance in goats is present at the codon 222 of the prion gene, with a lysine (K) in place of a glutamine (Q).To evaluate the prevalence of this 222K polymorphism in Sicilian goats we studied the "Girgentana",an autochthon breed which is at risk of extinction.

Methods: Blood samples were collected from 65 Girgentana goats. The isolated DNA was amplified for the coding region of the prion gene by PCR and then sequenced in Abi 3130 genetic analyzer. Sequence alignment was performed with the SeqScape software v2.5 (Applied Biosystems).

Results: The allele 222K was high frequent (0.30) in the Girgentana breed, whereas is less frequent in other breeds. This is important for the realization of a genetic selection based on 222K resistant allele for the autochthon goats.

Significance: Genetic selection for scrapie can eliminate the prion disease present in small ruminant flocks in Sicily and can assure the full eradication of the zoonotic bovine prion, which could persist in goats and sheep. The results on "Girgentana"goats showed how genetic biodiversity could be a great resource for the selection of resistance to diseases.

The work is supported by grant N. RF-2010-2318525 of Italian Ministry of Health.

P1-08 From Farm-to-Fork: Merck Millipore Singlepath® Direct Campy Poultry Rapid Test Kit for Farm-Based Direct Detection of Campylobacter spp. in Caecal-Type Samples from Live Chicken

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Introduction: The 2012 EFSA Scientific Opinion on Meat Inspection (EFSA Journal 2012;10(6):2741) proposed testing the *Campylobacter* status of live broiler flocks ≤ 3 days prior to slaughter, to identify the 'high shedding' flocks and allow segregation from low-shedding at slaughter, thereby avoiding cross-contamination of carcasses and reducing human consumption of *Campylobacter* spp. Such a strategy requires on-farm testing and a method which requires no specialized equipment or laboratory-trained personnel. Lateral Flow technology fulfills this requirement and offers a reliable, fast, user-friendly, alternative detection method to the laboratory-based cultural reference methods.

Purpose: To develop and evaluate a qualitative immunochromatographic assay for direct (non-enrichment) detection of high shedding (≥ 7.5 $\log_{10}$ CFU/g of caecal-type sample) *C. jejuni* and *C. coli* broiler chicken flocks, within 2 hours of sampling, as a rapid and farm-based alternative to standard cultural reference methods to monitor *Campylobacter*status of flocks and assist slaughter scheduling.

Methods: A sandwich Lateral Flow assay was developed, using gold labelled specific antibodies for *Campylobacter* spp. A non-enrichment sample preparation protocol was developed to enable a time-to-result of within 1 hour of sampling. Evaluation was by field studies conducted both on-farm (caecal droppings) and at slaughterhouse (caecal contents) using a cross-seasonal representative set of broiler chicken caecal-type samples. Reference method comparison was with ISO 10272 cultural method.

Results: In a field trial of caecal droppings collected on farm (n = 60), *Singlepath® Direct Campy Poultry* achieved a sensitivity of 96% (% correctly classified positive) and a specificity of > 99% (% correctly classified negative) based on a Limit of Detection of ≥ 7.5 $\log_{10}$ CFU/g of caecal content. In a field trial of caecal contents collected at slaughter (n = 60), the test kit achieved a sensitivity of 96% (% correctly classified positive) and a specificity of > 99% (% correctly classified negative) based on a Limit of Detection of ≥ 7.5 $\log_{10}$ CFU/g of caecal content.

Significance*:* Merck Millipore's Rapid Test Kit provides a unique, alternative, fast and simple method for detection of high shedding ≥ 7.5 $\log_{10}$ CFU/g of caecal-type sample) *C. jejuni* and *C. coli* broiler chicken flocks, on-farm or at slaughter, and can assist in monitoring *Campylobacter* spp. status of flocks and in slaughter scheduling.

P1-09 Evaluation of Adhesion and Invasion Properties of Different Listeria monocytogenes Isolates in Caco-2 Cell Line

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Introduction: *Listeria monocytogenes* is a foodborne pathogen responsible for important outbreaks and disease in humans. It is a Gram positive bacterium capable of binding to the epithelial host cells, and subsequently promoting its own internalization, replication and cell-to cell spread. *L. monocytogenes* interaction with the intestinal epithelium is a key step in the infection process.

Purpose: Our study aim was to investigate the ability of different *L. monocytogenes* isolates to adhere and to invade the human epithelial Caco-2 cell line. The strains used in this study have different origin such as human, food or environmental. The selected isolates showed an identical PFGE profile with other strains involved in recent cases of listeriosis. We performed adhesion and invasion assays *in vitro* in order to evaluate their bacterial virulence.

Methods: Adhesion and invasion assays were performed incubating *L. monocytogenes* with Caco-2 cells as it is described in literature. Bacteria were plated on BHI agar for enumeration by plate counting after 24 hours. Gentamicin was added in invasion assays. Bacterial internalization of the *L. monocytogenes* isolates was detected with fluorescence microscopy. CFSE and phallotoxins were used for visualization of bacteria and Caco-2 F-actin filaments respectively.

Results: The results of the adhesion and invasion assays were analyzed by one-way ANOVA and Tukey HSD test. It is important to underline that *L. monocytogenes* strains of human origin show a high adhesion and invasion index. In addition, there were differences in the adhesion and invasion index of several isolates. The fluorescence microscopy confirmed the high rate of internalization showed by the strains of human origin.

Significance: The adhesion and invasion process is the result of a fine-tuned gene expression, and the *in vitro* Caco-2 model represents a useful way to evaluate and compare the virulence potential of the selected strains.

P1-10 Protective Effect of Lactic Acid Bacteria on Host Defense of Caenorhabditis elegans against Yersinia Enterocolitica

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Introduction: *Yersinia enterocolitica* is listed in the annual reports of the European Food Safety Authority (EFSA) as the third-most-common enteropathogen. Infection with *Yersinia enterocolitica* e.g,. by ingestion of contaminated food or drinking water, can cause severe diarrhea, enterocolitis, and mesenteric lymphadenitis. For alleviation of the symptoms, antibiotic treatment has been used, but adverse effects on the composition of the intestinal microbiota was observed in both, human and ice subjects. Meanwhile, recent studies have shown that infection with *Y. enterocolitica* could be reduced or protected by sufficient numbers of Lactic Acid Bbacteria *in vivo* and *vitro* model. In this study, interaction between *Yersinia* and LAB was studied by using a *Caenorhabditis elegans* model, which is known as an alternative model host for virulence-associated factors of human pathogens. Consequently, some of LAB (*lactobacillus plantarum* LP133 and *lactobacillus fermentum* LF21) have revealed as having an inhibitory effect on *Yersinia Enterocolitica* infection by increasing of mean lifespan of *Yersinia* infected worms.

Purpose: LAB in this study was examined with regard to ability to protect against *Yersinia Enterocolitica*.

Methods: *C. elegans* mutant strain *glp-4* was used in the present study. To evaluate the effects of inhibitory effects of LAB on host life span against infection, *Caenorhabditis elegans* killing assay was used as an *in vivo* study. They fed on *Escherichia coli* OP50 for 2 days. 3-day-old from hatching worms were divided to either a control group that fed on OP50 or to a group fed on heat-killed LAB and OP50 for 1 days. 4-day old worms were then transferred on to *Y. enterocolitica* lawns. Each group of 24 worms assigned to one plates and incubated at 24°C. The numbers of live and dead worms were counted at least 24h. It was triplicated(n = 24 per plate).

Results: Nematodes fed heat-killed LAB and OP50 were clearly resistant to *Y. enterocolitica* infection compared with those fed only OP50. We observed that the mean life span of worms fed on (*L. plantarum* LP133, *L. fermentum* LF21) was about 20% greater than that of control in 4 day of infection.

Significance: Further understanding of the virulence-associated between probiotic bacteria and the host should clarify the contribution of these microorganisms and allow enhanced application of these approaches as prevention of *Yersinia Enterocolitica* -associated diseases.

P1-11 Study of the Microbial Flora of Steak Tartare by Metagenomic Approach

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Introduction: Steak tartare is a meat preparation prepared with bovine meat after grinding. It is eaten raw, hence representing a potential hazard for the consumer.

Raw meat like “steak tartare” is recognized to be sensitive perishable foods. Surveillance of spoilage during food storage is based primarily on the enumeration of total viable counts. However, microbial analysis alone might not be sufficient for understanding the modifications of the microbial ecology. Molecular technologies can elucidate microbial community structures. Among the culture-independent techniques, the metagenomic analysis targeting 16S ribosomal DNA has emerged as a powerful tool for studying bacterial composition of various ecosystems.

Purpose: This work describes the first application of metagenomic analysis on “steak tartare”.

Methods: Fifty-eight (58) samples of steak tartare were collected in butcheries (14), restaurants (6), sandwich shops (6), supermarkets marketing steak tartare provided by other establishments (SM1, 16 samples) and supermarkets preparing their own steak tartare (SM2, 16 samples). Total Viable Counts were performed on the same samples to compare the results. The bacterial populations were characterized at the day of receipt (day 0) and at the end of shelf life (day 2), except in the case of restaurants and sandwich shops where the samplings took place only at the date of production (day 0). Metagenomic analysis targeting the 16S rDNA was performed using the Roche GS junior. Raw sequences were treated by bioinformatics.

Results: The bacterial concentration (expressed as the median) was comprised between 4.5 and 5.5 log CFU/g. No statistical difference was observed. With the metagenomic approach, six species of bacteria were mainly recovered throughout the different sampling locations: *Brochothrix thersmosphacta, Lactococcus piscium, Leuconostoc gelidum, Pseudomonas* spp. and *Photobacterium kishitanii.*

Significance: Metagenomic analysis should be considered as a technique for quality control of the meat.

P1-12 **Listeria monocytogenes** ***Biofilm Formation and Dynamics in Multigenera Biofilms under Relevant Conditions for Food Processing***

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Introduction: Control and elimination of *Listeria monocytogenes* remains a major challenge in the food processing industry. *L. monocytogenes* is known to survive sanitation and persist in food processing environments. Their ability to adhere to surfaces and form biofilms are factors affecting survival properties. A better understanding of formation and persistence of *L. monocytogenes* in biofilms is important for improved control strategies.

Purpose: To study biofilm formation and strain dynamics of *L. monocytogenes* in multigenera culture biofilms under conditions relevant for food processing environments by combined use of microbiological and molecular analyses.

Methods: Isolates of *L. monocytogenes* and background flora were collected and selected after sampling in food industry. Biofilm studies were performed using stainless steel coupons in a laboratory model under conditions relevant for the salmon and meat processing industry. Biofilm experiments were performed at 12°C in Brain Heart Infusion (BHI) broth and Salmon broth to obtain relevant conditions. Both monospecies (*L. monocytogenes*) and multigenera (*L. monocytogenes* + background flora) biofilm formation and overall community dynamics were investigated by combinations of standard microbial plating, partial sequencing of a *L. monocytogenes* housekeeping gene and growth independent 16S rDNA sequencing.

Results: The study showed that the background flora suppressed biofilm formation of *L. monocytogenes* and that strains of *Pseudomonas* dominated in multigenera biofilms. Interestingly, the study indicated differences between *L. monocytogenes* strains in their ability to grow and compete in biofilms. The results were in accordance with what was found in sampling in the food industry, where *L. monocytogenes* is found in much lower numbers than other bacteria such as *Pseudomonas*.

Significance: The study illustrates that appropriate methods and combined microbiological and molecular analyses can be used to understand biofilm formation of important pathogens under relevant conditions. This is important for improved bacterial control strategies in the food processing chain.

P1-13 Relation between Microbiological and Serological Data of Human Pathogenic Yersinia enterocolitica in Pigs and Pig Batches

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Introduction: Pigs are the main reservoir of *Yersinia enterocolitica*, and the microbiological and serological prevalence of this pathogen differs between farms. The infection status of pig batches arriving at the slaughterhouse is largely unknown. Moreover, a link between the presence of *Y. enterocolitica* and the presence of antibodies is missing.

Purpose: A relation between the microbiological and serological prevalence could help to predict the contamination of the pigs prior to slaughter.

Methods: Pigs from 100 different batches were sampled. Tonsils and pieces of diaphragm were collected from 7,047 pigs (on average 70 pigs per batch). The tonsils were analyzed using a direct plating method and confirmed with a multiplex Polymerase Chain Reaction (*ail, yst, virF*). The meat juice of the pieces of diaphragm was analyzed by Enzyme Linked ImmunoSorbent Assay Pigtype Yopscreen (Labor Diagnostik Leipzig, Qiagen, Leipzig, Germany). The results of these prevalences were compared using a mixed-effects logistic regression at pig and batch level.

Results: *Y. enterocolitica* was found in 2,009 pigs, of which 1,872 also had antibodies against *Yersinia* spp. According to the logistic regression, the microbiological contamination could not be predicted by the presence of antibodies at pig level.

At batch level, a relation was observed (prevalence $=0.444/(1-e^{-0.063*(\text{Optical Density}-37.069)})$, cut-off value for a positive farm is 37OD%).

Significance: The given formula could predict whether a pig batch will contain infected pigs before they arrive at the slaughterhouse. This way, infected batches could be slaughtered last so cross-contamination in the slaughterhouse could be avoided or diminished.

P1-14 Monitoring the Expression of Genes Associated with Stress during Growth of Salmonella Typhimurium on a Plant Extract

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Introduction: Better understanding of bacterial pathogenicity is crucial for infectious control and treatment. However, several factors have to be checked, as infection is a process which requires the expression of genes not only coding virulence factors, but also physiological processes such as stress response and adaptation.

Purpose: Monitoring the expression of different genes associated with stress during growth of *Salmonella* Typhimurium (ST) on rocket extract regarding a laboratory medium

Methods: A low cells inoculum (10-15 cfu/mL) of *Salmonella* Typhimurium was used to contaminate a heat sterile rocket extract and a Luria – Bertani broth, (LB) growth medium. The expression of four different genes associated with stress along with the growth kinetics at 10 and 20°C was investigated.

Results: The final population of *Salmonella* Typhimurium was affected from the rocket extract as a difference of 1 log CFU/mL was observed regarding the laboratory medium. Regarding the expression of the four studied genes i.e. dps (starvation, stress response), dppA (adaptation to nutrient deficiency) rpoH (starvation) and osmY (osmotic stress), it was found to be more affected by the incubation temperature. In brief, these genes during growth on rocket extract were up-regulated and down-regulated at 10°C and 20°C, respectively.

Significance: The findings of the present study could show that ST reacts as exposed to different types stress when inoculated to a heat sterile plant extract at lower temperature.

This work was found by the action THALIS: "*Biological Investigation of the Forces that Influence the Life of pathogens having as Mission to Survive in various Lifestyles; BIOFILMS*", falls under the Operational Programme (OP) "Education and Lifelong Learning (EdLL)" and is co-financed by the European Social Fund (ESF) and National Resources

P1-15 Climate Change Impact on the Presence of Deoxynivalenol in Cereals Grown in The Netherlands

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Introduction: Climate change effects, such as elevated temperature and increased rainfall, are expected to affect the presence of mycotoxins in cereal grains. Quantitative estimates on the impact of climate change effects on mycotoxin occurrence are, however, scarce.

Purpose: The aim of the current study was to estimate the impacts of climate change effects on deoxynivalenol (DON) contamination of cereals (wheat and maize) grown in the Netherlands by 2040.

Methods: A series of quantitative models were applied such to consider both direct effects of changing climate on toxin contamination and indirect effects via shifts in crop phenology. The output of the one model was used as the input for the next model. The models used included two different combinations of a global and a regional climate model to produce climate data. Climate change projections for the IPCC A1B emission scenario were used for the scenario period 2031-2050 relative to the baseline period of 1975-1994. A weather generator was used for downscaling the generated climate data to local conditions. Crop phenology models for grain maize and winter wheat, and prediction models for DON contamination, in these crops were also used.

Results: Dates for flowering and full maturity of both wheat and maize will advance with future climate. Flowering advanced on average 5 and 11 days for wheat, and 7 and 14 days for maize. Full maturity was on average 10 and 17 days earlier for wheat, and 19 and 36 days earlier for maize. On the country level, contamination of wheat with deoxynivalenol decreased slightly, but not significantly. Variability between regions was large, and individual regions showed a significant increase in deoxynivalenol concentrations. For maize, an overall decrease in deoxynivalenol contamination was projected, which was significant for one climate model but not for the other one.

Significance: The use of quantitative models to estimate the impacts of climate change effects on food safety is very relevant, as well as considering both direct and indirect effects in the assessment of climate change impacts on crops and related food safety hazards.

P1-16 A Novel Molecular Tool for Verocytotoxin-producing Escherichia coli Identification and Pathogenicity Assessment

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Introduction: Verocytotoxin (VT)-producing *Escherichia coli* (VTEC) are zoonotic pathogens. On the basis of the pheno-genotypes and the epidemiological features, five seropathotypes (SPTs), from A to E, are identified with a decreasing rank of pathogenicity.

Purpose: To develop a tool for detecting VTEC strains in food and clinical samples and assessing their pathogenic potential.

Methods: A PCR screening of 147 *E. coli* strains, 61 VTEC, 59 of other pathogroups and 27 non-pathogenic, was conducted to identify targets associated with the most pathogenic VTEC. The gene, Z2121, was selected and a specific RealTime-PCR has been developed. The Z2121 reagents have been integrated in the iQ-Check STEC VirX kit targeting VT-genes (*vtx*) (Bio-Rad, Hercules, USA) and the composite assay was used to screen 188 VTEC of all the SPTs. Tests on artificially contaminated food matrices were performed by spiking ground beef samples with 10 and 100 CFU/g of VTEC belonging to O157, O26, O111, O103, O145 and O121 serogroups.

Results: Z2121 was present in the 41 VTEC belonging to the most pathogenic SPTs (A and B) and in 24 of 42 EPEC. The Z2121 iQ-Check STEC VirX kit specifically identified the SPTs A and B (135 strains) being simultaneously positive for *vtx*and Z2121 genes. The developed reagents correctly identified all the food samples, with the exception of half of those contaminated with 10 CFU of VTEC O26 and O111.

Significance: The Z2121 iQ-Check STEC VirX combined reagents efficaciously identify VTEC associated with the most severe forms of infection demonstrating to have a predicting value for pathogenicity. The inclusion of Z2121 improves the tools available facilitating the adoption of intervention measures when applied to food testing. The early identification of the most pathogenic VTEC is of utmost importance for human infections driving the approach to the management of the disease.

P1-17 Simultaneous Detection of 13 Foodborne Pathogens by MLPA-CE-SSCP

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Introduction: Illnesses caused by the consumption of food contaminated with pathogenic bacteria and/or their toxins are a major concern for public health and the economy. Therefore, the rapid and effective detection and identification of foodborne pathogens is important not only to control and investigate foodborne diseases but also to improve the management of food safety at an industrial scale.

Purpose: We aimed to develop a novel technique for the simultaneous identification of 13 important foodborne microbes including *Bacillus cereus, Campylobacter coli, Campylobacter jejuni, Clostridium perfringens, Cronobacter sakazakii, Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella* spp., *Shigella* spp., *Staphylococcus aureus, Vibrio vulnificus, Enterococcus* spp. and *Yersinia enterocolitica*using capillary electrophoresis-based single-strand conformation polymorphism (CE-SSCP) coupled with multiplex ligation-dependent probe amplification (MLPA).

Methods: Using the RAW program, we designed specific MLPA probes to target each of the 13 bacterial species, in accordance with the MRC-Holland instructions (http://www.mlpa.com). All MLPA experiments were performed using an MLPA reagent kit (MRC-Holland). Subsequently all MLPA products were amplified by Polymerase chain reaction (PCR), followed by an SSCP analysis of the PCR products. All CE analyses were performed with an ABI 3130 genetic analyzer using a 50-cm capillary array and a 15 wt% solution of Pluronic F108 polymer.

Results: All 13 targets were identified simultaneously in the electropherograms of the MLPA products with individual peaks corresponding to each microbe. Furthermore, these peaks corresponded with the peaks obtained for each strain run individually. Using this method of analysis, 50–500 pg/L of genomic DNA could be detected.

Significance: This MLPA-CE-SSCP-based diagnostic system can be used for the rapid detection of foodborne pathogens, which in turn, would improve the management of food safety at an industrial scale.

P1-18 PFGE-Typing of Enterotoxigenic Staphylococcus aureus Strains Isolated from Raw Milk in Poland
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Introduction: Raw milk may be contaminated with enterotoxigenic *S. aureus* which are responsible for staphylococcal food poisoning (SFP). These bacteria can access milk through their direct excretion from udders suffering clinical and subclinical staphylococcal mastitis or by environmental contamination during the handling and processing of raw milk.

Purpose: The purpose of this study was to determine the PFGE genotypes of enterotoxin-producing *S. aureus* isolated from raw milk.

Methods: A total of 390 *S. aureus* strains were isolated from 1,081 of raw cow's milk samples. Multiplex PCR assay was used to detect genes encoding SEs A, B, C, D, and E enterotoxins. The enterotoxigenic isolates were further characterized by PFGE after digestion of DNA with *Sma*I endonuclease. PFGE profiles were analyzed using BioNumerics software (version 6.6., Applied Maths, Belgium).

Results: Of the 390 strains studied, 49 (12.6%) were positive for one (91.8%) or two (8.2%) genes encoding SEs. The enterotoxin C gene (*sec*) was the most frequent (20 isolates, 40.8%), followed by *sea* (12 isolates, 24.5%), *sed* (10 isolates, 20.4%), and *seb* (3 isolates, 6.1%). Two strains (4.1%) had *sea* and *seb* genes while one (2.0%) harboured the *sea* and *sec* genes and one (2.0%) *sea* in combination with *sed*. None of analyzed isolates had enterotoxin E marker. PFGE investigation revealed a high degree of diversity of the analyzed strains. Using an 80% genetic similarity as a cut-off, the strains were grouped in 10 clusters. The two largest clusters contained 16 and 13 strains, respectively.

Significance: Enterotoxic *S. aureus* in raw milk posses a potential health hazard for consumers. PFGE patterns of such strains originated from Poland can be compared with patterns from other sources and give information about origin and genetic diversity of the strains.

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P1-19 Characterization of Vibrio parahaemolyticus Isolated from Raw Bivalve Molluscs in Poland
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Introduction: *Vibrio parahaemolyticus* is a widespread microorganism in the marine environment. The presence of these bacteria in food of marine origin is a potential threat to consumer health especially in countries where raw shellfish are eaten. In Europe, cases of infection appear sporadically, but every year their number increase.

Purpose: The aim of present study was to characterize *V. parahaemolyticus* isolated from live bivalve molluscs available in Poland.

Methods: A total of 400 samples from different species of raw shellfish were used in the study. *V. parahaemolyticus* was identified by the ISO 21872-1 standard. Suspected bacterial colonies were confirmed by PCR method for the species-specific target (*toxR* gene). *V. parahaemolyticus* isolates were tested for the presence of main virulence markers – the hemolysin *tdh* and *trh* genes. Moreover, other potential virulence properties as urease and protease activity were determined using conventional methods.

Results: The study showed that *V. parahaemolyticus* was identified in 71 out of 400 examined samples (17.8%). The PCR for the *toxR* gene confirmed the presence of these bacteria in 90.1% (64 out of 71) of the positive samples. Identification of virulence genes in the isolated *V. parahaemolyticus* showed that 3 of them had the *tdh* gene and 6 strains possessed *trh* marker. Urease activity was detected in all *trh*-positive isolates. Moreover, all *V. parahaemolyticus* strains showed protease activity.

Significance: The results indicate that *V. parahaemolyticus*quite frequently occurs in raw shellfish available in Poland. Additionally, some of these strains may be pathogenic for humans due to the presence of virulence markers.

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P1-20 Comparison and Molecular Characterization of Animal and Human Clostridium difficile Strains

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Introduction: *Clostridium difficile* is an important cause of infectious diarrhea in hospitals and nursing homes. The major risk factors for the development of nosocomial *C. difficile* associated disease include antibiotic therapy and increasing age. Additional potential risk factors are gastric acid suppression, inflammatory bowel disease or immunodeficiency, among others. In animals, such as pigs, calves and horses, *C. difficile* also seems to be an important cause of enteric disease. Moreover, recent isolation of *C. difficile* in a variety of meat products reinforces the hypothesis about a potential risk of foodborne transmission.

Purpose: The main objective of this study was to characterize and compare animal and human *C. difficile* strains with respect to the PCR-ribotype and the antibiotic resistance. Multilocus sequence typing were performed in order to study clonal relationships of the isolates.

Methods: Human *C. difficile* isolates were obtained from care home residents and hospitalized patients. Animal isolates were collected from stool samples and carcasses of pigs and cattle at slaughter. An identification of the strains was performed by PCR-ribotyping. Further characterization was performed by antibiotic resistance and MLST analysis. A neighbourd-joining phylogenetic three was constructed in order to determine the correlation between human and food isolates.

Results: A great variety of PCR ribotypes was found among the animal isolates, including PCR ribotypes 078 and 014. The most prevalent PCR-ribotypes in the nursing home were PCR-ribotypes 027 and 020. A high resistance to moxifloxacin, erythromycin, gentamicin and clindamycin was detected for some of the strains. Phylogenetic analysis showed that human and animal isolates with the same PCR-ribotype cluster in the same lineage.

Significance: The overlap between strains from animal and human host suggest a potential risk of interspecies transmission, including foodborne infections linked to this bacterium.

P1-21 PFGE Typing of Vibrio parahaemolyticus Isolated from Shellfish in Poland

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Introduction: *Vibrio parahaemolyticus* is a marine bacterium responsible for gastroenteritis in humans. Foodborne infections are associated with the consumption of contaminated raw or undercooked shellfish. Several molecular typing methods have been

developed for the differentiation of *V. parahaemolyticus*. Pulsed-field gel electrophoresis (PFGE) is a highly discriminatory technique which is used to determine the relatedness of *V. parahaemolyticus*strains.

Purpose: The aim of this study was to assess the PFGE-based relatedness of *V. parahaemolyticus*strains isolated from shellfish intended to consumption in Poland.

Methods: A total of 64 *V. parahaemolyticus* strains used in this study were isolated from raw shellfish (n = 400) available in Polish market, which originated from EU countries. PFGE was carried out according to the standard operating procedure of PulseNet. Bacterial DNA was digested with 40 U of *Sfi*I restriction enzyme. PFGE was performed using the CHEF DR III System (Bio-Rad, USA) and the macrorestriction profiles were analyzed by the BioNumerics software (version 6.6, Applied Maths, Belgium).

Results: The present study showed a high genetic diversity of *V. parahaemolyticus*tested. A total of 62 different PFGE patterns were identified, which were clustered into 14 groups containing at least two isolates. The remaining 17 profiles possessed only one strain. The largest cluster contained 7 isolates.

Significance: The obtained results indicate a great variety of *V. parahaemolyticus*as tested by PFGE. This method may be used for molecular comparison of macrorestriction profiles available in different databases.

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P1-22 Tools for Molecular Detection of Parasites

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Introduction: *Cryptosporidium* sp., *Giardia* sp., *Toxoplasma gondii*, *Naegleria fowleri* and *Entamoeba histolytica* are parasites responsible each year for millions cases of humans infection. Infection rates are from 1 to 10% in industrialized countries, and much higher in developing countries from 10 to 30%. Water is the main source of human contamination by these parasites. Thus, it's not uncommon to find parasites in the water pool or sea, introduced by infected individuals but also in food matrices like shellfish or vegetables.

Purpose: The methods for the diagnostic are currently based on immunofluorescence. They are tedious, poorly reproducible and difficult to implement by non-specialists. So we developed a method eliminating all these constraints. For this, we chose the real-time PCR, a rapid, sensitive and reliable tool.

Methods: Primers and probes were defined following the same phases of validation performance tests including specificity, sensitivity and robustness for each of the targeted parasites.

Results: Firstly, the specificity of the primers and probes was developed *in silico* and demonstrated by experiment for each of the parasites. Then, the sensitivity of the pairs was compared on dilution series up 1eqG/L. This corresponds to the sensitivity required for our diagnostic test (1 oocyst or cyst, 5 eqG/reaction). Finally, the robustness tests were realized to certify the performance, both repeatability and reproducibility of the result. Moreover, the detection tests were optimized with the incorporation of an internal control. The internal control PCR ensures that the reaction was successful in all wells, and to demonstrate the presence or absence of inhibitors of PCR.

Significance: These new emerging pathogens necessite the need to develop accessible and reproducible methods to be able to detect these parasites. The methods developed by the Ceeram for *Cryptosporidium* sp., *Giardia* sp., *Toxoplasma gondii*, *Entamoeba histolytica and Naegleria fowleri* meet the needs of simplicity, speed, specificity and sensitivity.

P1-23 Performances Assessment of the 3M^{TM} Molecular Detection Assay E. coli O157 (Including H7) Kit According to the ISO 16140 Standard for Escherichia coli O157 Detection in Raw Beef Meats, Raw Dairy Products, Raw Fruits and Vegetables

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Introduction: The 3M™ Molecular Detection Assay *Escherichia coli* O157 (including H7) kit uses isothermal amplification of specific DNA target sequences. The amplification is detected by bioluminescence.

Purpose: An independent study was conducted at ADRIA, to validate this new method in comparison to the ISO 16654 standard, as part of the NF VALIDATION approval process and according to the ISO 16140 standard

Methods: The Molecular Detection Assay E. coli O157 (including H7) test protocol includes a single enrichment step in pre-warmed Buffered Peptone Water (ISO) at 41.5°C. After lysis, DNA amplification is performed in the Molecular Detection Instrument.

Results: 185 samples were analyzed for relative accuracy, sensitivity and specificity study. The results demonstrate equivalent performances between the Molecular Detection Assay *E. coli* O157 (including H7) and the ISO 16654 methods. Depending on the tested (matrix/strain) pairs, the relative detection limits of the Molecular Detection Assay *E. coli* O157 (including H7) method vary from 0.2 to 1.0 CFU/25 g, those of the ISO standard vary from 0.2 to 1.3 CFU/25 g. The selectivity and the specificity of the alternative method were assessed by tested 50 target strains and 31 non target strains. The Molecular Detection Assay *E. coli* O157 (including H7) method was also evaluated in a ring trial involving 12 laboratories. The results of the calculated accuracy, accordance, concordance and odds ratio clearly show that the Molecular Detection Assay *E. coli* O157 (including H7) method precision is equivalent to the ISO 16654 standard.

Significance: The alternative method is a reliable method for *Escherichia coli* O157 (including H7) detection in raw beef meats, raw dairy products, raw fruits and vegetables, and offers important economic savings by reducing time to result and handling time.

P1-24 Prevalence of Salmonella spp. and Escherichia coli in Some Raw and Ready-to-Eat Foods Marketed in Kirsehir, Turkey

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Introduction: *Salmonella* spp. and *Escherichia coli* are two major pathogens, which are transmitted to human via food consumption. *Salmonella* serotypes are leading bacteria that are responsible for food poisoning around the world. *E. coli* strains are microbiological quality indicators of foods and also have highly pathogenic serotypes.

Purpose: The aim of this work was to investigate the hygienic quality of raw and ready-to-eat foods marketed in Kirsehir province of Turkey. For this aim, the prevalence of *Salmonella* spp. and *E. coli* were analysed.

Methods: 120 food samples were collected from local markets in Kirsehir during July 2012-March 2013. The food groups were raw milk (51), local cheese (5), raw meat or offal product (21), raw poultry meat or giblet (23) and ready-to-eat food (20). *Salmonella* isolation was done by following Turkish standard TSE EN ISO 6579 and *E. coli* isolation was done by following TS 6063 ISO 7251. Isolates were stored in Nutrient broth containing 15% glycerol at -80^{o}C. They were analysed under a microscope by using Gram staining and motility test. API 20 E test kits were used for biochemical identification of the isolates.

Results: Among 120 food samples collected, 16 *Salmonella* and 52 *E. coli* were identified. Distribution of *Salmonella* and *E. coli* respectively to analysed food groups were 0/51 and 21/51 in raw milk, 0/5 and 2/5 in local cheese, 1/21 and 11/21 in raw meat and offal product, 13/23 and 14/23 in raw poultry meat and giblet, 2/20 and 4/20 in ready-to-eat food. The highest prevalence of both pathogens was observed in raw poultry meat and giblets.

Significance: The prevalence of *Salmonella* spp. was 13.3% and *E. coli* was 43.3% in raw and ready-to eat foods marketed in Kirsehir province of Turkey. High prevalence of *E. coli* indicated poor hygienic conditions of the foods analysed. This work was financially supported by Ahi Evran University Research Fund with project No: PYO-ZRT-4001.12.003.

P1-25 A Multiplex PCR Assay for Simultaneous Detection of Seven Foodborne Pathogens in Korean Fresh Cut Product

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Introduction: Fresh cut product is increasingly recognized as a source of foodborne outbreaks in worldwide.

Purpose: A multiplex PCR assay was developed for the simultaneous detection of seven food-borne pathogens including *Escherichia coli* O157:H7, *Bacillus cereus*, *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Yersinia enterocolitica*, and *Enterobacter sakazakii*in fresh cut product

Methods: Seven specific primer pairs for multiplex PCR were selected based on the intimin adherence protein (*eae*) gene of *E. coli* O157:H7, DNA gyrase subunit B (*gyrB*) gene of *B. cereus*, invasion protein regulator (*hilA*) gene of *Salmonella* spp., invasion associated secreted endopeptidase (*iap*) gene of *L. monocytogenes*, thermonuclease (*nuc*) gene of *S. aureus*, putative GTPase (*bipA*) gene of *Y. enterocolitica* and internal transcribed spacer (SI) of *E. sakazakii*.

Results: The specificity and sensitivity of each primer were evaluated in testing different strains. When this multiplex PCR assay was applied to evaluate the validity of detecting seven foodborne pathogens in artificially inoculated lettuce, the detection limit of this simultaneous assay varied in the range from 10^3 to 10^0CFU/g by each pathogen after enrichment in 37°C for 18hr.

Significance: These results indicate that the developed multiplex PCR assay is and effective and informative supplement for practical use.

P1-26 Predictive Modelling of Microbial Interactions in Cheese: A Trade-Off etween Mathematical Exactness znd Empirical Pragmatism

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Introduction: In the RASFF database (ec.europa.eu), in the last decade, 40 notifications of *Listeria monocytogenes* related to blue-veined cheese were recorded. This cheese is characterized by a dynamic ecosystem, with protease- and pH-mediated interactions between lactic acid bacteria (LAB) and *Penicillium roqueforti* that can make the environment suitable for *L. monocytogenes* growth.

Purpose: The aim of the study is to explore two different approaches to model the behavior of *L. monocytogenes* in the above environment and to compare the predictions with data generated by challenge tests.

Methods: The first approach that was used was a system of differential equations to describe the ecosystem. This is a relatively advanced mathematical technique, but it has a disadvantage in that it is not easy to identify the coefficients of the system. The second approach replaces some of the differential equations with empirical quadratic polynomials, coefficients of which are estimated on data. For validation experiments, during cheese making and aging, LAB, mould, inoculated *L. monocytogenes*log cell concentrations, pH and free amino acid, were measured. Biochemical rates were taken from literature and derived from measurements. The system of differential equations was solved numerically by a software written in Visual Basic, as was the parameter estimation problem, using the Least Squares method.

Results: The differential equations reflect a mechanistic description of the dynamic system, but the practical applicability is hindered by the lack of relevant data. Empirical quadratic response surfaces can serve as a means to provide a trade-off between mathematical exactness and pragmatism. However, the latter is unsuitable for even the slightest extrapolation and it is important to keep the dynamic models at hand for times when more data will become available.

Significance: This study can serve as an example for both developers and users, by which to model complex dynamic interactions and evaluate the model's performance from a practical view point.

P1-27 Sampling for Heterogeneously Distributed Aflatoxins in Maize Lots from Large Shipments

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Introduction: The presence of aflatoxin B_1 (AFB1) in maize based feed has caused elevated levels of aflatoxin M_1(AFM1) of milk in Western Europe, including in the Netherlands in 2013. The levels, up to 250 mg/kg, were not detected during routine controls. Recently, EC legislation on sampling procedures of alflatoxins in large shipments have been adjusted (Commission Regulation No. 691/2013).

Purpose: The aim of this study was to study the inhomogeneity of maize lots for underpinning of sample procedures. Analytical results of sampling a large shipment of contaminated maize were compared to concentrations reported in scientific literature.

Methods: A literature review was conducted to obtain insights into aflatoxin concentrations in maize batches, as reported from previous studies, as well as the variation in these concentrations, both within and between lots. In addition, a large shipment of maize was sampled to obtain insight into the inhomogeneity of aflatoxin contamination of the load. In total, 70 different subsamples were collected and analysed individually for aflatoxin concentration. The obtained insights into the concentration and variation within the lot were compared with the literature results.

Results: Aflatoxin concentrations varied widely among the 70 subsamples of the shipment. This variation found was to be in agreement with the results from literature. More details will be given during the presentation.

Significance: Based on the obtained results a renewed sampling procedure was proposed. This renewed sampling procedures allows better insight in the actual contamination of large shipments of maize with aflatoxins.

P1-28 Evaluation of Motility and Biofilm Formation of Listeria monocytogenes Strains Isolated from Food, Environment and Human Case

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Introduction: *Listeria monocytogenes* is a ubiquitous foodborne human pathogen. Food products are important vehicles for the transmission of human listeriosis. Many *Listeria monocytogenes* strains are potentially highly pathogenic, but others are less virulent or even avirulent.

Purpose: Currently, available molecular typing data cannot evaluate the ability of *Listeria monocytogenes* strains to cause disease. Furthermore, there are no clear data about the role played by flagellar motility in biofilm formation and if these could be indicators of pathogenicity.

Methods: A total of 11 *Listeria monocytogenes* strains recovered from food (7), human clinical case (1) and environment (3), selected by serotype and PFGE profile, were examined for DNA sequencing, evaluation of motility on semi solid BHI agar (0,3%) and biofilm formation at 12°C and 37°C on steel, using cristal violet staining and scanning electronic microscopy. Strains optical densities at 492nm were compared through ANOVA, followed by Turkey's multiple comparison test ($P < 0,05$).

Results: Tested isolates, with the same serotype and similar PFGE profile, exhibited also similar patterns of the genes known to be involved in biofilm formation, motility and virulence. However, they showed different behavior in biofilm production and motility. The difference among strains in biofilm formation at 37°C and motility resulted statistically significant. The highest biofilm production was observed in food isolates, the lowest in the human isolate. On the other hand, the human strain showed the highest motility.

Significance: Our results did not report the presence of significant relationship between biofilm production ability and pathogenicity in humans, while this seems to be more strictly related to strain motility. Molecular similarity of strains did not result in the same behavior in motility and biofilm formation. This last aspect would be more representative of harbor capability and not for its pathogenic attitude. A larger set of genes and expression studies need to be addressed to clarify this issue.

P1-29 Modelling the Growth of Alternaria spp. on Tomatoes Used for Derived Tomato Products as Function of Temperature and Fungicide Concentration

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Introduction: A previous study showed the presence of alternariol and alternariol monomethylether (produced by *Alternaria* spp.) in molded tomatoes and derived tomato products, which can have serious health effects on consumers.

Purpose: The objective of the study was to fill the gaps in the knowledge on the parameters (i.e. temperature and fungicide concentration (Cu)) affecting the growth of *Alternaria* spp. on tomatoes used for derived tomato products.

Methods: Five *Alternaria* spp. strains were isolated from tomatoes and experiments were first performed on a simulation medium and later validated on tomatoes used for further processing. Growth rate was obtained by measuring the diameter of the molds, lagphase was evaluated by counting the total amount of germinated spores. Lagphase was considered when 90% of the spores were germinated.

Results: For the maximum growth rate different models were evaluated on their performance (RMSE, SEP, R^2 and graphical comparison with observed and fitted data) and the most suitable model for μ_{max} was the polynomial model with RMSE 0.0009-0.0022, SEP 6.2-29.5% and R^2 0.85-0.997. Fungicide concentration had no significant effect on the growth and was excluded from the model. Validation of the model on tomatoes resulted in a bias factor of 0.99-1.04 and accuracy factor of 1.23-1.4 indicating that the models were good predictors of the true mean colony growth diameter. Optimal growth was obtained at 22.6-32 °C at a μ_{max} of 0.206-0.50 mm/h. The longest lagphase was obtained at 5°C (29h – 107h) and the shortest at 23-30 °C (1.8h-12h).

Significance: The results can be used to predict *Alternaria* spp. growth on tomatoes used for further processing, and also to estimate the effect of climate change on mold growth and mycotoxin production. Application of the models is possible to develop control strategies against these important molds on tomatoes used for further processing.

P1-30 In-depth Proteomic Analysis of the Excretory/Secretory Antigen of Trichinella Spiralis

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Introduction: Trichinellosis is a zoonotic disease caused by parasites of the genus *Trichinella*. Raw or undercooked pork is the most common source of infection in humans. The EU reference method for detection of *Trichinella* larvae is based on microscopy of digested meat. However, serological methods (e.g., ELISAs) might be considered appropriate for pig production systems with negligible risk for *Trichinella* infection. Currently, most assays are based on detecting anti *Trichinella* IgG antibodies in the sample with the so-called "excretory/secretory antigen" (ESA). This antigen is a complex mixture of proteins which larvae release into the host, and its preparation is cumbersome and difficult to reproduce. Appropriately selected and defined antigens could allow equivalence of results and could enhance reliability of measurements, and are a prerequisite for standardization and developing quality assurance tools such as reference materials.

Purpose: The aim of the study was the in-depth proteomic characterisation of the *Trichinella spiralis* ESA.

Methods: Three ESA preparations from two different providers were analysed after in-solution digestion of proteins by label-free 2D-nano-UPLC-ESI-MS/MS on a Waters Synapt^{TM} HDMS^{TM} G2 system, combining high efficiency ion mobility spectrometry (IMS) with time-of-flight mass spectrometry (TOF-MS). Spiking of the peptide mixture with commercial internal standards provided for relative quantification of the peptides. Statistical analysis of the data was performed using the PLGS Mass-Informatics^{TM}platform and the UNIPROT *Trichinella* data base.

Results: More than 250 proteins (e.g., proteases, nucleases) could be unambiguously identified and quantified relative to the internal standard. The three preparations were comparable in terms of proteins identified; however, some differences were encountered in terms of relative protein abundances.

Significance: The results of the study constitute an important prerequisite for activities towards standardization of serological testing of *Trichinella* in pigs, e.g., development of reference materials for method validation and method performance verification.

P1-31 Comparison of Pathogenic Genes Occurrence in Campylobacter spp. isolated from Food and Environmental Samples

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Introduction: *Campylobacter* are recognized as a leading bacterial cause of food-related gastroenteritis in developed countries. Contaminated poultry meat is considered to be significant source of human campylobacteriosis. Other risk factor for infection include drinking contaminated water or contact with carriers animals.

Purpose: The aim of this study was to compare the occurrence of *Campylobacter* spp. pathogenic genes responsible for encoding virulence factors such as: the ability to move, adhesion to epithelium, toxins production and invasiveness in *Campylobacter* spp. isolated from food and environment.

Methods: The materials to investigate were 65 *Campylobacter* spp. strains isolated from poultry meat (34 *C. jejuni*, 31 *C. coli)* obtained in supermarkets in Bydgoszcz (Poland), 26 *Campylobacter* spp. strains obtained from domestic animals (18 *C. jejuni*, 8 *C. coli*) and 44 *Campylobacter* spp. (19 *C. jejuni*, 25 *C. coli*) isolated from surface water samples. The presence of the *flaA*, *cadF*, *cdtB* genes and *iam* sequence was determined with the PCR method with specific primers.

Results: All of the *Campylobacter* spp. isolates carried the *cadF* and *flaA* genes. The *cdtB* gene associated with toxin production was present in over 90% of *Campylobacter* strains. The *iam* gene occurrence was the highest in *Campylobacter* spp. strains isolated from chicken (94,1%). Lower prevalence of this gene was observed in *Campylobacter* strains from water samples (54%) and animal's isolates (66%).

Significance: High prevalence of *Campylobacter* virulence genes indicates an important role of these genes in pathogenesis process. Food (poultry meat) and environment (water, domestic animals) are source of invasive and pathogenic *C. jejuni* and *C. coli* strains and could be a reason for campylobacteriosis in humans.

Study was financed by the National Science Center (grant NN 404272540).

P1-32 Performances Assessment of the 3M™ Molecular Detection Assay* Salmonella *Kit According to the ISO 16140 Standard for* Salmonella *spp. Detection in Food Products and Environmental Samples

Justine Baguet, Muriel Bernard, Cecile Bernez, Claudie Le Doeuff, Sarah Peron, Maryse Rannou and **Daniele Sohier**, ADRIA Développement, Quimper, France

Introduction: The 3M™Molecular Detection Assay *Salmonella* kit uses isothermal amplification of specific DNA target sequences. The amplified sequence detected by bioluminescence.

Purpose: An independent study was conducted at ADRIA, to validate this new method in comparison to the ISO 6579 standard, as part of the NF Validation approval process and according to the ISO 16140 standard.

Methods: The *Salmonella* test protocol includes a single enrichment step in Buffered Peptone Water. Two incubation temperatures are available depending on the tested food categories (37°C and 41.5°C). After lysis, DNA amplification is performed in the Molecular Detection Instrument.

Results: 325 food and environmental samples were analyzed for relative accuracy, sensitivity and specificity study. The results demonstrate equivalent performances between the Molecular Detection Assay *Salmonella* and the ISO 6579 methods. Depending on the tested (matrix/strain) pairs, the relative detection limits of the Molecular Detection Assay *Salmonella* method vary from 0.3 to 2.0 CFU/25 g, those of the ISO standard vary from 0.3 to 1.8 CFU/25 g. The selectivity and the specificity of the alternative method were assessed by tested 50 target strains and 30 non target strains. The Molecular Detection Assay *Salmonella* method was also evaluated in a ring trial involving 15 laboratories. The results of the calculated accuracy, accordance, concordance and odds ratio clearly show that the Molecular Detection Assay *Salmonella* method precision is equivalent to the ISO 6579 standard one.

Significance: The alternative method is a reliable method for *Salmonella* spp. detection in food and environmental samples, and offers important economic savings by reducing time to result and handling time.

P1-33 Autochtonous Hepatitis E Cases: Where Does the Virus Come From?

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Introduction: In recent years, data indicate a high prevalence of hepatitis E virus (HEV) infections. A potential source of contamination is the consumption of porcine products or food contaminated by an environmental source.

Purpose: As suggested by the CDC and EFSA, the objective of the study was to evaluate the origin of HEV, not only evaluating pork products.

Methods: A method for HEV detection in environmental or food samples was set up. The kit hepatitisE@ceeramTools was used for real time RT-PCR detection. The kit was validated on 440 food samples collected worldwide in food companies in 2011, including pork liver sausages, shellfish, fruits, vegetables, herbs and spices, process water and ready-to-eat food. A study was also conducted on pig manure (100 samples) collected from 5 pig breedings positive for HEV to evaluate the persistence of HEV after manure treatments.

Results: The specificity of the kit was validated for the 4 different genotypes leading to a sensitivity of 5 copies/reaction. On food or environmental samples, a limit of quantification of the method of 500 genome copies was obtained whatever the samples. Below this limit, a sample is considered positive but not quantifiable with reliability. The results obtained for HEV demonstrate a prevalence of 0.9% in food samples. Concerning treated pig manure, 12% were positive with low level of contamination (£1000 genome copies/g of manure).

Significance: Our results demonstrate a prevalence for HEV in food samples, in the same range than HAV. The spreading of pig manure does not appear to be an agricultural practice at risk for HEV. All these results demonstrate that except pork liver products, other types of food do not seem to be a potential source of contamination. Such study could be helpful to explain the increase of human hepatitis E cases and better prevent HEV autochthonous cases.

P1-34 ISO 16140 Certification of a New Alternative to Detect Cronobacter spp. in Infant Formula and Environmental Samples

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Introduction: The iQ-Check™ *Cronobacter* method is based on the real PCR principle for *Cronobacter* spp. detection in infant formula and environmental samples. An ISO 16140 method comparison study was conducted, by analyzing 171 samples in the relative accuracy, sensitivity and specificity part and showing equivalent performances between the alternative method and the ISO/TS 22964 methods. Depending on the tested (matrix/strain) pairs, the relative detection limits of the Real-Time PCR method spp. method vary from 0.5 and 1.6 CFU/25g, those of the ISO standard from 0.5 to 1.5 CFU/25g. The selectivity and specificity of the alternative method was assessed by testing 52 target strains and 31 non target strains.

Purpose: An independent inter-laboratory study was conducted at ADRIA, to compare the alternative method precision to the ISO/TS 22964 one, as part of the NF Validation approval process and according to the ISO 16140 standard.

Methods: The new *Cronobacter* spp. protocol includes an overnight enrichment in BPW supplemented with vancomycin. An additional sub-culture is done in BPW for 4h ± 1h for infant formula analysis. After the DNA extraction step, the Real-Time PCR is run with a Bio-Rad automate. The presumptive positive results are confirmed by direct streaking onto RAPID'Sakazakii Agar for infant formula, and after a subculture in mLST prior to streaking for environmental samples.

Results: The alternative method was evaluated in a ring trial involving 13 laboratories. Probiotic infant formula was contaminated with the wild *C. sakazakii* Ad 940 strain. 8 blank samples, 8 samples contaminated at a fractional recovery level (0.8 cells/g) and 8 highly contaminated samples (20.6 cells/g) were sent to each collaborator. At the fractional recovery and high inoculation levels, the sensitivity values of the standard method were respectively 52% and 100%, those of the alternative method 55% and 99%.

Significance: This ISO 16140 study clearly shows that the new *Cronobacter* method is a reliable alternative method for *Cronobacter* spp. detection in infant formula and environmental samples, offering important economic savings by reducing time to result and handling time.

P1-35 Performance Assessment of the Thermo Scientific™ SureTec™ Listeria monocytogenes Real-Time PCR Assay - according to the ISO 16140 Standard for Listeria monocytogenes Detection in Food and Environmental Samples

Justine Baguet, Muriel Bernard, Cecile Bernez, Claudie Le Doeuff, Sarah Peron, Maryse Rannou, Melanie Streit and **Daniele Sohier**, ADRIA Développement, Quimper, France

Introduction: The Thermo Scientific™ SureTect™ *Listeria monocytogenes* Real-Time PCR Assay is a new detection method based on real-time PCR. The oligonucleotides target unique DNA sequences found only in the target micro-organism and use PCR technology to amplify and detect them. If present, the target DNA is amplified and the increasing fluorescent signal is detected by the PikoReal Real-Time PCR instrument and interpreted by the SureTect Software.

Purpose: An independent study was conducted at ADRIA, to validate this new method in comparison to the ISO 11290-1 standard, as part of the NF Validation approval process and according to the ISO 16140 standard.

Methods: The alternative method includes a single step enrichment in 24 LEB supplemented with both 24 LEB Buffer and selective supplements for 22-26 h at 37°C. After DNA extraction, PCR is run peformed using the PikoReal instrument

Results: 339 food and environmental samples were analyzed for relative accuracy, sensitivity and specificity. The results demonstrate equivalent performance between the alternative and ISO 11290-1 methods. Depending on the tested (matrix/strain) pairs, the relative detection limits of the alternative method vary from 0.2 to 1.2 CFU/25 g, those of the ISO standard vary from 0.2 to 1.1 CFU/25 g. The selectivity and the specificity of the alternative method were assessed by testing 50 target strains and 30 non target strains. The alternative method was also evaluated in a ring trial involving 10 laboratories. The results of the calculated accuracy, accordance, concordance and odds ratio clearly show that the alternative method precision is equivalent to the ISO 11290-1 reference method.

Significance: The SureTect *Listeria monocytogenes* Real-Time PCR Assay is a reliable method for *Listeria monocytogenes* detection in food and environmental samples, and offers important economic savings by reducing time to result and handling time.

P1-36 Performance Assessment of the Thermo Scientific™ SureTec™ Listeria species Real-Time PCR Assay according to the ISO 16140 Standard for Listeria spp. Detection in Dairy Products, Seafood, Vegetables and Environmental Samples

Justine Baguet, Muriel Bernard, Cecile Bernez, Claudie Le Doeuff, Sarah Peron, Maryse Rannou, Melanie Streit and **Daniele Sohier**, ADRIA Développement, Quimper, France

Introduction: The Thermo Scientific™ SureTect™ Listeria species Real-Time PCR Assay is a new detection method based on real-time PCR. The oligonucleotides target unique DNA sequences found only in the target micro-organism and use PCR technology to amplify and detect them. If present, the target DNA is amplified and the increasing fluorescent signal is detected by the Thermo Scientific PikoReal Real-Time PCR instrument and interpreted by the Thermo Scientific SureTect Software.

Purpose: An independent study was conducted at ADRIA, to validate this new method in comparison to the ISO 11290-1 standard, as part of the NF Validation approval process and according to the ISO 16140 standard.

Methods: The alternative method includes a single enrichment step in 24 LEB supplemented with both 24 LEB Buffer and selective supplements for 22-26 h at 37°C. After DNA extraction, PCR is run on the PikoReal instrument.

Results: 259 food and environmental samples were analyzed to determine the relative accuracy, sensitivity and specificity of the assay. The results demonstrate equivalent performance between the alternative method and the ISO 11290-1 method. Depending on the tested (matrix/strain) pairs, the relative detection limits of the alternative method vary from 0.3 to 1.5 CFU/25 g, those of the ISO standard vary from 0.3 to 1.3 CFU/25 g. The selectivity and the specificity of the alternative method were assessed by testing 52 target strains and 30 non target strains. The alternative method was also evaluated in a ring trial involving 10 laboratories. The results of the calculated accuracy, accordance, concordance and odds ratio clearly show that the alternative method precision is equivalent to the ISO 11290-1 standard.

Significance: The Real-Time PCR Assay is a reliable method for *Listeria spp.* detection in dairy products, seafood, vegetables and environmental samples, and offers important economic savings by reducing time to result and handling time.

P1-37 Performance Assessment of the Thermo Scientific™ SureTec™ Salmonella species Real-Time PCR Assay according to the ISO 16140 Standard for Salmonella spp. Detection in Food and Pet Food

Justine Baguet, Muriel Bernard, Cecile Bernez, Claudie Le Doeuff, Sarah Peron, Maryse Rannou, Melanie Streit and **Daniele Sohier**, ADRIA Développement, Quimper, France

Introduction: The Thermo Scientific™ SureTect™ *Salmonella* species Real-Time PCR Assay is a new detection method based on real-time PCR. The oligonucleotides target unique DNA sequences found only in the target micro-organism and use PCR technology to amplify and detect them. If present, the target DNA is amplified and the increasing fluorescent signal is detected by the Thermo Scientific PikoReal Real-Time PCR instrument and interpreted by the Thermo Scientific SureTect Software.

Purpose: An independent study was conducted at ADRIA, to validate this new method in comparison to the ISO 6579 standard, as part of the NF Validation approval process and according to the ISO 16140 standard.

Methods: The alternative method includes a single enrichment step. Different protocols are available depending on the tested food matrices. After DNA extraction, PCR is run on the PikoReal instrument.

Results: 442 samples were analyzed to determine the relative accuracy, sensitivity and specificity of the alternative method. The results demonstrate equivalent performance between the alternative method and ISO 6579. Depending on the tested (matrix/strain) pairs, the relative detection limits of the alternative method vary from 0.2 to 1.5 CFU/25 g, those of the ISO standard vary from 0.2 to 1.1 CFU/25 g. The selectivity and the specificity of the alternative method were assessed by testing 51 target strains and 30 non-target strains. The alternative method was also evaluated in a ring trial involving 10 laboratories. The results of the calculated accuracy, accordance, concordance and odds ratio clearly show that the alternative method precision is equivalent to ISO 6579.

Significance: The *Salmonella* species Real-Time PCR Assay is a reliable method for *Salmonella spp.* detection in food and pet food, and offers important economic savings by reducing time to result and handling time.

P1-38 Spore-forming Bacteria: A Characterized Collection of Industrial Strains for Taylor-made Food Testing

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Introduction: Aerobic and anaerobic Gram positive sporeformers exhibit a wide range of phenotypic and genotypic characteristics. These organisms are ubiquitous in the environment and have the ability to form endospores which enable them to survive heat treatments and sanitation, thus yielding to their persistence in food industries. Moreover the presence of spores in food may be associated to characteristic spoilage activity when bacterial germination and outgrowth is possible.

Purpose: Based on sporeformer biodiversity and occurrence in food industries, industrial strains from ADRIA's collection were selected for further characterization to group, trace and easily select strain of interest for specific and taylor made trials.

Methods: Industrial isolates were collected from raw ingredients, industrial environments and processed foodstuff and identified by 16SrDNA sequencing. A selection of 100 strains of more than 30 species of sporeforming bacilli, *i.e., Anoxybacillus, Aneurinibacillus, Bacillus, Brevibacillus, Clostridium, Geobacillus, Lysinibacillus, Paenibacillus, Ureibacillus*etc… are currently tested for industrial relevant features: -molecular typing: MLST, RAPD-PCR, Rep-PCR, PFGE fingerprints for isolate clustering or traceability -growth ability: minimal and maximal temperature and pH for growth, ability to grow in presence of salt -enzymatic activity and metabolite synthesis, -adhesion ability: microplates or on stainless steel coupons, -resistance parameters: heat or biocide resistance.

Results: For the wide range of samples tested, spore-forming bacteria contaminations were mainly associated to raw materials, deshydrated ingredients, RTE and surfaces. A large diversity of genus and species was recovered in contaminated products with a high prevalence of thermophilic sporeformers in ingredients and treated food. A set of screening methods is now available and even though data analysis is only partial, significant contribution is already possible for industrials, technical institute and research laboratory involved. Indeed for specific application, it is crucial to challenge real life microflora in order to highlight behavior of « super spoiler microorganisms ».

Significance: Within the frame of SPORE RISK network, a characterized collection of spore forming bacteria and tools are available to help detect, identify and trace spore-forming bacteria contamination along industrial production lines but also to help the selection of relevant strains for food testing.

P1-39 Using Chlorine Dioxide to Degrade Pesticide Residues in Fresh Vegetables and Fruits

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Introduction: Taiwan's warm and humid subtropical climate is suitable for the production of a wide variety of agricultural products; however, it also creates conditions favorable to the spread of plant disease and pests. Application of chemical pesticides has been adopted as an economical, rapid and effective means to deal with these problems, yet with the increase in use of

pesticides, many serious problems have appeared. Issues of food safety and environmental pollution, for instance, have arisen due to unapproved or excessive pesticides and residues.

Purpose: Using self-developed high purity chlorine dioxide to degrade pesticide residues in fresh vegetables and fruits

Methods: Six common pesticides, including Acetamiprid, Carbofuran, Dimethomorph, Ethion, Methomyl and Pencycuron, were selected as test agents. Also, electrolytic chlorine dioxide, commercial chlorine dioxide tablets (non electrolytic product) and sodium hypochlorite were used to investigate pesticide residues degradation levels. Sweet pepper, string bean, papaya and plum tomato were selected to serve as samples and were sprayed with the above pesticides under three different concentrations (100 mg/ L, 200 mg/ L and 500 mg/ L). After dipping, magnetic stirring and sonication methods were applied for 5, 10, 15, 20 and 30 minutes, HPLC and LC/MS was used to detect the degradation of pesticide residues.

Results: Results indicate that electrolytic chlorine dioxide (99.7% purity), when applied with dipping or stirring methods, could degrade Dimethomorph, Ethion and Methomyl by 96%, 99%, and 100% respectively. The results also show that removal of pesticide residues with electrolytic chlorine dioxide can be more effective than commercial chlorine dioxide tablets and sodium hypochlorite. Applying the sonication method to Carbofuran and Pencycuron was more effective than the other two methods and was able to reach 100% degradation.

Significance: This study validates the application of self-developed high purity electrolytic chlorine dioxide treatment as a safe and promising method for the degradation of pesticides when washing fresh vegetables and fruits.

P1-40 Rapid Detection and Identification of Pathogens by NIR Spectroscopy and Advanced Multivariate Statistics

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Introduction: The rapid detection and identification of pathogens play an important role in food production process. In the study, the implementation of NIR spectrometry as cheap, fast and sufficiently accurate technique for bacterial identification is described.

Purpose: Bacteria strains can be identified based on different cell chemical compositions. Differences in the proportion of macromolecules in living cells (nucleic acids, proteins, polysaccharides, lipids) have impact to NIR spectra. Because interpreting of NIR spectra is difficult, multivariate statistical methods are utilized.

Methods: Three bacterial strains (*Listeria ivanovii* CECT 913, *Escherichia coli* O157:H7 MB3885 pGFP, *Salmonella enterica* sv Thompson GFP STN pGT-Kan mB156) were grown overnight in tryptic soy broth with shaking at 37^{o}C. To remove of effect of culture medium, samples were centrifuged and resuspended in distilled water. Initial study included measuring of bacterial strains fixed and dehydrated on a glass fiber filter, allowing examination of pure spectra from bacteria. Raw spectra were treated by advanced pre-processing methods (extended multiplicative scatter correction) and models were created by canonical correlation and cluster analysis techniques. Accuracy of models was performed by CCR value (correct classification rate) computed by leave-one-label-out cross-validation technique. Effect of water and different type of membrane (nitrate of cellulos) was also examined.

Results: Results reveal that the proposed NIR-based method is able to detect and discriminate different food-borne pathogens. In addition, selected statistical procedures and models could drastically decrease CCR (from 99 to 60%). In the case of high concentrated pure samples, CCR reaches to 99%, with lower concentration, CCR is decreasing.

Significance: Results suggest the possibility of using NIR technology for rapid detection and identification of bacterial contamination in foods. It could lead to improve efficiency of existing traditional microbiological methods applied in food industry.

P1-41 Insight into the Level of Fumonisins in Serbian Maize Food Products

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Introduction: Fumonisins are a group of structurally related mycotoxins primarily produced by *Fusarium verticillioides* and *Fusarium Proliferatum*. Fumonisin B1 (FB1) and B2 (FB2) are the most abundant and often found as contaminants of maize products. FB1 is the most toxic and is related to an inhibition of sphingolipid synthesis and increased risk of oesophageal cancer.

FB1 is classified as possible human carcinogen. Provisional maximum tolerable daily intake of 2mg/kg bw/day was established for FB1, FB2 and FB3, independently or combined.

Purpose: Main objective of this study was preliminary insight into fumonisins contamination of maize products for direct human consumption, produced and marketed in Serbia in 2013. In total 10 samples, purchased as would be done by a consumer, included maize flour, grits and polenta of 5 different producers.

Methods: Analysis comprised of extraction, clean-up on immuno-affinity columns and quantification of fumonisins by HPLC with fluorescence detection using *o*-phthalaldehyde precolumn derivatization. Study of laboratory method performance preceded real sample analysis (limit of quantification 20ng/g and 15ng/g, recovery 94% and 72%, PT Fapas (maize flour) *z* scores -0.8 and 0.1, for FB1 and FB2, respectively).

Results: The results have shown a widespread presence of fumonisins in maize products, with 90% of tested samples being positive. Overall mean level of total fumonisins was 168ng/g, with maximum at 388ng/g. Maize flour had the highest level of contamination, with FB1 levels ranging from 184ng/g to 316ng/g, while FB2 was found in amounts from 42ng/g to 72ng/g. Only one polenta sample was negative, but not one sample exceeded the regulatory limit of 1000ng/g, established for the sum of FB1 and FB2.

Significance: These results should contribute to the assessment of exposure of the local population to fumonisins, as data for the occurrence of these compounds in foodstuffs marketed in Serbia are still limited and irregular.

P1-42 Analysis of Aflatoxins in Maize-based Foodstuffs in Serbia

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Introduction: Among mycotoxins, aflatoxins (AFs) are the most toxic and carcinogenic class, mainly produced as secondary metabolites of fungi *Aspergillus flavus* and *Aspergillus parasiticus*. The most toxic aflatoxin, AFB1, is an extremely potent carcinogen (IARC group 1). With the objective of a safe food supply, maximum levels of aflatoxins in maize foodstuffs have been established at 4ng/g for sum of AFs, and 2ng/g for AFB1.

Purpose: This study was undertaken to investigate aflatoxin presence in maize-based foodstuffs commercialised in Serbia at the end of 2013. A total of 20 samples were divided into two equal groups, one comprised of maize flour, grits and polenta, and second with maize-based snacks, representing 12 Serbian producers. The content of one packet, as purchased in supermarket, constituted a sample.

Methods: Samples were subjected to extraction and clean-up on immunoaffinity columns, followed by analytical determination using HPLC with fluorescence detection. The limit of quantification was established at 0.1ng/g for AFB1, 0.05ng/g for AFB2, and 0.5ng/g for AFG1 and AFG2. Application of the method on Proficiency test sample (Fapas, infant food, AFB1 0.12ng/g) resulted with zscore -0.7.

Results: Results of the study revealed the presence of aflatoxin B1 in 50% of samples in the first group, and 30% in second group. Level of AFB1 contamination was in compliance with regulatory criterion, ranging from 0.14ng/g to 2.0ng/g (mean at 0.61ng/g), and from 0.1ng/g to 0.24ng/g (mean at 0.16ng/g), in two groups respectively. Aflatoxin B2 was found only in one flour sample in quantity of 0.1ng/g, while AFG1 and AFG2 could not be quantified in any of the samples.

Significance: Reports of very high aflatoxin content in Serbian maize in 2012 and 2013, caused a lot of concern regarding human health. Hence, a survey on occurrence of these mycotoxins in foodstuffs could contribute to the assessment of margin of human exposure.

P1-43 Rapid Method for Detection of Salmonella spp. and Listeria spp. in Environmental Samples Using a Single Automated Workflow

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Introduction: During the past years, the food industry has been presented with increasing food safety concerns and a resulting demand for rapid, accurate, and easy-to-use pathogen detection systems for implementation as part of HAACP surveillance

programs. Increasingly, real-time PCR plays an important role in such rapid detection systems. We have developed and validated an automated workflow for pathogen DNA purification and real time PCR detection in environmental samples.

Purpose: The purpose of these studies was to examine the capabilities of the one-for-all automated QIAsymphony workflow for pathogen detection in environmental surface samples as part of an AOAC PTM^{TM}validation.

Methods: The ability of the protocol to detect *Salmonella* spp. or *Listeria* spp. from environmental surfaces was determined by inoculating stainless steel, ceramic tile, plastic or sealed concrete surfaces with the pathogen of interest, followed by swabbing the surface with swabs or sponges, incubating in BPW (*Salmonella*) for 18 + 2 h at 37+ 1^{o}C or in ONE broth plus supplement (*Listeria* spp) for 22 + 2 h at 30 + 1^{o}C . DNA was extracted from all cultures using a one-for all pathogen DNA protocol on a fully automated QIAsymphony platform. The resultant DNA was tested for the presence of pathogen with *mericon^{TM}* pathogen detection assays using the RotorGeneQ real-time PCR system. All results were compared to the ISO 6579 *Salmonella* spp or USDA/FSIS-MLG 8.08 *Listeria* spp. reference methods.

Results: Results obtained with the automated workflow were found to be no different from those obtained with the reference methods (POD statistical method), but time to results was shortened from 3 – 4 days to approximately 24 hours.

Significance: This new method using a one-for-all pathogen purification and detection workflow is an efficient and reliable alternative to the traditional reference methods of detecting *Salmonella* or *Listeria* spp. from environmental surfaces.

P1-44 Effect of Disinfection Technologies on Escherichia coli, Staphylococcus aureus, Salmonella enteritidis and Listeria innocua Inoculated on Lettuce, Strawberries and Cherry Tomatoes during a Refrigerated Storage Period

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Introduction: Several foodborne outbreaks have been reported due to the presence of pathogenic bacteria in fresh produce. *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp.* and *Listeria spp*. are the most common pathogens detected in vegetables and fruits. Disinfection remains one of the most important critical points along the processing line, in order to ensure the safety and quality of freshly cut vegetables and fruits for a defined period of shelf life.

Purpose: The purpose of the present study was to evaluate the effectiveness of different disinfection methods for their capacity to disinfect fresh, ready-to-eat products. The main goal was to study the effect of disinfection technologies during storage of the tested foods at 6°C for 15 days.

Methods: Four bacterial strains (*Escherichia coli* NCTC 9001, *Staphylococcus aureus* NCTC 6571, *Salmonella enteritidis* NCTC 6676 and *Listeria innocua* NCTC 11288) were inoculated on commercially available lettuce, strawberries and cherry tomatoes. Then, the foods were treated with 7 different disinfection methods: UV, Ultrasound, Sodium Hypochlorite solutions and combined technologies, such as UV followed by Ultrasound, UV followed by Sodium Hypochlorite and Ultrasound followed by Sodium Hypochlorite. Then the foods were stored during a 15-day period at 6°C.

Results: Populations of all the microorganisms in lettuce and strawberries products decreased from day 0 to day 3 of storage, whereas they increased from day 3 to day 15 of storage. Populations on cherry tomatoes exhibited an increase throughout the total storage period. The combined method of Ultrasound followed by Sodium Hypochlorite exhibited better results as far as microorganisms is concerned, compared to all other treatments ($P < 0.05$).

Significance: Storage conditions play a crucial role in commercial practice and do not prevent the growth or survival of pathogens on lettuce, strawberries and cherry tomatoes. These observations emphasize the importance of implementation of strict disinfection technologies during food processing.

P1-45 Detection of Salmonella in Feedstuffs and Environmental Samples Using an Integrated Automated Workflow

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Introduction: Food safety concerns and the demand for rapid, accurate and easy-to-use pathogen detection systems have resulted in increasing use of real-time PCR in the food industry. The automated *mericon*™ *Salmonella* spp. method combines a fully automated sample preparation method with real-time PCR detection on the Rotor-Gene Q platform.

Purpose: The purpose of these evaluations was to extend the ISO 16140 comparison of the fully integrated automated *Salmonella* spp. workflow to include environmental samples and animal feed.

Methods: The first study examined the relative accuracy of detection of *Salmonella* in 60 animal feed samples and 61 environmental samples. There were at least 30 positive and 30 negative samples in each group for a total of 121 samples analyzed. A relative detection level study was performed on one sample type from each group (cat food [feed products] and process water [environmental samples]). Each matrix was inoculated with a different serotype of *Salmonella* at four to five levels (from 0.2 to 5.4 CFU/25g) and an uninoculated control. For all studies, 25 g food samples were incubated in BPW for 18 +/- 2 h at 37 +/- 1°C. DNA was extracted from the enriched cultures using the automated *mericon* DNA extraction kits, analyzed by the RotorGene Q real-time PCR system and compared to the ISO 6579:2002 reference method.

Results: The method comparison demonstrated no significant differences in the number of positive samples detected between the integrated automated *mericon* method and the ISO 6579:2002 method for all samples studied.

Significance: This new method is an efficient and reliable alternative to the traditional reference methods of detecting *Salmonella* spp. in a variety of animal feed and environmental samples.

P1-46 The Influence of Ozonization and UV Irradiation on the Generation of Stable Organic Radicals in Grains and Flour Products

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Introduction: The use of ozone and UV irradiation as an anti-microbial agent for food treatment is considered as one of the most effective methods of ensuring the safety of food products. On the other hand, in these conditions, the generation of reactive oxygen species (ROS) can take place, which would lead to destruction of cell structures.

Purpose: The aim of the work was to investigate the influence of ozonization and UV irradiation on the food components and the possibility of radical formation during these treatments.

Methods: The EPR technique was applied to investigate the changes occurring in wheat and barley grains, as well as in wheat starch upon UV irradiation and interaction with ozone.

Results: Native starch is a diamagnetic material, inactive in EPR, grains and their parts exhibited EPR complex spectra with signals originating from transition metal ions such as Fe(III), Mn(II), Cu(II) and from stable organic radicals: semiquinone, phenoxyl and carbohydrate. The character and amounts of these species depended on the kind of grain (wheat or barley) and on the part of seed. The highest content of paramagnetic centers exhibited seed coats, the lowest one was found in endosperm. The main radical signal in the spectra of seed coats was attributed to semiquinone radicals, whereas phenoxyl and carbohydrate radical species were found mostly in embryos and endosperm, respectively. Upon ozonization and UV irradiation the amount of all radical species increased (about two times), whereas the signal intensities of transition metal ions changed only slightly. The carbohydrate radicals generated in starch material were similar to those formed during thermal treatment.

Significance: Our observations strongly suggest that reactive oxygen species formed upon ozone or UV treatment can damage the chemical structure of starch and grains and therefore are not neutral for food products.

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P1-47 An Evaluation of the Effectiveness of Water ATP Test Devices for Inter-Operator Repeatability

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Introduction: Water ATP testing is important in several food and drink processes. It helps to monitor the quality of water used for food processing, evaluate the effectiveness of Clean-in-Place (CIP) rinses and control fermentation processes. It is therefore important that the water ATP test device used is suitable for its intended purpose.

Purpose: To evaluate the performance of seven brands of ATP test devices for inter-operator repeatability.

Methods: The performance of the ATP test devices was evaluated by ten operators at 5 x 10^{-9} M ATP and 5 x 10^{-11} M concentrations. Testing was completed using the exact instructions provided by the device manufacturer. The Coefficient of Variation (%CV) of the results was used to categorise the devices into 'good' and 'bad' categories.

Results: 350 results at each ATP concentration were obtained to calculate the %CV for all devices. Using a 35%CV the devices were categorised into distinct groups of 'good' and 'bad'. Out of the seven devices evaluated four were categorised as 'good' for the 5 x 10^{-9} M ATP concentration and three were categorised as 'good' for the 5 x 10^{-11} M concentration.

Significance: The selection of a water ATP test device with a low %CV is critical to ensure the accuracy, reliability and repeatability of results.

P1-48 Reduction of Positive Rate of Non-O157 STEC in Raw Milk Using an Innovative Automated Sample Prep

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Introduction: Detection of non-O157 STEC in raw milk may lead to high positive rate. This is due to the co-amplification of virulent associated genes (*stx* and *eae*) and top6 O-groups carried by different strains present in the milk sample. The association of the VIDAS (ESPT, Phage Technology) for the capture of O-groups of interest upstream the PCR allows a better specificity for the detection of non-O157 STEC.

Purpose: Compare the VIDAS ESPT+PCR method to a commercial PCR for the detection of non-O157 STEC in raw milk.

Methods: 301 raw milk samples from different farms were analyzed. 25g were diluted 1/10 in BPW+Acriflavine (10mg/L), homogenized, and incubated 18h at 37°C. DNA was extracted using VIDAS ESPT1 protocol or commercial lysis kit from 800µL and 50µL of enrichment broth, respectively. DNA was analyzed by PCR screen 1 (*stx* and *eae*), and tested for O-groups (Top6) when positive for both *stx* and *eae*.

Results: 2/301 samples were tested positive for both *stx* and *eae* when DNA was prepared using VIDAS ESPT1. Those 2 samples were shown positive as well for O-groups. When tested with commercial PCR method on crude extract, 21 were positive for both *stx* and *eae*; and 13/21 were positive for O-groups PCR. Among those 301 samples, an O26 strain (*eae* and *stx* positive) was detected by both methods and successfully isolated.

Significance: The VIDAS ESPT automated DNA sample preparation prior to PCR enables a reduction of about 95% of positive rate for non-O157 STEC detection in raw milk, limiting the number of samples to be analyzed for O-Groups

P1-49 The Impact of Strain Competition on the Fitness and Virulence Potential of Listeria Monocytogenes

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Introduction: Inter-strain competition, in the same microenvironment affects the behavior of microorganisms. The coexistence of different *Listeria monocytogenes*strains under particular growth conditions, might influence their survival and virulence potential.

Purpose: To study the impact of co-culture on: (i) growth of *L. monocytogenes* strains in nutrient-rich broth, and (ii) invasion and intracellular proliferation of *L. monocytogenes*strains using human intestinal epithelial cells.

Methods: Growth of eight *L. monocytogenes* strains (serotypes 1/2a, 1/2b, 1/2c, 4b) was determined in single and two-strain mixed cultures (1:1 strain ratio). Resistance to rifampycin and streptomycin was induced for selective enumeration of strains. Populations of 3log CFU/ml were added to Tryptic Soy Broth and incubated at 10^{o}C. The growth was monitored at regular time intervals for up to 10 days. Based on the growth-data, four strain-combinations were chosen for *in vitro* virulence assay. Invasion efficiency and intracellular growth after 4h (37^{o}C) was determined in Caco-2 cells for strains in single or mixed cultures, previously incubated for one day at 10°C.

Results: Significant differences in growth kinetics of each strain were observed when grown alone as compared to the same strain in mixed cultures. For instance, strain ScottA showed growth rate of 0.35 day^{-1} when cultured alone, and 1.5 day^{-1} in a mixed culture. Strains that were outgrown by others, did not manage to reach 9 log CFU/ml, contrary to single cultures,

suggesting the growth cessation of each strain when its competitor reached the maximum growth levels. The invasion efficiency of one strain (e.g.*15162*) was 3-fold higher when grown with strain *C5* compared to single culture. In contrast, the number of intracellular bacteria of ScottA was reduced when co-cultured with strain 15162. The intracellular growth of strain 6179 was reduced when cocultivated with C5.

Significance: Competition between *L. monocytogenes* strains has a strain-dependent effect on fitness and virulence potential of the organism.

P1-50 Effect of Initial pH and Particle Concentration in Potato Salad on the Survival of Salmonella spp

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Introduction: The addition of particles, such as potato, in mayonnaise-based salads may increase the pH of the final products, especially close to the added particle. Such sites may constitute niches of survival for pathogenic bacteria.

Purpose: The objective of the study was to investigate the survival of *Salmonella* spp. in potato salad as it is affected by (i) the concentration of the potato and (iii) the initial pH of the cream salad.

Methods: Samples of cream salad (30% fat) were prepared with the addition of acetic acid as acidulant. Different concentrations of the acid were used in order to achieve initial pH of 3.6, 3.9, 4.1 and 4.4. Pre-boiled potato cubes (0.7 x 0.7 cm) were added in each sample in ratio of 0/1, 0.5/1, 1/1, 2/1, 3/1 (potato/cream salad) and inoculated (6 log CFU/g) with a five-strain composite of *Salmonella* spp. The samples were stored at 5^{o}C and periodically, the surviving population of the pathogen was enumerated on TSA and XLD plates.

Results: The initial pH of the cream salad affected the survival of the pathogen in samples without potato, with samples of pH 3.6 eliminating the pathogen within 24 hours. Depending on the concentration of the added potato, the pH of the final product increased by 0.1 to 0.5. This increase occurred during the first 24 - 36 hours, while no significant changes were observed during further storage. *Salmonella* spp. survived 5.8 log CFU/g in samples with 1/1 ratio of potato/cream salad, where the pH was adjusted at 3.98 after 24 hours of storage at 5^{o}C. However, the survival of the pathogen was limited at 4.6 log CFU/g in samples without potato and pH 3.94, suggesting that the viability of *Salmonella* is affected not only by the pH of the occurring environment, but also by the concentration of the potato that may decrease the acidity of the final product.

Significance: The results may be used to correlate the occurring pH in potato salads, as it is affected by the concentration of the potato, with the survival of *Salmonella* spp. Such correlation may assist in the determination of the formulation of such products, in order to ensure their microbial safety.

P1-51 The Houses of Cassava Flour from Copioba Vally, Nazare, Brazil: Tradition and Food Safety?

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Introduction: The cassava flour is a basic food for large population. The city of Nazaré, Brazil,, keeps alive the tradition of the production of cassava flour, especially the production of flour called "copioba", popularly known and recognized for being a finer flour and crunchy, kind of superior compared with other flours. However, just a little is known about the sanitary conditions and the preparation of this product.

Purpose: This study aimed to characterize the hygienic-sanitary profile of cassava flour houses of Copioba Valley, Nazaré, Brazil.

Methods: It is a quantitative study, conducted with 41 houses of cassava flour. The evaluation of the hygienic conditions was conducted using a check-list, which comprises five blocks: 1-Buildings, 2-Equipment and Utensils, 3-Individual and Manipulation, 4- Raw material/product exposed to sale, and 5-Flowchart of production and quality control.

Results: For the set of units evaluated, none achieved scores over 60, the limit below which characterizes poor sanitary condition. To equipment and utensils block, it was noted that this is the lowest score, which is associated with some aspects, especially the condition of equipments and utensils located in common areas of the entire manufacturing process, not attending the standards. The block concerning the Production Flow / handling / sale and quality control scored the highest - 9.10, but still at a poor level of performance. As a positive element, it was found that the product flow had no intersections, in the majority of

units, there is always a route between the root receiving, processing and roasting of mass. For all other blocks the results translate concern in a public health perspective.

Significance: The study showed problems of food safety in a traditional supply chain in the region, however, it is believed that simple changes is possible and would bring positive returns to the hygienic-sanitary profile of cassava flour houses.

P1-52 Evaluation of Food Safety Systems at Schools Foodservice in Hungary

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Introduction: Healthy childhood nutrition is extremely important as inadequate nutrition could result in negative consequences for a child's physical and mental development. Therefore, school catering programs have been scrutinized worldwide.

Purpose: The recently implemented education reform in Hungary resulted in centralized operation of school catering units given the advantages of better monitoring and adjusting the system when needed. Our aim is to develop a food safety assessment system designated for school catering units, in order to shed light on problems and determine the necessary interventions.

Methods: Checklists developed according to the Hungarian and European legislations, were applied to 68 school catering units, from which 19 were cooking units and 49 were distribution units. The inspection and data collection were carried out by HACCP auditors during personal visits. Data were analyzed by descriptive statistics, Student`s t-test and discriminant analysis. Scores were calculated separately for cooking and distribution units. The total catering point scores were divided by the maximum number of points possible and then converted into a percentage.

Results: Based on their overall final score, 17 units were classified as 'good' (above 75%), 32 units were classified as 'average' (60-74%) and 19 were classified as 'inadequate' (below 60%). Inadequate handling of food waste, lack of food warmer and reheating equipment and poor quality management rendered low scores for most places. Distribution units obtained significantly lower scores. Discriminant analysis revealed that the main problems in school kitchen classified as 'average' or 'inadequate' were related to food handling practices. Based on our results the training of food handlers and improving their attitudes is a primary task.

Significance: Our food safety checklist give us the chance to shed light on the main food safety problem in school catering units moreover the improvement strategy for each unit can be also determined.

P1-53 Risk of Shiga Toxin-producing* Escherichia coli *(STEC) in Game Meat and Meat Products

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Introduction: The role of wildlife as a reservoir of STEC has been investigated in different countries, detecting a high rate of STEC excreting animals, in particular wild ruminants, with isolation of STEC belonging to different serotypes, including the major human pathotypes; however only a few reports have been published on STEC in game meat and meat products.

Purpose: We describe the results of STEC analyses performed on game meat and ready-to-eat products, mostly imported from EU countries, sampled by Official Veterinarians in 2013 and sent to our Institute.

Methods: The samples were tested according to ISO/TS 13136:2012 (E) for the detection of STEC and the determination of O157, O111, O26, O103 and O145 serogroups.

Results: Twenty-five game meat samples (20 red deer, 2 roe deer, 3 wild boar) and 8 smoked dry sausages (made with red deer, roe deer, chamois meat mixed with pork) were analysed. Twenty out of 25 (80%) meat samples and 3 out of 8 dry sausages (37.5%) tested positive for *stx* genes. Overall, 19 samples were also positive for the *eae* gene and 21 for at least one of the "top five" serogroup genes, the most frequent being O103 (82.6%) and O145 (78.3%). STEC strains were isolated from 13 *stx* positive samples (56.5%), 4 of them belonging to highly pathogenic serogroups: *Escherichia coli* O145 *stx*1 *eae+*, O103 *stx*1 *eae+* and O157 *stx2 eae+* from 3 deer meat samples and *E. coli* O103 *stx*1 *eae+* from one sausage.

Significance: In spite of the rather small number of tested samples, the high rate of STEC isolation, including highly pathogenic strains, highlights the risk associated to the consumption of game meat and RTE products, that can be regarded as an important

vehicle of STEC for humans. Prevention should mainly rely on appropriate hunting practices, avoiding carcass faecal contamination and good hygienic practices during meat processing.

P1-54 Tracing Sources of Listeria monocytogenes Contamination in Traditional Italian Cheese Associated with a U.S. Outbreak

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Introduction: In 2012, a USA multistate outbreak of listeriosis with 22 cases and 4 deaths was related to the consumption of "ricotta salata" (salt-cured ricotta cheese) imported from Italy. Intense sampling activities were then conducted by Italian competent authorities.

Purpose: Trace the source of *Listeria monocytogenes* contamination at production level and assess the possible persistence of strains in processing environments.

Methods: The USA outbreak was linked to a product from an Italian establishment (plant A) that processed semi-finished products supplied by five plants (B, C, D, E, F). During the first stage of sampling, 187 environmental and 198 cheese samples were taken. Other 179 and 35 samples were collected 8 months later. Specimens were examined by ISO 11290-1 and 2 for detection and enumeration of *Listeria monocytogenes*. Strains were serotyped (US/FDA method) and characterized by PFGE (CDC protocol).

Results: During the first sampling stage, *Listeria monocytogenes* was found in environmental samples of plants B and D. Sixty-three end-products (31.8%) were positive. Genetic similarity was shown by PFGE between strains isolated from USA clinical cases and products. Follow-up activities showed the persistence of environmental contamination in plants D and E: strains from processing environment were highly similar to strains from cheese sampled several months before. Environmental strains from plant D were found in end-products processed in plant A, even if they were produced from semi-finished products of other suppliers. On this basis, the environment of plant D seems to be the origin of contamination that could have spread to a variety of end-products through the environment of plant A.

Significance: Our study was yielded from the collaboration of different Italian competent authorities, allowing to traceback the origin of contaminations that caused a severe outbreak in USA. The persistence of strains in environmental niches of processing plants, due to inadequate hygiene, is probably the cause of product contamination.

Poster Session 2 - Food Defense, General Microbiology, Meat & Poultry, Microbial Food
Thursday, 8 May 2014: 10.00–17:00

P2-01 Smart and Sustainable Food Packaging Utilizing Flexible Printed Intelligence and Materials Technologies – SusFoFlex Project

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Introduction: The European food packaging industry needs active, intelligent and sustainable food packaging materials in combination with flexible packaging technologies to stay competitive on the global market. The new active, intelligent and sustainable solutions have to be consumer-oriented, ensure the safety and quality of food, reduce food losses, and reduce the environmental impact of food packaging.

Purpose: SusFoFlex project focus on some specific food categories well-known for problems of short shelf life, safety and food losses, such as fresh-cut fruits and salads and other vegetables and fish, even though a methodology approach is followed and developed which could be flexible applied also to other food categories characterized by high and increasing market volumes.

Methods: SUSFOFLEX will identify the key areas where improvement in terms of barriers and smart functions can be made by using natural additives, filler and nanomaterials, by PLA films, and by developing sensor materials that can gain information on the condition of the product. A nanomaterials based sensor that can gain information on the condition of the product will be

developed and the sensor will be an integrated part of a smart label/sticker. On the other hand the sensor will monitor the packaged food, and communicate the information to the user and Hybrid, RFID based near field communication will be used for communication.

Results: In the scope of the project, up to now plant extracts for potential application in food packaging were prepared from different agro-food by-products and antimicrobial effects of these compounds have been tested. Plant extract preparation, PLA-film development and antimicrobial effect results will be presented.

Significance: Seven industrial companies from Spain, Ireland, Norway, Finland and Turkey and 8 European RTD units participated in the project to share their knowledge and experiences. To reward their endeavor the EU decided to fund the project through the Seventh Framework Programme.

P2-02 The Effect of Double Pasteurization on the Microbiological Properties of Liquid Egg

Csaba Németh[1], László Friedrich[2], Csaba Balla[2], Ildikó Zeke[2] and Luis Alberzóto Castillo[2], (1)Capriovus Ltd., Szigetcsép, Hungary, (2)Corvinus University of Budapest, Budapest, Hungary

Introduction: Nowadays, people are developing numerous new liquid egg preservation methods and testing them in industrial settings, but such methods are beyond most small producers of egg products, mainly because of the high cost of investment and implementation. On the other hand, everyone has access to simple pasteurization equipment.

Purpose: In this study, we have examined how conventional double pasteurization with a plate heat exchanger affects the microbiological nature of liquid egg.

Methods: For our experiment, liquid egg was prepared within two hours of starting the tests and kept at 4°C. Then it was pasteurized in a plate heat exchanger for 5 minutes at 65°C. Following this, the material was cooled back down to 4°C and kept for one hour in a buffer container while the heat exchanger was cleaned. Afterward the pasteurization was repeated. We then prepared a series of 1ml decimation dilutes of three types: raw liquid egg, a once-pasteurized sample, and a twice-pasteurized sample, after which we determined the microbe count of the samples with nutrient agar poured plates. Additionally, we sequenced the 16S rDNS gene of the isolate obtained from the twice-pasteurized liquid egg.

Results: Our results, which are in agreement with the literature, show the first pasteurization caused a more significant reduction in spore count. Whereas the first pasteurization reduced by a magnitude of approximately 2 the total live spore count of aerobes in the egg liquid, the second pasteurization only reduced the count by a further 1 order of magnitude. There was yet another difference. In the double-pasteurized samples, the diversity of microflora was considerably less: only 3 populations/species could be identified: *Rhodococcus erythropolis/Rhodococcus qingshengii/Rhodococcus baikonurensis/Rhodococcus boritolerans, Nocardia coeliaca, Chryseobacterium sp., Brevundimonas nasdae/Brevundimonas vesicularis.*

Significance: Based on our results, it can be stated that double pasteurizing liquid egg enables manufacture of microbiologically stable products. This may be a feasible alternative for smaller plants operating at less than 100% capacity, and is preferable to the more modern and more costly technologies.

P2-03 Effects of Die Material on Physical, Textural and Sensory Characteristics of Pasta

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Introduction: The popularity of pasta is ever increasing in all around the world due to its nutritional properties particularly its glycemic index, convenience and various shapes. Pasta quality can be affected by a vast variety of factors such as physical characteristics, color, desired shapes and sizes which some of them are able to be affected by the material of the dies.

Purpose: The objective of this research was to evaluate the impact of bronze and Teflon die on physical, textural and sensory properties of pasta.

Methods: Durum wheat semolina was supplied by Zar Flour Co., and manufactured to pasta by ZarMacaron Co., by extruder equipped with 0.9 mm bronze or Teflon die. Then pasta was dried under controlled condition at 80°C for 4 hours. Thickness of pasta samples was measured by colis (Mitutoyo, Japan). Cooked weight and cooking loss were determined by the AACC Method 66-50 (2000). The L*, a*, and b* values of pasta samples were measured by a Hunter Lab Colorimeter (D25-9000 USA). Sensory evaluation (5-point hedonic scale) of pasta samples was made by 15 trained panelists.

Results: Findings showed that extrusion by using bronze die caused an increase in pasta thickness compared to Teflon die. Cooked weight and cooking loss in both pasta samples had no significant difference for bronze and Teflon die. It was found that manufactured pasta with Teflon die caused higher yellow color; moreover higher b* value in comparison with pasta extruded with bronze die. In sensory evaluation, panelists preferred the texture and taste of the pasta produced with Teflon die while bronze die caused coarse and rough texture of pasta.

Significance: Physical properties play a significant role in general acceptability of pasta products. In addition of raw material quality, production technology like die material (including bronze or Teflon) can have a principal impact on customer attractiveness.

P2-04 Consumer Preference and Willingness to Pay for Sheep Meat Quality and Safety in Addis Ababa
Aga Neme Doba, Ambo University, Ambo, Ethiopia

Introduction: The main objective of this study was to assess consumer preference and willingness to pay for sheep meat quality and safety attributes in Addis Ababa city.

Purpose: The main objective of this study was to assess consumer preference and willingness to pay for sheep meat quality and safety attributes in Addis Ababa city.

Methods: Two hundred (200) households were selected using multi-stage sampling procedure. Rapid market appraisal was held to select attributes. These were derived using an orthogonal fractional factorial design to create profile scenarios. The data from conjoint experiment were estimated using rank-ordered logit model. Relative importance and willingness to pay were estimated from the coefficient of rank-ordered logit result.

Results: All parameters were significantly different from zero with the expected signs. Accordingly, hygiene is highly valued by all consumers regardless of income strata, as indicated by part-worth utility, relative importance, and willingness to pay and the least preferred attribute is fat content. Respondents from high income households were more concerned about hygiene and fat content than medium and low income households. Conversely, respondents from the low income households were more concerned about price, freshness and official stamp than their medium and higher income counterparts. A large percent of consumers were willing to pay a premium for hygiene; where individual consumers were willing to pay a premium of 23.35 ETB for better and improved cleanliness. Freshness was ranked second with a WTP premium of 11.53 ETB and official stamp, and fat content were 8.92 ETB and 8.61 ETB, respectively. The WTP result showed that hygiene, freshness, official stamp, and fat content were ranked from most to least valued attributes.

Significance: This finding would have a positive implication for forming product differentiation strategies within the animal source food policy in general and the sheep meat industry in particular.

P2-05 Production and Evaluation of Mango Pulp by Using Ohmic Heating Process
Tarek abd El-Maksoud, Cairo University, Giza, Egypt

Introduction: This research aimed to study the use of ohmic heating in the processing of mango pulp comparing to conventional method. Mango pulp was processed by using ohmic heating under the studied suitable conditions.

Purpose: The present investigation was carried out to evaluate the quality characteristics and optimal conditions for processing of mango pulp by ohmic heating which is compared to a conventional heating method.

Methods: Physical, chemical and microbiological properties of mango pulp were studied.

Results: The results showed that processing of mango pulp by using either ohmic heating or conventional method caused a decrease in the contents of TSS, total carbohydrates, total acidity, total sugars (reducing and non-reducing sugar) and an increase in phenol content, ascorbic acid and carotenoids compared to the conventional process. The increase in electric conductivity of mango pulp during ohmic heating was due to the addition of some electrolytes (salts) to increase the ions and enhance the process. The results also indicate that mango pulp processed by ohmic heating contained more phenols, carbohydrates and vitamin C and less HMF compared to that produced by conventional one. Total pectin and its fractions had slightly reduced by ohmic heating compared to conventional method. Enzymatic activities showed a reduction in poly phenoloxidase (PPO) and polygalacturonase (PG) activity in mango pulp processed by conventional method. However ohmic heating completely inhibited PPO and PG activities.

Significance: Ohmic heating is a viable new technology in food preservation.

P2-06 Pediococcus Acidilactici Probiotic Effect on the Fatty Acid Profile of Chicken Meat in Algeria
Naima Sahraoui, Blida University, Blida, Algeria

Introduction: In general, the fatty acid profile of chicken tissues reflects the composition of lipids ingested.

Purpose: This study was conducted to determine the effect of probiotic *Pediococcus acidilactici* on the fatty acid profile of the meat of broilers.

Methods: The fatty acid composition was determined by gas chromatography.

Results: The use of probiotics may have some potential to improve carcass yield and reduce the rate of fatty acids. While the levels of MUFA were significantly lower in the supplemented lot compared to the control group at 28th and 42 days (22.61 ± 1.30 vs. 29.36 ± 1.27 and 19.38 ± 2.67 vs 26.37 ± 0.72), the percentage of polyunsaturated fatty acids were significantly higher in probiotic lot (40.07 ± 2.01 vs. 34.20 ± 0.77) to 42th day, and the S / P (0.99 ± 0.04 vs. 0.87 ± 0.02).

Significance: To assess the potential benefits in terms of health, research should also be encouraged to study the effect of the use of probiotics on the total.

P2-07 The Standard of Food Safety and Control
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Introduction: Food safety standards require a significant implementation of specific standards from production to consumption.

Purpose: Basic Analytical Prevention Point Control (BAPPC) has a wide range of acceptability and methodological standard in risk analysis for industrially processed foods.

Methods: BAPPC is a bigger challenge, especially in developing countries, where food marketability and comparative exchange of commodity channels are less formal. This particular study adapted a BAPPC methodology to assess health risks at different points in the informal milk marketing network today. The critical control points identified for high total bacterial counts were channels with multiple transaction points, which took considerable time from the farm without refrigeration facilities. High coliform counts were associated with the use of plastic versus metal containers.

Results: Approximately 14% of samples were adulterated with added water sample.

Significance: Recommendations for procedures to improve milk quality and how these can be communicated to farmers, market agents and consumers are proposed and discussed.

P2-08 'Something in the Air' - Assessing Microbial Air Quality in Production Facilities
Edward Stuttard, Strategic Sampling Pty Ltd, Runaway Bay, Australia and Margaret Tentser, DTS Food Laboratories, Kensington, Australia

Introduction: This presentation will review microbiological air sampling methods, and their relevance for food production facilities.

Purpose: Industry update

Methods: Quantitative air sampling using impaction samplers.

Results: Presented as Case studies

Significance: Environmental monitoring for microorganisms in the production facility is incomplete without testing the two critical air sources present in all facilities

Ambient air is exactly that, and it's now considered in BRC assessments, but there are no test methods or guidance limits presented for this ubiquitous medium.

Compressed air, often a major source of contamination, is difficult to sample, and difficult to decontaminate when issues arise. Neither of which are valid reasons for ignoring the impact of the microorganisms entrained in compressed air.

This presentation will discuss the tools of the trade, suggest some guideline limits, and present some recent case studies.

P2-09 Exposure to Nitrate and Nitrite in Finland: Quantitative Risk Assessment

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Introduction: The aim of this study was to determine the main sources of dietary exposure to nitrite and nitrate in Finland and the levels of exposure for children and adults.

Purpose: There was reason to suspect the acceptable daily intake (ADI) can be exceeded for some population groups, and a quantitative risk assessment was needed to define the part of the population possibly exceeding the ADI.

Methods: Concentrations of nitrate and nitrite were measured in foods sold in Finland, comprising additive sources (sausages, cured meat, cheeses and herring products) and natural sources (vegetables, fruit, berries and tap water). Nitrate was measured in 1011 food and 1502 water samples, nitrite in 247 food and 2947 water samples between 2000 and 2012.

Finnish individual consumption data of adults (25 to 74 years, N=2038) were gathered in the FinDIET 2007 study and that of children (1, 3 and 6 years, N=1471) in the DIPP study. Monte Carlo approach was exploited in the stochastic exposure assessment carried out with MCRA program v. 7.1.

Results: Dietary nitrate exposure of the Finnish population comes mainly from vegetables and fruit. However, while the main source for adults was leafy vegetables, children were exposed from several vegetable groups. Nitrite exposure was only studied from additive sources and tap water. The main nitrite source for all age groups was sausages such as frankfurters and grilled sausages. For the average user, 65% of the adults' and 34-82% of the children's exposure from additive sources was from sausages. Among the 3- and 6-year-olds, one child out of ten was in danger of exceeding the ADI of nitrite from additive sources only.

Significance: Based on the risk assessment results [1], new food use recommendations for children have been published in Finland in December 2013.

[1] Evira Research Reports 2/2013 (in Finnish). ISBN 978-952-225-126-8.

P2-10 Control of Tetracycline Residues in Meat Traded in North Sinai Egypt Using Traditional Techniques

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Introduction: Antibiotic residues appear to be the most important chemical hazard in meat. The bioaccumulation of tetracycline in the meat predisposes it to development of drug resistant bacteria and allergic reactions in consumers.

Purpose: The objectives of the current study were to determine the concentration levels of tetracycline in muscle, liver and kidney of cattle. Another aim was to control the tetracycline residues in meat using a marinating and simmering technique.

Methods: A total of 330 meat, liver and kidney samples (110 of each) were randomly collected from El-Arish abattoir to evaluate their tetracycline residual content. The samples were divided based on animal age as follows: group I, 30 veal (< 6 months), group II, 30 bull (7-18 months) and group III, 30 cow (> 5 years) age carcasses and group IV, 20 imported frozen meat samples. Positive meat samples for tetracycline were treated using marinating and simmering technique to study their effects on tetracycline levels.

Results: Tetracycline residues were not detected in all samples of group I. The mean concentration tetracycline values for meat, liver and kidney of group II were 11.296, 15.984 and 21.032 µg/g, respectively. Meanwhile, for group III, concentrations were 23.116; 41.255 and 45.022 µg/g and for group IV were 8.596, 12.684 and 16.832 µg/g, respectively. All examined samples (100%) were within the permissible limits sets by the Egyptian Organization for Standardization and Quality Control. Marinating

technique significantly reduced ($P > 0.05$) the concentrations of tetracycline in meat in groups I; II and IV by levels 12.2%; 45.1% and 11.4%, respectively.

Significance: The results of this study concluded that meat and offal from adult cattle, cow and imported meat had considerable tetracycline residues but did not exceed Egyptian permissible limits. Veterinarians must be well aware of the importance of drug residues in meat and the possible risk to the general public. They must have updated information about the proper withdrawal times of all drugs used in their areas of practice. Marinating and simmering techniques were effective on reducing tetracycline concentration levels in treated meat samples.

P2-11 Establishment of Cardinal Parameter Model of Pseudomonas aeruginosa as a Function of Temperature, pH and Sodium Lactate

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Introduction: Sodium lactate has proved to be a possible preservative against several spoilage and pathogen bacteria in food, and is categorized as a Generally Regarded As Safe (GRAS) food additives in many countries.

Purpose: In order to verify the bacterial activation of sodium lactate combined with other environmental factors, the cardinal parameter model (CPM), one of important models of predictive microbiology, might be established in the study.

Methods: An automated turbidimetric system, Bioscreen C, was used to monitor the growth of *Pseudomonas aeruginosa*, one of Specific Spoilage Organisms (SSO), which was separated from spoilage pork at a range of temperature (25~40°C), pH (5.0~7.5) and mass concentration of sodium lactate (0~0.035 g/ml). The CPM was fitted on the basis of nonlinear least squares method using *fminsearch* function of Matlab software, and CPM represented the lag time of *P. aeruginosa* as a function of temperature, pH and sodium lactate mass concentration.

Results: The CPM was fitted to determine the cardinal parameters $T_{\min}$, T_{opt}, $T_{\max}$, $pH_{\min}$, pH_{opt}, pH_{max} and the minimum inhibitory concentration (*MIC*) of sodium lactate, respectively, and then the established model was validated with ten random data within above ranges. The results showed that the growth parameters were predicted well by CPM with R^2= 0.9291, B_f = 1.0975, A_f= 1.3936, and *RMSE*= 1.5989, respectively. The validation parameters were 0.8546, 1.1225 and 1.2117 for R^2, B_f and A_f, respectively.

Significance: The CPM could provide the technological reference for predicting and controlling the growth of *P. aeruginosa.*

P2-12 Modelling the Growth of Single Cells and Cell Colonies of Pseudomonas aeruginosa

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Introduction: In the research of quantitative microbial risk assessment (QMRA), it is vital to understand how lag times of individual cells are distributed over a bacterial population.

Purpose: To verify the effect of inoculum size on the lag time of *Pseudomonas aeruginosa*, one of Specific Spoilage Organisms (SSO) in meat products, based on the theory of predictive microbiology.

Methods: A flow chamber image system was used to study the single cell growth of *P. aeruginosa*. A stochastic modeling process was applied to connect the growth of *P. aeruginosa* single cells and cell colonies, and made it possible to simulate the population growth of *P. aeruginosa*. Experimental bacteria growth viable counts using different initial inoculum sizes were detected to validate the simulation process. Meanwhile, many simulations were used to verify the effect of inoculum size on the lag time of *P. aeruginosa*.

Results: Results indicated that the agreement between simulations and viable counts were good at both 25°C and 35°C, and the simulation process could be one method for predicting the population growth of *P. aeruginosa*. Through many simulations, it was demonstrated that the lag time decreased from 12.52h to 6.33h at 25°C and 8.61 h to 4.01h at 35°C as the initial inoculum size increased from 0 to 100 CFU/ml. Uncertainty and variability were shown clearly in the simulation process.

Significance: This method used in the study could be used as reference to predict bacterial population growth and to study the lag behavior of microorganism.

P2-13 Cross-contamination Risks during Tomato Hand Harvesting

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Introduction: Current guidelines for fresh Florida tomatoes state that tomatoes that touch the ground should not be harvested; tomatoes that touch plastic bed mulch are often harvested.

Purpose: The relative cross-contamination risk of tomatoes touching new or used plastic bed mulch was compared to that of tomatoes touching the ground under varying inoculation conditions and contact times.

Methods: A five-strain, rifampicin resistant *Salmonella* cocktail was (100 µl) spot inoculated onto 5 x 5cm new and used plastic bed mulch pieces or soil (10 g) to obtain ca. 6 log CFU/item. All experiments were done at ambient temperature. Surfaces were either touched immediately to tomatoes (wet) or allowed to dry for 1 or 24h before contact; two contact times (touch (1-2s) and 24h contact), were investigated (n = 10). Bacterial populations were enumerated on tryptic soy agar supplemented with rifampicin; when counts fell below the limit of detection, enrichments were performed following standard protocols. The transfer direction was then reversed by inoculating tomatoes and measuring transfer to contacting surfaces.

Results: *Salmonella* transfer from surfaces to tomatoes was lower when the inoculum was allowed to dry for 24h prior to contact. *Salmonella* transfer from soil to tomatoes (0.1–2.0%) was significantly lower than from new (11.3–36.5%) or used (17.9–33.5%) plastic bed mulch, under all conditions. *Salmonella* transfer from tomatoes to surfaces was lower when the inoculum was allowed to dry for 24h prior to contact, especially with longer contact times. When contact times increased to 24h, transfer to new plastic bed mulch increased (35.3-73.4% wet; 40.9-61.6% 1h dry); transfer to used plastic bed mulch remained consistent (49.4-30.6% wet; 42.5-42.6% 1h dry); and transfer to soil decreased (84.5%-29.8% wet; 61.4-36.5% 1h dry).

Significance: *Salmonella* transfer between tomatoes and soil or plastic mulch is dependent on moisture; potential cross-contamination of tomatoes from plastic mulch is not less than from soil.

P2-14 Isolation and Partial Characterization of Proteolytic Lactic Acid Bacteria with Potential for Application in the Reduction of Cow Milk Allergenicity

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Introduction: Cow milk allergy (CMA) is a serious problem that affects 2.5% of children under 3 years of age, representing 9% of food allergy cases. This immunological reaction is triggered by the binding of Immunoglobulin E (IgE) to specific epitopes, present in milk proteins. An alternative to reduce this problem could be the hydrolysis of milk principal allergens by microbial fermentation. However, the bacterial strains used and the process conditions influence the success of this application. Therefore, the study of new proteolytic lactic acid bacteria (LAB) strains, capable to hydrolyze the proteins responsible for CMA, is essential for the development of novel hypoallergenic dairy products.

Purpose: The objective was to screen for new proteolytic LAB from bovine milk and characterize their proteolytic activity.

Methods: Potential proteolytic LAB were isolated from raw bovine milk. The proteolytic activity was confirmed in UHT skim milk and partially characterized using purified fractions of Na-caseinate and whey proteins, in non-proliferative cell system. Strains were differentiated by RAPD-PCR and identified by 16S rRNA sequencing. The hydrolysis profiles were compared after SDS-PAGE electrophoresis.

Results: Nine LAB strains presenting proteolytic activity on skim milk were selected and identified as *Enterococcus faecalis*. Preliminary characterization of the proteases produced by these strains indicated that they belong to the group of metalloproteases. The optimal conditions for hydrolysis were achieved at 42°C and pH 6.5. All hydrolyzed milk casein fractions (α_{S1}, α_{S2}-, and β-caseins), but to different extents. *Ent. faecalis*VB63F presented the strongest proteolytic activity, achieving complete hydrolysis of native Na-caseinate and partial hydrolysis of whey proteins.

Significance: Our results suggest that *Ent. faecalis*VB63F presents a good potential to be applied in the reduction of milk proteins allergenicity. Further tests on the safety of this strain will indicate its potential application in the manufacture of new hypoallergenic dairy products.

Acknowledgment: FAPESPS-2013/11168-0

P2-15 Effects of High-intensity 405 nm Light Emitting Diode on Inactivation of Gram-negative Foodborne Pathogens
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Introduction: A light emitting diode (LED) has recently received increased attention due to its antibacterial effect. Some researchers have reported that 405 nm LED had bactericidal effect, demonstrating the potential of novel food preservation technology. However, little information is available on its effectiveness on foodborne pathogens and its antibacterial mechanism.

Purpose: The aim of this study was to investigate the antimicrobial effect of LED on the selected Gram-negative foodborne pathogens and to elucidate its antibacterial mechanism by determining bacterial membrane damage.

Methods: *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Shigella sonnei* were treated with 405 nm LED. A 10 ml volume of bacterial suspension (about 10^8 CFU ml^{-1}) was exposed to 945 J cm^{-2} of LED at 4°C. The irradiance was 35 ± 5 mW cm^{-2}. The percent of sublethal injuries were determined by plating tryptic soy agar (TSA) and TSA including 2-3% NaCl and 1% bile salts as selective agents, respectively. A Live/Dead®Cell Viability assay was used to examine the membrane damage using fluorescence microscopy.

Results: *E. coli* O157:H7, *S.* Typhimurium, and *S. sonnei* were inactivated by 1.0-, 2.0-, and 0.8-log CFU ml^{-1}, respectively, at 945 J cm^{-2}. Regardless of bacterial strain and selective agent, longer exposure time resulted in an increase in the injury percent, indicating that LED-treated cells became more susceptible to NaCl and bile acid than untreated control cells. A Live/Dead®Cell Viability assay also clearly showed that LED-treated cells underwent loss of the physical integrity of the membrane, whereas untreated cells were intact.

Significance: These results indicate that 405 nm LED might be effective to inactivate the selected Gram-negative foodborne pathogens in foods, exhibiting the potential of novel food preservation technology. In addition, this study suggests that the antibacterial effect of the LED would be due to membrane damage.

P2-16 Arsenic, Cadmium, Copper and Lead Residues in Meat and Edible Offal of Cattle from North Sinai, Egypt
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Introduction: Environment pollution by heavy metals is a serious global problem. Risks of metal residues in meat are serious, as reflected by the high metal concentrations recorded in the air, water and animal feed.

Purpose: Therefore a total of 330 meat, liver and kidney samples (110 of each) were randomly collected from El-Arish abattoir, Sinai Peninsula, Egypt, to evaluate their Arsenic (Ar), cadmium (Ca), copper (Cu) and Lead (Pb) residual content.

Methods: The samples were divided based on animal age as following; group I veal, group II adult bull, group III, old cow and group IV, imported meat samples. Samples of group I had a lowest Ar, Ca, Cu and Pb residual levels in compared to other groups.

Results: Age of the slaughtered cattle had a significant effect *($P > 0.05$)* on the metal levels in the examined samples. All examined samples for Ar, Ca, Cu and Pb residual levels fell within the permissible limits set by the Egyptian Organization for Standardization and Quality Control; they were considered safe for human consumption.

Significance: There were considerable amounts of arsenic, cadmium, copper and lead residual recorded in meat and edible offal in this study. More governmental efforts are still needed to control the environmental pollution and improve the environment quality of El-Arish Zoon.

P2-17 Effect of the Use of Water in the Decontamination of Smooth and Modular Conveyor Belts in Poultry Cutting Rooms
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Introduction: Conveyor belts are widespread in Brazilian poultry processing plants certified for exports. Operational cleaning of these conveyor belts is carried out with water under pressure, but the efficiency of this procedure on reducing bacterial contamination may be questioned.

Purpose: To evaluate the effect of water in cleaning conveyor belts in poultry cutting rooms of Brazilian slaughterhouses.

Methods: Samples of the surfaces of smooth and modular conveyor belts, with or without cleaning by water, were collected in four poultry slaughterhouses. Samples were analyzed for mesophilic (n = 959), enterobacteria counts (n = 959), and *Listeria monocytogenes* (n = 640).

Results: Mean mesophilic counts in smooth conveyor belts were 1.62 log CFU/cm^2 (with cleaning), and 1.65 log CFU/cm^2 (without cleaning) (P = 0.94). In modular, cleaning was not efficient (P < 0.001) in reducing bacterial counts (with cleaning 2.07 log CFU/cm^2, and without cleaning 1.85 log CFU/cm^2). In enterobacteria counts, smooth conveyor belts that were cleaned presented lower counts (0.57 log CFU/cm^2) when compared with those that were not cleaned (0.76 log CFU/cm^2) (P < 0.001), and modular that were cleaned or not presented counts of 1.32 and 0.88 log CFU/cm^2, respectively, which were statistically significant results (P < 0.001). *L. monocytogenes* was isolated from 23% (smooth conveyor belts with cleaning) and 31% (without cleaning) (P = 0.0002; OR = 0.57). In modular conveyor belts with cleaning, *L. monocytogenes* was found in 12% and conveyor belts without cleaning 23% (P = 0.0002; OR = 2.53). Results showed that water influenced the cleaning process, suggesting that the use of water should be constantly evaluated, once when it is not used, the level of contamination of the surfaces may be influenced as well as the innocuousness of products of animal origin.

Significance: Cleaning processes should be routinely evaluated in order to ensure the hygiene of the surfaces that contact with food and, therefore, their safety. Acknowledge: FAPESP.

P2-18 Effect of Different Marinades on the Indigenous Microflora of Chicken Breast Fillets under Various Marinating Conditions

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Introduction: Marination is a common practice for enhancing quality while prolonging the shelf life of meat. Therefore, studies on the marinating conditions are of great importance.

Purpose: Evaluation of the effect of different marinades, marinating time and temperature on the microbial counts, physicochemical profiles and sensory quality of marinated chicken fillets

Methods: Chicken breast fillets were immersed in five marinades (lemon and pomegranate juice, apple cider vinegar, and combinations) for 1, 3, 6, 9 and 24 hours, at 4, 10 and 20°C. After marination, Total Viable Counts (TVC), *Pseudomonas* spp., *Brochothrix thermosphacta*, Enterobacteriaceae and lactic acid bacteria populations were determined. Sensory assessment (odor, flavor and tenderness of oven-cooked samples), pH and Fourier Transform Infrared (FT-IR) spectroscopy measurements were performed. Data were analyzed using ANOVA, Principal Component Analysis (PCA) and Canonical Discriminant Analysis (CDA).

Results: Marination reduced TVC and *Pseudomonas* spp. counts by 1.0-3.0 log units, while caused a 2.0-5.0 log decrease in *Br. thermosphacta* populations. Except for one hour marinating, both temperature and time did not seem to affect microbial counts. Limited time interval marination (1 and 3h) led to organoleptically more satisfactory results. PCA and CDA applied to the FT-IR spectral data, showed discrimination of the samples based on different marinades and marinating conditions

Significance: This study suggests that even short time marination has a significant effect on chicken breast fillets spoilage bacteria besides enhancing sensory quality.

Acknowledgment: The action THALIS: "Development, mathematical modeling and optional design of non-thermal technologies for processing, packaging, distribution and storage of safe high quality food products", has been co-financed by the European Union (European Social Fund - ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: THALES: Reinforcement of the interdisciplinary and/or inter-institutional research and innovation.

P2-19 Assessment of the Efficacy of Fourier Transform Infrared Spectroscopy in Predicting the Microbiological Quality of Pasteurized Vanilla Cream

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Introduction: Although numerous methods have been applied to assess food quality, their value is usually limited due to time consuming and retrospective information provided. Hence, the implementation of rapid analytical methods, capable of identifying and quantifying microbial spoilage of perishable food products, are still of great significance for the food industry.

Purpose: The evaluation of Fourier transform infrared (FT-IR) spectroscopy as a means of monitoring the microbiological quality (i.e., total viable counts, TVC) of pasteurized vanilla cream, a perishable ready-to-eat food product.

Methods: Vanilla cream samples were stored aerobically at 4, 8, 12 and 15°C for 40 days. At appropriate time intervals, duplicate samples were analyzed using both conventional microbiological analysis and FT-IR spectroscopy. Feature (i.e., wavenumbers) selection of the acquired spectra was performed based on communality values as derived from principal components analysis. The selected spectra, after a pre-processing step, were then evaluated against the corresponding TVC using Partial Least Squares (PLS) regression.

Results: No microbial growth was observed during storage of vanilla cream at 4 and 8°C, while TVC reached approximately 6 log CFU/g at 13 and 5 days of storage at 12 and 15°C, respectively. PLS regression revealed a good correlation between the microbiological data at 12 and 15°C and the corresponding FT-IR spectra, with the cross-validation R^2 and root mean squared error being 0.95 and 1.16, respectively

Significance: The results of this study demonstrate that FT-IR spectroscopy is a promising analytical technique for the rapid assessment of the microbiological status of pasteurized vanilla cream.

This work has been supported by the project "Efficacy of NOVEL analytical techniques to predict the quality and safety of newly developed perishable food products 11SYN_2_1528" co-financed by the EU (European Social Fund – ESF) and Greek national funds through the O.P. "Competitiveness and Entrepreneurship (OPC II)" ROP Macedonia – Thrace, ROP Crete and Aegean Islands, ROP Thessaly – Mainland Greece – Epirus, ROP Attica, Framework NSRF 2007-2013, COOPERATION 2011.

P2-20 Coaggregation among Rhodococcus and Acinetobacter from the Food Industry

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Introduction: Different types of bacteria may bind specifically to each other and form coaggregates, which may have implications for the spatial organisation of biofilms.

Purpose: In the present study coaggregation between *Acinetobacter* and *Rhodococcus* isolated from the food industry was investigated.

Methods: Three strains of *Rhodococcus* and seven strains of *Acinetobacter* were grown in tryptic soy broth at 30°C, washed, the resulting suspensions mixed, and formation of coaggregates was observed visually, spectrophotometrically and by scanning electron microscopy. The mechanisms of coaggregation were studied by treatment of suspensions with heat and enzymes and in competitive binding assays with different sugars.

Results: Strain-specific coaggregation was observed. One strain of *Rhodococcus* formed coaggregates with two strains of *Acinetobacter* and another strain of *Rhodococcus*. Stronger coaggregation was observed for cells cultivated at 30 vs 20°C, in TSB vs R2A growth medium and for cells from exponential or early stationary phase vs late stationary phase. The formation of coaggregates was promoted in the presence of mineral salts. For three of the strains the coaggregation factor seems to be proteinaceous.

Significance: Coaggregation was observed between bacteria from food industry, and has previously been shown to be important for structure of oral biofilms. Further studies are needed to determine the impact of coaggregation on survival of bacteria in the food industry.

P2-21 Polycyclic Aromatic Hydrocarbons in Food: Dietary Intake in Austria

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Introduction: Polycyclic Aromatic Hydrocarbons (PAHs) are contaminants in food. In this study the term "PAHs" refers to the sum of benzo[a]pyrene, benzo[a]anthracene, benzo[b]flouranthene and chrysene (PAH4). These compounds are carcinogenic and genotoxic and no toxicological threshold can be derived. So there is a need to assess the risk for the Austrian population.

Purpose: The objective of this study is to evaluate the dietary exposure to PAH4 for the Austrian population through different foodstuffs and to identify the highest contributors. Furthermore, a potential concern for consumer health has to be evaluated.

Methods: Estimates of dietary exposure are based on analytical results of food samples from Austrian retail (2007-2011) and on Austrian food consumption data. The exposure assessment was done on the basis of a mathematical sampling technique called Monte Carlo simulation. In this probabilistic exposure assessment the distribution of PAH4 concentrations was combined with the distribution of food consumption data. For risk characterisation the Margin of Exposure (MOE) approach was used.

Results: The mean dietary exposure to PAH4 for women is 0.53 ng/kg bw/d [0.51; 0.55] and for men 0.41 ng/kg bw/d [0.39; 0.42]. Regarding the 95th percentiles the dietary exposure for men is 5.88 ng/kg bw/d [5.52; 6.26] and for women 5.94 ng/kg bw/d [5.57; 6.30]. For male and female adults, the main contributors to the PAH4 exposure are roasted coffee, chocolate and preserved (salt) meat. MOE–values are in a range of 644000 (mean) – 57000 (P95) for women and of 837000 – 58000 for men.

Significance: These results indicate a "Risk of low Concern" for the Austrian population. Presently, PAHs are not at the top of a risk ranking list. Nevertheless, in terms of food safety and consumer protection monitoring of PAHs should be continued. Furthermore, all efforts should be done to reduce the presence of PAHs in food.

P2-22 Risk Factors for a High Batch-level Bacteriologic Prevalence of Human Pathogenic Yersinia enterocolitica in Belgian Pigs at Slaughter

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Introduction: Pigs are the main reservoir of *Y. enterocolitica*, but the prevalence of this pathogen differs between farms. This variation of the infection status allows determination of risk factors.

Purpose: When risk factors for a high batch-level bacteriologic prevalence should be known, farmers could apply them and create intervention strategies to reduce the within-batch prevalence prior to slaughter.

Methods: One hundred farms were visited and data concerning housing, ventilation, biosecurity, management, feeding and disease control were collected using a face-to-face questionnaire. At the slaughterhouse, tonsils of on average 70 slaughter pigs per batch were sampled to determine the number of positive animals per batch. First, variables were submitted to a univariable analysis using a mixed effect logistic regression, with farm as random effect. Variables which were related to the *Yersinia* prevalence ($P < 0.05$) were included in a multivariable model, excluding at each step the non-significant variable until only significant main effects and interactions remained.

Results: *Y. enterocolitica* was found in 85 pig batches. In the multivariable model, three risk factors and two protective factors remained significantly associated with *Y. enterocolitica* carriage in the tonsils ($P < 0.1$). More piglet suppliers, a high density of pig farms in the surroundings and a semi slatted floor in the fattening pig stables were positively associated with a higher infection level whereas the use of a disinfection bath before entering the stables was a protective factor. A poor biosecurity level was a second protective factor, although a higher prevalence may be caused by a significant interaction between the presence of pets in the stables and a poor biosecurity level.

Significance: The farms with the smallest risk of infection with *Y. enterocolitica* are farrow-to-finish farms in a low-density area using a fully slatted floor and clean disinfection baths. A poor biosecurity level combined with the presence of pets increases the prevalence.

P2-23 Salmonella: Effect of Chilling Pork on the Sampling Method

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Introduction: Salmonellosis is the second most important bacterial zoonosis in Europe. The consumption of pork is an important source for human infection. Sampling pork in the slaughterhouse is a useful control point to evaluate the entrance of *Salmonella* in the food chain. The moment of sampling and the kind of sampling is responsible for an observed decrease in recovery before and after chilling in the slaughterhouse.

Purpose: If there is a difference between sampling before and after chilling due to the sampling method, this method should be changed according to the moment of sampling.

Methods: In two slaughterhouses with a different cooling method, four strains were used in two different dilutions and transferred to pork skin. The skin pieces were sampled before and after chilling with two different sampling methods: swabbing and destruction. Both slaughterhouses were visited three times and each sample was made in triplicate. All samples were analyzed using the ISO-method.

Results: There was no difference observed between the cooling methods. The best method before chilling was swabbing, whereas after chilling the destruction method yielded the highest number of positive samples. There was no significant difference between swabbing before chilling and destruction after chilling.

Significance: A real decrease in recovery of *Salmonella* before and after chilling was absent. The sampling method should be adapted to the moment of sampling.

P2-24 Probabilistic Models for the Effect of Temperature, Water Activity and Sodium Metabisulphite Concentration on the Growth and OTA Production Boundaries of Aspergillus carbonarius Isolated from Greek Wine Grapes
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Introduction: Black aspergilli and in particular *A. carbonarius* have a central role in OTA contamination of grapes. Climatic factors could lead to fungal contamination and increase the risk of mycotoxins in these products.

Purpose: To develop a probabilistic modeling approach to determine the growth and OTA production boundaries of an *Aspergillus carbonarius* isolate on a synthetic grape juice medium as a function of water activity (a_w), temperature and sodium metabisulphite (NaMBS) concentration.

Methods: A full factorial design was implemented to assess the effect of diverse combinations of a_w (0.88, 0.90, 0.93, 0.98), temperature (15, 20, 25, 30, 35°C) and NaMBS (0, 50, 100, 150 ppm) on the growth rate and OTA production for a period of up to 28 days. Fungal growth was measured as changes of fungal diameter over time whereas OTA was determined by HPLC.

Results: No fungal growth and OTA was detected at 15°C and 0.88 a_w, irrespective of NaMBS concentration. The highest level of NaMBS was effective to suppress growth and OTA production at all temperatures and a_w in the range of 0.88-0.93. In lower NaMBS levels growth and OTA production can occur at progressively decreasing a_w at all temperatures assayed. The degree of agreement between predictions and observations was 94.3% and 86.1% for OTA and growth boundaries, respectively.

Significance: Information on fungal-food ecosystem relations is indispensable to assess the risk of contamination of grapes by *A. carbonarius* and it could be employed in HACCP implementation plans.

P2-25 Factors for Uncertainty in Mercury Risk Evaluation of Seafood Consumption
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Introduction: When addressing contaminant or more specifically mercury risk evaluation of seafood consumption, there are several factors which should be taken into account in order to assure the accuracy of the results. National dietary surveys are designed as multipurpose, and do not fit the necessity for specific categorization of food products (based on these factors) in order to appropriately match occurrence and consumption data. Consumption survey and seafood classification divergences among countries become critical when attempting risk assessments at an international level[i].

[i] ***[i]*** *Sand S., Héraud F., Arcella D. (2013).The use of chemical occurrence data at European vs. national level in dietary exposure assessments: A methodological study. Food and Chemical Toxicology 62 (2013) 7–15.*

Purpose: This study aims at documenting the existence of certain factors that have been underestimated in recent mercury risk assessments, detected when attempting to match internally compiled data with consumption rates from recent Spanish dietary surveys (ENIDE-ENALIA). It also aims at the generation of discussion on whether present risk evaluations are accurate enough, and the difficulties of international approaches to mercury risk assessment for such a complex category as currently valid for seafood products. A proposal for a new detailed seafood categorization is also given.

Methods: Approximately 12.000 total mercury analytical results in seafood have been compiled from the ANFACO-CECOPESCA laboratory database (2005-2013). Data classification has been carried out based on a variety of factors like; taxonomic link, group homogeneity concerning mercury levels, seafood commercial categories and their market importance, and

descriptive statistical data obtained for derived groups. Additional information has been collected from bibliography for underrepresented groups as well as for comparison purposes.

Results: A total of 85 seafood categories have been laid out covering the enormous variety of seafood products in Spain. For each category descriptive statistics on mercury levels are *given (Average, STD, median, minimum/maximum, percentage of results beyond legal levels, percentile 95 and percentage of left censored data).* The analysis of results highlights the importance of appropriate and detailed categorization either for occurrence and consumption data, as is the case of tuna products. Differentiation or appropriate weighing methods for wild and aquaculture products should be considered since dramatic differences have been found in mercury levels for several species. Comparison of internal results with data from different macro-datasets *(EFSA[i], FDA[ii], Karimi et al.2012[iii])*has been made underlining similarities and differences in occurrence data across studies for seafood categories.

[i] *EFSA Panel on Contaminants in the Food Chain. (2012).Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal 2012; 0(12):298.*

[ii] *FDA 1990-2010 , "National Marine Fisheries Service Survey of Trace Elements in the Fishery Resource" Report 1978,*

[iii] *Karimi R, Fitzgerald TP, Fisher NS (2012) A Quantitative Synthesis of Mercury in Commercial Seafood and Implications for Exposure in the United States. Environ Health Perspect 120: 1512–1519.*

Significance: Results highlight the importance of some variables when realizing mercury risk evaluation of seafood products, and the need for an appropriate dietary survey design in order to match occurrence and consumption data at a national level. It also underlines the difficulties of a common international approach for risk evaluation.

P2-26 Metabolomic Analysis during Growth of Salmonella on Rocket Medium

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Introduction: Current trends indicate an increase in produce-based outbreaks caused by e.g., *Salmonella* spp., while their persistence in the plant environment is due to biofilm formation either on or within the plants.

Purpose: Comparison of metabolomics, on laboratory medium and on rocket extract of S. Typhimurium (*ST*) CDC 6516-60

Methods: Luria – Bertani (LB) growth media and extract from rocket, were inoculated with *ST* and the samples were incubated at 20°C. The growth kinetics along with the metabolic profile was investigated.

Results: The final population on LB was about 1 log CFU/ml higher than rocket extract, while the metabolomic analyses with GC-MS resulted in 86 and 109 compounds for LB and rocket extract, respectively. According to PLS-DA using the web server Metaboanalyst 2.0, 50 from the 132 different compounds were selected as important (VIP>1.0) and were used for further analysis. After this analysis, the samples were hierarchical clustered according to the growth medium and phase. Additionally, the compounds 2-butanodiol, 2,3,5-trimethylpyrazine, pyrazine, 2-ethyl-5-methyl-, acetyl propionyl were the five compounds which high associated with LB. Similarly, in the case of rocket extract, the five compounds with the highest VIP score were amyl alcohol, 2-Hexen-1-ol(trans), butanamide, N-methyl-4-(methylthio)-2-(2,2-dimethylpropylidene)amino-, Pyrazine,2,5-dimethyl- and 2-Penten-1-ol,(Z)-.

Significance: The knowledge of the different metabolic compounds and the correlation with the different growth conditions of the microorganism could be fundamental for understanding its growth and the possible actions to be taken for controlling the probability of survival on food chain or food processing environments.

Acknowledgments: This work was found by the action THALIS: "*Biological Investigation Of the Forces that Influence the Life of pathogens having as Mission to Survive in various Lifestyles; BIOFILMS*", falls under the Operational Programme (OP) "Education and Lifelong Learning (EdLL)" and is co-financed by the European Social Fund (ESF) and National Resources

P2-27 A Modular Software Framework Supporting Predictive Microbial Model Generation (PMM-Lab) and Application (FoodProcess-Lab)

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Introduction: Modelling the tenacity of microorganisms in food matrices and food process chains is a small but important part of quantitative microbial risk assessments. Models and modelling software helping to predict the fate of bacteria can help to improve food safety.

Purpose: The purpose of this research was to develop a modular framework supporting users in modelling and predicting bacterial tenacity along food process chains.

Methods: The open-source software programs PMM-Lab and FoodProcess-Lab are newly developed Java plug-ins to the open source software framework Konstanz Information Miner (KNIME, www.knime.org). They contain an integrated database for experimental test results, mathematical formulas, estimated predictive models, food process chains and corresponding parameters. As a proof-of-concept application the fate of *Salmonella* spp. during the production of raw, ready-to-eat (Mettwurst) sausages was calculated using FoodProcess-Lab and models generated in PMM-Lab.

Results: A new open-source software framework for application of predictive microbial models on food process chain has been developed. In a proof-of-concept application the *Salmonella* spp. concentration model calculated an increase from an initial concentration of 3.0 log (CFU/g) to 5.5 log (CFU/g) over the course of the Mettwurst production process. Increasing the nitrite content from 50 to 100 ppm markedly reduced bacterial growth.

Significance: The software introduced is the first open-source solution in the field. It is moreover the first system that also provides features to create a model repository and a food process chain database. With this PMM-Lab/FoodProcess-Lab enables users to predict bacterial tenacity in a short period of time, for example during crisis situations. Workflows used for model generation and prediction can be exported and freely shared with others including raw data, formulas and estimated model parameters.

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P2-28 Storage Temperature Rupture in Air Catering: A Validation Study with a Risk Evaluation Based on Probabilistic Approach

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Introduction: This study was conducted for an airline catering company to validate temperature exemptions to transport meal trays from their production site to the aircraft.

Purpose: The purpose of this study is to validate a temperature rupture of 2h00 at +8°C considering the storage temperature applied for ready-to-eat products is 72h00 at +3°C. The methodology developed allows covering a wide range of products by focusing on the most sensitive products per category.

Methods: The validation method is divided into several steps:

- Classification of the different food products covered by this designation according to their level of risk: realization of durability studies and growth simulation according to intrinsic factors (pH, a_w) for 2 microorganisms selected by risk analysis *Listeria monocytogenes* and *Bacillus cereus* using Sym'Previus models.

- Determination of the growth potential of *Listeria monocytogenes* and *Bacillus cereus*: challenge tests are performed on 6 target products.

- Determination of the maximum growth rate μmax and lag phase of *Listeria monocytogenes* and *Bacillus cereus* on the most sensitive matrix: full challenge test are performed on the most sensitive target product.

- Probabilistic approach to evaluate the risk due to the temperature rupture of 8°C 2h00: Using historical results database indicating prevalence of microbiological contamination and the growth characteristic of the most sensitive product, probabilistic approach is applied using Monte Carlo simulations (Sym'Previus models).

Results: The challenge test performed on the concerned products demonstrated no significant bacterial growth (< 0.5 log) during the microbiological shelf life of the products including a temperature rupture of 2h00 at 8°C (conservation during 60h at 3°C, 2h at 8°C, and 10h at 3°C). The study demonstrates that the temperature rupture of 2h00 at 8°C has no significant effect on the

validated shelf life of the products of the most sensitive products delivered by the catering company at the stage of airplane delivery. Indeed, in the worst case scenario, the percentage of products reaching critical limits for *Listeria monocytogenes* and *Bacillus cereus* remains close to 0 %.

Significance: Based on this study, the temperature designation of 2h00 at 8°C has been validated by the French Food Safety Agency (ANSES).

P2-29 Assessing the Food Safety of "Pesto alla Genovese" Sauce, an Italian Artisanal Product, vs. Clostridium botulinum.
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Introduction: *Clostridium botulinum* is an anaerobic, sporeforming bacterium. Although botulism is rare, its mortality rate is very high. Isolates of *C. botulinum* belong to two main groups: proteolytic (mesophilic) and non proteolytic (psycrophilic) strains. The "pesto alla genovese" is an artisanal Italian product which, from a chemical-physical point of view, could support the growth of proteolytic strains.

Purpose: A *C. botulinum* risk assessment on the "pesto alla genovese" sauce was conducted by means of a challenge study and a stress test.

Methods: The study was composed by 2 steps. The first one consisted in verifying *C. botulinum* growth on a batch by inoculating a mix of proteolytic strains. The samples were incubated at 20°C and the microbiological and chemical-physical analysis were conducted from time 0 for 15 days in 6 different dates. The second step aimed to define a safety threshold of the product by means of a stress test at different water activity (a_W) values by diluting the single samples.

Results: The first phase of the study showed no growth of *C. botulinum* in the batches of pesto alla genovese because of the hurdle represented by the pH and the a_W. The second phase showed a large variability inside and across the studied batches suggesting that a_W is the critical parameter affecting the growth of *C. botulinum in the "pesto"*. Further to this, the plate count obtained in this phase showed that the product, although at different a_W values, does not support the growth of *C. botulinum* because of the antimicrobial effect of the indigenous lactic flora which reached high concentrations.

Significance: A good risk assessment vs. *C. botulinum* should consider the product and its manufacturing process steps, the experimental data obtained by a challenge and a stress test, the indigenous microflora and the critical parameters of growth-non growth (pH and a_W).

P2-30 Inactivation of Salmonella spp. in Raw Pork Meat by Using UV-C Illumination
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Introduction: *Salmonella* is becoming an increasing concern for the swine industry all over the world since despite attempts to control foodborne pathogens, a significant number of pork products are still contaminated, leading to potential public health issues and economic losses for industries.

Purpose: Study the effect of the illumination of raw pork meat with UV-C light to inactivate *Salmonella* spp.

Methods: Raw pork pieces of meat weighting about 25 g were cut and inoculated with different loads of *Salmonella* bacteria. UV-C illumination was carried out using Osram HNS 6W lamps with a light flux in the range from 14 to 42 W/m^2. Samples were homogenized in 25 ml of sterile buffered water peptone and concentration of viable *Salmonella* and microflora naturally present in meat was quantified by dilution and plating in agar culture media.

Results: Illumination with a UV-C light flux of 14 W/m^{2}during 5 to 15 min leads to a reduction of 90% in the concentration of viable *Salmonella*. The efficiency of the process is not negatively affected by the presence of a high load of *Salmonella* bacteria, achieving comparable percentage inactivation that for lower bacterial loads after similar illumination time. The high variability in the concentration of natural microflora present in meat does not seem to affect the efficiency of the UV-C treatment against *Salmonella*. The UV-C illumination also allows reducing microbial load in non-inoculated pieces of meat, extending the shelf life of the raw product and reducing the numbers of pork testing positive for *Salmonella* in the industry.

Significance: UV-C treatment reduces not only the concentration of pathogenic *Salmonella*, but also of spoilage bacteria naturally present in pork, without affecting its fresh appearance. This technology improves safety and shelf life of the product, guaranteeing its natural flavor and taste.

P2-31 Fate of the Mycotoxins DON and Enniatins when Cooking Pasta
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Introduction: Knowledge on the fate of mycotoxins during processing may assist risk assessors on the interpretation of data on occurrence of mycotoxins in raw materials. It is unknown to what extent cooking influences the content of enniatins in the pasta.

Purpose: Aim of this study was to assess the influence of pasta cooking on the occurrence of the mycotoxins: deoxynivalenol (DON), enniatin A (ENNA), enniatin A1 (ENNA1), enniatin B (ENNB) and enniatin B_1(ENNB1).

Methods: Three contaminated dry pasta samples containing both DON and ENNB and a blank dry pasta were selected. The samples were cooked (100 g to 500 ml tap water) for 10 min in a laboratory setting according to a pre-set protocol. Cooked pasta, the cooking water and the cooked pasta after rinsing were sampled. Samples were stored at -20°C and ground under liquid nitrogen before analysis. Samples were analysed in duplicate for DON, ENNA, ENNA1, ENNB and $ENNB_1$ using an in-house validated method (extraction with acidified acetonitrile/water (80%/20%) followed by filtration and separation/identification using LC-MS/MS with acidified eluents).

Results: The mass balance showed that all the mycotoxins were recovered either in the pasta or in the cooking/rinse water. Roughly 40% of the DON was transferred from the pasta to the cooking water while the enniatins remained virtually completely in the pasta. This is according to expectation and in agreement with literature since DON is much more hydrophobic than the enniatins. It can however, not be excluded that changes in cooking conditions (times and rinsing intensity) may slightly modify the mycotoxin retention percentages.

Significance: The results will assist in the assessment of the exposure of humans to mycotoxins.

P2-32 Survey of Pathogens Isolated from Clinical Mastitis Cases
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Introduction: Clinical mastitis is one the major reasons for veterinary drugs use in dairy farms with implications to milk production, quality and safety.

Purpose: Here, a survey was conducted during January to May 2013 (rainy season) to evaluate the pathogens isolated from milk samples originating from cows with clinical mastitis.

Methods: Thus, 116 milk quarter samples were aseptically collected. Clinical mastitis was assessed by udder examination and strip cup test. Bacterial analysis was conducted by culturing 0.01 ml of each sample on 5 % ovine blood agar plates and MacConkey agar. The plates were incubated for 48 hours at 35°C, which was followed by Gram staining, observation of colony morphology and biochemical testing.

Results: In 58.62% (n = 68) of milk samples from clinical mastitis, bacteria fail to grow even after 48h of conventional culture. Here, we isolated *Escherichia coli* (6.04%, n = 7); coagulase negative staphylococci (CNS) (6.70%, n = 8); *Streptococcus uberis* (4.31%, n = 5); yeast (4.31%, n = 5); *Streptococcus* sp. (4.31%, n = 5), which exclude *S. agalactiae*, *S. bovis*, *S. uberis* and *Enterococcus* sp.; *Corynebacterium* sp. (3.45%, n = 4); *Staphylococcus aureus* (2.59%, n = 3); *S. equinus* (1.75%, n = 2); *Klebsiella* sp. (0.86%, n = 1); *Pseudomonas* sp. (0.86%, n = 1); *S. uberis* and CNS (2.59%, n = 3); *Streptococcus* sp. and CNS (0.86%, n = 1); *S. aureus* and *E. coli* (0.86%, n = 1); *S. aureus* and *Streptococcus* sp. (0.86%, n = 1) and *S. aureus* and *S. uberis* (0.86%, n = 1). No sample was regarded as contaminated (yielded ≥ 3 colonies from different bacterial species).

Significance: We suppose that pathogens could not be isolated due several factors such as the inflammatory response that implies on bacteria survival, and other methods such as real time PCR are needed to detect pathogens from clinical mastitis cases.

P2-33 Environmental Contaminants, Pesticides and Natural Toxins in Biobased Food Contact Materials
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Introduction: Biobased packaging materials based on polymers from agricultural sources (crops) are interesting from a sustainable point of view. New biobased materials are being developed and should comply with European food packaging regulations. However, European legislation does not cover the risks of chemical contamination of the raw agricultural sources used.

Purpose: The aim of this work was to study the content of pesticides, natural toxins (mycotoxins and plant toxins) and persistent organic environmental contaminants in both biobased packaging materials and the raw materials used for their manufacturing.

Methods: A set of nine samples of biobased plastic materials from polylactic acid (PLA), corn starch, potato starch and renewable polyethylene, a sample of PLA pellets and three samples of potato starch were tested. Pesticides, and natural toxins were analysed by extraction with water and acidified acetonitrile (1% formic acid) and analysed by LC-MSMS. PAHs, PBDEs and PCBs were extracted with ethyl acetate, cleaned up silica SPE columns and analysed by GCxGC-ToFMS.

Results: Fenhexamid, pendimetralin, bendiocarb and pyridaben were detected in corn-starch based films at levels ranging 1.7-50 mg/kg. Alternariol (0.1-1.1 mg/kg), alternatiol methyl ether (0.1-0.3 mg/kg) and coumarin (11.6-52.6 mg/kg) were detected in almost all the samples, while fumonisins, solanine, lupaine, lupinine and sparteine were only present in potato starch raw material. This may mean that the latter toxins are removed during the manufacturing process. Bio-PE contained traces of PCB28 and PCB52.

Significance: The process of manufacturing biobased packaging materials does not always eliminate contaminants originating from the raw materials. The levels of contaminants found in this study were compliant with European food legislation, therefore, no migration tests needed to be carried out. The crops used for production of these biobased packaging materials originated from Europe, with a strict legislation framework.

P2-34 A Novel Diffusion-based Time-temperature Indicator to Monitor Microbial Quality of Angelica Juice

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Introduction: Non-pasteurized angelica juice (NPAJ) is preferred despite a short shelf life because thermal pasteurization can cause deterioration of its beneficial effects and sensory quality. Time-temperature indicators (TTI) can monitor changes in temperature and time during distribution and storage of NPAJ to ensure its microbial quality.

Purpose: This study focused on characterization of a novel diffusion-based TTI system for monitoring the microbial safety of NPAJ during distribution and storage. A mathematical kinetic model was established by measuring time-temperature dependent diffusion distances of the TTI at various temperatures.

Methods: Diffusion of isopropyl palmitate (IPP) injected into the TTI was measured at various temperatures, and a mathematical model based on relationships between diffusion and time-temperature was established. Predicted diffusion distances were compared with measured values to validate the kinetic model. The relationship between TTI response and microbial growth (total aerobe counts) in NPAJ was investigated under both isothermal and dynamic temperature conditions.

Results: Predicted results from the established model were in good agreement with experimental results. The IPP diffusion distances were 9.7 mm and 7.2 mm when total aerobe counts in NPAJ reached a critical bacterial level (6 log CFU/ml) during storage at 15°C and 25°C, respectively. Neither bacterial growth nor IPP diffusion in the TTI was observed at 5°C storage for 48h. During dynamic temperature storage, the bacterial counts reached a critical point after 27.4h while IPP in the TTI diffused a distance of 7.6 mm. IPP diffusion of 7.0 mm in the TTI is considered to be a threshold point for the bacterial quality of NPAJ.

Significance: The IPP-based TTI system responded well when refrigerated foods are left at higher than recommended refrigeration temperatures. The TTI characterized in this study can be considered as a useful device to monitor microbial quality and temperature abuse during storage of chilled foods.

P2-35 Inactivation of Bacillus subtilis Spores Using TiO_2-UVC Photocatalysis

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Introduction: *Bacillus subtilis* is known to have the ability to form a tough and protective endospore, allowing it to tolerate harsh environmental conditions, and is frequently implicated in the spoilage of foods. There is an increasing interest in the

application of TiO_2-UVC photocatalysis (TUVP) for the disinfection of food surfaces. However, there is no attempt to inactivate *B. subtilis* spores using the TUVP.

Purpose: The objective of this study was to evaluate the TUVP to inactivate *B. subtilis* spores.

Methods: *B. subtilis* spore suspensions were treated with TUVP, UVC, and heat (80°C). Dipicolinic acid (DPA) release was measured with HPLC to clarify the inactivation mechanisms by the TUVP. Propidium iodide staining method was used to observe inner membrane damages in the *B. subtilis* spore.

Results: Heat treatment at 80°C could not inactivate *B. subtilis* spore, regardless of processing time. However, a 6-log reduction of *B. subtilis* spores was obtained by TUVP when the UV dosage was 9.6 J/cm^2. DPA release under TUVP was increased rapidly as the UV dosage increases.

Significance: This study showed that the TiO_2-UVC photocatalysis is the most effective method to inactivate the *B. subtilis* spore.

P2-36 A Concept for Standardized Description of Experimental Microbial Data and Predictive Microbial Models
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Introduction: In the food quality and food safety domain there is an increasing demand for reliable, well annotated predictive microbial models and data. Several groups and researchers have successfully applied and published mathematical models that predict growth, survival or inactivation of microorganisms in different food matrices under various environmental and processing conditions. In order to support scientists in identifying models that fit to their specific needs, a standardized data format to describe experimental data and predictive models would be beneficial.

Purpose: This research proposes standardized data exchange formats capable of describing experimental microbial data as well as predictive microbial models. Such standards are of high relevance to software developers, as its implementation would ease the exchange of data and models between different software tools. Such data formats would additionally support public data collections and facilitate the development of predictive microbial model repositories. Finally it would improve transparency, quality, validity and annotation of these resources.

Methods: It is proposed to adopt two existing data exchange formats from the field of systems biology:

- Numerical Markup Language (NuML) for experimental data
- Systems Biology Markup Language (SBML) for predictive microbial models

Results: A proof-of-concept will be provided demonstrating that data and meta data relevant for model generation and model application can be transferred with the proposed formats. Moreover a software tool supporting the import and export of NuML / SBML-encoded files will be presented.

Significance: NuML and SBML can be used as open data exchange formats in the domain of food safety and food quality modelling. It is demonstrated that these standards can easily be adapted and applied to this domain and thus might support existing community resources.

P2-37 New Chromatographic Methods of Analysis for Qualitative Assessment of Fatty Acids and Chlorophyll in Pistachio
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Introduction: With the opening of non-European markets food is often imported from countries with low-quality management and control systems. Developing certified analysis systems enables both to protect food products and to safeguard consumer's health. A method for detection analysis of pistachio was developed in the Food Chemistry and Technology Area laboratories of Istituto Zooprofilattico Sperimentale della Sicilia.

Purpose: The aim of this work is validating an analytical method (classified as accredited method) to identify some of the main chemical and organoleptic properties of pistachio (fatty acids and chlorophyll) to certificate the quality and specificity of the product.

Methods: The results show a specific percentage composition pattern of the identified fatty acids (linoleic, oleic, palmitic, and stearic acids) contained in the fruit. Indeed, the extracted chlorophyll values showed distinctive qualitative characteristics associated to the food product.

Results: Fat extraction is carried out using an Accelerated Solvent Extraction system. The esterification of fatty acids to fatty acid methyl esters is performed using an alkylation derivatization reagent. Analysis of fatty acid methyl esters is performed using a gas-chromatic technique with a FID detector [Thermo Trace GC Ultra] using a 100% PoliethylenGlycole capillary column. Chlorophyll sample were extracted and purified by QuEChERS method. Extracts were then filtered through 25 mm diameter polypropylene syringe filter (MFS HP020, 0.2 µm pore size) to remove cell and filter debris. Chlorophylls were detected by HPLC UV diode-array spectroscopy (430 nm).

Significance: Detecting the percentage of fatty acid methyl esters and the chlorophyll content make it possible to attribute specific marks to the product which is useful to accurately identify its species and typization. This method provides new tools to protect and certify pistachio.

P2-38 Analysis of Toxoplasma gondii in Pork Meat Destined to the Processing of Dry-cured Ham

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Introduction: Toxoplasmosis is a worldwide-distributed zoonosis caused by the protozoan *Toxoplasma gondii*. Infection can produce a severe disease in immunocompromised people and abortions in pregnant women, as well as perinatal death, fetal abnormalities, or reduced quality of life in children who survive prenatal infection. Epidemiological studies confirm ingestion of raw or undercooked meat products containing tissue cysts as a risk factor associated with toxoplasmosis. Pork, which is routinely used in the production of meat products, has been considered as an important source of *T. gondii* infection in humans. Seropositivity in general is a good indicator of the presence of viable parasites in tissues and the level of isolation increases with antibody titer. However, viable *T. gondii* has been isolated from seronegative pigs.

Purpose: With the aim of contributing to the risk assessment process, the present study was carried out to estimate the presence of infective *T. gondii* in raw ham from farm pigs with low serological titers destined to the processing of dry-cured ham. To assure risk evaluation, target organs (tongue and heart) were also studied.

Methods: Eleven pigs with a titer ≤ 1:40 were killed in a commercial slaughterhouse and their hams, tongue and heart were analyzed. A concentration bioassay technique was used to demonstrate viable bradyzoites in the tissues (n = 88 analyses). Blood samples were drawn from mice 60 days after inoculation, and serum was examined for *T. gondii* antibodies by indirect immunofluorescence assay

Results: No viable parasites were detected in raw hams or in target organs.

Significance: These results indicate that raw hams from pigs with low serological titers are suitable for the processing of cured ham with an insignificant risk of acquiring toxoplasmosis.

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P2-39 Evaluation of an In-house Laboratory for Listeria Self-monitring of a Dairy Production Plant

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Introduction: Self-control for the occurrence of *Listeria monocytogenes* is mandatory for dairy plants in Europe. Nevertheless these laboratories are often driven by experts from the dairy production and not according to good laboratory practise. In this study a laboratory for detection of *Listeria* using enrichment and miniVidas® was investigated.

Purpose: The laboratory of a dairy production plant was analysed on a scientific basis. Testing on *Listeria* was performed and the bacteriophage P100 was tested in the lab due to use of this phage for decontamination in the plant. The use of controls of all steps of the protocols was checked and the workflow and handling was investigated.

Methods: The detection of *Listeria* was performed according to ISO-11290 and according to *Rossmanith (2006)*, when qPCR was performed. Bacteriophage-P100 was detected according to *Kim (2008)* by plaque assay and amplifying the gp104 region by qPCR.

Results: The analysis of the workflow of the laboratory showed that false-positive results are mainly to expect due to defective consumables. Nevertheless the probability of false-negative results is low in this laboratory. False-positive results are from a higher probability. This results from the analysis of sampling, laboratory hygiene and handling.

The analysis of the *Listeria* monitoring during a nine-month period showed that positive samples in the production plant are linked 100% to *Listeria*-positive raw milk samples from the suppliers thus indicating contaminations in the lab. This confirms the expectation of false-positive results in the lab. The occurrence of P100 in the environment of the plant suggests a high risk for false-negatives.

Significance: The use of state-of-the-art technology is not sufficient if the surrounding parameters and the environment are not included in structural design of the workflow and methodology. Moreover the use of bacteriophages increases the risk for false-negative results due to inhibition of growth of the target bacteria in enrichments.

P2-40 Food Safety Impact of Greenhouse Management Practices: Delphi Expert Elicitation Approach
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Introduction: Over one half of fresh tomato sold in the US is grown in greenhouses. Management practices in tomato greenhouses in North America were documented previously.

Purpose: Delphi expert elicitation was performed to quantify food safety impact of several greenhouse management factors (water management, workers, environment and greenhouse design, equipment sanitation, animals, waste, and traceability).

Methods: The pre-tested questionnaire was completed by 20 US and International experts in food safety of fresh fruits and vegetables. Two rounds of Delphi process were completed with the expert agreement of $\geq$ 70%.

Results: Greenhouse irrigation was rated by experts to be a highly important source of contamination with human pathogens (n = 20; 78.95%) and the majority tough that testing for both pathogens (73.71%) and indicator organisms (60%) was important in preventing the contamination of fruit. However, after the detection of pathogens in irrigation water, it was not clear what corrective would be best implemented relating to the time before the product can be safely sold on market. Although experts agreed that people only occasionally may introduce pathogens into the greenhouse (n = 16; 92.9%), handwashing and effective health policy were ranked the most effective in preventing contamination from employees and visitors (n = 20; 100% and 72.22%, respectively). The presence of human pathogens in the greenhouse environment presented an important source of contamination for edible tomato fruits (n = 20; 84.23%). Experts also agreed that livestock and poultry operations should be at least 250 feet away from the greenhouse (88.24%). Experts considered greenhouse size, construction materials and growing medium to have little impact on food safety of greenhouse grown produce.

Significance: The findings of this study enhanced the understanding of the factors that affect food safety of greenhouse produced vegetables and should be considered in designing and developing risk based food safety systems for tomato greenhouse production.

P2-41 Decision Support Tool for Producers of Advanced Ready-to-Eat Foods to Ensure Safe, Tasty and Nutritious Products
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Introduction: Food business operators must make daily decisions about food safety and quality, often based on limited data.

Purpose: To map the topics where decision support is needed and develop a prototype of a multidisciplinary decision making tool.

Methods: Three companies were visited by researchers for several days to identify the real industry challenges, learn where deviations occurred, as well as how and when decisions were made. Afterwards, experiments were carried out in the lab with simulated industry products. More than 40 variations of potato and pasta salads with chicken meat, vegetables and dressing

inoculated with *Listeria monocytogenes* and *Staphylococcus aureus* were investigated. The pathogen levels during storage were categorized in red, yellow and green based on "significant growth," "no growth or slight reduction" and "100 % inactivation."

Results: The production of advanced ready-to-eat products was very complex. Decision support was desired by industry within topics related to multidisciplinary dilemmas like:

- The demand by customers for many different products versus the increased risk of mistakes by differentiated production.
- The desire of high productivity per "person and square meter," particularly the use of pre-cooked raw materials from other suppliers versus in-house processing and control.

The laboratory experiments indicated that the salad compositions, process and storage scenarios, could be used to predict the pathogen level categories by using the pH, the lactic acid bacteria content in the dressing, the storage temperature and the content of meat as input parameters in a log linear growth model. The data visualized how the salad composition could be adapted to improve the safety, how replacement of an ingredient could be done without compromising quality or safety, and which deviations that lead to a change of food safety category and thereby a need for corrective action.

Significance: Beside the results themselves, the main significance of the work is how changes and deviations can be categorised and presented in an easy and rapid way for people with limited training in microbiology who have to make decisions. The results represent a part of a prototype multidisciplinary decisions support tool for practical use in industry currently being developed in the EU project STARTEC. The cost/benefit and nutrition parts of the tool are not presented here.

P2-42 Integrated Shelf Life: a New Approach to Predict the Shelf Life of Food Products

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Introduction: Appropriate tools for the prediction of shelf life of food products should be available for the food business operators, in order to not only assess the evolution of the organoleptic characteristics but also evaluate the microbiological profile which ultimately may require to perform an integrated shelf life study.

Purpose: The shelf life of liver pâté crostini was evaluated taking into account the sensory analysis results and the microbiological profile of the prevalent microbial population, thus obtaining a shelf life prediction for other time-temperature profiles.

Methods: Three batches of the product were incubated at +4°C for 7 days and for the following 38 days at 8°C. Microbiological and organoleptic data were collected at given times. Using the log counts average of the batches, a single growth curve was created. The product sensory reject time was captured on the curve and the shelf life was estimated to find a statistical correlation between the sensory and the microbiological data at other time-temperature profiles.

Results: The prevalent microbial population (lactic flora) started from an initial concentration of about 2 log CFU/g, stopped growth after 16 days (data average of 3 batches). The product sensory reject time was in average estimated around 30 days with the appearance of an abnormal coloration. Starting from the latest time in which the product was fully edible (22 days) and considering an additional margin of safety, a shelf life was estimated around 19 days. The average times between the beginning of the stationary phase and the appearance of an abnormal coloration were similar. Although it is unclear if there is definitive link between the microbiological profile of the products and the onset of the abnormal pigmentation, the time correlation was used to predict shelf life at other time-temperature profiles.

Significance: The integrated shelf life is a great opportunity for FBO because it allows to set up a shelf life prediction approach for any time-temperature profile.

P2-43 Ultrasounds: A Potential Eco-friendly Intervention Strategy to Maintain Quality of Irrigation Water

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Introduction: GAP guidelines recommend growers to test their irrigation water for microbial and chemical contaminants as a preventive measure. In the case of an insufficient water quality; intervention strategies based on water treatment should be taken. Water from difference sources can be used in the production of fruit and vegetable crops, which includes the use of reclaimed

water. Considerable seasonal or climatic variations in water quality are also possible. Irrigation water quality should be also controlled due to the risk of algae accumulation which harms irrigation systems.

Purpose: The purpose of this study was to evaluate the capacity of ultrasound to maintain the microbial quality of irrigation water, including treated wastewater.

Methods: Five reservoirs belonging to five intensive farms from Spain (37º44´N, 0º57W) were selected. Each reservoir was subjected to one type of disinfection treatment: (i) untreated surface water (control); (ii) surface water treated with chlorine; (iii) surface water treated with ultrasounds (20 KHz); (iv) surface water treated with ultrasounds (40 KHz); (v) wastewater treated with ultrasounds (20 KHz). The physico-chemical and microbiological quality of irrigation water was monitored.

Results: Ultrasound treatments did not reduced the organic matter content of irrigation water when compared to chlorine. *Enterococcus* and total coliforms were significantly higher in untreated water than treated irrigation water. *E. coli* levels of irrigation water were significantly reduced by the disinfection treatments. However, no significant differences were observed between the different disinfection treatments. Algae levels were also reduced by all the tested disinfection treatments, which reduced the risk of filter collapse from high (control reservoir) to medium (treated reservoirs). The quality of the water significantly affected the effectiveness of the ultrasound treatments, especially when reclaimed was treated.

Significance: These results suggest ultrasounds as a potential eco-green alternative to chemical treatments for the control of the microbial quality of irrigation water.

P2-44 Monitoring the Kinetics of the Germination and Activity Recovery of Bacillus Spores after a Heat-treatment by Flow Cytometry

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Introduction: Heat treatment is the major hurdle used to eliminate spore populations in food products. However, spores can resist this treatment and are able to germinate and recover activity during storage. Using culture-based methods, bacterial population is estimated by counting the colony forming units that account for the population ability to germinate and recover a growth activity without quantifying late germinating or hyper-dormant spores.

Purpose: The aim of this work is to develop a method to monitor the germination and activity recovery of *Bacillus* spores over time and quantify the effect of recovery conditions on germination and activity recovery kinetics.

Methods: Subpopulations of spores of *B. weihenstephanensis* KBAB4 and *B. licheniformis* Ad978 after a heat treatment have been identified using flow cytometry. Intact spores, damaged spores, germinated spores, outgrowing cells were clustered using fluorescent markers: Syto9 (live cells), PI (dead cells) and CFDA (esterase activity) in conjunction with classic methods (OD 600nm, cultural methods and phase-contrast microscopy). The transition through each sub-population, corresponding to physiological stages, has been observed over time by flow cytometry.

Results: The intact and heat-inactivated spores were not permeable to Syto9 or PI and were dimly fluorescent; germinated spores could be marked by Syto9 or PI, and had the same size as intact spores; the vegetative cells were well marked with Syto9 or PI but were bigger than germinated spores. Finally, CFDA marking allows following the activity recovery since the germination stage. Thanks to monitoring over time, germination and activity recovery kinetics have been determined.

Significance: This work allows a description of the behavior of hyper-dormant or late germinating spores after a heat-treatment. The germination and activity recovery behavior of individual spores after a heat-treatment could improve the control of spore-forming bacteria in food.

P2-45 A Comparison of the Quantitative Enumeration of Yeast and Molds Using 3M Rapid Yeast and Mold Plates and Other Media Used in Traditional Microbiology Testing

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Introduction: Yeast and mold contamination is a major concern to food producers and current methods of detection for this group of microbes, a 5-day incubation, results in time constraints to food producers as they are forced to wait for this important microbiology test result.

Purpose: The purpose of the trial was to compare the 3M TM Rapid Yeast & Mold plates to the ISO 21527: Parts 1 and 2, method for the enumeration of viable yeast and mold in a range of food samples.

Methods: Twelve food types were purchased, from retail food outlets, and the indigenous level of yeast and mold tested using both the 3M TM Rapid Yeast & Mold plates & ISO 21527: Parts 1 and 2 reference methods. These foods having low levels of indigenous yeast and mold contamination were artificially contaminated with 4 yeast spp. and 4 mold spp. The samples were then retested using both the 3M TM Rapid Yeast & Mold plates & ISO 21527: Parts 1&2 reference methods and the results compared.

Results: The quantitative results, showed no statistical differences between the 3M TM Rapid Yeast & Mold plates, after 60 hrs and the results seen on Dichloran rose bengal chloramphenicol agar (DRBC), Oxytetracycline-Glucose Yeast Extract (OGYE) Agar and the Dichloran 18% glycerol agar (DG18), after a 5-day incubation.

Significance: The 3M TM Rapid Yeast & Mold plate allows a faster detection of yeast and mold levels in food samples than the traditional ISO 21527: Parts 1 and 2 reference methods.

P2-46 Effect of Gamma Irradiation on the Mirobiological, Chemical and Sensory Properties of Cottage Cheese Dairy Products

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Introduction: Among several types of foods, dairy products (cottage cheese, cream cottage cheese desserts) are often requested but not allowed in low microbial diets for immuno-compromised patients. Based on their nutritional properties, these products would be highly recommended also for this consumer group.

Purpose: The aim of our studies was to determine the radiation doses provide microbiological safety of selected dairy products without diminishing the quality/nutritional/sensory parameters.

Methods: Microbiological parameters of cottage cheese chocolate bar (Túró Rudi), the “national snack of Hungary”, and cream cottage cheese desserts were determined by traditional culturing techniques. Fatty acid analysis was carried out by gas chromatographic method. Effect of irradiation on conjugated dienes was determined spectrophotometrically. Malonaldehide (MDA) analysis was performed according to the method described by Menoyo et al., 2003.

Results: Total aerobic bacteria, aerobic and anaerobic spore counts in Túró Rudi and cream cottage cheese desserts ranged from 2 to 3 logs CFU/g. Radiation treatment with 2.0 kGy dose reduced the low initial total aerobic plate count by about one log-cycles. Results of fatty acid analysis indicate that undesirable lipid oxidation did not occur in irradiated samples up to 3 kGy dose. The radiation dose of 3 kGy caused rancidity in the conjugated dienes of chocolate part of cottage cheese desserts. Statistically significant differences in organoleptic properties (colour, odour, taste and texture) were determined only in the taste of Túró Rudi irradiated with 2 kGy dose.

Significance: Results suggest that low dose irradiation can improve the microbial quality of these products, providing a wider selection of foods for immuno-compromised patients.

Acknowledgment: Assistance of AGROSTER Co. Ltd Budapest in irradiation of samples is highly acknowledged. This work was supported by IAEA Nr.16243 project.

P2-47 Effect of High Pressure Processing on the Survival of Listeria monocytogenes and Shelf Life of Chicken Fillets

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Introduction: High pressure processing (HPP) is a non-thermal inactivation technology, to control spoilage and foodborne pathogens in several foods.

Purpose: To evaluate the effect of HPP on *Listeria monocytogenes* (LM) and indigenous microbiota of chicken breast fillets (CBF).

Methods: CBF were inoculated with LM (5 strains) at three different initial levels of inoculum (10^2, 10^4, 10^6 CFU/g), packaged under vacuum subjected to HPP of 500 MPa for 10min. The samples were stored at 4 and 12°C. TVC, LM, pseudomonads, *Brochothrix thermosphacta*, lactic acid bacteria, *Enterobacteriaceae*and yeasts populations were determined, whereas enrichment was followed to ensure the presence/absence of LM. Sensory analysis of non-inoculated samples was conducted to determine the shelf life of the product.

Results: After applying HPP, counts of LM were reduced to the detection limit of the enumeration method, irrespective of the inoculum. There was an absence of the pathogen after the 2nd day of storage for the low inoculum and presence for the medium inoculum cases for both temperatures. In the case of high inoculum, the pathogen population increased, reaching 3 and 7 log CFU/g at 4 and 12°C, respectively. Regarding the control samples (without HPP treatment), the pathogen population remained stable at 4°C, but increased 1.9-3.5 log CFU/g after storage at 12°C depending on the inoculum.

Significance: It was shown that HPP may increase the shelf life of CBF enhancing the safety of the product.

Acknowledgment: *The action THALIS:* "Development, mathematical modeling and optional design of non-thermal technologies for processing, packaging, distribution and storage of safe high quality food products", has been co-financed by the European Union (European Social Fund - ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: THALES: Reinforcement of the interdisciplinary and/or inter-institutional research and innovation.

Poster Session 3 - Antimicrobials, Applied Lab, Communication Outreach and Education, Dairy and Other Food Commodities, Epidemiology, Food Toxicology, Seafood
Friday, 9 May 2014: 10.00–14.00

P3-01 Improving Food Safety Practice in Meat Sector "An Innovative e-learning" Approach

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Introduction: The EU's primary concern is to improve the health and wellbeing of European citizens through higher quality and safe food. By considering this priority, there is a strong need to follow up the new technologies in food safety sector and update the knowledge on new and changing EU legislations. To fulfill this need there should be an integrated, sustainable training module based on the demands of each country and EU legislations. However there is not such an online training specialized for food safety in the meat sector which can cover these needs, so SAFEMEAT_EU project will aim to promote e-learning applications in EU as an example of easy learning.

Purpose: SAFEMEAT_EU Project is an innovative e-learning program focusing on the meat sector which is going to be piloted across all European countries. SAFEMEAT_EU aims to enhance the qualifications and skills of meat sector workers and experts-trainers to make all meat and poultry processors compatible with and conforming to the present EU regulations on food safety.

Methods: To achieve this, one candidate and three European member countries participated in the project to share their knowledge and experiences on food safety issues and e-learning technology.

Results: The training program will be developed by a pan European committee of top professionals as to bring about a state of the art curriculum. The effects of the program will be measured by evaluation questionnaires and surveys. The project will start on December 1st 2013. An introduction of the project and the first 2-3 months results will be presented in IAFP's European Symposium on Food Safety.

Significance: To reward their endeavor the EU decided to fund the project through the European Program "*Leonardo da Vinci*" (2013-1-TR1-LEO05-47583).

P3-02 Production of High Value-added Compounds from Food Processing Wastes

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Introduction: Food processing wastes could be considered as a source of valuable nutraceuticals and the valorization of fruit by-products as a source of dietary fiber, pectin and phytochemicals (phenols, tannins, flavonoids and carotenoids) and natural coloring and clouding agents.

Purpose: The present work was conducted to study the physical and chemical properties of wastes (solids and liquids) generated during processing of some fruits and vegetables and utilization as a value-added products.

Methods: The determination of phytochemical profiles and dietary fiber composition of dried solid wastes was also studied as well as evaluation of their antioxidant activity. In addition, the utilization of solid wastes as value-added products was studied.

Results: The results also indicated that the use of methanolic extracts of dried solid wastes had antifungal effect against *Aspergillus niger*, *Fusarium solani*, *Aspergillus paraseticus* and *Aspergillus flavus*. Moreover, the results showed that the percent of fungal inhibition increased by increasing the extract concentration. A complete growth inhibition (100%) of *Fusarium solani* and *Aspergillus flavus* was achieved by using 1250 ppm of dried state of mango kernel extract or apricot pomace extract or strawberry pomace extract. Meanwhile, dried orange peel extract caused 100% inhibition for *Aspergillus flavus* at the same concentration.

Significance: The utilization of the separated wastes for the production of value-added compounds such as food thickeners pectin, dietary fiber and phytochemicals compounds (phenolic compounds, flavonoids, ascorbic acid, fiber sources and tannins) as well as the use in biogas production or animal feed.

P3-03 Use of Moringa Oleifera (Drumstick) Seed as Natural Coagulant and Antimicrobial Agent for Nile River Water Treatment

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Introduction: Water quality and treatment is becoming an increasing concern, especially in developing nations, where water quality is poor and proper treatment is lacking. *Moringa oleifera* is a tropical plant whose seeds contain water soluble substances that have coagulation activity in water.

Purpose: The coagulation and antimicrobial efficiency of the *Moringa oleifera* seed solution at different concentrations in turbid surface water (Nile River) were studied and compared with alum, which is presently the most widely used industrial coagulant.

Methods: The physicochemical and microbial analysis of the turbid surface water

Results: The water sample has turbidity of 28 NTU and the presence of 30 × 100 MPN/ml coliform bacteria, 286 × 10^2 CFU/ml mesophilic bacteria and 70 × 10^2 CFU/ml mesophilic fungi respectively. However, microbial reduction of 70-93.3 % for coliform bacteria, 93.7-98.3 % for mesophilic bacteria and 97-100 % for mesophilic fungi was obtained following coagulation of the water sample with Moringa oleifera seed solution, at the concentration of 2 %. Moringa seed is non-toxic and environmentally friendly, and unlike alum does not significantly affect the pH and conductivity of the treated water.

Significance: *Moringa oleifera* seed may be a potentially viable substitute to alum in both home and pilot water treatment especially in the rural areas of the developing countries.

P3-04 Changes of Bioactive Compounds, Antioxidant Capacity and Natural Sweetener Content of Dehydrated Stevia rebaudiana Bertoni Leaves

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Introduction: During the last few years, one of the raw materials claiming great interest and demand, both in international and national markets, has been the *Stevia rebaudiana Bertoni*, a plant from which products with sweetening, medicinal, pharmaceutical and feeding purposes can be obtained

Purpose: To determine if *Stevia* leaves will be a useful ingredient to use in food products.

Methods: *Stevia* leaves were dehydrated at six temperatures from 30 to 80°C, and the changes in total phenolic, flavonoid contents, vitamin C, antioxidant capacities (DPPH and ORAC) and sweetener content were researched.

Results: The *Stevia* leaves showed a high content of sweetener content vitamin C, which increased and decreased during drying temperature from 40 to 80°C, respectively. Phenolic and flavonoid contents in the dried *Stevia* leaves showed a higher decrease at lower temperature due to longer drying time. The radical-scavenging activity also showed higher antioxidant activity at higher drying temperatures (70–80°C) than at lower drying temperatures (40–50°C). Total phenolic content (TPC) and flavonoids showed good correlation with antioxidant capacity.

Significance: *Stevia* leaves proved to be an excellent source of antioxidants and natural sweetener compounds and are therefore a potential ingredient for new functional food products.

P3-05 Application of Liquid Freezing Method to the Inactivation of Kudoa septempunctata in Olive Flounder Meat

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Introduction: *Kudoa septempunctata* is a myxosporean parasite of olive flounder and causes a foodborne illness that causes more than 100 cases in Japan each year. The consumption of raw olive flounder meat containing a high concentration of *K. septempunctata* spores induces severe diarrhea and emesis. Although *K. septempunctata* is inactivated easily by freezing at -80°C, the meat texture deteriorates by freezing. In Japan, the commercial value of raw olive flounder depends on its food texture. Therefore, *K. septempunctata* inactivation by conventional freezing method is unsuitable due to deterioration of the meat texture. However, the newly-developed liquid freezing method uses an alcoholic liquid refrigerant that serves as an effective heat exchanger and enables rapid cooling. In addition, it is suggested that the food quality of meat treated with the liquid freezing method is comparable to that of unfrozen meat.

Purpose: In this study, we evaluated the effect of the liquid freezing method on the survival of *K. septempunctata* and the meat texture of olive flounder.

Methods: *K. septempunctata* infected olive flounder were frozen by using the liquid freezing method for 1 min to 5h. After thawing, the changes in the meat texture were evaluated by using a creep meter. The survival of spores were determined by the toxicity to Caco-2 human intestinal cells.

Results: The fracture curve of olive flounder meat subjected to liquid freezing resembled that of meat stored at 4°C, indicating that the structure of olive flounder muscle was well preserved. Liquid freezing preserved the transparency of olive flounder meat to the same degree as that of meat stored at 4°C. The treatment of olive flounder with the liquid freezing for 5 min completely inactivated *K. septempunctata*.

Significance: These results indicated that the liquid freezing method could be used for *K. septempunctata* inactivation without affecting the meat quality.

P3-06 Heavy Metal and Mineral Contents of Several Wild Fruits Collected from Roadsides

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Introduction: Mineral contents of wild fruits were determined by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES).

Purpose: The potassium content of fruits was found high compared with other mineral results.

Methods: Sulfur and selenium could not be detected at all in some samples. While rose (Mut (Sertavul)) sample contains Ca at the high level (9148.59 mg/Kg), Silverberry sample contained at the low level (549.79 mg/Kg). In addition, K values of samples ranged from between 3926.12 mg/Kg (Silverberry) to 24097.56 mg/Kg (Wild plum).

Results: While magnesium content changes between 211.70 mg/Kg (Silverberry) to 2751.00 (Rose (Karaman)), P contents were found between 248.5 mg/Kg (Silverberry) to 1380.38 mg/Kg (Rose (Karaman)), respectively. As heavy metal, Co, Mo, Cd, Cr, Ni and Pb contents ranged from 35.38 mg/Kg (Hawthorn) to 117.11 mg/Kg (Wild plum).

Significance: Cr content of Ahlat pear provided from Agrý location was found at high level (6.47 mg/Kg) compared with fruits provided from other locations.

P3-07 Development of a Biochip Array for Simultaneous Screening of Endophyte Alkaloids in Flour

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Introduction: Ergot alkaloids are mycotoxins produced by fungi of all species of the *Claviceps* genus which parasitize the seed heads of living plants at the time of flowering. Infections are prevalent in cereals and wild grasses and upon infection the fungus replaces the developing grain or seed with an alkaloid containing wintering body called sclerotium or ergot. The determination of ergot alkaloids has gained importance for food safety as these compounds are undesirable contaminants of cereal products and can cause adverse health effects in humans and animals.

Purpose: Biochip array technology enables the simultaneous detection of multiple analytes from a single sample, which increases the screening capacity. This study reports the development of a biochip array for the multiplex screening of endophyte alkaloids in flour.

Methods: Simultaneous competitive chemiluminescent immunoassays were employed. The capture in-house made polyclonal antibodies were immobilised and stabilised on the biochip surface defining discrete test sites. The assays were applied to the Evidence Investigator analyser. Samples (n = 27) were extracted from flour by liquid/liquid extraction and only 50µl of the test sample was required for the multi-analytical assessment.

Results: Initial analytical evaluation showed immunoassay 1: ergot alkaloids (generic) detected all six predominantly present ergot alkaloids, i.e., ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, ergocornine and their related –inines required by the European Union. Immunoassay 2 was specific for paxilline. The Limit of Detection for flour samples was <2 ppb and <0.5ppb for immunoassay 1 and 2, respectively. Both assays presented an intra-assay precision typically <10% and a % recovery ranging from 75% to 115%.

Significance: The results from this initial evaluation indicate that this biochip array enables the simultaneous detection of endophyte alkaloids in flour and represents a fast, reliable multi-analytical tool for the screening of these compounds from a single, low volume test sample.

P3-08 Development and Validation of a Food Safety Climate Assessment Tool

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Introduction: Up to now scientific research mainly focused on the development and implementation of Food Safety Management Systems (FSMS) in food (processing) companies. However, in practice, a well elaborated and 'fit for purpose' FSMS, does not always guarantee the highest level of food safety and hygiene and a stable system output. This might be due to co-determining factors, such as the Food Safety Climate (FSC) prevailing in the company. Indeed human behavior (e.g., the actual execution of procedures), and decision making is influenced by the perceived organizational FSC.

Purpose: The goal of this research was to define the concept Food Safety Climate and to develop and validate a tool for the measurement of the Food Safety Climate in companies.

Methods: The development phase was executed by means of a comprehensive literature study and discussion with experts in the field. Next, twenty experts with expertise in audits concerning food safety/quality (such as governmental agencies, third party certification bodies, sector associations, universities and industry) were asked to evaluate the relevance, reliability and validity of our initial Food Safety Climate assessment tool.

Results: Food Safety Climate is defined as employees' (shared) perception of the leadership, communication, engagement, resources and risk perception concerning food safety and hygiene within their current work organization. A self-assessment survey with 27 indicators was developed and adjusted based on the expert validation.

Significance: Our Food Safety Climate assessment tool enables companies to go beyond traditional food safety management and aims to mirror the human dimension in food safety. In further research we want to test our tool in food companies and make a link to microbiological and/or chemical monitoring results and to psychological indicators of food safety performance.

P3-09 Quality of Seafood Captured in Fishing Communities in Sao Francisco do Conde, Brazil

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Introduction: Seafood is an excellent food source. However, under inadequate capture and storage conditions, it can present a risk to public health due to microbial contamination and chemical changes. Artisanal fishing is a tradition in the Baía de Todos dos Santos, Brazil, even though environmental concerns.

Purpose: This study aimed to evaluate the sensory, microbiological and physicochemical quality of fish freshly caught in São Francisco do Conde, *Baía de Todos dos Santos*, Brazil.

Methods: Eighty-three samples, including *sururu* (mussel) (*Mytella guianensis*), mullet (*Mugil brasiliensis*), shrimp (*Penaeus* spp) and snook (*Centropomus undecimalis*) from eight different locations were subjected to sensory analysis, microbiological count of mesophilic aerobic bacteria, staphylococci coagulase testing, total coliform counting, detection of *Escherichia coli* and *Salmonella* spp., and pH analysis.

Results: All samples were compliant with Brazilian legislation based on sensory observation. Mesophilic aerobic bacteria counts were found to be less than 6.00 log CFU/g. For total coliforms, scores ranged between 1.00 and 6.22 log CFU/g, with 23% (n = 19) of the samples testing positive for *E. coli.* Only 2.5% (n = 2) of the samples exceeded the standard established for fresh fish for the presence of coagulase positive staphylococcus. However, all the samples were negative for *Salmonella* spp. The pH values ranged from 5.68 to 7.30. A total of 14% of the samples were classified as non-compliant with Brazilian legislation.

Significance: The results showed contamination and changes in fish, even immediately post-capture, intensifying concerns about the safety of fish caught in the municipality. These results emphasize the need to apply and maintain good hygienic practices when catching and processing fish.

P3-10 Post-harvest Fungi Diversity and Level of Aflatoxin Contamination in Stored Maize: Cases of Kitui and Nakuru Counties and Kitale District in Trans-Nzoia County in Kenya

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Introduction: Aflatoxin contamination of maize in Africa poses a major threat to food security and the health of many African people. In Kenya, aflatoxin contamination of maize is high due to environmental, agricultural and socio-economic factors.

Purpose: This research was carried out to gather scientific information on the fungi population, diversity and aflatoxin level during post-harvest period. The study was conducted in three geographical locations of Kitui, Kitale and Nakuru. Samples were collected from storage structures of farmers and transported to the Biosciences eastern and central Africa (BecA)- International Livestock and Research Institute (ILRI) hub laboratories.

Methods: Mycoflora were recovered using the direct plating method. A total of five fungal genera (*Aspergillus, Penicillium, Fusarium, Rhizopus* and *Bssyochlamys* spp.) were isolated from the stored maize samples.

Results: The aflatoxin producing fungi *A. flavus* was recovered in 82.03% of the samples. Moisture content determination helped to correlate the mycoflora recovered. Kitui recorded the highest moisture content with a mean of 19.33 followed by Nakuru and Kitui at 17.23 and 16.39, respectively. The means were not significant ($P = 0.23 > 0.05$) across the three locations. When individual samples were analysed using Vicam fluorometer method, aflatoxin analysis revealed that most of the samples (58.4%) had been contaminated with aflatoxins. The means were significantly different ($P = 0.00 < 0.05$) in all the three locations. Genetic relationships of *A. flavus* isolates were determined using 13 Simple Sequence Repeats (SSRs) markers. The results were used to generate a phylogenetic tree using DARwin5 software program. A total of 5 distinct clusters were revealed among the genotypes. The isolates appeared to cluster separately according to the geographical locations. Principal Coordinates Analysis (PCoA) of the genetic distances among the 91 *A. flavus* isolates explained over 50.3% of the total variation when two coordinates were used to cluster the isolates. Assessment of genetic diversity of the *A. flavus* isolates using Analysis of Molecular Variance (AMOVA) showed high variation of 87% within populations and 13% among populations showing the isolates also differed in terms of geographical locations.

Significance: This study showed that aflatoxin contamination of stored maize is still a major problem and prevention strategies are required to mitigate the menace.

P3-11 Viability of Escherichia coli O157:H7 During Fermentation and Storage of Laban (Ayran) Manufactured with Different Spices

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Introduction: Laban (Ayran) is a traditional fermented liquid milk product that is made from cow's milk. It has a sharp acid taste and is widely consumed at lunch with main meal.

Purpose: The growth of *Escherichia coli* O157:H7 during processing and storage of Ayran manufactured with different spices was investigated.

Methods: Preheated rehydrated milk was inoculated with a cocktail culture of *Escherichia coli* O157:H7 (10^5 CFU/ml of milk) and with thermophilic yogurt lactic starter culture. A 1% of Ginger powder-*Zingiber officinale*, Green tea-*Camellia sinensis* and Fenugreek-*Trigonella foecum-graecum* were separately added to inoculated milk. The inoculated milk was incubated at 42°C for 5h, and then the samples were cooled and subsequently stored at 4°C for 7 days.

Results: The results showed that *Escherichia coli* O157:H7 grew at an early stage of fermentation but declined at the end of the process. There was no significant difference between the populations of *Escherichia coli* O157:H7 in the presence of spices during the first 4h of the incubation period. Ayran samples with green tea and Ginger had a slight effect on the microbe. The populations of *Escherichia coli* O157:H7 in all samples decreased significantly during storage of Ayran (pH 4.2-4.7).

Significance: The results obtained from this study indicate that the pH of Ayran and storage temperature was critical to the survival and growth of *Escherichia coli* O157:H7 in the manufacture of Ayran.

P3-12 Inactivation of Stressed Escherichia coli O157:H7 Cells on the Surface of Rocket Salad Leaves by Chlorine and Peroxyacetic Acid

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Introduction: *Escherichia coli* O157:H7 has been frequently associated with many foodborne outbreaks caused by consumption of leafy greens (lettuce, spinach and celery).

Purpose: Investigate the ability of deionized water, chlorine and peroxyacetic acid to detach or inactivate stressed and unstressed cells of *E. coli* O157:H7 contaminating the surface of rocket salad leaves.

Methods: *E. coli* O157:H7 cells stressed by acid, cold, starvation, or NaCl exposure, as well as unstressed cells were inoculated on the surface of rocket salad leaves at 4°C. The effectiveness of two sanitizers (200 ppm chlorine and 80 ppm peroxyacetic acid) and deionized water for decontaminating the leaves treated with stressed and unstressed *E. coli* O157:H7 were evaluated during storage at 10 or 25°C for 0.5, 1, 3, and 7 d.

Results: It was found that washing with 80 ppm peroxyacetic acid was more effective and reduced unstressed and stressed cells of *E. coli* O157:H7 by about 1 log CFU/leaf on the leaves. There was no difference in the ability of stressed and unstressed cells to survive surface disinfection with the tested agents. Treatments reducing unstressed and stressed cells of *E. coli* O157:H7 on rocket leaves stored at 25°C were more effective than when used on those stored at 10°C.

Significance: Washing with peroxyacetic acid or chlorine solution does not ensure the safety of rocket leaves, but such treatments could restrict the likelihood of water-mediated transfer of *E. coli* O157:H7 during washing and subsequent processing.

P3-13 Real-time PCR Method Combined with Immunomagnetic Separation for Detecting Healthy and Heat-injured Salmonella Typhimurium on Raw Duck Wings

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Introduction: Salmonellosis has been reported from consumption of duck meat; therefore, an effective detection method is important to ensure safe consumption. However, the conventional culture detection method is time consuming and labor-intensive.

Purpose: This study investigated an alternative rapid detection method combining real-time PCR and immunomagnetic separation (PCR-IMS) to determine healthy and heat-injured *Salmonella* Typhimurium on raw duck wings.

Methods: Immunocapture method using Dynabeads® was optimized on *Salmonella* cells with different reaction and separation times. Three Taqman primers (*Sal*, *invA* and *ttr*) were evaluated with five parameters [inclusivity, exclusivity, PCR efficiency, detection probability, and limit of detection (LOD)] to optimize the real-time PCR protocol. The optimized PCR-IMS assay was compared with ISO and a real-time PCR method by analyzing artificially inoculated raw duck wings with healthy and heat-injured *Salmonella* cells at 10^1 and 10^0 CFU/25g levels. The optimized PCR-IMS assay was also validated with naturally contaminated samples.

Results: Under optimal IMS conditions (30 min reaction and 3 min separation), 85 and 64% of *Salmonella* were captured from pure culture and food suspensions, respectively. Although 100% detection was observed at 10^3 and 10^4 CFU/ml using *Sal* and *invA* with and without IMS, respectively, *Sal* showed lower LOD (10^3 CFU/mL) and higher PCR efficiency (94.1%) than *invA*, therefore *Sal* was chosen for PCR protocol. The optimized PCR-IMS method was significantly ($P = 0.0011$) better to detect healthy *Salmonella* after 7-h enrichment than PCR method alone, however there was no significant ($P > 0.05$) difference between two methods with longer enrichment time (14h). The diagnostic accuracy of PCR-IMS was shown to be 97.5% through the validation study.

Significance: The optimized PCR-IMS method could provide a sensitive, specific and rapid detection for *Salmonella*, enabling 10-h detection. However, a longer enrichment time should be needed for reliable detection of heat-injured cells.

P3-14 Milk Quality Parameters Associated with the Occurrence of Antimicrobial, Macrocyclic Lactone and Pyrethroid Residues in Bulk Tank Milk

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Introduction: Antimicrobials, pyrethroids and macrocyclic lactones are the most widely used veterinary drugs in dairy cattle management for disease therapy and the control of infections. Extensive and improper use of these active compounds can lead to the presence of their residues in milk, causing human health risks, the development of microbial drug resistance, the spread of resistant pathogens, loss of industrial output and technological problems in dairy products.

Purpose: Identify milk quality parameters that are associated with the occurrence of antimicrobial, macrocyclic lactone and pyrethroid residues by using a multivariate principal components analysis (PCA).

Methods: A total of 132 raw milk samples were collected at dairy farms in Minas Gerais State in Brazil and analyzed for 42 analytes, comprising pyrethroids, macrocyclic lactones and antibacterials, using liquid chromatography coupled with mass spectrometry in tandem mode and gas chromatography with electron capture detection.

Results: Forty milk samples were positive for at least one analyte (above detection limit) and 11 milk samples were proven non-compliant, with an analyte concentration above the maximum residue limit set by Brazilian legislation. The milk parameters that were significantly associated with antimicrobial residues by confirmatory tests were lactose and nonfat contents by PCA. Furthermore, bulk tank milk samples with antimicrobial residues had higher lactose, total solids, nonfat solids and fat contents. PCA showed that the fat, protein and total solids contents and the somatic cell count and total bacteria count were associated with macrocyclic lactone residues in bulk tank milk. PCA for pyrethroid residues in bulk tank milk showed that the lactose and nonfat solid contents and titratable acidity were inversely associated with pyrethroid residues.

Significance: Thus, the veterinary drugs residues detected by confirmatory tests were associated with some milk quality parameters which can be used for monitoring mammary health, milk hygiene and safety.

P3-15 Development of Bioactive Food Packaging on Artificially Contaminated, Sliced Food

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Introduction: Recently, increased research attention has been given to the use of bacteriocins in coating packaging material to inhibit, reduce or delay the growth of pathogenic or spoilage microorganisms upon contact with the food.

Purpose: To evaluate the effect of plastic film activated with culture cell free to inhibit *Listeria monocytogenes* ATCC 19115 on the agar plate and on the cheese surface.

Methods: The agar well method was used to evaluate the anti-listeria activity of cell free supernatant of *L. lactis* ATCC 11454 and CRA 26 grown in M17, MRS at 100% or at 25% of their standard concentrations plus milk. Films activated by spraying of twofold concentrated supernatant, were used to pack sliced cheese inoculated with *L. monocytogenes* and stored at 12°C for 20 days.

Results: Milk added to either Ml7 or MRS provided the highest levels of anti-listeria activity assayed (800 AU ml^{-1}) for both *L. lactis* strains. A significant decrease of *L. monocytogenes* ATCC 19115 counts were observed in cheeses packed by films treated twofold concentrated supernatant of *L. lactis* ATCC 11454 and CRA 26 (Tukey's test), with an average decrease of 2.11 and 2.13 log CFU g^{-1}respectively, after 15 days of the storage. The activated films didn't cause changes on the indigenous lactic bacteria of the cheese.

Significance: The use of films treated with bacteriocins represents a useful tool for the control of the development of pathogens in foods during storage.

P3-16 Situational Risk Perception

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Introduction: Consumption of hamburgers with core temperatures below 70°C constitutes a risk of *E. coli* O157:H7 infection. Despite widespread media coverage of *E. coli* outbreaks, many consumers still seem to prefer rare hamburgers.

Purpose: Previous studies on risk perception and communication have shown that merely informing consumers about food risks does not usually result in improved handling and usage behaviour. To develop more effective interventions, we need better explanations of why many consumers still appear to prefer risky food. Our research focuses on possible explanations related to situational aspects. Although many studies in consumer science and behavioural nutrition have shown that where and with whom we eat has a decisive influence on behaviour, only little research exists on the role of such situational aspects in risk perception and communication.

Methods: A representative sample of 1046 Norwegian consumers participated in a web experiment. Participants were randomly divided into four groups. Each group was told to imagine a specific eating situation (at their friends place, at home, at a restaurant in the Mediterranean, at a Norwegian restaurant). Four pictures of hamburgers (rare, medium rare, medium, well-done) were presented in randomised order, and participants rated their intentions to eat each hamburger.

Results: The results show that participants generally perceive their own home to be the safest place to consume a hamburger, but that they are significantly more likely to consume an undercooked hamburger when it is served at a friend's place.

Significance: The findings indicate that situational factors such as social norms are important and need to be considered when developing food safety strategies.

P3-17 Comparative Analysis of Antimicrobial Resistance and Virulence Genotypes of Escherichia coli from Poultry Meat and Young Chicks

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Introduction: Recent studies indicated that some highly resistant strains of *E. coli* can be common contaminants of broiler meat, and resistance determinants can be of importance for the food production and human health. However, much less is known about

their virulence determinants, and detailed genetic analyses of antimicrobial resistance and virulence are especially missing in *E. coli* from newly hatched broiler chicks.

Purpose: We aimed to provide a comparative description of antimicrobial resistance and virulence of *E. coli* isolates from young chicks from farms and from fresh broiler meat.

Methods: A total of 70 *E. coli* isolates characterized here derived from different poultry sources: raw meat (28), young chicks from farms (represented by 11 intestinal- and 11 extraintestinal strains) and 20 *E. coli* isolates from newly hatched chicks. Resistance and virulence genotyping was performed using high throughput PCR microarray systems, AMR05 and Ec03, respectively.

Results: The tetra-resistant phenotype of streptomycin-nalidixic acid-sulfonamide-tetracycline commonly occurred among *E. coli* strains from different chicken sources. The antimicrobial resistance genotype of *E. coli* strains from raw meat showed the highest similarity with the intestinal strains from young chicks. The high prevalence of antimicrobial resistance genes related to the flexible genome indicates the commonly high distribution of certain mobile genetic elements in poultry *E. coli*. Obviously, the predominance of the virulence genes in the extraintestinal *E. coli* strains was not surprising; however, some of the virulence genes (*iss*, *tsh*, *iutA*) showed high prevalence in commensal isolates from newly hatched chicks and from the meat.

Significance: Results indicate that *E. coli* from newly hatched chicks may represent a reservoir for multi-resistance and virulence for both pathogenic and commensal *E. coli* strains of young chicks and of poultry meat.

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P3-18 Pyocins - Novel and Highly Specific Antimicrobial to Prevent* Escherichia coli *O157:H7 Contamination in Food

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Introduction: DuPont has announced food security goals to increase safety and quality of the global food supply by 2020. Parts of the goals entail developing new solutions to increase safety by providing products targeting various pathogens. The pyocin technology developed by AvidBiotics is based on R-type bacteriocins which are high molecular weight phage tail like protein complexes produced by *Pseudomonas aeruginosa*. Pyocins are retargeted R-type bacteriocins which are altered to specifically kill e.g., *E. coli* O157:H7. The advantage of pyocins compared to other food decontamination technologies is their designed specificity to kill selected organisms (Scholl et al., 2009).

Purpose: Product development of the pyocin technology is currently proceeding as a partnership between AvidBiotics and DuPont.

Methods: The R-type pyocin, used as a platform for development of an *E. coli* O157 specific antimicrobial, is the R2-pyocin from *P. aeruginosa* PAO1. Specificity was retargeted by fusing tail fibers with *E. coli* O157-specific P2 phage tail fibers. The inhibition spectrum and potency was assessed by a semi-quantitative agar-based assay and an OD based potency assay. SDS-PAGE and immunoblotting was used to test LPS degradation by the pyocin. *In situ* efficacy was assessed in beef decontamination/contamination experiments and Shiga toxin production assessed in *E. coli* exposed to the pyocin (Scholl et al., 2009).

Results: The developed pyocin was highly specific for O157 strains when testing 49 *E. coli* O157:H7 and 15 non-O157 isolates. Retargeted pyocin both recognized and degraded LPS from O157 in contrast to wild type R2 pyocin. First application studies showed that pyocins add in helping to improve safety and quality of food products.

Significance: The pyocin technology is useful for developing pyocins with specific activity against pathogens like *E. coli* O157:H7. The pyocin technology fits well with DuPont's food security goal about improving safety and quality of the global food supply.

P3-19 Prevalence of Foodborne Bacterial Pathogens in Molluscs at Retail in Poland

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Introduction: Molluscs, as filter-feeders, may represent an important public health risk resulting from accumulation of potential pathogenic bacteria. Existing epidemiological data indicate growing trends in outbreaks of foodborne illnesses due to consumption of shellfish, especially eaten raw.

Purpose: The aim of this study was to evaluate the prevalence of foodborne bacterial pathogens in molluscs at retail in Poland.

Methods: A total of 410 samples of live molluscs (mussels, oysters and clams) were tested during years 2009-2013 for the presence of *Salmonella* spp., *Vibrio parahaemolyticus*, coagulase-positive *Staphylococcus* spp., and spore-forming anaerobes. The identification analyses were performed according to ISO standards and suspected bacterial colonies were identified biochemically using API systems.

Results: The study showed that among 410 samples tested *Salmonella* spp. were identified in 13 shellfish (3.2%). *V. parahaemolyticus* and coagulase-positive *Staphylococcus* spp. were detected in 84 (20.5%) and 62 (15.1%) samples, respectively. Spore-forming anaerobes were the most frequently identified microorganisms (293 samples, 71.5%). Moreover, the study revealed that 109 shellfish (26.6%) were contaminated with more than one bacterial pathogen. Most of the molluscs were positive for spore-forming anaerobes and *V. parahaemolyticus* (42 samples, 38.5%) and spore-forming anaerobes together with coagulase-positive *Staphylococcus* spp. (30 samples, 27.5%). Eighteen samples (16.5%) were contaminated with three pathogens. Spore-forming anaerobes, coagulase-positive *Staphylococcus* spp. and *Salmonella* spp. were found at the same time in 7 samples, whereas 10 samples were positive for spore-forming anaerobes, *V. parahaemolyticus* and coagulase-positive *Staphylococcus* spp. Furthermore, spore-forming anaerobes, *Salmonella* spp. and *V. parahaemolyticus* were simultaneously identified in one shellfish.

Significance: The results of the study indicate a significant microbiological contamination of shellfish available at retail in Poland. Consumption of raw molluscs contaminated by foodborne pathogens may pose a significant risk for human health.

P3-20 The Comparison of Consumer Preferences and Nutritive Value of Sea Bream (Sparus aurata) from Two Different Culture Conditions

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Introduction: Turkey is one of the biggest countries that export aquaculture products (sea bream and sea bass) to Europe. Also, sea bream is one of the most preferred species from Europe.

Purpose: In this study, fisheries which grow two different culture conditions are investigated as nutritive value and sensory analysis during a year.

Methods: Proximate analyses were done by AOAC (1990); Sensory Analyses were done by Consumer Preferences Test.

Results: Differences between two groups protein, lipid, moisture, ash and sensorial analyses were investigated. Moreover, according to statistical analyses, differences were obtained at the end of this study.

Significance: Results of this study indicated that differences in culture conditions and harvesting time are so important to have more tasty and healthy products for consumers.

P3-21 Integrons and Antimicrobial Resistance Genes of Multidrug Resistant* Escherichia coli *and Coliform Bacteria from Foods of Animal Origin Confiscated at the Hungarian Borders

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Introduction: The import of contaminated food may represent a food safety risk by the spread of pathogenic and/or multidrug resistant (MDR) bacteria and their determinants for virulence and antimicrobial resistance.

Purpose: Here we aimed to isolate and characterize MDR *E. coli* and coliform bacteria from food samples from non-Schengen countries confiscated at the Hungarian borders.

Methods: *E. coli* and coliform colonies were isolated based on their phenotype on Chromocult® Coliform selective media. Furthermore, API®, PCR and 16S rDNS sequencing were used for species identification. Resistance phenotypes were determined by disc diffusion method for 18 antimicrobials with animal and human clinical relevance. Corresponding

antimicrobial resistance and virulence gene patterns were identified using PCR microarray systems AMR05 and Ec03, respectively. The gene cassette arrangements of the integrons were defined by amplicon sequencing.

Results: From the total of 207 confiscated food samples, 833 coliform isolates were collected. Among them 17 (13 *E. coli* and 4 coliforms identified as *Enterobacter spp.*) showed resistance to at least three different antimicrobial classes thus were designated as MDR. The 17 strains represented 14 different food samples. Resistance genes *strA, strB, sul2, $bla_{TEM-1,}$ tet* (A) predominantly occurred, but in general the prevalence of the virulence genes was low. The identification of genes *qnrB, aac(6')-Ib, bla_{OXA-7}* in some of the isolates indicated the presence of certain emerging antimicrobial resistance plasmids. Class 1 integrons were found in 10 of the 17 MDR isolates (9 *E. coli*, 1 coliform), and in the majority of them the *sul1* gene was absent from their 3' conserved segment (CS). Interestingly, in one of the pork samples we detected a non-typical class 1 integron carrying the *sul3*gene on its 3'CS.

Significance: Above results showed that these illegal foods may frequently carry MDR *E. coli* and coliform bacteria with some unusual or new antimicrobial resistance traits.

P3-22 Which Virus may be Used as Process Control for Diagnosis of Hepatitis A Virus and Norovirus in Food and Water?
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Introduction: The two main viruses most frequently involved in foodborne infections worldwide are norovirus (genogroup I (NoV GI) and genogroup II (NoV GII) and hepatitis A virus (HAV). They are mainly transmitted through fecal–oral route, by person-to-person contact or by consuming contaminated water and foods. Detection methods used in the field of food virology are currently based on a final detection of the viral genome using real-time reverse transcriptase PCR (RT-qPCR). One of the general requirements for viral diagnosis in food concerns the use of a process control virus to monitor the quality of the entire viral extraction procedures as described in the CEN/ISO/TS 15216-1 standard published in 2013. The selected virus should exhibit morphological and physicochemical properties similar to the pathogen viruses, thus providing comparable extraction efficiency.

Purpose: The aim of this study was to determine which virus is most likely to choose as process control, murine norovirus (MNV-1) or mengovirus, for detecting HAV, NoV GI and NoV GII in bottled water, salads and semi-dried tomatoes.

Methods: Food samples were spiked with HAV, NoV GI or NoV GII alone or in presence of MNV-1 or mengovirus. Recovery rates of each pathogen virus were compared to those of both potential process controls using a multiple comparison procedure.

Results: Both process control viruses did not influence the recovery of pathogen virus regardless of the food matrices. MNV-1 was the most adapted to validate the detection of HAV and NoV GII in the three food matrices as well as NoV GI in salads. Mengovirus seems the most adapted for NoV GI detection in bottled water and semi-dried tomatoes.

Significance: The process control virus is essential for validation of viral diagnosis in food and its choice is dependent of food type and pathogen virus.

P3-23 Comparison of the New TEMPO® BC Method with the ISO 7932 Method for Rapid Enumeration of Bacillus cereus Group in Food and Environmental Samples
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Introduction: *Bacillus cereus* is a Gram positive, aerobic, spore-forming bacterium which, when present in foods improperly stored, can lead to food poisoning. TEMPO BC is an automated method which answers this emerging risk, by allowing rapid enumeration of *Bacillus cereus* group in food and environmental samples. The TEMPO system comprises an innovative card with a medium adapted for the rapid enumeration of quality indicators. It replaces serial dilutions and tedious plate reading with a simple 1/10 dilution, followed by an automated enumeration based on the Most Probable Number method.

Purpose: The purpose of this dual site study was to compare the new method to ISO 7932 method on a variety of food products.

Methods: More than 280 samples were tested, including milk and egg powders, baby food, cereals, and prepared foods, some of them naturally contaminated with *Bacillus cereus*. Test portions were diluted 1:10 using Peptone Salt or Buffered Peptone Water. TEMPO cards were inoculated with 1 ml or 0.2 ml of the 1:10 dilution (enumeration range 10 to 49,000 CFU/g or 50 to 250,000 CFU/g), with results available after 22 – 27 hours incubation at 30°C. Test portions were plated onto MYP media according to ISO 7932 method, and presumptive colonies confirmed by hemolysis test on blood agar plate, leading to a 48 – 72 hour enumeration for positive samples.

Results: More than 150 samples were positive by at least one method, most of samples being contaminated with naturally occurring *B. cereus*. The TEMPO BC results were comparable to reference results, showing > 95% concordance within 1 log. Moreover, for samples with high background flora, the new method was found to be more efficient at enumerating *B. cereus*.

Significance: The new method provides reliable and accurate results for the rapid quantitation of *Bacillus cereus* group in a large variety of foods.

P3-24 Use of UV-C Light to Reduce Escherichia coli and other Facultative Aerobic Mesophilic Bacteria in Fresh Minimally Processed Lettuces

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Introduction: Processing of vegetables promotes a faster physiological and microbial degradation of the product compared to the raw products, and increases the risk of foodborne disease outbreaks.

Purpose: Study the effect of the illumination of fresh-cut lettuce leaves with UV-C light to slow down physiological and microbial degradation and reduce microbial risk of outbreaks.

Methods: Lettuce leaves were inoculated with a load of *E. coli* of 10^8 CFU by spotting 100 µl taken from a fresh liquid culture. UV-C illumination was carried out using Osram HNS 6W lamps, with an emission peak at 254 nm. The maximum light flux tested was of 42 W/m^2 when using 3 lamps. All experiments were conducted in duplicate and quantified in the same day of the UV-C treatment. Samples were homogenized in 23.5 ml of sterile buffered water peptone with a stomacher Bag Mixer 400 for 2 min. Concentration of viable bacteria was quantified by dilution, plating on LB nutrient agar and incubation at 37°C for 24h.

Results: *Staphylococcus* spp. and Enterobacteriaceae (mainly coliforms, different from *E. coli*) were analyzed to be naturally present in lettuce. Their quantification showed the presence of $(2.0 \pm 0.2) \times 10^5$ CFU/g. After 15 min of UV-C treatment a reduction of 99 ± 0.1 % of viable bacteria is achieved. For highly contaminated lettuces inoculated with *E. coli*, a 6-log reduction in viable bacteria has been achieved after 15 min by applying 46 W/m^2 of UV-C light. Longer illumination times do not lead to a notable increase in bacterial inactivation, whereas can negatively affect sensorial properties of the product.

Significance: UV-C treatment reduces the concentration of bacteria naturally present in minimally processed lettuces, contributing not only to extend the shelf life of the product but also to reduce microbial risks of outbreaks and consequently economic losses for the industry.

P3-25 Use of Weibull Distribution to Determine the Bactericidal Activities of Methyl and Dodecyl Rosmarinate against Staphylococcus carnosus LTH1502

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Introduction: Rosmarinate esters (REs), a novel class of 'phenolipids,' are derived from rosmarinic acid and a series of alcohols (1-18 methyl groups). The antimicrobial activities of the compounds show a cutoff phenomenon as a function of carbon chain alcohols. It appears to be strongly related to their specific affinity for phospholipid bilayers, which the threshold is with the dodecyl ester. The varying carbon chain length among REs alters not only the antimicrobial activity but could also change their mechanism.

Purpose: This study aimed to investigate the relationship between the antimicrobial efficiency and mechanisms of selected methyl (RE1) and dodecyl (RE12) rosmarinates by using mathematical model, Weibull distribution.

Methods: RE1 (0.2-6.4 mM) and RE12 (0.00625-0.2 mM) were tested against 10^4 CFU/ml *Staphylococcus carnosus* (LTH1502). The treated cells were incubated at 37°C, collected over 48h and plated for enumeration. The colonies were counted using a colony counter. The efficiency of each REs was determined the inactivation curve at minimum inhibitory concentration

(MIC) by using Weibull distribution and performing probability density function (PDF). The 10^8 CFU/ml cells were further treated with RE1 and RE12 at minimum bactericidal concentration (6.4 mM and 0.05 mM, respectively) and collected after 24h incubated. The treated cells were freeze dried and observed under scanning electron microscope (SEM).

Results: The MICs of RE1 and RE12 were 0.8 and 0.05 mM. The PDF profiles at MIC demonstrated that the killing activity of RE1 depended on time application whereas of RE12 started right after application. The results were supported by the SEM images. The SEM image of treated cells indicated that RE1 involved in the transportation into the cells whereas the cell membrane was entirely destroyed by RE12.

Significance: This study introduces a new aspect of using Weibull distribution to categorize the antimicrobial efficiency and presents the substantial explanations of the structure-activity relationship.

P3-26 Co-occurrence of Multiple Mycotoxins in Dry Chili (Capsicum annum L.) Samples from the Markets of Sri Lanka and Belgium

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Introduction: Most countries have reported only the occurrence of "classical mycotoxins" aflatoxins and ochratoxin A in chilies. This study applies a multi-mycotoxin analytical method for the first time to screen several toxicologically significant mycotoxins in chili samples.

Purpose: The main objective of the study is to determine the mycotoxin contamination in chili samples from the markets in Sri Lanka. These contaminations were compared with the chilies from the markets in Belgium. These contamination data will be further used in risk assessment studies.

Methods: An in-house developed and validated high performance liquid chromatography triple quadruple tandem mass spectrometry method was used for multiple mycotoxin analysis in chilies. A modified QuEChERS (quick, easy, cheap, effective, rugged and safe) based extraction procedure was applied.

Results: In addition to aflatoxins (<LOQ-687 µg/kg) and ochratoxin A (OTA; <LOQ-282 µg/kg), the chili samples (Sri Lanka (n = 86) and Belgium (n = 35)) were also found to be contaminated with sterigmatocystin (STERIG; <LOQ-32 µg/kg), fumonisin B2 (FB2; <LOQ-87 µg/kg), citrinin (<LOQ-2.1 mg/kg) and alternariol methyl ether (70 and 222 µg/kg). Aflatoxin B1 (AFB1) was the predominant mycotoxin contaminating almost 77% of the Sri Lankan samples; while 67% of them exceeded EU maximum level for AFB1 and 44% of the samples exceeded the EU ML for total aflatoxins. Co-occurrence of different mycotoxins, AFB1-OTA (36%), AFB1-STERIG (28%), OTA-AFB1-STERIG (17%) and AFB1-FB2 (14%) was found in different forms of chilies. Nine Belgium samples exceeded the EU ML.

Significance: A high mycotoxin contamination of this single spice chili already indicates the necessity of mycotoxins analysis in other food products and the assessment of the overall health risk associated with mycotoxins exposure. Though evaluating the combined toxicity is foreseen highly complex, this study emphasizes the importance on the development of novel strategies in order to perform toxicological research on the "cocktail effect" of multiple mycotoxins present in foods.

P3-27 Food Safety Enforcement Does Little to Improve Food Safety, Most Businesses Needs a Lighter Touch, Education-based Inspection

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Introduction: Food safety in retail business remains a contemporary public issue health. For over 100 years, environmental health officers have relied on enforcement as the main incentive to improve food safety in food retail businesses. However, enforcement strategies combined with educational approach may provide knowledge and motive businesses to comply with the legislation.

Purpose: This study challenges this preconception and introduces a new process that suggests structured enforcement with an educative focus is much more sustainable and supportive of businesses and protects the public's health more effectively.

Methods: Jan 2013, in a rural authority in NSW, Australia undertook a review of current practice and introduced a new educative approach to food inspections. Food retail businesses that had not been fully complying with the legislation were given educational advices that were practical to the needs and the operation of the business. Routine inspection also followed.

Results: This change has resulted in a higher degree of compliance in less than a year. Businesses need more than a list of 'works to do.' They need to understand why things are wrong and even more importantly know why they need to change practices. Focused educative interventions required a longer time and effort but resulted in higher levels of compliance in the 180+ inspections carried out.

Significance: This new approach is less intrusive to small businesses and encourages sustained culture and compliance change to improve food safety across a range of different types of food businesses.

P3-28 Food Safety in Home and Consumer Studies Education

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Introduction: In Swedish compulsory school, the mandatory school subject Home- and Consumer Studies (HCS) is an opportunity to educate students about Food Safety. It is defined in the Curriculum-2011 as "Hygiene and cleaning in handling, cooking and storage of food." By having Food Safety knowledge, the students might influence their future health. In Sweden it has been estimated that approximately five percent of the population get foodborne infections each year, which has an impact on public health.

Purpose: To investigate Food Safety as a part of HCS education by providing insights regarding perception and behavior connected to Food Safety among students in the 9th grade in Swedish Compulsory School.

Methods: A national questionnaire survey was conducted in autumn 2013 among 529 students in the 9th grade, at 18 schools in different parts of Sweden by using a Student Response System. The content of the questionnaire focused on Curriculum 2011 and the hygiene Hot Spots summarized in the four C's: *Cooking, Cleaning, Cooling* and *Cross-contamination.*

Results: The results indicate positive behavior regarding hand washing, cooking and best before date, while there were uncertainties associated with reheating, cross-contamination, minced meat and dishcloths. Mother was considered the largest source of knowledge and trust, while HCS education was an important source for those who do not get the opportunity to cook at home, which was more common among boys. The students were uncertain regarding what can be considered as an appropriate refrigerator temperature.

Significance: Even if a mother is suggested to be the most important source of knowledge related to Food Safety this study indicates that HCS education fulfills an important function, especially for boys who seemed to more rarely get their knowledge from home. Present data indicate that some issues, e.g., to avoid Cross-contamination and Cooling need to be more highlighted in HCS education, especially from a health perspective.

P3-29 Antibacterial and Anti-quorum Sensing Activity of Dietary Plants Cultivated in Korea

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Introduction: The discovery of new agents is demanded by the appearance of multidrug resistant bacteria, like super bacteria, for many years. Also, quorum sensing, cell-to-cell communication, is regarded as remarkable target for development of new antibiotics in recent.

Purpose: We investigated the antibacterial and anti-quorum sensing (anti-QS) capacity of five dietary plants: *Punica granntum* L., *Citru junos, Prune mume, Boehmeria nivea* (L.) GAUDICH, and *Zingiberaceae* plant cultivated in Korea.

Methods: The crude extracts of five plants were evaluated on antibacterial activity against five major foodborne pathogens, *Staphylococcus aureus* (*S. aureus*), *Listeria monocytogenes* (*L. monocytogenes*), *Bacillus cereus* (*B. cereus*), *Escherichia coli* (*E. coli*), and *Salmonella* Enteritidis (*S.* Enteritidis), by bi-layer agar well method. To test for anti-QS activity, five crude extracts were screened using biomonitor strain *Chromobacterium violaceum* (*C. violaceum*). Interference with biolacein (purple pigment) production in *C. violaceum* was used as indication of anti-QS activity.

Results: All of the extracts showed antibacterial activity against *S. aureus*, *L. monocytogenes*, and *B. cereus* (gram positive bacteria). The extract of *Punica granntum* L. also had high activity against *E. coli* and *S.* Enteritidis (gram negative bacteria). The extract from *Zingiberaceae* plant had lower minimal inhibitory concentration (MIC) than that of *Punica granntum* L. against gram positive bacteria. The extract of *Zingiberaceae* plant had the lowest MIC of 50ug/ml against *S. aureus* and *L. monocytogenes*. Four crude extracts, except *Boehmeria nivea* (L.) GAUDICH, displayed high anti-QS activity in *C. violaceum*

producing violet pigment as a result of QS. Especially, the extract of *Zingiberaceae* plant inhibited production of violet pigment in *C. violaceum* at 10mg/ml. In addition, the extracts of *Punica granntum* L. and *Prune mume* showed anti-QS activity against *C. violaceum*.

Significance: These results exhibit the potential of five dietary plants to be used as food preservatives and new antibacterial agents

P3-30 Occurrence of Vibrio vulnificus and Its Specific Phages in Seawater, Sediment, and Seafood Samples from West and South Costal Area of Korea

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Introduction: *Vibrio vulnificus*, a virulent human pathogen, is responsible for many cases of seafood-related deaths related to the ingestion of raw or undercooked seafood in many countries. Bacteriophages causing lysis of *V. vulnificus* have potential as an alternative to antibiotics or antibacterial agents in controlling bacterial food contamination.

Purpose: The purpose of this study was to examine the occurrence of *V. vulnificus* and its specific phages in seawater, sediment, and seafood samples from west and south costal area of Korea.

Methods: A total of 269 samples were collected from five provinces in March to September, 2013. *V. vulnificus* was isolated by using selective media, such as TCBS and Chrom agar and identified by Vitek2 system. Plaque assay (agar overlay method) was used to identify the presence of phage in enrichment culture.

Results: Seven strains of *V. vulnificus* were isolated mostly in August and September in Gyeonggi province costal area. *V. vulnificus* phages specific for a *V. vulnificus* reference strain were found in only two marine samples, while *V. vulnificus* phages specific for *V. vulnificus* strains isolated in this study were detected in thirteen samples including sea snail. The occurrence of *V. vulnificus* didn't show a tendency to correspond to that of *V. vulnificus* phage.

Significance: To examine the occurrence and distribution of *V. vulnificus* considering environmental factors, such as geographic position, season, seawater temperature, salinity, etc and to isolate newly discovered *V. vulnificus* strains are important to research on securing seafood safety. Searching for *V. vulnificus*-specific phage with highly lytic activity is prerequisite to potential application as a biocontrol agent.

P3-31 TOS MUP Method For the* Bifidobacterium *Enumeration According ISO 29981/IDF 220:2010 : The First Ready-to-Use Method

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Introduction: TOS MUP selective medium is the mandatory method for the enumeration of presumptive bifidobacteria in milk products according ISO 29981/IDF 220:2010. bioMérieux proposed the first ready-to-use method including the 5 x 100ml TOS MUP base and a lyophilized Mupirocin supplement.

Purpose: The aim of the study was to evaluate the performances of the bioMérieux TOS MUP kit versus a commercial method with dehydrated medium.

Methods: Inclusivity study was performed by enumerating 30 *Bifidobacteria* strains usually used in milk products (Bifidobacterium *adolescentis, B. animalis, B.bifidum, B. breve, B. longum*) and exclusivity tests were done with 10 non-*Bifidobacterium* lactic acid bacteria. To complete the comparative study, enumeration of *Bifidobacterium* in 10 commercial milk products and yogurts was carried out on ready-use TOS MUP media and dehydrated current method. The samples were prepared according to the international standards ISO 6887-2 (dried milk products) or ISO 7889/IDF 117 (yogurt products). Each sample was suspended in 90 ml of diluent (¼-strength Ringer's solution). Additional decimal dilutions were prepared in ¼-strength Ringer's solution. Aseptically add 2 ml of MUP-Selective Supplement to 100 ml of liquefied base at 48°C ± 1°C. The complete TOS MUP medium contains 50 mg/l Lithium-Mupirocin.

Results: On the ready-to-use medium, the productivity rate was comprised between 70% and 120% for the recovery of the *Bifidobacterium* compared to the control method. Selectivity study showed an inhibition of lactic acid bacteria commonly used in fermented and non-fermented dairy products. For products analysis, no significant differences were observed between the ready-use method and the dehydrated medium for the enumeration of *Bifidobacteria*.

Significance: The new ready-to-use TOS MUP medium will help the end-user by improving laboratory workflow for *Bifidobacterium* enumeration.

P3-32 Applying Pseudocereals in Product Development

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Introduction: Cereal products represent a large amount of the human diet. It is known that the amount of nutritionally beneficial components in conventional grain grists is not satisfying; therefore, fortifying them is strongly recommended. Pseudocereals are a good choice as an improving agent due to their high poliphenolic component and vitamin content.

Purpose: Developing a healthier bakery product based on optimal mixture of wheat flour and pseudocereal flour by increasing its antioxidant capacity. Since pseudocereals' natural flavour might be unpleasant for consumers, a correctly chosen spice could have a positive impact on organoleptic features, as well as on their nutritional values.

Methods: Pseudocereal flour levels were raised by 10% in the flour fraction to aim for the optimal dough texture. The appropriate spices were determined, and then these mixtures were exposed to cooking and baking. Effects of these operations on dough textures and antioxidant capacity were compared. Antioxidant capacity was measured by the FRAP method. A product based on 100% wheat flour was used as a control sample.

Results: A mixture containing less than 30% pseudocereal portion does not affect significantly the nutritional quality, above 30% it leads to an inferior texture quality at cooking, whereas at baking even a 50% ratio can be applied. Cooking decreased the FRAP values while baking increased them which was enhanced by the applied spices. Based on rheological experiments the product is consumable for more days.

Significance: The addition of pseudocereal flours due to their gluten-free nature decreases the dough texture quality. Although several food additives could reduce this phenomenon, consumers would then associate the product with artificial foods, quite the contrary, we managed to develop an additive-free, healthier product.

P3-33 Construction of a LC-MS/MS Library for Screening 35 Steroids in Foods or Dietary Supplements

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Introduction: The sales cases of foods containing unauthorized substances have been increased worldwide. So its safety management problems have been issued.

Purpose: It is needed to develop and utilize a MS library to analyze steroids in health or dietary supplements rapidly and accurately for the safety management. Therefore, identifying 35 steroids in health or dietary supplements through LC-MS/MS library searching are the purpose of this study.

Methods: A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method is presented for the qualitative screening for 35 steroids in foods or dietary supplements, which is considerably more than in previous methods. A Waters ACQUITY UPLC BEH C_{18} column (100 × 2.1 mm^2I. D., 1.7 μm) kept at 35°C in an oven was used, and the mobile phases consisted of DW (v/v, A) and ACN (v/v, B) containing 0.1% formic acid. The compounds were introduced into a triple quadrupole mass spectrometer equipped with electro-spray ionization (ESI) spray ion source operating in the positive ionization mode. Identification was based on the compound's absolute retention time, protonated molecular ion, and one fragment ion obtained by multiple reaction monitoring (MRM) at an individually selected collision energy.

Results: Based on MRM ratio and retention time, the screening library was constructed, including all 35 steroids. The advanced analytical method for 35 steroids in dietary supplements was developed and it contributes to have a rapid and accurate library for searching unauthorized substances. The library is successfully applied to screen in dietary supplements. This might help preventing foods-related incidents and clamping down on illegally circulated foods.

Significance: The high selectivity and sensitivity of a triple quadrupole LC-MS/MS instrument combined with library searching offers a new approach for a rapid and accurate the screening steroids in foods and dietary supplements.

P3-34 Rapid Sample Preparation for Molecular Biological Food Analysis Based on Magnesium Chloride

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Introduction: Due to the implementation of critical pathogen levels, direct quantification of foodborne pathogens from food is going to become standard in food risk analysis. Until now major challenges for molecular biological detection and quantification (such as qPCR) of foodborne pathogens are heterogeneous food matrices and large sample quantities. Therefore a major research topic is the development of sample treatment methods prior to subsequent molecular detection and quantification methods, which allow the separation, concentration and purification of the target organisms from the sample matrix.

Purpose: *I*n this study a new chemical concept is introduced for the sample preparation method Matrix-Lysis, based on solubilization of proteins through the preferential interaction with $MgCl_2$. The underlying chemical principles of $MgCl_2$ based solubilization are described and possible pitfalls shown and countermeasures pointed out.

Methods: Molecular biological (qPCR) and microbiological methods (Plate Count Method) are used to quantify different Gram-positive and Gram-negative bacteria from various artificially contaminated foodstuffs and naturally contaminated acid curd cheese samples, from a recent *L. monocytogenes* outbreak in Austria and Germany, after Matrix-Lysis.

Results: Artificial contamination experiments show that all bacteria were efficiently recovered from 6.25g - 12.5g food to allow for accurate quantification with detection limits of 10 CFU/g. Examination of naturally contaminated samples resulted in 100% relative accuracy, 100% relative specificity and 100% relative sensitivity compared to the ISO 11290-1 standard method. Overall 135 individual samples for two different microorganisms and five different foodstuffs were analyzed with the new $MgCl_2$ based buffer system, which resulted in equivalent recovery rates.

Significance: The results demonstrate the excellent applicability of Matrix-Lysis as sample preparation for the direct quantification of foodborne pathogens with molecular biological as well as microbiological methods and the new chemical concept described enables widespread use of this method for routine applications to enhance food safety and risk assessment along the food production chain.

P3-35 Bioactive Extracts From Agro-food Wastes Inhibit Adhesion of Campylobacter jejuni on Polystyrene and Intestinal Epithelial Cells

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Introduction: Prevention of bacterial adhesion, as a pre-step of microbial biofilm formation on the surfaces of materials, is crucial for safety of industrial processes and in protecting human health. Bacterial attachment to host surfaces is a critical step in the pathogenesis of most infections, including adhesion of enteric pathogens to intestinal epithelium. Thus, new anti-adhesive compounds are highly needed in many medical and industrial applications. Agro-food by-products and waste materials from olive oil production, wine and essential oil production, which otherwise present a high economic burden and environmental problem could present a reasonable source of bioactive phytochemicals.

Purpose: The aim of our study was to investigate the *in vitro* anti-adhesive properties of chemically characterized ethanolic extracts from several plant materials – olive leaves (*Olea europea*, OE), grape skins and seeds of Pinot noir (*Vitis vinifera* L., GE) and thyme (*Thymus vulgaris*, TE, TW) prior and after hydrodistillation of essential oil.

Methods: It was tested on a model abiotic surface (polystyrene) and animal and human intestine epithelial cells (PSI, H4) against *Campylobacter jejuni*– the most common cause of foodborne gastroenteritis worldwide.

Results: All plant extracts exhibited antimicrobial activity, with minimal inhibitory concentrations (MICs) from 0.625 – 1.25 mg/ml. However, anti-adhesive activity was expressed at much lower concentrations that have influenced growth inhibition. The most efficient anti-adhesion activity was shown for *Thymus* waste material (TW>TE>OE>GE) and was influenced by extract concentration and phenolic content. The extracts gave similar patterns on polystyrene and intestine cell models with relative adhesion reduction rate up to 30-50 % in the concentration range from 0.2- 50 µg/ml.

Significance: Added value of some food production by-products with bioactivity and beneficial effects in preventing pathogen contamination and infection.

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P3-36 Preliminary Study of the Impedance Method for Rapid Detection of Listeria monocytogenes in Dry-cured Ham

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Introduction: Listeriosis is one of the most important foodborne diseases, caused by *Listeria monocytogenes*. Its ability to multiply in various foods at temperatures as low as 2 to 4°C results in the occurrence of *L. monocytogenes* in ready-to-eat foods with a relatively long shelf life. *L. monocytogenes* is an ubiquitous microorganism in the meat industry. Food producers and distributors have great interest in rapid methods to ensure safety of their meat products. Therefore it is necessary to develop rapid detection methods in microbiology that allow compliance with microbiological criteria established in European legislation.

Purpose: The impedance method is based on change in the conductivity of the culture medium owing to the microorganisms metabolism during growth. The objective of this study was to carry out the preliminary stages to set point this method for detection of *L. monocytogenes* in dry-cured ham.

Methods: Six strains of *Listeria spp*. (four strains of *L. monocytogenes* and two strains of *L. innocua*) were assayed. The concentrations (CFU/ml) obtained through plate count method after cultivation in brain heart infusion agar at 37°C/24h were correlated with the detection time (DT) obtained with µ-Trac 4200 of SY-LAB systems.

Results: The calibration curve revealed a great coefficient of correlation ($R^2 > 0.98$) for all strains assayed in pure culture. The results showed differences in DT between strains of *L. monocytogenes* at similar CFU/ml, and differences among this pathogenic species and *L. innocua*. This method showed a high sensitivity because it was able to detect 1 CFU/ml, with a DT from 16 to 22 hours depending on the strain assayed.

Significance: The method offered good results in this preliminary step with pure cultures. These results allow its evaluation in dry-cured ham.

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P3-37 The Combined Effect of Ambient Air Temperature and Relative Humidity on Monthly Counts of Bacterial Foodborne Diseases Using Seasonal ARIMA Models in South Korea from 2008–2012

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Introduction: Evaluating the influence of air temperature and relative humidity as climate variability on bacterial foodborne illness cases incidence may improve the ability to predict how climate change may affect these diseases.

Purpose: To examine the associations between air temperature and relative humidity as regional climate variability and bacterial foodborne disease monthly incidence in South Korea.

Methods: The weather variables (air temperature and relative humidity) and number of cases of 11 bacterial foodborne diseases during the period 2008–2012 has been studied on a monthly basis. The Pearson correlation between each weather variable and foodborne diseases cases was conducted. Seasonal autoregressive integrated moving average (ARIMA) models were used to perform the regression analyses.

Results: *Salmonella* spp. was positively associated with average temperature of the same month ($B = 0.040$, $SE = 0.109$, $P < 0.001$). By contrast, *Vibrio parahaemolyticus* was positively associated with relative humidity one month previously ($B = 0.092$, $SE = 0.025$, $P < 0.001$). No climatic factors were significantly associated with other pathogens (*Shigella* spp., *Yersinia enterocolitica*, *Staphylococcus aureus*, *Clostridium botulinum*, *Clostridium perfringens*, *Bacillus cereus*, and *Listeria* spp.).

Significance: These results suggest that temporally lagged relationships between climate variables and national bacterial foodborne disease incidence data can contribute to disease prediction models and implication for future patterns of diseases in South Korea with regard to climate change.

P3-38 Evolution of Microbiological Analytical Methods for Dairy Industry Needs

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Introduction: Fermenting microorganisms play a pivotal role in the development of physicochemical and sensory properties of food products but also contribute to product safety by limiting the growth of pathogenic and spoilage microorganisms. Therefore, evaluation of cell viability is of great importance for the fermented food industry in general, and more specifically for the dairy sector.Traditionally, culture-based methods have been used to enumerate microbial populations in dairy products. Recent developments in molecular methods now enable faster and more sensitive analyses than classical microbiology procedures. These molecular tools allow a detailed characterization of cell physiological states and bacterial fitness and thus, offer new perspectives to integration of microbial physiology monitoring to improve industrial processes.

Purpose: Review of existing methods described to enumerate and characterize physiological state of technological microbiota in dairy products. Discussion of current deficiencies related to specific needs of the dairy industry for fast, efficient, reliable and standardized methods.

Methods: In addition to global Internet search, a total of four international databases were screened for journal articles, books, patents, conferences and symposia proceedings in the field of food science, food industry, life science and biomedical information (FSTA®, BIOSIS® Preview, Medline®, Foodline®).

Results: Strikingly, the use of chromogenic media has been developed for the analysis of pathogenic and spoilage bacteria and validated and normalized methods are available, but nothing of the kind was set up for technological microbiota. Recent studies show that PCR-based methods, flow cytometry and omics technologies show interesting analytical potentialities to quantify fermenting microbes and probiotics in dairy products. However, they still suffer from a lack of validation and standardization for quality control analyses, as reflected by the absence of performance studies and official international standards.

Significance: While standard methods have been developed and validated for food-borne pathogens, lactic acid bacteria and probiotics seem to be the poor relations of the diagnosis industry and method standardization. This is surprising when considering the amount of quality controls performed by the dairy industry.

P3-39 Characterization of Two Bacteriocins Active against Listeria monocytogenes and Listeria innocua Produced by Lactobacillus spp.

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Introduction: Lactic acid bacteria (LAB) were isolated from Portuguese fermented sausages, which were produced by natural fermentation. Application of LAB could inhibit foodborne pathogens in the fermented meat industry.

Purpose: The aim of our study was to characterize stable anti-listeria bacteriocins produced by *Lactobacillus plantarum* ESB 202 and *Lactobacillus sakei* ESB 153.

Methods: Stability of bacteriocins was examined by using different environmental conditions (pH, temperature, enzymes). The size of bacteriocins was determined by tricine-SDS-PAGE gel. Adsorption studies were also done. *Listeria monocytogenes* (serogroup IIb) and *Listeria innocua* NCTC 11288 were used as target organisms.

Results: *Lactobacillus plantarum* ESB 202 and *Lb. sakei* ESB 153 produce anti-listeria bacteriocins. The activity of both bacteriocins was slightly reduced after treatment at different environmental conditions. Bacteriocins produced by *Lb. plantarum* and *Lb. sakei* were small peptides (lower than 14.5 kDa). The bacteriocins did adhere to the surface of the *Lb. plantarum* and *Lb. sakei* cells.

Significance: Bacteriocins produced by *Lb. plantarum* and *Lb. sakei* may be used in the food industry as bio-preservative agents since they are proved to be stable in unfavourable environmental circumstances.

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P3-40 Influence of Environmental Factors on Phage-bacteria Interaction and on Efficacy and Survival of the Phage P100

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Introduction: Effectiveness of bacteriophages for elimination of food pathogens was described in several publications. Most of these experiments were done using restricted growth conditions on laboratory scale. However, host-virus interaction, adsorption and replication of phages are dependent on the growth rate of the host and the physical and chemical environment.

Purpose: The aim of the study was to investigate the influence of temperature, salt concentrations, pH, and detergents on survival of P-100 phage and host-virus interaction under these conditions.

Methods: Bacterial growth over a time of ≥100 days at different temperature was determined with and without infection using plate counts and measurement of optical density. The survival of phages at and their ability to attach to and to replicate in host cells at different pHs, salt and detergent concentrations was monitored using plaque assays and adsorption tests. Additional the survival of P-100 in smear water was tested at 4°C and 10°C.

Results: Efficacy of the phages was dependent on temperature and host-virus ratio and best results were achieved at 4°C with high phage concentrations. At 10° and 20°C re-growth of bacteria was observed. Survival of bacteriophages was not depended on temperature. Within the first 37 days the phage-titer was decreasing 1.5 log in SM Puffer and 3-4 log in smear water. Phage infectivity was lost at pH 2 and pH 12 within one hour, but not at high salt or detergent concentrations. Attachment of the phages to the host was observed under all tested conditions, whereas replication was dependent on growth of the bacteria in these environments.

Significance: The high efficacy of phage treatments in food production plants is the basis of their use. This investigation demonstrates that environmental factors do significantly influence the performance of such treatments. This has to be considered when bacteriophages are used as emergency treatment.

P3-41 Optimization and Validation of Quantitative and Confirmatory Test Method to Determine Multiresidues of ß-lactams and Tetracycline in Kidney Using UPLC-MS/MS

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Introduction: Antimicrobials are widely used in veterinary medicine, mostly in livestock as therapeutic, prophylactic, and growth promoters. The indiscriminate use of them may lead to the presence of their residues in foods. Most methods recommended for β-lactam and tetracycline detection uses UPLC-MS/MS.

Purpose: The aim of this work was to optimize and validate a test method to determine quantitative and confirmatory multiresidues of β-lactams and tetracyclines in poultry, bovine, equine and swine kidney, using UPLC-MS/M.

Methods: The validated methodology was based on the extraction, using a mixture water /acetonitrile (8:2), of analytes tissue and extract purification with hexane and phase dispersive BOND ELUT C18. The extract was concentrated to 2 ml at 40°C, filtered through 0.22 µm membrane and analyzed by UPLC-MS/MS with electrospray ionization source in positive mode. The method has wide linear range of work, being evaluated were selectivity, matrix effect, CCα, CCβ, precision, trueness, LOD, LOQ and robustness. The detection limit was set between 2.5 to 25.0 mg/kg and the quantification limit between 5.0 to 50.0 mg/kg.

Results: The method showed repeatability and intermediate reproducibility conditions tested. Trueness was evaluated by the recovery values ranging between 98 and 107%. In assessing the robustness, the changes made in the extraction procedure were relevant level of concentration studied. The measurement uncertainty was estimated taking into account the uncertainty of the calibration curve and intermediate reproducibility of the data.

Significance: The proposed method is easy to apply with a capacity analysis of many samples in a single analytical run. All validation requirements have been assessed as suitable criteria of Commission Decision 2002/657/EC EU and Codex 2009, showing selectivity and specificity, precision and accuracy appropriate.

P3-42 Comparison of Six Agar Media for the Isolation of Non-O157 Shiga toxin-producing Escherichia coli

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) is a foodborne pathogen of increasing importance which can cause severe illness in humans. While non-sorbitol fermenting O157 STEC strains can be isolated without great difficulty, the isolation of non-O157 serotypes remains problematic. Bacterial isolation greatly depends upon the use of an adequate selective isolation media. While several STEC isolation media have been developed in the recent years, still little is known about their performance.

Purpose: The aim of this study was to evaluate the growth characteristics and morphology of a variety of 16 non-O157 STEC serotypes on six selective isolation media.

Methods: A minimum of two strains of the different common and less common non-O157 STEC serotypes were tested. In addition several commensal *E. coli* were included in the study. All the strains (10^4 CFU/ml) were plated onto six different selective and one non-selective isolation medium. All plates were enumerated after 24 hours of incubation at 37°C.

Results: Tryptone Soy Agar (TSA), Trypton Bile X-glucuronid agar (TBX) and Rainbow O157 agar were able to support the growth of all strains. However, Rainbow O157 agar showed a broad variety of different colours. Modified MacConkey agar as described by Possé (2008), Rapid *E. coli* O157, and CHROMagar STEC diminished the growth capability substantially. Furthermore, the commensal *E. coli* were mostly able to grow on a majority of the selective media.

Significance: Results clearly show that the isolation media do not meet the necessary requirements, namely to allow unhindered growth of all STEC strains and to inhibit all other interfering organisms, including commensal *E. coli*.

P3-43 Screening and Characterisation of Bacteriophage P-100 Insensitive* Listeria monocytogenes *Isolates in Austrian Dairy Plants

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Introduction: *Listeria monocytogenes* is one of the most important foodborne pathogens. Although there are different ways for decontamination, the use of bacteriophages is an option that becomes more and more popular. A phage to combat *Listeria* is P-100 which is also commercially available. P-100 is frequently used in food production nowadays but there is not much known about occurrence and the development of resistance against it.

Purpose: *L. monocytogenes* isolates obtained from Austrian dairies were screened for P-100 resistance and the frequency of occurring resistances before and after P-100 treatments was examined. Moreover the efficacy of different phage to bacteria ratios and the development of resistance were investigated and the detected insensitive isolates were molecular biological subtyped.

Methods: Cross-streak-tests (Miller 1998) were used for the screening of phage insensitive isolates. Measurement of optical density and CFU determinations using different phage to bacteria ratios, adsorption tests (Wendlinger et al. 1996) as well as PCR (Rossmanith et al. 2006 and Dourmith et al. 2004) and PFGE were carried out.

Results: Thirteen out of 502 isolates were found to be insensitive to P100. Seven of these isolates, all from 2001, derived from four different plants. Six insensitive isolates have been found in 2011 and 2012 in one dairy in which P100 was introduced in 2010. Before 2010 no insensitive isolates were found in this facility. None of the insensitive isolates showed significant changes in cell number or in growth curves compared to uninfected controls regardless of bacteria to virus ratio. The phage P-100 was not able to attach or replicate in insensitive isolates.

Significance: Incorrect use of bacteriophages can enhance the development of resistances and render treatment with phages less effective. Therefore parameters regarding efficacy of phages, occurrence and development of phage resistance have to be observed to avoid the development in future.

P3-44 Effect of High Hydrostatic Pressure Processing on Quality and Safety of Dairy and Meat Products

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Introduction: High hydrostatic pressure (HHP) treatment is an encouraging alternative to traditional thermal treatments for food preservation. HHP treatment can improve the safety and stability of HHP-processed foods by inactivating microorganisms as well as enzymes responsible for shortening the shelf life of a product. Using this technology nutritional attributes of the product remain virtually unaffected, providing better quality products.

Purpose: The aim of this work was to study the effect of high hydrostatic pressure treatment on the formation of biogenic amines (responsible for many pseudo-allergic food related reactions) in cheese and sausages, as well as to investigate the effect of HHP on the survival of *Listeria monocytogenes* after HHP treatment in milk and minced meat.

Methods: Samples were pressurized at 500 MPa/10 min. Biogenic amines were measured using an automatic amino acid analyzer. Aerobic plate count was determined on TPC agar. Number of *Listeria monocytogenes* (total and injured cells) was determined on Palcam agar using TAL method.

Results: 500 MPa/10 min HHP treated samples contained less biogenic amines compared to that of untreated cheese and Hungarian fermented sausage samples. High hydrostatic pressure reduced the microbial load of samples. High pressure values resulted considerable reduction of *Listeria monocytogenes* inoculated into minced pork meat.

Significance: HHP treatment improved the microbial quality and safety of the selected food products and it was effective in the reduction of biogenic amine content.

P3-45 High-Throughput QIAxcel-based Subtyping: Application to Enhanced Epidemiologic Surveillance of Campylobacter jejuni

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Introduction: Campylobacteriosis is the most common bacterial foodborne disease worldwide, with up to 1% of the population afflicted annually. Although Multi-Locus Sequence Typing (MLST), the gold standard for *C. jejuni* subtyping, has yielded important insights into epidemiology, population genetics and ecology of this pathogen, substantial knowledge gaps remain. We have developed Comparative Genomic Fingerprinting (CGF) which uses differences in accessory genome content for high resolution strain subtyping. CGF generates data highly concordant with MLST but with the discriminatory power required for a range of epidemiologic applications, including case cluster detection.

Purpose: In this study, we have adapted CGF to the QIAxcel Advanced capillary electrophoresis system to improve CGF data analysis and streamline the CGF workflow.

Methods: Direct comparison of agarose gel and capillary electrophoresis data was performed by analyzing the same set of eight 5-plex PCRs in the CGF assay for 96 samples. The presence/absence of each gene was scored and global concordance was assessed.

Results: A global concordance of 97.4% was obtained (3740 vs. 3840 data points). Critically, 100% of mismatches were due to problems with agarose gel data, including false positives/negatives and mis-identified bands. Integration of the QIAxcel Advanced system into the CGF workflow has improved CGF data analysis by increasing accuracy and sensitivity that led to development of CGF40 assay that increased discriminatory power by doubling the number of markers originally used.

Significance: In addition to improved CGF data analysis, integration of the QIAxcel Advanced platform into the CGF workflow resulted in a high-throughput and low-cost subtyping assay. Automation of documentation, data analysis and data reporting should facilitate its implementation into routine surveillance. The CGF method for *C. jejuni* subtyping has made it feasible to envision a national network for Campylobacter surveillance, representing the first step in a cohesive strategy for the prevention and control of campylobacteriosis.

P3-46 Comparision of the Baird-Parker Agar, Baird-Parker RPF and Petrifilm Staph Express in the Detection and Enumeration of* Staphylococcus *Coagulase-positive in Raw Milk

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Introduction: The reference methodology for *Staphylococcus* spp. enumeration recommends the use of Baird-Parker agar (BP); however, other culture media may produce results in shorter time, as Baird-Parker - RPF agar (bioMérieux, Marcy l'Etoile, France) and Petrifilm™ Staph Express Count Plate - PSE - (3M Microbiology Products, St. Paul, USA).

Purpose: This work was carried out in order to compare the efficiency of the aforementioned culture media for the enumeration of *Staphylococcus* spp.

Methods: The experiment was designed in random blocks and the media were compared by the *t* test. Thirty-six samples of raw milk were analyzed using Baird-Parker agar (BP), Baird-Parker - RPF agar and Petrifilm™ Staph Express Count Plate – PSE.

Results: Mean *Staphylococcus* spp. count obtained by PSE (2.50 log CFU/ml) was lower ($P < 0.05$) than those by BP (4.12 log CFU/ml) and RPF (3.86 log CFU/ml), being the latter two values considered similar ($P > 0.05$).

Significance: The results showed viable the use of RPF, but not PSE, replacing BP for *Staphylococcus* spp. enumeration without altering the accuracy of the analysis.

P3-47 Time and Order Application Effects of Mild Temperatures and Eugenol on the Growth Inhibition of Escherichia coli K12 and Staphylococcus carnosus

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Introduction: Food preservation requires sequencing the application of hurdles; few studies focus on the effects of order application and time delays over their antimicrobial effectiveness.

Purpose: The influence of sequential application on the effectiveness of preservation hurdles was investigated when populations of *Escherichia coli* K12 and *Staphylococcus carnosus* were exposed to the combination of mild heating (42°C), mild chilling (20°C) and eugenol applied in different order and time.

Methods: Strains were exposed either to 42 or 20°C initially and to eugenol 3 and 8h later. Sub-inhibitory concentrations of eugenol were applied at 2.25 mM against *E. coli* K12 and 2.30 mM against *S. carnosus*. When eugenol was initially applied, strains were at optimal growth temperature (37°C), and then the incubation temperature was raised to either 42°C or lowered to 20°C after 3 and 8h. Bacterial survival was monitored by CFU enumeration with direct plate counts. The inactivation kinetics of the treated strains were compared by calculating the area between the control curve and the inactivation curve, denominated antimicrobial effect parameter (I_{AE}).

Results: The sequential application of eugenol and 20°C with 8h delay was more effective than faster hurdle application (3h delay and simultaneous) against *E. coli* K12. The intensity of the antimicrobial efficiency dropped against *E. coli* K12 when eugenol was sequentially applied at 42°C, but against *S. carnosus* changes in the effectiveness were not observed. Measurement of the concentration of eugenol in the aqueous phase and calculation of eugenol partition coefficient (K) at 37, 20 and 42°C suggested the effects of temperature on the relative permeability of eugenol across the lipid bacterial cell membrane.

Significance: Food processors should consider time delays and order application of mild preservation hurdles to accurately evaluate their effectiveness and optimize their application along food production lines.

P3-48 Observational Comparison of Food Safety Behaviours Implemented by Young-Adult and Older-Adult Consumers

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Introduction: Food safety research suggests young-adults (18-25 years) and older-adults (≥ 60 years) implement more food safety malpractices during domestic food preparation than other consumer groups. However, data detailing actual behavioral malpractices and comparisons between these consumers is lacking. Such data would allow for the development of targeted consumer food safety education.

Purpose: The study aim was to determine the actual food safety behaviours of these two consumer groups commonly implicated with foodborne illness.

Methods: A model domestic kitchen equipped with ceiling-mounted digital cameras allowed for the observation of food preparation sessions by young-adults (aged 18-25 years) (YA) (n = 60) and older-adults (aged ≥ 60 years) (OA) (n = 100). The preparation of a recipe handling foods commonly associated with pathogen contamination allowed for the observation of food safety practices using a predetermined behavioural-checklist.

Results: Cumulatively, food safety malpractices were observed to be widespread among both groups. With the majority (85% YA, 83% OA) failing to adequately implement hand washing/drying immediately after handling raw chicken and a comparable proportion (15% YA, 20% OA) were observed washing raw chicken. Differences in behavior were determined between consumer groups. Failure to adequately clean/change chopping board between preparation of raw chicken and ready-to-eat food was determined to be significantly greater ($P < 0.05$) among older-adults (64%) than young-adults (28%), whereas, failure to wash salad produce was significantly greater ($P < 0.05$) among young-adults (78%) than older-adults (24%). Furthermore, in both consumer groups, significant differences ($P < 0.05$) were determined between gender and observed behavior, with males implementing more food safety malpractices than females.

Significance: Consequently, young-adult and older-adult consumers may be at risk of foodborne illnesses due to observed food safety malpractices. Results may suggest a need for educational strategies to not only target different consumer groups due to observed variations in behaviour, but also according to gender. Overall, this study increases our understanding of the food safety behaviours of two consumer groups and provides important information to inform future food safety initiatives.

P3-49 Partial Purification and Characterization of a Bacteriocin Produced by Lactobacillus paraplantarum FT259, Isolated from Brazilian Semi-hard Artisanal Cheese

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Introduction: Bacteriocins (antimicrobial peptides synthesized by bacteria) are of interest in the food industry to control foodborne pathogens such as *Listeria monocytogenes*. These peptides can be degraded by digestive enzymes, representing a potentially safe alternative to classical food preservatives. The bacteriocinogenic *Lactobacillus paraplantarum* FT259 has probiotic potential, as demonstrated by previous studies, and this stimulates researches on the antimicrobial peptide produced.

Purpose: The aim of this study was to partially purify and characterize the bacteriocin produced by *L. paraplantarum* FT259.

Methods: *L. paraplantarum* FT259 bacteriocin was purified from 1400 ml of neutralized cell-free supernatant of a MRS broth culture (37°C / 24h). Amberlite® XAD-16 resin (Sigma-Aldrich, USA) was used for bacteriocin purification, followed by solid phase extraction – SPE (HF Mega Bond Elut C18 10g-60ml, Varian, USA), with isopropyl alcohol as eluent. Extracts containing bacteriocins were analysed by glycine SDS-PAGE (sodium dodecyl sulfate - polyacrylamide gel electrophoresis) and revealed by silver staining and biological indicator. Antimicrobial activity in each step was measured by critical dilution assay using *L. monocytogenes* as indicator strain and expressed as arbitrary units per ml (AU/ml). Genomic DNA was obtained using Illustra bacteria genomicPrep mini spin kit (GE Healthcare, UK) and polymerase chain reaction (PCR) was carried out to detect genes related to plantaricin production (*plan*A, *plan*B, *plan*C, *plan*D, *plan*EF, *plan*G, *plan*I, *plan*J, *plan*K, *plan*N, *plan*NC8, *plan*S, *plan*W). Amplicons were analysed in agarose gel electrophoresis and stained with ethidium bromide.

Results: The partially purified bacteriocin produced by *L. paraplantarum* FT259 was obtained with a yield of 45.7% (1,024,000 AU). By SDS-PAGE, the molecular weight of the bacteriocin was estimated to be between 3.5 and 8.5 kDa. Only the cluster *plan*ABCD (signal transduction pathway) and the plantaricin NC8 structural gene were detected by PCR, suggesting the production of plantaricin NC8 by this strain.

Significance: Characterization of the bacteriocin produced by *L. paraplantarum* FT259, as well as its purification process and the knowledge of genes related to bacteriocin production will help to design strategies for its biotechnological application. Acknowledgments: FAPESP (process # 2011/11983-0), CNPq (process # 480772/2011-8).

P3-50 Antimicrobial Resistance of Campylobacter jejuni and Campylobacter coli Isolated from Children and from Poultry Meat in Poland

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Introduction: *Campylobacter jejuni* and *Campylobacter coli* belong to the leading bacterial cause of human gastroenteritis in the world. Epidemiological data suggest that contaminated products of animal origin, especially poultry, contribute significantly to campylobacteriosis. Antibiotic resistance in *Campylobacter* is emerging globally and recognized by the WHO, as a problem of public health importance.

Purpose: The aim of this study was to determine the antimicrobial resistance *C. jejuni* and *C. coli* isolates from hospitalized children and from poultry meat in Poland.

Methods: Research was conducted on 60 *Campylobacter* spp. strains (48 *C. jejuni*, 12 *C. coli*) obtained from stool samples from children with diarrhea at Infectious Diseases Hospital in Bydgoszcz and on 65 *Campylobacter* spp. strains (34 *C. jejuni*, 31 *C. coli*) isolated from raw chicken meat from retail trade in Bydgoszcz. MIC value for erythromycin, azithromycin, ciprofloxacin, and tetracycline were determined with the E-test method.

Results: The prevalence of resistance among *C. jejuni* and *C. coli* isolates from poultry meat was as follows: ciprofloxacin (61%, 71%), tetracycline (46.4%, 43.6%), erythromycin and azithromycin (3.0%, 1.8%). All of the analyzed from children isolates were susceptible to macrolides. 50% of them were resistant to tetracycline and 64% to ciprofloxacin. A higher prevalence of multidrug resistant *Campylobacter* strains has been observed for meat isolates than for human isolate.

Significance: The present study pays attention to the appearing problem of macrolide (erythromycin, azithromycin) resistance of *Campylobacter* strains isolated from poultry. Contaminated food can be the transmission vehicle for these strains on people. The resistant *Campylobacter* has been detected in products of animal origin and the food chain, posing a risk to human health. The increasing resistance to (fluoro)quinolones, tetracycline and erythromycin of *C. coli* and *C. jejuni* strains, might compromise the efficacy of the treatment.

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P3-51 ESIA ONE DAY: A Unique and Simple Method for Cronobacter spp. Detection in Large Sample Size

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Introduction: *Cronobacter* spp is an enterobacteria which was blamed in cases of neonatals infections conveyed by infantile dried milk. It is therefore mainly detected in infant food products, such as powdered milk formula and in the manufacturing environment of the food sector. bioMérieux propose a new simplified protocol for samples from 50g to 300g with a new range of confirmation tests. The final result could be in 48h with a proprietary confirmation test.

Purpose: The aim was to evaluate the performances of ESIA One Day™ versus the reference method for the detection of *Cronobacter spp*in powder formula (PIF) and raw material on 50g to 300g samples.

Methods: The comparative study was performed by detecting Cronobacter in PIF and raw materials with ESIA One Day™ method and ISO/TS 22924 standard protocol. With the ISO standard method, samples were diluted to one tenth when the alternative method allowed a quarter enrichment factor in buffered peptone water supplemented with vancomycin. After an incubation time of 18 ± 2 hours at 37 ± 1°C, enriched broth was isolated on ESIA™ medium. Plates were incubated 21 hours at 44±1°C. Typical colonies were confirmed directly by conducting Fast Crono Confirmation test, by performing a subculture by spot or streak on agar ESIA Confirmation, by inoculating ID 32E gallery or by Rapid ID 32E.

Results: The study proved that there were no significant differences between ESIA One Day™ method and ISO/TS: 22924 standard for large samples. Sensitivity and specificity of the alternative method are equivalent to the reference method. Moreover, the shorter confirmation protocol could give a result in 5 minutes.

Significance: ESIA One Day™ method provides a simple and rapid solution for an accurate detection of *Cronobacter* spp in large sample size with a two-day solution.

P3-52 Exposure of Ready-to-Eat Pomegranate Arils to Vapors of 'Brandy' or Distillery Ethanol

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Introduction: The limited shelf life of ready-to-eat pomegranate arils necessitates the use of antimicrobial packaging.

Purpose: Evaluation of the effect of brandy and ethanol vapors on microbial, physical, and sensorial attributes of pomegranate arils stored at different temperatures.

Methods: Arils (10 g) and cotton/cellulose absorbent cloths (2 x 2 cm) supplemented with ethanol or brandy (36% v/v alcohol) were placed into compartmentalized Petri-dishes in two sections. Samples were divided into 5 experimental assays: (a) arils + water (control); (b) arils + 1 ml of ethanol; (c) arils + 2 ml of ethanol; (d) arils + 1 ml of brandy; (iv) arils + 2 ml of brandy, were packaged under aerobic conditions in perforated bags and stored at 4, 10, and 20°C (n = 4). Total viable counts, lactic acid bacteria, and yeasts and molds were enumerated during storage. Changes in gases, pH, color and multi-spectral image attributes, weight loss, texture, and sensory properties of arils were also evaluated.

Results: LAB and yeasts-molds co-dominated during storage. Vapors produced by the two antimicrobials significantly inhibited ($P < 0.05$) the growth of the above three microbial groups, at all storage temperatures. At 4°C, when population of TVC on controls was 6.9 log CFU/g (day 23), the respective counts on arils treated with ethanol or brandy followed the order: 4.9 log CFU/g (1 ml of ethanol)> 3.9 log CFU/g (1 ml of brandy)> 2.2 log CFU/g (2 ml of ethanol)> 1.2 log CFU/g (2 ml of brandy). Moreover, arils exposed to brandy and ethanol vapors showed lower weight loss (%) and limited variation in firmness compared to controls regardless of storage temperatures, also resulting in delayed quality decay. Color measurements revealed that arils exposed to brandy vapors showed more intense red color compared to controls and samples exposed to ethanol.

Significance: Such preservation methods may raise new perspectives on mild antimicrobial packaging in order to extend shelf life of perishable minimally processed fruits like pomegranate arils.